Psyche

Coleoptera

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Research Article

Integrative Taxonomy, Phylogeny, and New Species of the Weevil Genus *Onyxacalles* Stüben (Coleoptera: Curculionidae: Cryptorhynchinae)

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A molecular phylogeny of the western Palearctic weevil genus *Onyxacalles* Stüben, 1999 is presented, combining two mitochondrial genes (COI and 16S) in a Bayesian analysis. Based on molecular data, *Onyxacalles pyrenaeus* Boheman, 1844 is transferred into the genus *Kyklioacalles* Stüben 1999 (*K. fausti* group) and—in an integrative taxonomy framework—the interaction between morphology and molecular analysis is illustrated. The species of *Onyxacalles* s. str. are assigned to three new species groups, *O. henoni*, *O. luigionii*, and *O. portusveneris* groups. The distribution of the related species in the Mediterranean area is illustrated with values of COI and 16S p-distances. Three new species are described and distinguished from their related species: *Onyxacalles nuraghi* Stüben sp.n. from Italy (Sardinia), *Onyxacalles torre* Stüben and Astrin sp. n. from France (Corsica) and *Onyxacalles vilae* Stüben sp. n. from Croatia (Velebit Mts.). A catalogue of all 20 species of *Onyxacalles* is given, and a key is finally presented combined with image stacking of the habitus and aedeagus for all species.

1. Introduction

Together with a number of other genera, the genus *Onyxacalles* Stüben, 1999 (Curculionidae: Cryptorhynchinae) was separated by Stüben [1] from the formerly excessively broadly circumscribed genus *Acalles* Schoenherr, 1825 as a group with initially 8 species. Since then, many new species of *Onyxacalles* have been described, mainly from Spain and North Africa. These discoveries were supported by the morphological finding that the three species from the Canary Islands belong to this genus [1, 2], a thesis that gained support from recent molecular analysis and has contributed to the new subgenus *Araneacalles* Stüben and Astrin [3]. This closed a "biogeographical gap" (between the Pyrenees and northwestern Africa) as a direct consequence of target-oriented collecting activities and descriptions of many new *Onyxacalles* species over the past decade [4–9]. Thus, including the new species presented in this work, the genus now comprises 20 valid species.

Most species of *Onyxacalles* are found in the west Mediterranean area and on the Macaronesian Islands. Only one species, *Onyxacalles croaticus* (H. Brisout, 1897) [10], reaches Eastern Europe (Carpathians); another species, *O. amasyaensis* Wolf, 2001, was described from Turkey, but could be a synonym of *Onyxacalles denominandus* A. and F. Solari, 1907. This species richness in the west Mediterranean is well founded in the ecological preferences of *Onyxcalles*.

As "nocturnal goblins of the last primeval forests" [7], the species of *Onyxacalles* are not common in the often disturbed landscapes of the Iberian Peninsula and North Africa [11, 12]. These conditions provide good maps of relictual vegetation and information about the habitats, allowing us to trace these nocturnal Cryptorhynchinae in the dark and humid relicts of natural forests under big

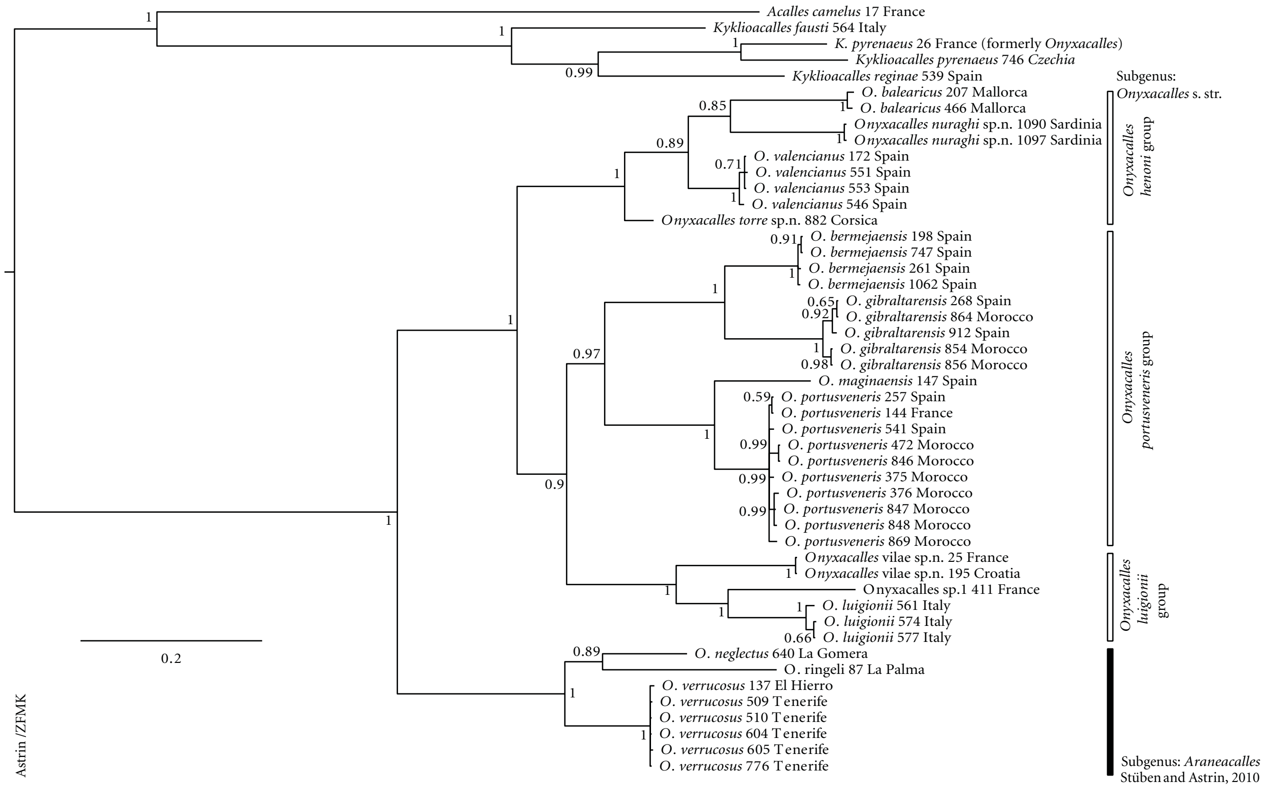

1
Acalles camelus 17 France
1
Kyklioacalles fausti 564 Italy
1
K. pyrenaeus 26 France (formerly Onyxacalles)
Kyklioacalles pyrenaeus 746 Czechia
0.99
Kyklioacalles reginae 539 Spain
Subgenus:
Onyxacalles s. str.
0.85
O. balearicus 207 Mallorca
1
O. balearicus 466 Mallorca
Onyxacalles nuraghi sp.n. 1090 Sardinia
1
Onyxacalles nuraghi sp.n. 1097 Sardinia
0.89
Onyxacalles henoni group
0.71
O. valencianus 172 Spain
1
O. valencianus 551 Spain
O. valencianus 553 Spain
1
O. valencianus 546 Spain
Onyxacalles torre sp.n. 882 Corsica
0.91
O. bermejaensis 198 Spain
O. bermejaensis 747 Spain
1
O. bermejaensis 261 Spain
1
O. bermejaensis 1062 Spain
0.65
O. gibraltarensis 268 Spain
0.92
O. gibraltarensis 864 Morocco
O. gibraltarensis 912 Spain
1
1
O. gibraltarensis 854 Morocco
0.97
0.98
O. gibraltarensis 856 Morocco
O. maginaensis 147 Spain
0.59
O. portusveneris 257 Spain
O. portusveneris 144 France
O. portusveneris 541 Spain
1
0.99
O. portusveneris 472 Morocco
O. portusveneris 846 Morocco
0.99
O. portusveneris 375 Morocco
0.9
O. portusveneris 376 Morocco
0.99
O. portusveneris 847 Morocco
O. portusveneris 848 Morocco
O. portusveneris 869 Morocco
Onyxacalles portusveneris group
1
Onyxacalles vilae sp.n. 25 France
1
Onyxacalles vilae sp.n. 195 Croatia
1
Onyxacalles sp.1 411 France
1
O. luigionii 561 Italy
1
O. luigionii 574 Italy
0.66
O. luigionii 577 Italy
Onyxacalles luigionii group
0.2
0.89
O. neglectus 640 La Gomera
O. ringeli 87 La Palma
1
O. verrucosus 137 El Hierro
O. verrucosus 509 Tenerife
O. verrucosus 510 Tenerife
1
O. verrucosus 604 Tenerife
O. verrucosus 605 Tenerife
O. verrucosus 776 Tenerife
Subgenus: Araneacalles
Stüben and Astrin, 2010
Astrin /ZFMK

Figure 1: Bayesian consensus tree (50% majority rule) for COI and 16S.

oak trees and behind a dense jungle of *Smilax aspera* L. Therefore, sifting by day is not the ideal method of catching *Onyxacalles*. With their long legs, *Onyxacalles* species climb trees by night and were fogged by us from the canopy of the *laurisilva* on the Canary Islands of Tenerife and La Gomera [13, 14]. The flightless *Onyxacalles* are highly specialised woodlander inhabitant, and because of their restricted dispersal (above all on the European continent), they are an ideal bioindicator of original forest, highlighting that protection and sustainable development should concern us as entomologists and conservationists.

Moreover, with the study of the cryptic and similar *Onyxacalles* species, the taxonomist, especially the morphologist, enters a minefield. Due to intraspecific variability, the few external characteristics that are suitable for a differential diagnosis do not guarantee a reliable (re)identification of species: if the tufts of bristles exist or not, if the elytra are short oval or elongated, or if the midgroove of pronotum is more or less distinct—all these are important pointers, but are not conclusive. All *Onyxacalles* species have the hooked apex of the aedeagus in common, as the genus name implies (cf. Figures 9(a)–9(l)). However, a complex internal sac of the aedeagus (endophallus) does not exist—as it is typical for *Kyklioacalles*, *Dichromacalles,* or most genera from Macaronesia. Here, we are quickly stretched to our limits of a descriptive morphology.

With the "**M**olecular **W**eevil **I**dentification" Project (see below), we tread a path towards an integrative taxonomy [15–21]. Molecular taxonomy is not only an addition or an accessory "immunisation" to confirm morphological results. The integration of the two approaches must be done with the intention of *falsifying* and, if so, it can lead to a new way of viewing morphology: *interspecific* characteristics are discovered that are not regarded as belonging to *intraspecific* variability, or—more often—substituting the "human eyes," the way we look at things. Instead of "anthropocentric conspicuities" (e.g., forms, colours, sizes), we focus on constitutive characteristics (e.g., apomorphies and homologies) that were previously overlooked in our diagnostic keys. An example is the latest history of the science behind the original species *Acalles pyrenaeus* Boheman, 1844 (see below).

Another telling example is the present classification of *Onyxacalles* s. str. into two species groups: the *O. luigionii*- and *O. pyrenaeus*-groups, based on the bristles of the elytral intervals (cf. [1, 8]). This hypothesis is no longer tenable, and this has nothing to do with superficial diagnosis of affinities or inaccurate observations. First, molecular analysis of related species reveals new informal species groups (see Figure 1) and makes evidence available to the morphologist, who then looks for new external characteristics. These are inconspicuous paradigm changes with a high impact [22], because the "puzzles," in this case the *species*, which were previously pressurised within the framework of "normal science" (and more and more frequently caused difficulties) continue, but the emphasis of the characteristics under the new molecular phylogenetic paradigma has changed (see "Catalogue of *Onyxacalles*"), and new characteristics are discovered (see "Key to the species of *Onyxacalles*").

The assumption that the morphologist should have seen a characteristic must be abandoned in favour of the question: would the morphologist be able to "*see*" it?

2. Catalogue of *Onyxacalles*

Species included in the molecular analysis are printed in bold (l.t. = type locality).
Genus: Onyxacalles Stüben, 1999a.
177 type species *Acalles luigionii* A. and F. Solari, 1907.
Subgenus: *Onyxacalles* s. str.
Onyxacalles henoni Group

balearicus Stüben, 2005: 115, Spain: Majorca (l.t.)

croaticus H. Brisout de Barneville, 1867: 62 (*Acalles*), Croatia (l.t.), Austria, Czech Republic, Germany, Poland, Slovakia, Slovenia

hannibali Germann, 2004: 118, Tunisia (l.t.)

henoni Bedel, 1888: 36 (*Acalles*), Algeria: Mt. Edough (l.t.).

nuraghi Stüben **sp. n.**, Italy: Sardinia (l.t.).

torre Stüben and Astrin **sp. n.**, France: Corsica (l.t.).

valencianus Germann, 2005: 104, Western Spain (l.t.).

Onyxacalles portusveneris Group

bermejaensis Stüben, 2001: 145, Spain (l.t.).

gibraltarensis Stüben, 2002: 206, Morocco (l.t.), Spain.

maginaensis Stüben, 2004: 120, Southern Spain (l.t.).

portusveneris Mayet, 1903: 74 (*Acalles*), France (l.t.), Spain, Morocco

seguraensis Stüben, 2003a: 201, Spain.

Onyxacalles luigionii Group

luigionii A. and F. Solari, 1907: 521 (*Acalles*), Central (l.t.) and Southern Italy.

vilae Stüben **sp. n.**, Croatia (l.t.), France: Isère (perhaps Austria and Slovenia).

cf. luigionii, France: Alpes Maritimes.

Incertae Sedis

denominandus A. and F. Solari, 1907: 523 (*Acalles*), Turkey (l.t.).

porcheti Hoffmann, 1935: 162 (*Acalles*), France: Pyrenees (probably a synonym of *A. luigionii,* see also Stüben 2007: 149).

amasyaensis Wolf, 2001: 150, Turkey (l.t.). (probably a synonym of *Acalles denominandus*)

Subgenus: *Araneacalles* Stüben and Astrin, 2010: 78 type species *Acalles verrucosus* Wollaston, 1863.

neglectus Kulbe, 1999: 193, Canary Islands: La Gomera (l.t.), El Hierro.

ringeli Kulbe 1999: 196, Canary Islands: La Palma (l.t.).

verrucosus Wollaston, 1863: 219 (*Acalles*), Canary Islands: Tenerife (l.t.), El Hierro.

3. Materials and Methods

The molecular analysis is based on 45 (43 after transfer of *O. pyrenaeus*, see below) individuals in 15 species or putative species of *Onyxacalles* and on 5 outgroup species (Cryptorhynchinae from 4 other genera; only the two closer genera are shown in the tree for better visualisation, while the 2 more distant ones were removed; see Table 1). Most sequences have been published in Astrin et al. (2012). Collecting and vouchering information as well as GenBank accession numbers are given in Table 1. Voucher specimens and extracted genomic DNA are deposited at the Biobank of the ZFMK (Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany).

DNA extraction was carried out on samples preserved in ethanol or on dried material, using Macherey-Nagel Nucleo Spin Tissue kits (Dueren, Germany) or BioSprint 96 kits (Qiagen, Hilden, Germany). We extracted DNA from either 2-3 legs, head and prothorax, or sometimes also the whole weevil, depending on size and conservation of the sample. PCR reaction mixes (50 μL) contained 125 nmol $MgCl_2$, 5 μL 10x PCR-buffer, 25 pmol of forward and reverse primer each, 5 pmol dNTPs, 1.75 units of *Taq* polymerase, and 5 μL total undiluted DNA template. The lab chemicals were purchased from Sigma-Aldrich (Steinheim, Germany). We used the Qiagen (Hilden, Germany) Multiplex PCR kit in cases where the regular protocol failed. PCR primers were taken from Astrin and Stüben (2008; COI is based on the Folmer et al. [23] region; 16S is based on the Crandall and Fitzpatrick (1996) region). Primer sequences were as follows: LCOI490-JJ (COI forward, fw) 5′-CHACWAAYCATAAAGATATYGG-3′, HCO2198-JJ (COI reverse, rev) 5′-AWACTTCVGGRTGVCCAAARAATCA-3′; 16S-ar-JJ (16S fw, erroneously as "rev" in [24]) 5′-CRCCTGTTTATTAAAAACAT-3′, 16S-1472-JJ (16S rev) 5′-AGATAGAAACCRACCTGG-3′. Thermal cycling was performed on blocks of the type GeneAmp PCR System 2700 (Applied Biosystems, Foster City, CA, USA). PCR program for 16S: first cycle set (15 repeats): 35 s denaturation at 94°C, 35 s annealing at 55°C (−1°C per cycle) and 60 s extension at 72°C. Second cycle set (25 repeats): 35 s denaturation at 94°C, 35 s annealing at 40°C, and 50 s extension at 72°C. PCR program for COI: same as for 16S, but annealing temperatures at 70°C and 55°C, with a decrease of 2°C per cycle in the first cycle set. Double-stranded sequencing was carried out by a sequencing facility (Macrogen, South Korea, and Netherlands) using the same primers as in PCR.

DNA sequence alignment was performed manually (COI) or using the MUSCLE ver. 3.6 programme [28] (16S), run with default parameters. Sequence length was 554 bp for 16S (aligned; longest sequence: 544 bp; shortest: 533 bp) and 658 bp for COI, for concatenated sequence data 1212 bp. The 16S alignment comprised 29 positions with

Table 1: Collecting data, vouchers, and GenBank accession numbers for the material analysed in this study. All specimens determined by P. E. Stüben, 2010 and 2011. Vouchers (DNA, morphology) are kept at the ZFMK Biobank. Most sequences have been published (or are reviewed) in Astrin et al. 2012 [21]. GenBank accession numbers of new sequences in this study start with "JN…". Taxonomic changes with regard to this publication are printed in brackets (old name).

Taxon	Collecting data	DNA voucher	COI 16S
Acalles camelus (Fabricius 1792)	France: Isère, 2 km SE Lans en Vercors, Montagne de Lans; N45°06′45″ E05°36′21″, 1352 m; Abies, Fagus, Fraxinus, 2005, Stüben	ZFMK-DNA-JJ0017, ZFMK-TIS-cI0026cam	EU286282 EU286447
Acallorneuma doderoi A. and F. Solari 1908	Italy: Sicilia (PA), 6 km SW Godrano, Bosco Ficuzza, Mte. Rocca Busambra; N37°51′38″ E13°23′24″, 1200 m; Quercus, Fraxinus, 2002, Stüben	ZFMK-DNA-JJ0065, ZFMK-TIS-cS0082dod	EU286292 EU286457
Cryptorhynchus lapathi (Linné 1758)	Germany: Bienen bei Rees, Altrheinarm, 2004, Scharf	ZFMK-DNA-JJ0214, ZFMK-TIS-cD0354lap	EU286360 EU286523
Kyklioacalles fausti (Meyer 1896) [25]	Italy: Campania, Cilento, 6 km SE Vallo d. Lucania, M. Sacro o Gelbison; N40°12′41″ E15°19′42″, 1544 m; Fagus, 2008, Stüben	ZFMK-DNA-JJ0564, ZFMK-TIS-cI625fau	GU213776 GU213772
Kyklioacalles pyrenaeus (Boheman 1844) [26] (gen. Onyxacalles)	France: Isère, 14 km N Grenoble, Massif de la Chartreuse, NW Col de Porte; N45°18′40″ E05°45′17″, 1649 m; Abies, Fagus, Fraxinus, 2005, Stüben	ZFMK-DNA-JJ0026, ZFMK-TIS-cI0035pyr	GU988172 GU987762
Kyklioacalles pyrenaeus [26] (gen. *Onyxacalles*)	Czech Republic: W Bohemia (KT), Balkovy, Doubrava Hill (6545), 2008, Kresl	ZFMK-DNA-JJ0764, ZFMK-TIS-cCz798pyr	GU981555 GU981506
Kyklioacalles reginae Stüben 2003	Spain: Teruel, S. Javalambre, Fuente la Risca near Arcos de las Salinas; N39°59′56″ W01°01′21″, 1121 m; Amelanchier ovalis, Acer monspessulanum, Erinacea anthyllis, Ulex, 2008, Stüben	ZFMK-DNA-JJ0539, ZFMK-TIS-cE600reg	GU981544 GU981495
Onyxacalles balearicus Stüben 2005	Spain: Mallorca, 3 km SE Lluc, Sra. de Tramuntana, Sa Maleta; N39°48′47″ E02°53′23″, 571 m; Quercus ilex, 2004, Stüben	ZFMK-DNA-JJ0207, ZFMK-TIS-cE0168bal	EU286357 EU286521
Onyxacalles balearicus Stüben 2005	Spain: Mallorca, 11 km NE Lluc, Sra. de Tramuntana; N39°52′03″ E02°58′20″, 107 m; PT, Smilax aspera, Quercus ilex, 2004, Stüben	ZFMK-DNA-JJ0466, ZFMK-TIS-cE0294bal	GU988348 ---------
Onyxacalles bermejaensis Stüben 2001	Spain: Andalucía, 11 km S Ronda, Sierra de las Nieves; N36°39′51″ W05°05′01″, 1047 m; Quercus ilex, 2005, Stüben	ZFMK-DNA-JJ0198, ZFMK-TIS-cE0167ber	EU286350 EU286514
Onyxacalles bermejaensis Stüben 2001	Spain: Málaga, 9 km SE Ubrique, Sierra de Líbar; N36°36′52″ W05°23′16″, 663 m; Quercus ilex, Ceratonia, 2007, Stüben	ZFMK-DNA-JJ0261, ZFMK-TIS-cE0194ber	GU988244 GU987827
Onyxacalles bermejaensis Stüben 2001	Spain: Málaga, NW Marbella, Sierra de las Nieves; N36°39′52″ W05°04′57″, 1043 m; Quercus ilex, Echinodera spinosa, 2009, Stüben	ZFMK-DNA-JJ0747, ZFMK-TIS-cE778ber	GU988506 GU988066
Onyxacalles bermejaensis Stüben 2001	Spain: Prov. Málaga, Algatocín, near Opayar; N36°34′39″ W05°18′13″, 576 m, Quercus sp., 17.8.2010, Stüben	ZFMK-DNA-JJ1062, ZFMK-TIS-cES1062	JN121398 ---------
Onyxacalles gibraltarensis Stüben 2002	Spain: Cádiz, 10 km SW Algeciras, El Bujeo; N36°04′10″ W05°31′48″, 257 m; Quercus suber, 2007, Stüben	ZFMK-DNA-JJ0268, ZFMK-TIS-cE0206gib	GU988249 GU987832
Onyxacalles gibraltarensis Stüben 2002	Morocco: Rif, SW Oued-Laou, river, O. Laou; N35°17′47″ W05°13′38″, 210 m; Quercus suber, Smilax, Arbutus, 2009, Stüben	ZFMK-DNA-JJ0854, ZFMK-TIS-cE897gib	GU988577 GU988137
Onyxacalles gibraltarensis Stüben 2002	Morocco: W Sebta, vir. Biutz; N35°53′04″ W05°24′08″, 337 m; Quercus suber, Smilax, Arbutus, 2009, Stüben	ZFMK-DNA-JJ0856, ZFMK-TIS-cE899gib	GU988578 GU988138
Onyxacalles gibraltarensis Stüben 2002	Morocco: S Ksar-es-Seghir; N35°45′16″ W05°30′49″, 278 m; Pistacia, Quercus suber, 2009, Stüben	ZFMK-DNA-JJ0864, ZFMK-TIS-cE907gib	GU988584 GU988144
Onyxacalles gibraltarensis Stüben 2002	Spain: Cádiz, Los Barrios, Alcornocales N.P., between Facinos, Río Las Cañas and Mantera Torero; Olea europaea, 2009, Torres	ZFMK-DNA-JJ0912, ZFMK-TIS-cE949gib	GU988608 ---------
Onyxacalles luigionii (A. & F. Solari 1907)	Italy: Campania, Cilento, 6 km SE Vallo d. Lucania, M. Sacro o Gelbison; N40°12′41″ E15°19′42″, 1544 m; Fagus, 2008, Stüben	ZFMK-DNA-JJ0561, ZFMK-TIS-cI622lui	GU988407 GU987967

Table 1: Continued.

Taxon	Collecting data	DNA voucher	COI 16S
Onyxacalles luigionii (A. & F. Solari 1907)	Italy: Campania, Monti Picentini, 9 km N Acerno, Piano Laceno; N40°48′58″ E15°07′35″, 1210 m; Fagus, 2008, Stüben	ZFMK-DNA-JJ0574, ZFMK-TIS-cI635lui	GU988417 GU987977
Onyxacalles luigionii (A. & F. Solari 1907)	Italy: Basilicata, Monte Pollino, 9 km SE Rotonda, Rif. de Gasperi; N39°54′37″ E16°07′15″, 1486 m; Fagus, 2008, Stüben	ZFMK-DNA-JJ0577, ZFMK-TIS-cI638lui	GU988418 GU987979
Onyxacalles maginaensis Stüben 2004	Spain: Andalucía, 28 km E Jaén, Sierra Magina; N37°43′21″ W03°29′11″, 1600 m; Quercus ilex, 2005, Stüben	ZFMK-DNA-JJ0147, ZFMK-TIS-cE0169mag	EU286327 EU286491
Onyxacalles maginaensis Stüben 2004	Spain: Almería, 11 km NW Laujar de Andarax, Sierra Nevada, Bayárcal; N37°02′27″ W03°00′12″, 1291 m; Quercus ilex, 2007, Stüben	ZFMK-DNA-JJ0257, ZFMK-TIS-cE0187mag	submitted to GenBank
Onyxacalles maginaensis Stüben 2004	Spain: Teruel, S. Javalambre, Fuente la Risca near Arcos de las Salinas; N39°59′56″ W01°01′21″, 1121 m; Amelanchier ovalis, Acer monspessulanum, Erinacea anthyllis, Ulex, 2008, Stüben	ZFMK-DNA-JJ0541, ZFMK-TIS-cE602mag	GU988390 GU987950
Onyxacalles neglectus Kulbe 1999	Spain: Canary Islands, La Gomera, S Hermigua, El Cedro, Las Mimbreras; N28°07′27″ W17°13′26″, 901 m; laurisilva, 2008, Astrin and Stüben	ZFMK-DNA-JJ0640, ZFMK-TIS-cE713neg	FJ716525 GU988014
Onyxacalles nuraghi sp.n.	Italy: W-Sardinia, E Macomer: above Lei; N40°19′54″ E08°53′49″, 1020 m; Quercus, Acer monspessulanum, 4.10.2010, Stüben	ZFMK-DNA-JJ1090, ZFMK-TIS-cIT1090	JN642097 JN121399
Onyxacalles nuraghi sp.n.	Italy: W-Sardinia, E Macomer: above Lei; N40°19′17″ E08°53′52″, 586 m; Quercus ilex, 7.10.2010, Stüben	ZFMK-DNA-JJ1097, ZFMK-TIS-cIT1097	JN642098 JN121300
Onyxacalles portusveneris (Mayet 1903) [27]	France: Gard, 15 km NE Nimes, Pont du Gard, Collias; N43°57′03″ E04°28′59″, 68 m; Quercus ilex, 2006, Stüben	ZFMK-DNA-JJ0144, ZFMK-TIS-cF0166por	EU286326 EU286490
Onyxacalles portusveneris (Mayet 1903) [27]	Morocco: Rif Mts., 10 km W Ketama = Issague; N34°57′40″ W04°40′51″, 1600 m; Prunus lusitanica, 2001, Stüben	ZFMK-DNA-JJ0375, ZFMK-TIS-cM480por	GU988311 ---------
Onyxacalles portusveneris (Mayet 1903) [27]	Morocco: M-Atlas, 10 km S Ain-Leuh; N33°13′48″ W05°20′50″, 1700 m; Quercus ilex, Rubus, Cedrus, 2002, Stüben	ZFMK-DNA-JJ0376, ZFMK-TIS-cM481por	GU988312 ---------
Onyxacalles portusveneris (Mayet 1903) [27]	Morocco: High Atlas, E Marrakech, N Taddert, (near Tazouguerte); N31°28′07″ W07°24′59″, 1727 m; Quercus, 2009, Stüben	ZFMK-DNA-JJ0846, ZFMK-TIS-cE889port	GU988573 GU988133
Onyxacalles portusveneris (Mayet 1903) [27]	Morocco: Middle Atlas, S Azrou, Äin Leuh; N33°16′50″ W05°20′18″, 1582 m; Quercus ilex, Euphorbia, 2009, Stüben	ZFMK-DNA-JJ0847, ZFMK-TIS-cE890port	GU988574 GU988134
Onyxacalles portusveneris (Mayet 1903) [27]	Morocco: Middle Atlas, S Azrou, S Äin Leuh; N33°14′57″ W05°21′04″, 1715 m; Quercus ilex, 2009, Stüben	ZFMK-DNA-JJ0848, ZFMK-TIS-cE891port	GU988575 GU988135
Onyxacalles portusveneris (Mayet 1903) [27]	Morocco: Rif, 10 km W Ketama; N34°57′40″ W04°40′51″, 1600 m; Cedrus, Prunus, 2009, Stüben	ZFMK-DNA-JJ0869, ZFMK-TIS-cE912port	GU988587 GU988147
Onyxacalles portusveneris (Mayet 1903) [27] (*Onyxacalles* sp.)	Morocco: High Atlas, 59 km SE Marrakech; N31°28′19″ W07°24′22″, 1500 m; Quercus ilex, Quercus suber, 2002, Stüben	ZFMK-DNA-JJ0472, ZFMK-TIS-cM482mag	GU988350 GU987922
Onyxacalles ringeli Kulbe 1999	Spain: Canary Islands, La Palma, Cumbre Nueva, 4,5 km SE El Paso, El Pilar; N28°37′37″ W17°49′45″, 1432 m; laurisilva, 2006, Stüben	ZFMK-DNA-JJ0087, ZFMK-TIS-cC0171rin	EU286300 EU286465
Onyxacalles sp. 1 (*O. luigionii*)	France: Alpes-Maritimes, 3 km W Sospel, Col de Braus; N43°52′34″ E07°24′17″, 1051 m; Quercus pubescens, Ostrya carpinifolia, broom, 2007, Stüben	ZFMK-DNA-JJ0411, ZFMK-TIS-cF440lui	GU988325 GU987897
Onyxacalles torre sp. n. (*O. henoni*)	France: Corsica, Col de Vizzavona, 22 km S Corte; N42°06′45″ E09°06′49″, 1100 m; Fagus, 2001, Stüben	ZFMK-DNA-JJ0882, ZFMK-TIS-cF479hen	GU988592 ---------

Table 1: Continued.

Taxon	Collecting data	DNA voucher	COI 16S
Onyxacalles valencianus Germann 2005	Spain: Alicante, 7 km SW Alcoi, Sierra de Menechaor, Santurio de la Font Roja; N38°39′34′′ W00°32′29′′,1296 m; Quercus ilex, 2007, Stüben	ZFMK-DNA-JJ0172, ZFMK-TIS-cE0180val	EU286331 EU286495
Onyxacalles valencianus Germann 2005	Spain: Castellón, Morella, Barranco de la Bota; N40°33′12′′ W00°00′27′′, 814 m; Quercus ilex, Hedera helix, 2008, Stüben	ZFMK-DNA-JJ0546, ZFMK-TIS-cE607val	GU988393 GU987953
Onyxacalles valencianus Germann 2005	Spain: Barcelona, above dry river bed, near Vallirana; N41°22′36′′ E01°55′02′′, 245 m; Quercus ilex, Ficus carica, Smilax aspera, 2008, Stüben	ZFMK-DNA-JJ0551, ZFMK-TIS-cE612val	GU988398 GU987958
Onyxacalles valencianus Germann 2005	spain: Barcelona, S. Montseny, Tordera valley, near St. Marçal; N41°48′01′′ E02°25′15′′, 1060 m, 2008, Stüben	ZFMK-DNA-JJ0553, ZFMK-TIS-cE614val	GU988400 GU987960
Onyxacalles verrucosus (Wollaston 1863)	Spain: Canary Islands, El Hierro, 7 km W La Frontera, Pista Derrabado; N27°44′29′′ W18°03′24′′, 895 m; Laurus azorica, 2006, Stüben	ZFMK-DNA-JJ0137, ZFMK-TIS-cC0170ver	EU286324 EU286488
Onyxacalles verrucosus (Wollaston 1863)	Spain: Canary Islands, Tenerife, 6 km N La Laguna, Monte de las Mercedes; N28°31′50′′ W16°17′09′′, 950 m; laurisilva, 2003, Stüben	ZFMK-DNA-JJ0509, ZFMK-TIS-cE570ver	--------- GU987937
Onyxacalles verrucosus (Wollaston 1863)	Spain: Canary Islands, Tenerife, 4 km S Los Silos, Teno Mts., Monte del Aqua; N28°19′20′′ W16°49′14′′, 700 m; laurisilva, 2003, Stüben	ZFMK-DNA-JJ0510, ZFMK-TIS-cE571ver	GU988373 GU987938
Onyxacalles verrucosus (Wollaston 1863)	Spain: Canary Islands, Tenerife, NE La Laguna, Anaga Mts. near Moquinal; N28°31′55′′ W16°17′24′′, 840 m; laurisilva, 2008, Astrin and Stüben	ZFMK-DNA-JJ0604, ZFMK-TIS-cE677ver	GU988433 GU987995
Onyxacalles verrucosus (Wollaston 1863)	Spain: Canary Islands, Tenerife, NE La Laguna, Anaga Mts. near Chinobre; N28°33′21′′ W16°10′46′′, 808 m; Laurus, Ixanthus viscosus, 2008, Astrin and Stüben	ZFMK-DNA-JJ0605, ZFMK-TIS-cE678ver	GU988434 GU987996
Onyxacalles verrucosus (Wollaston 1863)	Spain: Canary Islands, Tenerife, SW Los Silos, Teno Mts., Monte del Agua, Chupadero; N28°19′23′′ W16°49′12′′, 940 m; Laurus novocanariensis, 2008, Astrin, Stüben, Behne and Floren	ZFMK-DNA-JJ0776, ZFMK-TIS-cE813ver	GU988524 GU988085
Onyxacalles vilae sp.n. (*O. luigionii*)	France: Isère, 2 km SE Lans en Vercors, Montagne de Lans; N45°06′45′′ E05°36′21′′, 1352 m; Abies, Fagus, Fraxinus, 2005, Stüben	ZFMK-DNA-JJ0025, ZFMK-TIS-cI0027lui	EU286286 EU286451
Onyxacalles vilae sp.n. (*O. luigionii*)	Croatia: Dalmatian, 8 km E Karlobag, Velebit Mts., Stupacinovo; N44°32′41′′ E15°09′58′′, 1049 m; Fagus, 2007, Stüben	ZFMK-DNA-JJ0195, ZFMK-TIS-cHR0339lui	EU286348 EU286512

gaps. All of these were included into phylogenetic analysis. We implemented the GTR+I+Γ [29] model of nucleotide substitution for both genes in Bayesian MCMC analyses, run in MrBayes ver. 3.1.2 [30]. Only COI was included for the new sequences. We ran two independent replicates for 10 million generations per analysis (each with 1 cold chain and 3 chains of different temperature). Every 1′000th tree was sampled (20′000 trees retained). Negative log-likelihood score stabilisation was determined in a separate visualisation (in MS Excel). Accordingly, we retained 19.800 trees (after discarding burn-in), of which a 50%-majority rule consensus tree was built, with posterior probabilities (Figure 1).

4. Results and Discussion

4.1. A Gestalt Switch—Changing the Way You See: Kyklioacalles pyrenaeus (Boheman, 1844). The species of *Onyxacalles* are characterised by a particularly long and slender rostrum which is at least 3-4 times as long as wide between the insertions of the antennae. A further conspicuous feature (not typical for western Palearctic Cryptorhynchinae—excepting the species of the Macaronesian Islands) is the unusually long and slender (arachnoid) legs. The name of this genus refers to the hook-shaped tip of the aedeagus. No further species of the former accumulative genus *Acalles* or of other western Palearctic genera of the Cryptorhynchinae exhibit such a characteristic hook-shaped tip of the aedeagus (*onyx*; greek: hook, hook-shaped tool). Complex-sclerotised structures of the internal sac are absent or reduced to simple line- or bar-like structures. These structures have been significant, for instance, for the partly phylogeny-based classification and determination of the genera and species of *Dichromacalles* and *Kyklioacalles*. The strongly sclerotised median lobe exhibits either only unclear structures or none at all (see [31]).

As early as the beginning of the last century, in their ground-breaking revision of the western Palearctic species of *Acalles* s.l., A. and F. Solari placed the species *A. pyrenaeus*

Boheman, 1844, *A. henoni* Bedel, 1888, *A. croaticus* Brisout, 1867, and *A. luigionii* Solari, 1907—although together with further species—into the same group (see IV. group; [32]). Together with initially 7 further species (among it the above species denominated by A. and F. Solari, as well as 3 further species from the Canary Islands), they were transferred into the new genus *Onyxacalles* [1, page 186], a genus that currently comprises 20 species.

However, *Onyxacalles pyrenaeus* is a polymorphic species, with regard to the outline of the aedeagus as well as the more or less ovally rounded elytra [1, page 188]. This is—among others—the reason why we could not ascertain definitively whether the subspecies *Acalles pyrenaeus germanicus* Letzner, 1882 (= *Onyxacalles boehmei* Košťál & Holecová, 2001 [33] syn.) is really a junior synonym or not (even if the first author considers it a synonym; cf. [7, page 123]).

In addition to *Onyxacalles portusveneris* (Mayet, 1903) (see Figure 4), *O. pyrenaeus* has an exceptionally large distribution area (cf. [31]). This could explain the high genetic distances of the mitochondrial COI and 16S gene (e.g., France: Lans en Vercors—Austria: Merkersdorf, p-distances: COI = 8,5%, 16S = 2,4%). This species can be found from the Pyrenees to the mountains of Western, Central, and Eastern Europe to the Carpathians and can be beaten from the branches of different conifers, especially larch, but can also be sifted under deciduous trees (e.g., *Fagus*).

This does not coincide with the other *Onyxacalles* species, which live on different deciduous trees and never prefer conifers. Apart from these ecological conditions, we can establish that the above-mentioned differences to the other genera of the western Palearctic Cryptorhynchinae point to a closer relationship to the species of *Onyxacalles*, a view with which most authors concur (e.g., [33]).

In any case, the taxonomists did not pay attention to the distinct ecology and—in comparison with the other species of *Onyxacalles*—the clearly narrower aedeagus (cf. Figure 6(k) versus Figures 9(a)–9(l)). Are these peripheral characteristics? And in which genus of Cryptorhynchinae is it possible to place this species (without the need for a monotypic genus)? The first author had never imagined assigning this species to the genus *Kyklioacalles* Stüben, 1999. There was no reason for such a review, not even an "initial suspicion" (see below).

However, this presumed "*Onyxacalles*" species appears deeply nested within the genus *Kyklioacalles* in the dendrogram [34, Figure 1]. This inclusion within *Kyklioacalles* is maximally supported. Furthermore, it is obvious that *Acalles pyrenaeus* forms a clade with *K. fausti* (Meyer, 1896), *K. reginae* Stüben, 2003, *K. saccoi* (Colonnelli, 1973) [35], and *K. reinosae* (H. Brisout de Barneville, 1867) [10, 34]. Two species, *K. reginae* and *K. reinosae*, are distinguished from the other species by the completely different habitus, but chiefly belong and were allocated early on to the *K. fausti* group "only" on the basis of the endophallus. This allocation has been shown to be justified by molecular data [34].

But what would have happened if *Kyklioacalles* had not been defined initially on the basis of the cyclical structure of the endophallus, as the name implies [36], and as an immediate consequence, *Acalles pyrenaeus* would not have been eliminated? Putting it the other way round: what if the similarity between this species *Kyklioacalles saccoi* and *Kyklioacalles fausti* had been considered in a habitus-to-habitus comparison? That is not only a simple, retrospective, and dispensable "what-if" question, because in this case one brings the fact to mind that *A. pyrenaeus* must now be coercively placed among the *Kyklioacalles* species, Figures 8(f)–8(h).

The definition especially of a higher taxon is an arbitrary supposition. As taxonomists we always operate with *constructs* and as morphologists we *find* characteristics that are prominent to our eyes (and sometimes "we like to see"). It must be admitted that extremely rarely do we look for homologies, which in theory constitute the best criterion (e.g., [37]), but are in practice often difficult to find when dealing with cryptic and similar-looking species.

This change of mind and perception is similar to a Gestalt switch [22], a figure spinning in two directions: the contour line of the species is the same, but the species is not (as in the Gestalt psychology, the vase-face and duck-rabbit illusions). We cannot explain this Gestalt switch based on morphological research alone, and we cannot build and establish it *within* this framework. But we know the cause for this inconspicuous paradigm change: only extrinsic evidence from DNA analysis has *opened* the morphologist's *eyes* in this case—and this in the true sense of the word.

4.2. Integrative Taxonomy: Changing the Way You Look for Species. Integrative taxonomy sounds like an accumulation of different disciplines: morphology, molecular biology, ecology, ethology, and biogeography deliver the ingredients. But this is not invariably the case, and not so simple. It is a more eventful, reciprocal exchange of *evidence*, which an initial suspicion either confirms or rejects (comparable to an unsolved criminal case).

An initial morphological suspicion was already available when we discovered the new species *Onyxacalles nuraghi* (*O. henoni* group) in the humid *Quercus/Acer* forest in the mountains of Marghine on Sardinia. The differences from the well-known species of this group are obvious and easy to assemble (see below differential diagnosis of *O. nuraghi*). In the case of *Onyxacalles vilae* sp. n. (*O. luigionii* group) from the Velebit Mountains (Croatia), it was morphologically more ambiguous, but in case of the third species *Onyxacalles torre* sp.n. (*O. henoni* group) from Corsica, the specimens of the current type series remained completely unnoticed in the collection for years.

But the molecular results in view of these three (resp. four) species are obvious (see also Figures 1–4). With maximal support, *O. vilae* is widely separated in the tree topology from *O. nuraghi* and *O. torre* (*O. henoni* group; Figure 1). Together with *Onyxacalles* sp. 1, *O. vilae* groups with *O. luigionii* (*Onyxacalles* sp. 1 as sister taxon of the latter), but all these species are separated by considerable p-distances (>9% COI and >4% 16S; Figure 3). Interestingly, the *O. vilae* specimen from Croatia shares the haplotype of the French specimen. *O. torre* and *O. nuraghi* are genetically closer to each other (1,8% 16S; Figure 2), but not sister species.

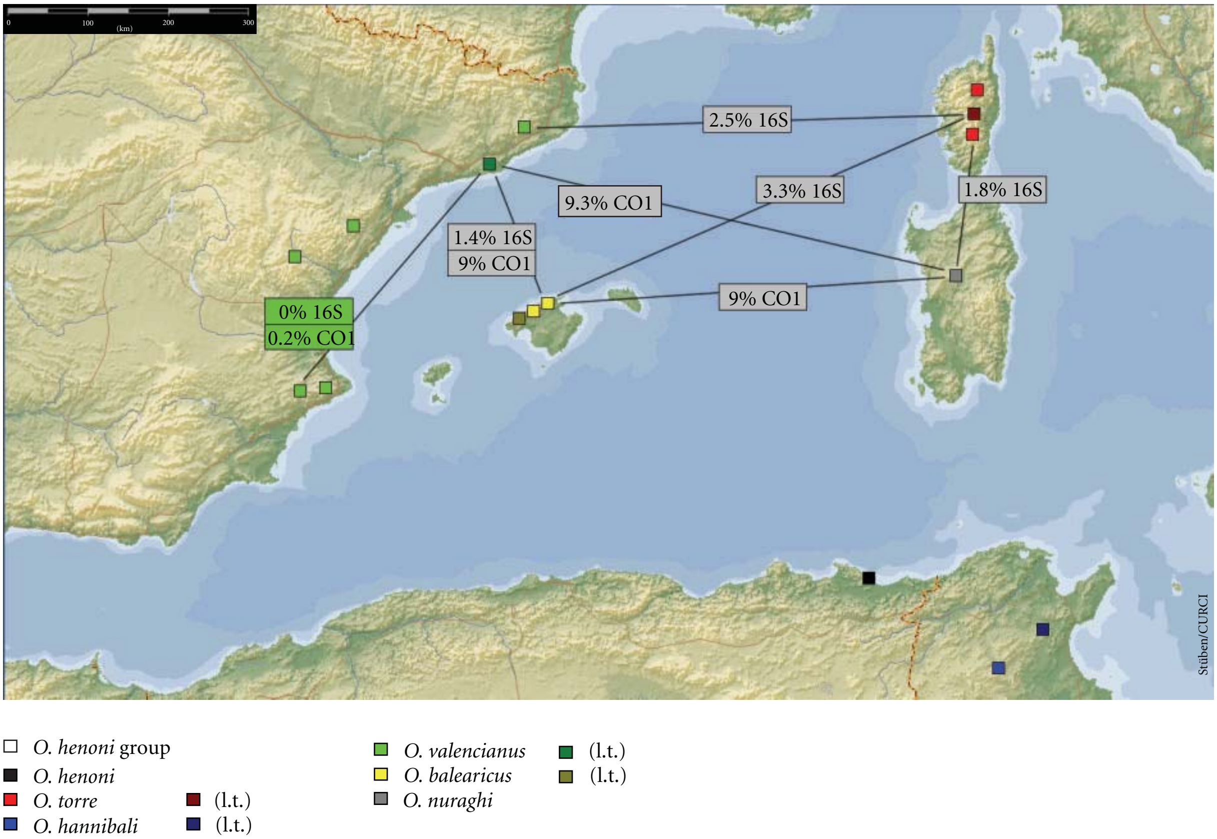


Figure 2: Distribution of related species of the West Mediterranean species of the ***O. henoni*** group (without *O. croaticus*, Eastern Europe) with values of COI and 16S p-distances.

The unambiguous molecular evidence (cf. Figures 2 and 3) prompts the morphological reinvestigation of the material and forces the morphologist to look for new characters (see below "taxonomy")—if they exist!

On the one hand—in the case of *Onyxacalles* species—we currently give more attention to the apex of the rostrum and its punctures, or of the more or less curved and hook-shaped apex of the aedeagus. Vice versa, we see in the conspicuous and serially placed elytral bristles and its erected tufts on the intervals "seductive anthropomorphisms" and analyses of yesteryears. The perception does not become more precise, and the tuning is not finer adjusted—rather the way we view characters has changed: the perspective regarding criterion and weighting has undergone a shift influenced by extrinsic data.

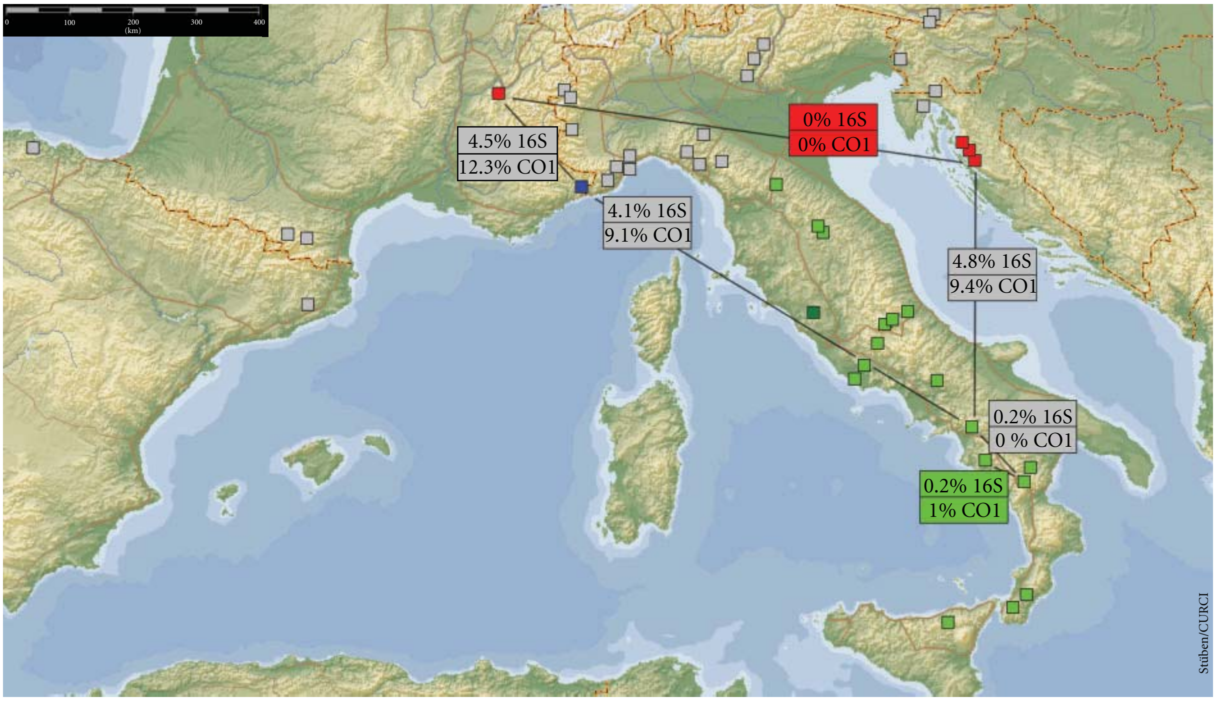
On the other hand, however, the few specimens in the immediate vicinity of the French village Sospel (Maritime Alps) attest that it does not succeed in all cases (see: Figure 1: *Onyxacalles* sp. 1). The high p-distances of the mitochondrial COI and 16S genes of these specimens compared to the Middle and South Italian populations of *O. luigionii* and to *O. vilae* from the Velebit Mountains and the French Isère indicate the plausibility of a new species (cf. Figure 3). However, morphological differences could not be diagnosed. More findings and larger series of comparison material should establish a clearer picture in future. Therefore, it is premature to postulate a cryptic species and contrast it in a differential diagnosis by nothing more than molecular characters at this stage. Furthermore, this action would have the direct disadvantage that all previous existing records from the French and Italian Maritime Alps could not be allocated to the above-mentioned species. Nevertheless, this could shift in the future, should in depth "DNA barcodes" become available for all applied entomological disciplines.

The first step in this development has already been taken: the **M**olecular **W**eevil **I**dentification-Project (ZFMK, CURCI), which is going to establish a molecular (DNA barcodes) and photographic database (stacked images) as well as the highly important associated reference collections for European Curculionoidae (ca. 6000 species). Only by meticulous cross-vouchering can misidentifications be corrected, and molecular results can be linked to the more than 250 years history of entomology. Integrative taxonomy is not just an accumulative or encyclopaedic "furthering of knowledge," but rather—as in this case—an interactive process in an interdisciplinary dialogue.

5. Taxonomy

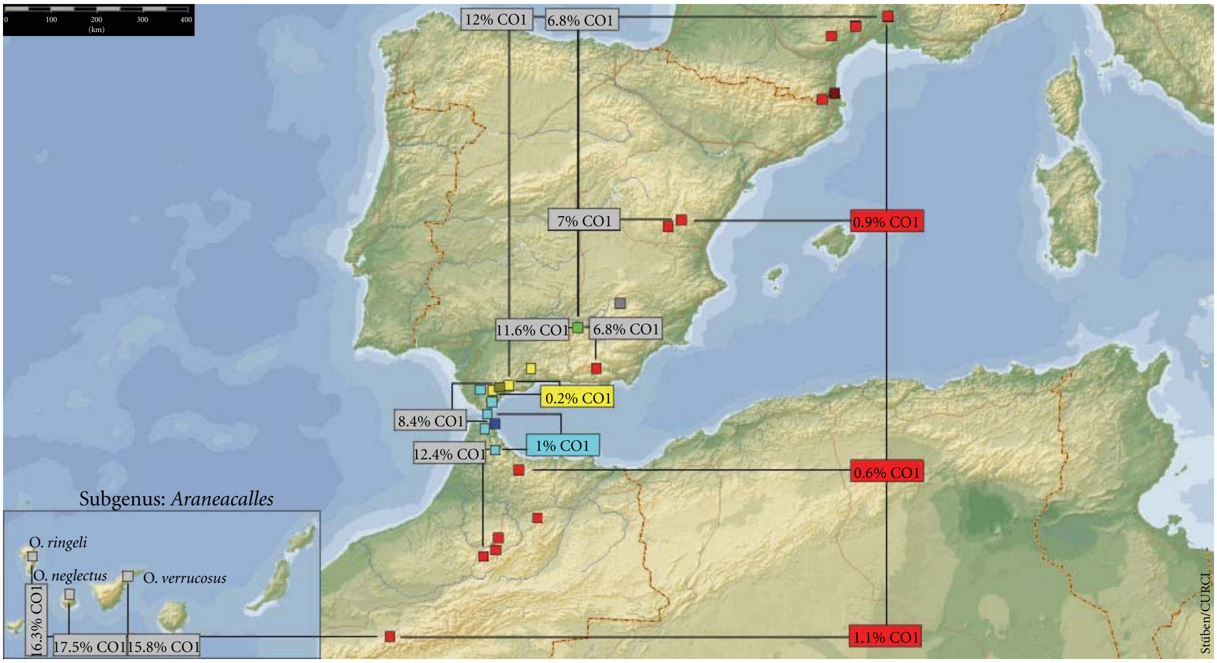
Family: Curculionidae Latreille, 1802.

Subfamily: Cryptorhynchinae Schoenherr, 1825.


0
100
200
(km)
300
400
4.5% 16S
12.3% CO1
0% 16S
0% CO1
4.1% 16S
9.1% CO1
4.8% 16S
9.4% CO1
0.2% 16S
0 % CO1
0.2% 16S
1% CO1
Stüben/CURCI

▨ *O. luigionii* group 🟩 *O. luigionii* ■ (l.t.)
🟥 *O. vilae* 🟦 *O.* sp. 1

FIGURE 3: Distribution of related species of the ***O. luigionii*** group in the Mediterranean area with values of COI and 16S p-distances.


0
100
200
(km)
300
400
12% CO1
6.8% CO1
7% CO1
0.9% CO1
11.6% CO1
6.8% CO1
0.2% CO1
8.4% CO1
1% CO1
12.4% CO1
0.6% CO1
Subgenus: *Araneacalles*
O. ringeli
O. neglectus
O. verrucosus
16.3% CO1
17.5% CO1
15.8% CO1
1.1% CO1
Stüben/CURCI

☐ *O. portusveneris* group 🟩 *O. maginaensis*
🟥 *O. portusveneris* ■ (l.t.) ▨ *O. seguraensis*
🟦 *O. gibraltarensis* ■ (l.t.) 🟨 *O. bermejaensis* 🟫 (l.t.)

FIGURE 4: Distribution of related species of the ***O. portusveneris*** group in the Mediterranean area with values of COI p-distance (658 bp).

Genus: *Onyxacalles* Stüben, 1999, Type species: *Acalles luigionii* A. and F. Solari, 1907 (L.t.: Central Italy).

5.1. New Species of the Onyxacalles henoni Group

5.1.1. Onyxacalles nuraghi Stüben sp. n.

(Figures 5(a)–5(c), 5(i), 6(l)).

Type Material

Holotype (1♂). Italy: Sardinia, Macomer, Lei, N40°19′54″ E08°53′49″, 1020 m, *Quercus, Acer monspesulanus*, 4.10.2010, leg. Stüben-27-, coll. CURCULIO-Institut, D-Mönchengladbach.

Paratypes (1♂). Data as for holotype, coll. Stüben; **4♂**, **1♀**: Italy: Sardinia, Macomer, Lei, N40°19′17″ E08°53′52″, 586 m, *Quercus* ilex, 7.10.2010, leg. Stüben-33-, coll. Stüben, CURCULIO-Institut, D-Mönchengladbach (1♀), coll ZFMK, 1♂: ZFMK-DNA-JJ1097, ZFMK-TIS-cIT1097.

DNAtype (1♂). Data as for holotype, coll. ZFMK: ZFMK-DNA-JJ1090, ZFMK-TIS-cIT1090; GenBank Acc. no COI: JN121399, 16S: JN642097.

Differential Diagnosis. The new species from the south-facing slope of the Chain of Marghine (Italy: Sardinia) belongs—from a morphological and molecular perspective—to the *Onyxacalles henoni* group and should be compared with the most closely related species from Majorca (Spain): *Onyxacalles balearicus* Stüben, 2005.

Onyxacalles nuraghi

(1) Disc of pronotum with a channel from the base towards the flat sector in front of the fore-margin; with tufts of bristles on both sides of the channel (Figure 5(i)).

(2) Bristles on the elytral intervals at least 2x as long as wide; shaping tufts with big gaps; their distances range from 3x the length of bristles.

(3) Elytra of male with parallel sides in the middle sector (dorsal view); contour line of elytra forms almost a semicircle in lateral view (Figure 5(a)).

(4) Apex ("hook") of the aedeagus (in ventral view) smaller (Figure 5(c)).

Onyxacalles balearicus

(1*) Disc of prontoum without a channel and without tufts of bristles (Figure 5(h)).

(2*) The free-standing bristles on the intervals shorter, 1.3x as long as wide; placed in a single row, not forming tufts.

(3*) Elytra of male broader and stronger (short ovally) rounded (slightly "egg shaped"); contour line of elytra flatter or slightly rounded behind the base in lateral view (Figure 5(g)).

(4*) Apex (hook) of the aedeagus (in ventral view) broader (Figure 5(j)).

The new species from Sardinia is different from *Onyxacalles henoni* (Bedel, 1888) [38] (Algeria: Mt. Edough, *loc. typ.*), with which it has the tufts of bristles on the elytral intervals in common, by (1) darker elytral integument (Figure 5(a) versus 5(k)), (2) finer and longer white bristles on the femora, and (3) longer apex ("hook") of the aedeagus (lateral view, see Figure 5(c) versus 5(n)). It can be distinguished from *Onyxacalles valencianus* Germann, 2005 from the Spanish mainland (Barcelona: Villarana, *loc. typ.*) by (1) elytral tufts of bristles (versus single bristles), (2) deep channel of the pronotum (versus without channel), and (3) longer apex of aedeagus (lateral view, see Figure 5(c) versus 9(h)).

For a comparison with all other species see below the "Key to the species, of *Onyxacalles* Stüben, 1999".

Description

Length. 2.60–3.40 mm (without rostrum).

Head and Rostrum. Eyes large; rounded ovally towards front and acuminate towards underside of rostrum; frons between eyes more slender than the base of rostrum; rostrum reddish brown, closely covered with white scales at the base; rostrum of male reaching 3/4 length of pronotum and finely punctuated towards apex; rostrum of females reaching 4/5 length of pronotum, slender, shiny, and even more finely punctuated. The last three funicles of antennae short ovally rounded; the first two funicles elongated; the club clearly separated from funicles.

Pronotum. Widest at the end of the first third of the pronotum (holotype: 1.17x as wide as long); well rounded laterally towards the fore-margin and the base; with a deep depression at the sides directly behind the fore-margin; disk of pronotum strongly arced, with a channel in the middle from the base towards the flat sector in front of the fore-margin. The integument is rich in contrast consisting of round black scales on the disk and oval, white/brown scales on the flanks of the pronotum. Elongated and black bristles in an upright position in the middle of the disk on both sides of the channel; with a similar, but white tuft of bristles on each side of the pronotum; the deep punctures always covered with scales.

Elytra. Oblong (holotype: 1.29x as long as wide); widest in the middle and there with nearly parallel sides; only slightly rounded directly in front of the base; short ovally rounded towards the apex. Contour line of elytra strongly arced, almost forming a semi circle. The shiny and predominantly dark brown integument with a beige/white crescent-shaped fascia in front of the base and on the elytral slope. Bristles on the first and third interval (excluding the suture stripe)

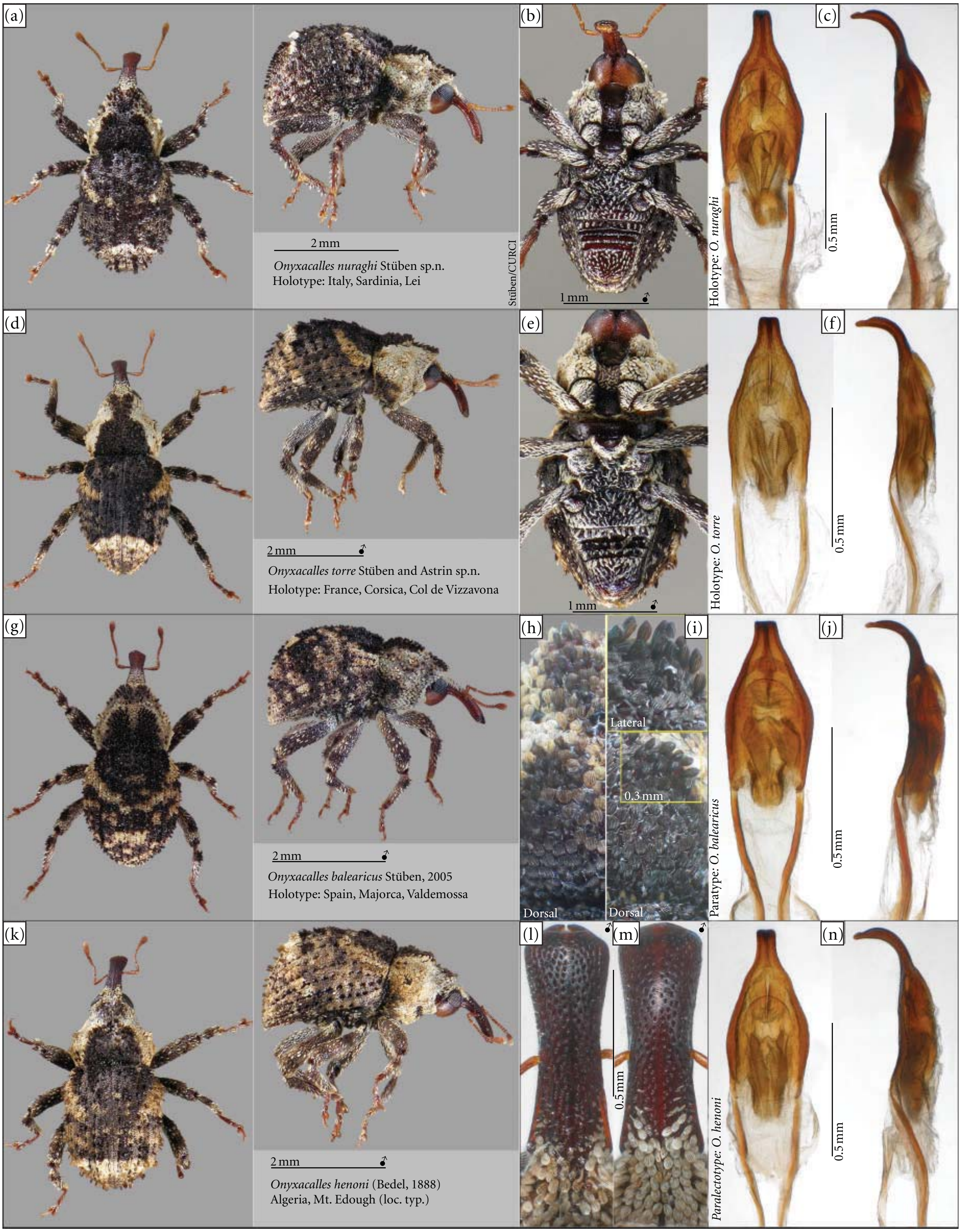

(a)
2 mm
Onyxacalles nuraghi Stüben sp.n.
Holotype: Italy, Sardinia, Lei
Stüben/CURCI
(b)
1 mm ♂
Holotype: O. nuraghi
(c)
0.5 mm
(d)
2 mm ♂
Onyxacalles torre Stüben and Astrin sp.n.
Holotype: France, Corsica, Col de Vizzavona
(e)
1 mm ♂
Holotype: O. torre
(f)
0.5 mm
(g)
2 mm ♂
Onyxacalles balearicus Stüben, 2005
Holotype: Spain, Majorca, Valdemossa
(h)
(i)
Lateral
0.3 mm
Dorsal
Dorsal
Paratype: O. balearicus
(j)
0.5 mm
(k)
2 mm ♂
Onyxacalles henoni (Bedel, 1888)
Algeria, Mt. Edough (loc. typ.)
(l) ♂
(m) ♂
0.5 mm
Paralectotype: O. henoni
(n)
0.5 mm

FIGURE 5: (a)–(c) *O. nuraghi* sp.n.—habitus (dor./lat./ven.), aedeagus (ven./lat.). (d–f) *O. torre* sp. n.—habitus (dor./lat./ven.), aedeagus (ven./lat.); (g, j) *O. balearicus*—habitus (dor./lat.), aedeagus (ven./lat.); (k, n) *O. henoni*—habitus (dor./lat.), aedeagus (ven./lat.). By comparison, bristle of pronotum—*O. balearicus* (h) versus *O. nuraghi* (i); rostrum—*O. henoni* (l) versus *O. torre* (m).

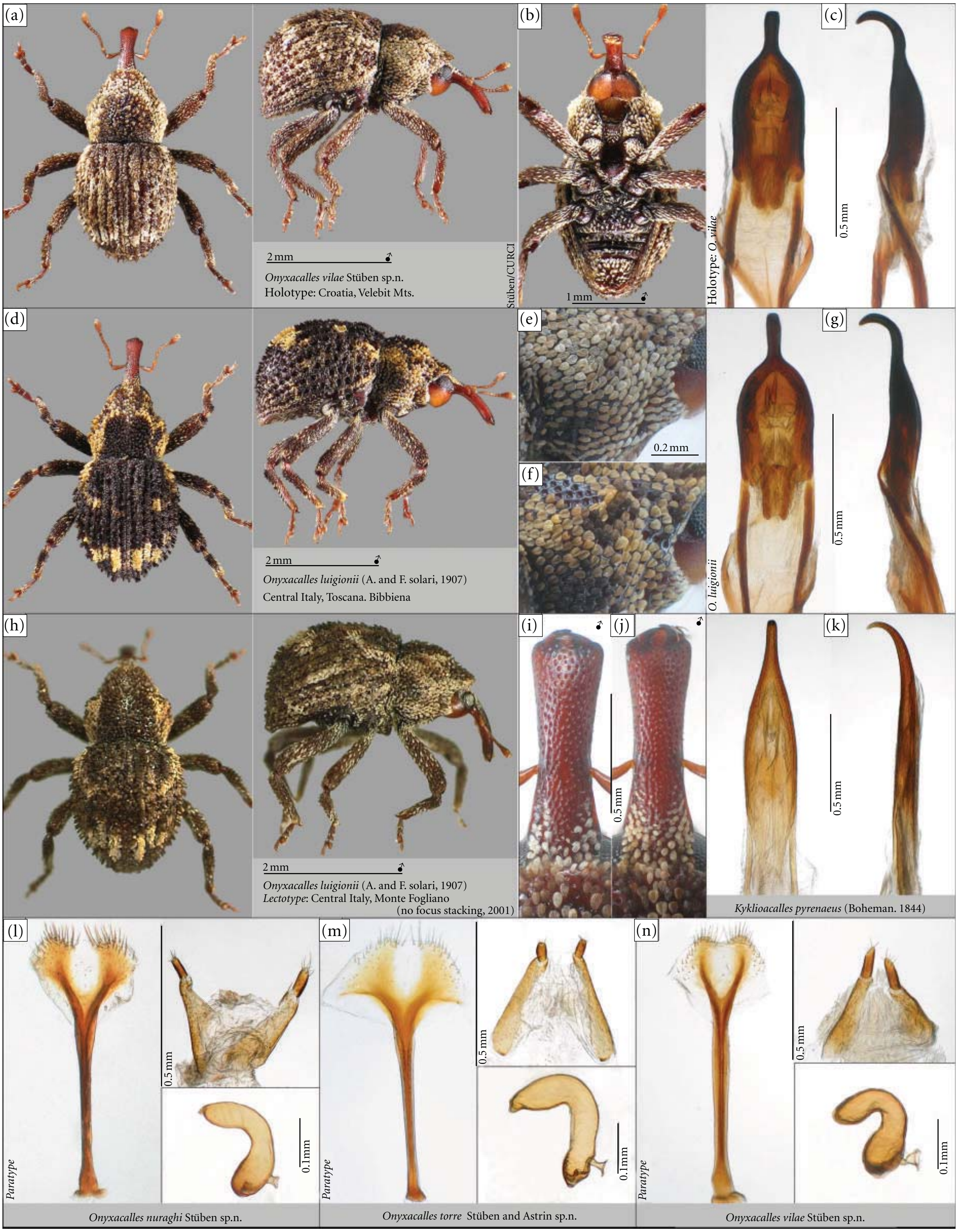

(a)
2 mm ♂
Onyxacalles vilae Stüben sp.n.
Holotype: Croatia, Velebit Mts.
Stüben/CURCI
(b)
1 mm ♂
(c)
Holotype: *O. vilae*
0.5 mm
(d)
2 mm ♂
Onyxacalles luigionii (A. and F. solari, 1907)
Central Italy, Toscana. Bibbiena
(e)
0.2 mm
(f)
(g)
O. luigionii
0.5 mm
(h)
2 mm ♂
Onyxacalles luigionii (A. and F. solari, 1907)
Lectotype: Central Italy, Monte Fogliano
(no focus stacking, 2001)
(i) ♂
(j) ♂
0.5 mm
(k)
0.5 mm
Kyklioacalles pyrenaeus (Boheman. 1844)
(l)
0.5 mm
0.1mm
Paratype
Onyxacalles nuraghi Stüben sp.n.
(m)
0.5 mm
0.1mm
Paratype
Onyxacalles torre Stüben and Astrin sp.n.
(n)
0.5 mm
0.1mm
Paratype
Onyxacalles vilae Stüben sp.n.

FIGURE 6: (a–c) *O. vilae* sp. n.—habitus (dor./lat./ven.), aedeagus (ven./lat.); (d, g) *O. luigionii*—habitus (dor./lat.), aedeagus (ven./lat.); (h) *O. luigionii* (lectotype)—habitus (dor./lat.); (k) *Kyklioacalles pyrenaeus*—aedeagus (ven./lat.); (l–n) female genital (spiculum ventrale, ovipositor, spermatheca) of *O. nuraghi* sp.n. (l), *O. torre* (m) and *O. vilae* (n). By comparison, bristle of pronotum (lat.)—*O. vilae* (e) versus *O. luigionii* (f); rostrum—*O. vilae* (j) versus *O. luigionii* (j).

at most 2x longer than wide, forming flattened tufts, which have big gaps between them (their distances range from three times the length of bristle); bristles sparse on the second and fourth intervals; striae on the disc and at the sides of elytra clearly narrower than intervals, punctures oblong.

Legs. Long; the marginal front femora reach the base of the rostrum; the hind femora reach the end of the elytral apex. They are covered with predominantly dark brown scales; tibia with long, white, and laterally protruding bristles.

Venter. The 2nd strip-type sternite only a little bit longer than the 3rd, but not longer than sternites 3 and 4 together. 1st sternite of male with a broad depression (Figure 5(b)).

Female Genital. See Figure 6(l).

Aedeagus. Apex ("hook") of the aedeagus (in ventral view) small, see Figure 5(c).

Etymology. The species name refers to the Nuragic civilization of Sardinia, lasting from the Bronze Age (18th century BC) to the 2nd century AD.

Ecology. Onyxacalles nuraghi was discovered by the first author on Sardinia near Lei (Macomer) in the mountains of Marghine in 2010. The seven specimens were shifted under *Quercus* and *Acer* between 500 and 1000 m above sea level.

Distribution. This species is so far only known from the Chain of Marghine, Figure 2.

5.1.2. Onyxacalles torre Stüben and Astrin sp. n.

(Figures 5(d)–5(f), 5(m), and 6(m)).

Type Material

Holotype (1♂). France, Corsica (Haute-Corse): Col de Vizzavona, 22 km S Corte, 1100 m, 8.10.2001, 42°06′45′′N 09°06′49′′E, *Fagus* (sift), leg. Stüben-4-, coll. CURCULIO-Institut, D-Mönchengladbach.

Paratypes (8♂, 9♀). Data as for holotype, coll. Stüben, CURCULIO-Institut, D-Mönchengladbach (1♀), Zoologisches Forschungsmuseum Alexander Koenig, D-Bonn (1♂, 1♀).

DNAtype (1♀). Data as for holotype, coll. ZFMK: ZFMK-DNA-JJ0882, ZFMK-TIS-cF479; GenBank Acc. no 16S: GU988592.

Further Material (1♂). France, Corsica (Haute-Corse): Caporalino 10 km N Corte, 350 m, 7.10.2001, 42°23′08′′N 09°11′37′′E, *Alnus, Fraxinus*, *Quercus* (sift), leg. Stüben-2-, coll. Stüben; **2♂**: France, Corsica (Haute-Corse): Tattone, 18 km S Corte, 750 m, 8.10.2001, 42°09′21′′N 09°09′43′′E, *Castanea* (sift), leg. Stüben-3-, coll. Stüben; **1♂**: France, Corsica (Corse-du-Sud): Radicale, 20 km E Ajaccio, 400 m, 9.10.2001, 41°55′31′′N 08°58′10′′E, 9.X.2001, *Quercus ilex* (sift), leg. Stüben-9-, coll. Stüben; **2♂**: France, Corsica (Corse-du-Sud): Cozzano 2 km NE Zicavo, 750 m, 10.10.2001, 41°55′31′′N 09°08′31′′E, *Castanea* (sift), leg. Stüben-12-, coll. Stüben; **4♂**, **4♀**: France, Corsica (Corse-du-Sud): Coll de La Vaccia N, 9,5 km S Zicavo, 1150 m, 10.10.2001, 41°49′19′′N 09°05′04′′E, *Fagus* (sift), leg. Stüben-14-, coll. Stüben.

Differential Diagnosis. The new species from Corsica (France) belongs—morphologically and molecularly—to the *Onyxacalles henoni* group and is distinguished from *Onyxacalles henoni* (Bedel, 1888) [38] from Algeria (Mt. Edough, *loc. typ.*) by the following characteristics.

Onyxacalles torre

(1) Rostrum finely punctuated towards apex (Figure 5(m)).
(2) Scales of the elytra predominantly dark brown or black (Figure 5(d)).
(3) Apex ("hook") of the aedeagus broader in ventral view and strongly curved (nearly rectangular) in lateral view (Figure 5(f)).

Onyxacalles henoni

(1*) Rostrum coarsely and densely punctuated towards apex (Figure 5(l)).
(2*) Scales of the elytra predominantly bright: white, beige, or brown (Figure 5(k)).
(3*) Apex ("hook") of the aedeagus smaller in ventral view and not so strongly curved in lateral view (Figure 5(n)).

The new species from Corsica can be distinguished from *Onyxacalles nuraghi* from Sardinia (see above) by (1) contour line of elytra behind the base flatter (in lateral view, Figure 5(d) versus 5(a)), (2) elytra more egg-shaped towards the apex (Figure 5(d) versus 5(a)), and (3) apex ("hook") of the aedeagus (in ventral view) shorter and wider (Figure 5(f) versus 5(c)). For a comparison with all other species, see the "Key to the species of *Onyxacalles* Stüben, 1999" below.

Description

Length. 3.00-4.00 mm (without rostrum).

Head and Rostrum. Eyes large; rounded towards front and acuminate towards underside of rostrum; frons between eyes as wide as the base of rostrum; rostrum reddish brown, closely covered with white scales at the base; rostrum of male reaching 2/3 length of pronotum and finely punctuated towards apex (Figure 5(m)); rostrum of females reaching 3/4 length of pronotum, slender, shiny, and even more finely punctuated. The last three funicles of the antennae short ovally rounded, the fourth 1.5x, the third 2x, the second 4.5x, and the first conical funicle 2x longer than wide; the elongated club clearly separated from funicles.

Pronotum. Widest at the end of the first third of the pronotum (holotype: 1.17x as wide as long); strongly rounded laterally towards the fore-margin and the base; with a depression at the sides directly behind the fore-margin; disk of pronotum arced, in the middle sometimes with a slight channel-like depression in front of the base. The integument is rich in contrast consisting of round black and dark-brown scales on the disk and in front of the fore-margin, and more or less oval, white scales on the flanks of the pronotum. In the middle of the disk on both sides of the flat depression with elongated, studded, and black bristles in an upright position; with similar placed, but shorter and white bristles on each side of the pronotum; the punctures always covered with scales.

Elytra. Oblong (holotype: 1.31x as long as wide); widest in front of the middle, here with parallel sides or slightly egg-shaped towards the apex; strongly curved in front of the base. Contour line of elytra flatter behind the base in lateral view, the contour line of the elytral slope forming an arc towards the apex. The shiny and predominantly dark brown or black integument with a beige/white crescent-shaped fascia in front of the base and on the elytral slope; sometimes the whole apex can be light brown. Bristles on first and third interval (excluding the suture stripe) 1.5x longer than wide, flattened, and shaping tufts; their distances range from the 2x length of bristles. Bristles sparse on the second and fourth intervals; striae on the disc and at the sides of elytra are small strips, clearly narrower than the intervals, punctures on the disc oblong, round at the sides.

Legs. Long; the marginal front femora reach the base of the rostrum, and the hind femora reach the end of the elytral apex. They are covered with predominantly dark brown and elongated scales; tibia with white/brown and laterally protruding bristles forming fasciae.

Venter. The 2nd strip-type sternite only a little bit longer than the 3rd, but not longer than sternites 3 and 4 together. 1st sternite of male with a broad depression (Figure 5(e)).

Female Genital. See Figure 6(m).

Aedeagus. Apex (hook) of the aedeagus broad in ventral view and strongly curved (nearly rectangular) in lateral view; see Figure 5(f).

Etymology. The species name refers to the Torrean civilization in Corsica during the second millenium BC. The characteristic building of this culture is the “Torre” (tower), the Corsican counterpart of the Sardinian “Nuraghe.”

Ecology. *Onyxacalles torre* was sifted by the first author in the mountains of Corsica and is a nocturnal inhabitant of the dark and shady forests like all other *Onyxacalles*.

Distribution. This species is so far only known from Corsica (France); Figure 2.

5.2. New Species of the Onyxacalles luigionii Group

5.2.1. Onyxacalles vilae Stüben sp. n.

(Figures 6(a)–6(c), 6(e), 6(i), and 6(n)).

Type Material

Holotype (1♂). Croatia: 20 km S Krasno Polje, Northern Velebit Mts., N44°38′14′′ E15°05′13′′, 1185 m, limestone: *Fagus*, 26.7.2004, leg. Stüben-10-, coll. CURCULIO-Institut, D-Mönchengladbach.

Paratypes (1♂, 2♀). Data as for holotype, coll. Stüben, CURCULIO-Institut, D-Mönchengladbach (1♀), Zoologisches Forschungsmuseum Alexander Koenig, D-Bonn (1♀); **1**♀: Croatia: Krasno Polje, Northern Velebit Mts., N44°49′40′′ E15°01′49′′, 838 m, limestone: *Fagus, Quercus*, 25.7.2004 leg. Stüben-7-, coll. Stüben; **1**♂: Croatia: 5 km W Krasno Polje, Northern Nord-Velebit Mts., N44°48′49′′ E14°58′36′′, 1534 m, limestone: *Fagus*, 26.7.2004, leg. Stüben-8-, coll. Stüben; **1**♀: Croatia: 6 km W Krasno Polje, Northern Velebit Mts., N44°48′56′′ E14°58′08′′, 1494 m, limestone: *Fagus*, 26.7.2004, leg. Stüben-9-, coll. Stüben; **1**♂: Croatia: 12 km S Krasno Polje; Northern Velebit Mts., N44°43′00′′ E14°59′42′′, 1414 m, limestone: *Fagus*, 27.7.2004, leg. Stüben-14-, coll. Stüben.

DNAtype (1♂). Croatia: 8 km E Karlobag, Velebit Mts., Stupacinovo, N44°32′41′′ E15°09′58′′, 1049 m, limestone: *Fagus*, 14.07.2007, leg. Stüben-27-, coll. ZFMK: ZFMK-DNA-JJ0195, ZFMK-TIS-cHR339; GenBank Acc. no COI: EU286512, 16S: EU286348.

Further Material (9♂, 7♀). France, Isère, 2 km SE Lans en Vercors, Montagne de Lans, 45°06′40′′N 05°36′25′′E, 1391 m, 23.7.2011, Kalk: *Fagus* (beaten), leg. Stüben-2-, coll. Stüben; **3♂, 3**♀: France, Isère, NW Lans en Vercors: near Autrars, Parc Regional du Vercors, 45°14′12′′N 05°34′58′′E, 1370 m, 23.7.2011, Kalk: *Fagus* (beaten), leg. Stüben-5-, coll. Stüben.

Differential Diagnosis. The new species from Croatia (Velebit Mts., *loc. typ.*) belongs—morphologically and molecularly—to the *Onyxacalles luigionii* group and is distinguished from *Onyxacalles luigionii* (A. & F. Solari, 1907) [32] from Central Italy (Monte Fogliano, *loc. typ.*) by the following characteristics.

Onyxacalles vilae

(1) Rostrum of the male broader, 2.8x as long as wide (as measured by apex); punctures not so densely packed

in front of the apex (separated by flat intervals) Figure 6(i).

(2) Bristles of the low-contrast elytra on first and third intervals (excluding the sutural stripe) longer and more slender; their distance is larger (Figure 6(a)).

(3) Scales of the white fascia at the sides of the pronotum (behind the base) predominantly oblong (Figure 6(e)).

(4) Median lobe of aedeagus smaller, 1.64x as long as wide; apex ("hook") a little bit shorter (in ventral view), flatter, and not so strongly curved in lateral view (Figure 6(c)).

Onyxacalles luigionii

(1*) Rostrum of the male more slender, 3.1x as long as wide; punctures mainly dense towards the apex (only separated by small ridges) Figure 6(j).

(2*) Bristles of high-contrast elytra on the on first and third intervals shorter, broader (towards the apex of bristle), and more dense, clearly visible on the white fascia of the elytral slope (Figures 6(d) and 6(h)).

(3*) Scales of the white fascia at the sides of the pronotum (behind the base) predominantly round (Figure 6(f)).

(4*) Median lobe of aedeagus broader, 1.93x as long as wide; apex ("hook") of the aedeagus longer (in ventral view), strongly (nearly rectangular) curved in lateral view. Internal structure (endophallus) of the sac different (Figure 6(g)).

For a comparison with all other species see below the "Key to the species of *Onyxacalles* Stüben, 1999."

Description

Length. 2.40–3.20 mm (without rostrum).

Head and Rostrum. Eyes large; rounded ovally towards front and acuminate towards underside of rostrum; frons between eyes as wide as the base of rostrum; rostrum reddish brown, closely covered with white and oval scales at the base; rostrum of male 2.8x as long as wide (as measured by apex) and finely punctuated towards apex, here separated by flat intervals (Figure 6(i)); rostrum of female clearly longer, slender, shiny, and even more finely punctuated (without punctures in front of the apex). The last funicles of antennae nearly trapezoidal, the funicles 4–6 short oval, the third funicle 1.3x, the second 3x, and the first conical funicle 1.5x longer than wide; the elongated club not clearly separated from the 7th trapezoid funicle.

Pronotum. Widest at the end of the first third of the pronotum (holotype: 1.12x as wide as long); well rounded laterally towards the fore-margin and the base; with a slight depression at the sides directly behind the fore-margin; disk of pronotum arced, without a channel or a flat depression in the middle. The integument not so rich in contrast, consisting of round, brown scales on the disk and off-white, predominantly oblong scales at the sides of the pronotum (behind the base, Figure 6(e)). In the middle of the disk with elongated brown bristles in an upright position; with a similar, but white tuft of bristles on each side of the pronotum; the deep and dense punctures covered with scales.

Elytra. Short oval (holotype: 1.19x as long as wide); widest at the end of the first fourth in front of the elytral base; here laterally strongly rounded directly in front of the base; ovally rounded towards the apex. Contour-line of elytra flatter behind the base in lateral view, the contour line of the elytral slope forming a circular arc towards the apex. Bristles of the low-contrast elytra on first and third intervals (excluding the sutural stripe) slender, 2x-3x as long as wide, their distance reaching the double length of bristle, and forming tufts only in front of the base and on the elytral slope; the uprightly protruding bristles on the second and fourth intervals have wider gaps between them (their distances range from three- to fourfold length of bristle); the scales on the intervals do not cover the underground completely; striae on the disc broad, but not broader than the intervals, reaching the width of the intervals at the sides of elytra (but often covered by scales); punctures deep and rounded.

Legs. Long; the marginal front femora reach the base of the rostrum, and the hind femora reach the end of the elytral apex. They are covered with predominantly brown scales; tibia with short, white, and laterally protruding bristles.

Venter. The 2nd strip-type sternite only a little bit longer than the 3rd, but not longer than sternites 3 and 4 together. 1st sternite of male with a broad depression (Figure 6(b)).

Female Genital. See Figure 6(n).

Aedeagus. Median lobe of aedeagus small, 1.64x as long as wide; see Figure 6(c).

Etymology. The species name refers to a "Vila" (*fairy*) in the Velebit Mts. This massif has a similar relevance for Croatians to Olympus for Greeks or the Fuijiyama for the Japanese. In Croatia, the mystical Velebit Mts. range is famous for its fairies, the most celebrated called "Vila Velebita" (*The Fairy of Velebit*).

Ecology. Onyxacalles vilae was sifted by the first author in the Velebit mountains of Croatia under *Fagus* and *Quercus* between 800 and 1600 m above sea level.

Distribution. A complete distribution map will be given in a separate faunistic study in the future, but this species was also sifted by the author in the Montagne de Lans near Lans en Vercors (France: Isère)—on limestone and under *Fagus*, too (N45°06′45″ E05°36′21″, 1352 m). Working hypothesis: It could be possible that all specimens of the "Alpine Arc"—between Grenoble and the Velebit mountains—belong to this new species and can be separated from the Central Italian populations of *Onyxacalles luigionii* (cf. Figure 3).

6. Key to the Species of *Onyxacalles* Stüben, 1999

(1) Smaller species with an ovally rounded habitus, legs shorter, femora reach the base of the rostrum; rostrum shorter and broader; if tufts of bristles exist on the elytral slope, these are only densely placed (not tapered). Distribution: continent of Western Palaearctic.

Subgenus: *Onyxacalles* s. str 2

(1*) Larger species with a more "elliptical" habitus and with long legs, femora reach the insertions of the antennae; rostrum very long and slender; the tapered tufts of bristles on the elytral slope strongly protruding (Figures 8(b)–8(d)). Distribution: Western Canary Islands.

Subgenus: *Araneacalles* Stüben and Astrin, 2010 . . . 16

(2) Apex of the aedeagus regularly rounded in lateral view: Figures 6(c) and 6(g) (both species without distinctive tufts of bristles on the uneven elytral intervals, only with densely placed bristles in one or two rows).

***Luigionii* group 2**

(2*) Apex of the aedeagus with a second, separated peak in lateral view (cf. Figures 9(a)–9(l)) (most species with more or less characteristic tufts of bristles on the elytra).

. . . . 4

— *Luigionii* group —

(3) Rostrum of the male more slender, 3.1x as long as wide; punctures mainly dense towards the apex (only separated by small ridges) (Figure 6(j)). Bristles of high-contrast elytra on the 1st and 3rd intervals shorter, broader (towards the apex of bristle), and more dense (Figures 6(d) and 6(h)). Median lobus of aedeagus broader, 1.93x as long as wide; apex ("hook") of the aedeagus longer (in ventral view), strongly (nearly rectangular) curved in lateral view. Internal structure (endophallus) of the sac different (Figure 6(g)). Distribution: Central and Southern Italy (Figure 3).

***Onyxacalles luigionii* (A. & F. Solari, 1907) [32]**
= ? *Onyxacalles porcheti* [39] (Figure 8(e), Pyrenees)

(3*) Rostrum of the male broader, 2.8x as long as wide (as measured by apex); punctures not so densely packed in front of the apex (separated by flat intervals) (Figure 6(i)). Bristles of the low-contrast elytra on the 1st and 3rd intervals (excluding the sutural stripe) longer and more slender; their distance larger (Figure 6(a)). Median lobus of aedeagus smaller, 1.64x as long as wide; apex ("hook") a little bit shorter (in ventral view), flatter, not so strongly curved in lateral view (Figure 6(c)). Distribution: Croatia (l.t.), France: Isère (Figure 3).

***Onyxacalles vilae* Stüben sp. n.**

—

(4) Pronotum levelled, "triangular" and stubby, strongly broadened just behind the base; the base 2x longer than the fore-margin (this characteristic is not so pronounced in *O. gibraltarensis*; however, this species can be clearly separated from all other *Onyxacalles* s. str. by the completely rounded sides of the aedeagus (Figure 9(a)), see digit 5). Distribution: Southern France, Iberian Peninsula, Morocco.

***Portusveneris* group 5**

(4*) Pronotum more arched and marginally broader than long, widest at the end of the first third; the base at most 1.5x longer than the fore-margin. Distribution: Algeria, Tunisia, Western Spain, West Mediterranean Islands, Southeastern Europe, Turkey.

. . . . 9

— *Portusveneris* group —

(5) Pronotum more slender, clearly separated from elytra; body outline broadly similar to the species of the *henoni* group (see digit 4*), but easy to distinguish from these species by the completely rounded sides of the aedeagus. Habitus (Figure 7(a)). Aedeagus (Figure 9(a)). Distribution: Southern Spain, Northern Morocco (Figure 4).

***Onyxacalles gibraltarensis* Stüben, 2002**

(5*) Pronotum widest directly behind the base and elytra widest directly in front of the base; therefore, pronotum and elytra do not seem separated (Figures 7(b)–7(e)).

. . . . 6

(6) Elytra egg-shaped towards the apex (Figure 7(b)); Aedeagus (Figure 9(b)). Distribution: Southern France, Iberian Peninsula, Morocco (Figure 4).

***Onyxacalles portusveneris* (Mayet, 1903) [27]**

(6*) Elytra oval or with more or less parallel sides (Figures 7(c)–7(e)).

. . . . 7

(7) Male with a split midtibia spine at the apex; uneven elytral intervals without tufts of bristles. Habitus (Figure 7(c)). Aedeagus (Figure 9(c)). Distribution: Southern Spain (Figure 4).

***Onyxacalles seguraensis* Stüben, 2003**

(7*) Male without a split midtibia spine at the apex; uneven elytral intervals with tufts of bristles.

. . . . 8

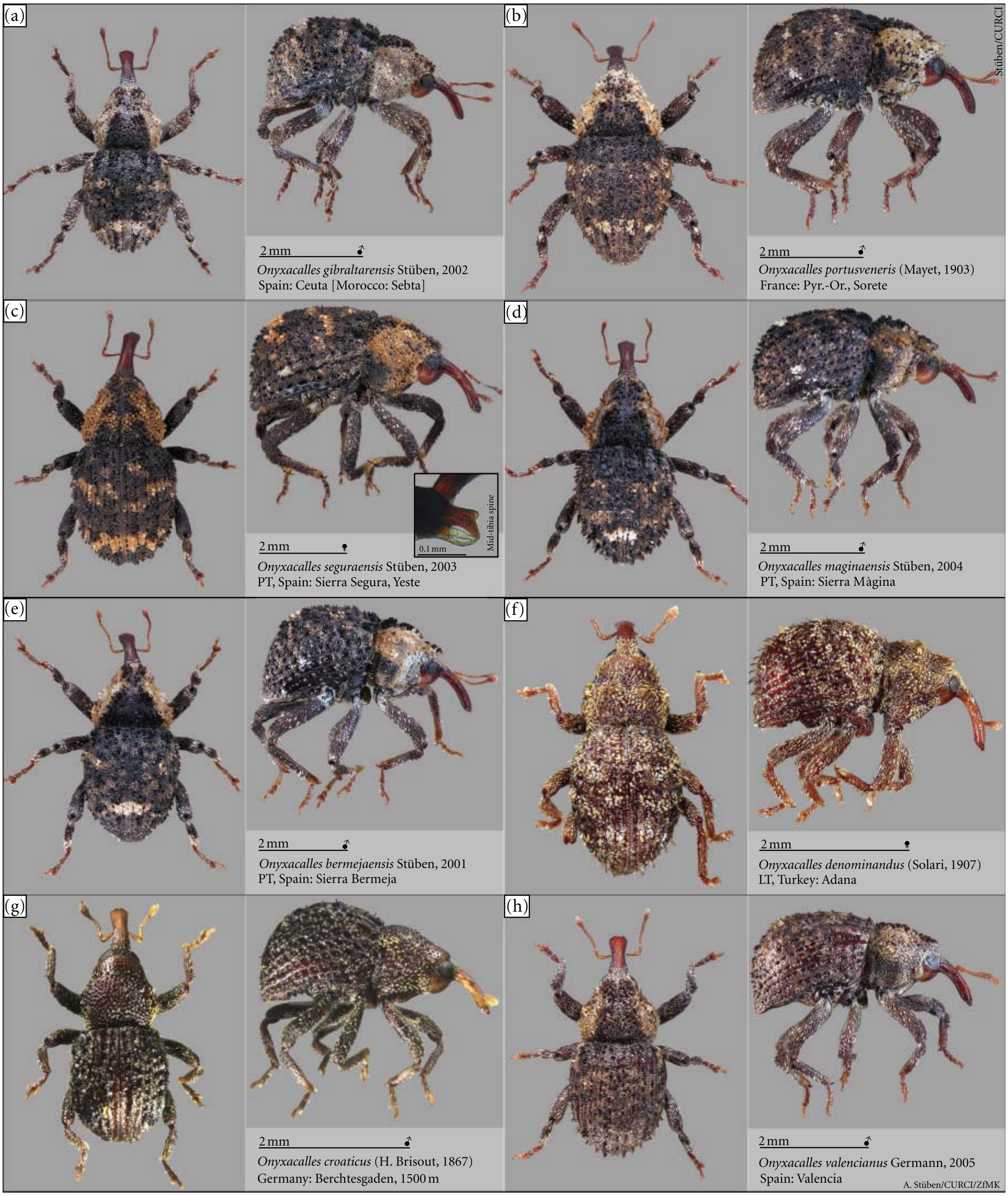

(a)
2 mm ♂
Onyxacalles gibraltarensis Stüben, 2002
Spain: Ceuta [Morocco: Sebta]
(b)
Stüben/CURCI
2 mm ♂
Onyxacalles portusveneris (Mayet, 1903)
France: Pyr.-Or., Sorete
(c)
Mid-tibia spine
0.1 mm
2 mm ♀
Onyxacalles seguraensis Stüben, 2003
PT, Spain: Sierra Segura, Yeste
(d)
2 mm ♂
Onyxacalles maginaensis Stüben, 2004
PT, Spain: Sierra Màgina
(e)
2 mm ♂
Onyxacalles bermejaensis Stüben, 2001
PT, Spain: Sierra Bermeja
(f)
2 mm ♀
Onyxacalles denominandus (Solari, 1907)
LT, Turkey: Adana
(g)
2 mm ♂
Onyxacalles croaticus (H. Brisout, 1867)
Germany: Berchtesgaden, 1500 m
(h)
2 mm ♂
Onyxacalles valencianus Germann, 2005
Spain: Valencia
A. Stüben/CURCI/ZfMK

Figure 7: (a–e): ***Portusveneris* group**—*O. gibraltarensis* (a), *O. portusveneris* (b), *O. seguraensis* (c), *O. maginaensis* (d), and *O. bermejaensis* (e); (f): **Incertae sedis**—*O. denominandus*; (g-h): ***Henoni* group** (see also next figures)—*O. croaticus* (g) and *O. valencianus* (h)*;* all habitus (dor./lat.).

(8) Punctures at the sides of elytra fine and slender; bristles on the first four intervals of the elytral slope in single row. Habitus (Figure 7(d)). Aedeagus (Figure 6(d)). Distribution: Southern Spain (Figure 4).

***Onyxacalles maginaensis* Stüben, 2004**

(8*) Punctures at the sides of elytra broader and deeper; bristles on the third interval of the elytral slope densely placed, forming a pronounced tuft at the level of the white fascia. Habitus (Figure 7(e)). Aedeagus (Figure 9(e)). Distribution: Southern Spain (Figure 4).

***Onyxacalles bermejaensis* Stüben, 2001**

—

(9) Pronotum with a deep midgroove and with strong concavities on each side; a species from Turkey.

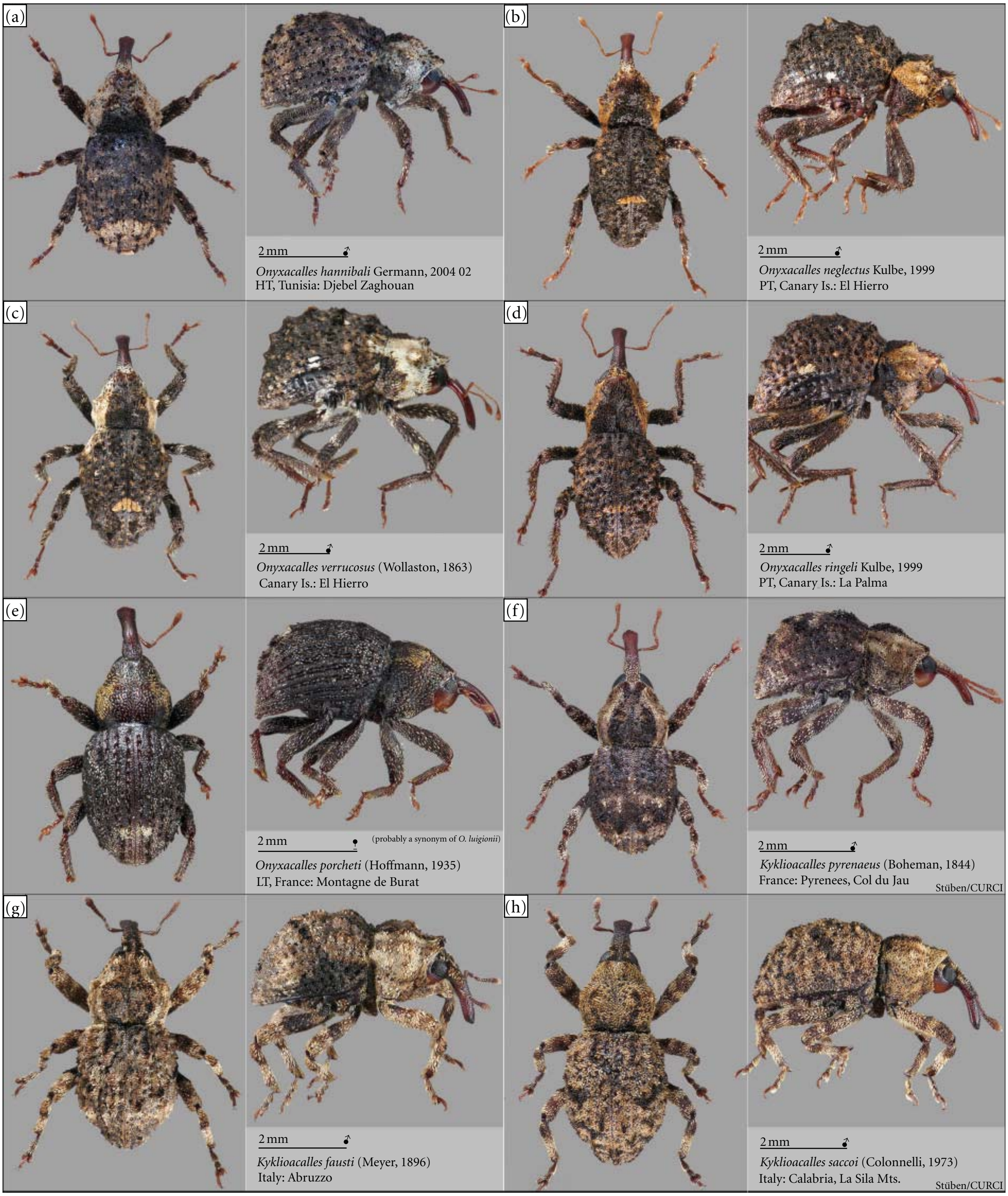

(a)
2 mm ♂
Onyxacalles hannibali Germann, 2004 02
HT, Tunisia: Djebel Zaghouan
(b)
2 mm ♂
Onyxacalles neglectus Kulbe, 1999
PT, Canary Is.: El Hierro
(c)
2 mm ♂
Onyxacalles verrucosus (Wollaston, 1863)
Canary Is.: El Hierro
(d)
2 mm ♂
Onyxacalles ringeli Kulbe, 1999
PT, Canary Is.: La Palma
(e)
2 mm ♀
(probably a synonym of *O. luigionii*)
Onyxacalles porcheti (Hoffmann, 1935)
LT, France: Montagne de Burat
(f)
2 mm ♂
Kyklioacalles pyrenaeus (Boheman, 1844)
France: Pyrenees, Col du Jau
Stüben/CURCI
(g)
2 mm ♂
Kyklioacalles fausti (Meyer, 1896)
Italy: Abruzzo
(h)
2 mm ♂
Kyklioacalles saccoi (Colonnelli, 1973)
Italy: Calabria, La Sila Mts.
Stüben/CURCI

FIGURE 8: (a): ***Henoni* group**—*O. hannibali* (see also the other species of this group: Figures 5(a), 5(d), 5(g), 5(k)); (b–d): subgenus: ***Araneacalles***—*O. neglectus* (b), *O. verrucosus* (c), and *O. ringeli* (d); (e): *O. porcheti* (perhaps *O. luigionii*); (f): *Kyklioacalles pyrenaeus*; (g) *K. fausti*; (h) *K. saccoi*; all habitus (dor./lat.).

Habitus (Figure 7(f)). Aedeagus (Figure 9(f)). Distribution: Turkey.

***Onyxacalles denominandus* (A. & F. Solari, 1907) [32]**
= ? *Onyxacalles amasyaensis* Wolf, 2001

(9) Pronotum behind the base at most with a flat depression or a hinted channel; mainly West Mediterranean species, only one species from southeastern Europe.

***Henoni* group** **10**

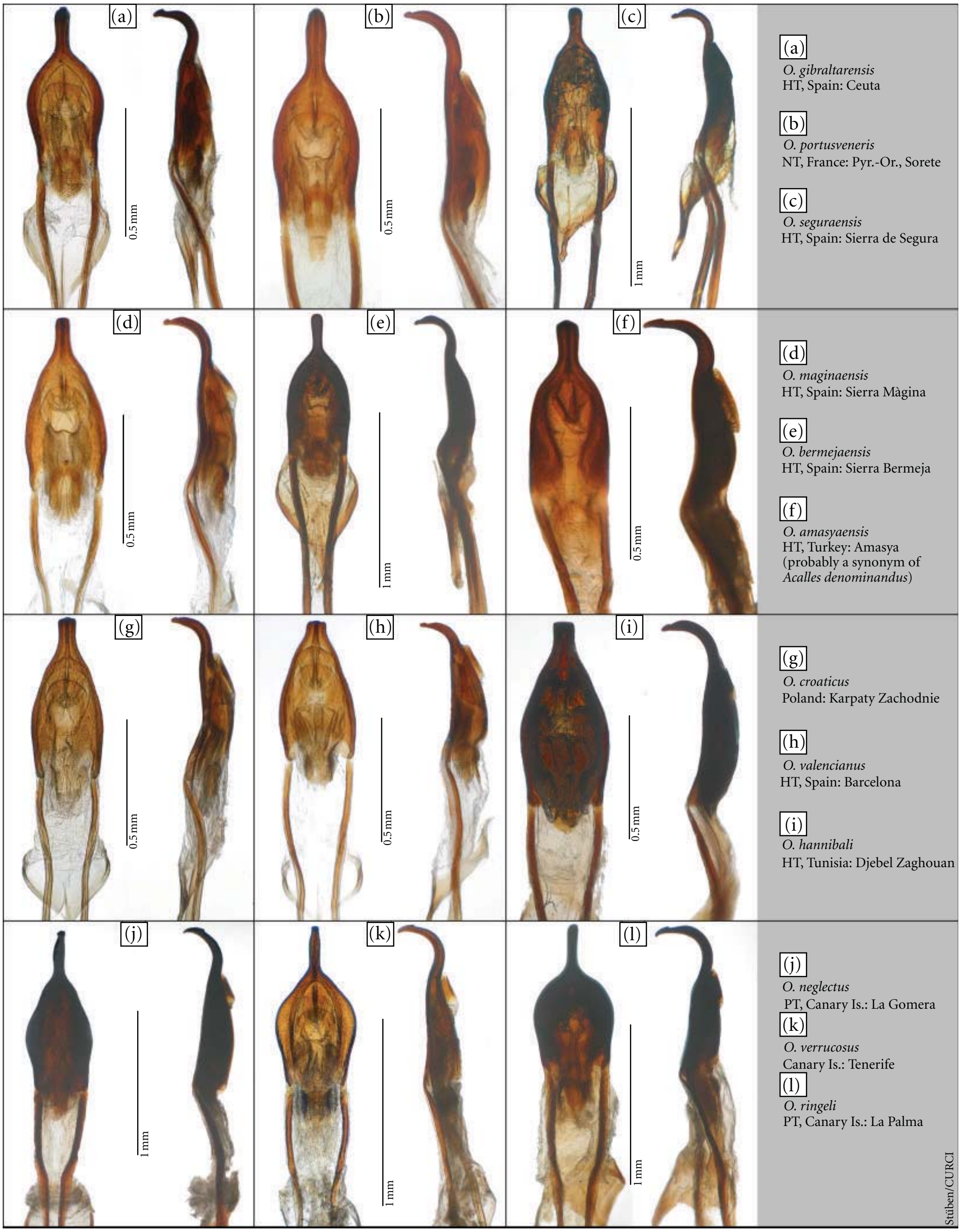

(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
(i)
(j)
(k)
(l)
0.5 mm
1 mm
(a) O. gibraltarensis HT, Spain: Ceuta
(b) O. portusveneris NT, France: Pyr.-Or., Sorete
(c) O. seguraensis HT, Spain: Sierra de Segura
(d) O. maginaensis HT, Spain: Sierra Màgina
(e) O. bermejaensis HT, Spain: Sierra Bermeja
(f) O. amasyaensis HT, Turkey: Amasya (probably a synonym of Acalles denominandus)
(g) O. croaticus Poland: Karpaty Zachodnie
(h) O. valencianus HT, Spain: Barcelona
(i) O. hannibali HT, Tunisia: Djebel Zaghouan
(j) O. neglectus PT, Canary Is.: La Gomera
(k) O. verrucosus Canary Is.: Tenerife
(l) O. ringeli PT, Canary Is.: La Palma
Stüben/CURCI

FIGURE 9: (a–d): ***Portusveneris* group**—*O. gibraltarensis* (a), *O. portusveneris* (b), *O. seguraensis* (c), *O. maginaensis* (d), and *O. bermejaensis* (e); (f): **Incertae sedis**—*O. amasyaensis*; (g–i): ***Henoni* group**—*O. croaticus* (g), *O. valencianus* (h), *O. hannibali* (i) (see also the other species of this group: Figures 5(c), 5(f), 5(j), 5(n)); (j–l): subgenus: ***Araneacalles***—*O. neglectus* (j), *O. verrucosus* (k), and *O. ringeli* (l).

— *Henoni* group —

(10) Elytra with superelevated and in tubercles dissected intervals; a species from southeastern Europe, which is added to the *henoni* group preliminary on the basis of a similar form of the aedeagus. Habitus (Figure 7(g)). Aedeagus (Figure 9(g)). Distribution: East and Southeast Europe [31].

Onyxacalles croaticus **(H. Brisout de Barneville, 1867) [10]**

(10) Elytra flat, without tubercles.

.... **11**

(11) Elytra and pronotum (almost) without tufts of bristles; these single, beaded bristles placed in a row.

.... **12**

(11*) Elytra on the intervals 1 and 3 and pronotum at the sides with tufts of bristles; these bristles densely placed in 2-3 rows, forming tufts at regular intervals.

.... **13**

(12) Elytral bristles short, shovel-shaped and densely placed (at most 1.3x as long as wide); elytra rich in contrast (colored); apex of aedeagus strongly curved in lateral view. Habitus (Figure 5(g)). Aedeagus (Figure 5(j)). Distribution: Spain, Majorca (Figure 2).

***Onyxacalles balearicus* Stüben, 2005**

(12*) Elytral bristles more slender, at least 2x as long as wide and their distance large; elytra poor in contrast; apex of aedeagus flatter curved in lateral view. Habitus (Figure 6(n)). Aedeagus (Figure 9(h)). Distribution: Eastern Spain (Figure 2).

***Onyxacalles valencianus* Germann, 2005**

(13) Disc of pronotum with a channel from the base towards the flat sector in front of the fore-margin; elytra of male with parallel sides in the middle sector (dorsal view). Habitus (Figure 5(a)). Aedeagus (Figure 5(c)). Distribution: Italy, Sardinia (Figure 2).

***Onyxacalles nuraghi* Stüben sp. n.**

(13) Disc of pronotum at most with a flat hallow behind the base; elytra of male broader and stronger (short ovally) rounded (slightly "egg-shaped").

.... **14**

(14) Elytral intervals only with a few bristles and small tufts; elytra poor in contrast. Habitus (Figure 8(a)). Aedeagus (Figure 9(i)). Distribution: Tunisia (Figure 2).

***Onyxacalles hannibali* Germann, 2004**

(14*) Elytral intervals studded with bristles and with numerous distinctive tufts; elytra rich in contrast.

.... **15**

(15) Rostrum coarsely and densely punctuated towards apex (Figure 5(l)); scales of the elytra predominantly bright: white, beige, or brown (Figure 5(k)); Apex ("hook") of the aedeagus smaller in ventral view and not so strongly curved in lateral view (Figure 5(n)). Distribution: Algeria, Mt. Edough (Figure 2).

***Onyxacalles henoni* [38]**

(15*) Rostrum finely punctuated towards apex (Figure 5(m)); scales of the elytra predominantly dark brown or black (Figure 5(d)); apex ("hook") of the aedeagus broader in ventral view and strongly curved (nearly rectangular) in lateral view (Figure 5(f)). Distribution: France, Corsica (Figure 2).

***Onyxacalles torre* Stüben and Astrin sp. n.**

— Subgenus: *Araneacalles* —

(16) Punctures of the 1st and 2nd elytral striae rounded, pothole-like, and as wide as the intervals; the underground of elytra in front of the middle on the 8th and 9th intervals with scales, not shiny; fore-margin of pronotum with a curved up collar. Habitus (Figure 8(b)). Aedeagus (Figure 9(j)). Distribution: Canary Is., La Gomera (l.t.), El Hierro (Figure 4).

***Onyxacalles neglectus* Kulbe, 1999**

(16*) Punctures of the 1st and 2nd elytral striae elongated, clearly smaller than intervals; the underground of elytra in front of the middle on the 8th and 9th intervals without scales, shiny; fore-margin of pronotum without a curved up collar.

.... **17**

(17) Punctures at the extreme striae slender, the intervals broader; elytra widest in front of the middle, egg-shaped. Habitus (Figure 8(c)). Aedeagus (Figure 9(k)). Distribution: Canary Is., Tenerife (l.t.), El Hierro (Figure 4).

***Onyxacalles verrucosus* (Wollaston, 1863) [40]**

(17*) Punctures at the extreme striae larger and rounded, not broader than intervals; elytra oval, widest in the middle. Habitus (Figure 8(d)). Aedeagus (60). Distribution: Canary Is., La Palma (Figure 4).

***Onyxacalles ringeli* Kulbe, 1999**

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Research Article

Contribution to the Knowledge of the Genus *Linda* Thomson, 1864 (Part I), with the Description of *Linda* (*Linda*) *subatricornis* n. sp. from China (Coleoptera, Cerambycidae, Lamiinae)

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Linda (Linda) subatricornis n. sp is described from Sichuan (holotype locality), Fujian, Shaanxi, Hebei, Ningxia of China. It is separated from the most similar species *L. atricornis* Pic by differences in genitalia and antennal insertions. Detailed descriptions, photographs of habitus and genitalia, distribution of the two sibling species and short discussion on the related species are presented.

1. Introduction

Linda Thomson, 1864 [1], includes two subgenera, *Linda* and *Dasylinda*, mostly confined to China [2]. While studying more than 150 specimens of *Linda* (*Linda*) *atricornis* Pic from different localities, we were surprised to observe two very different kinds of male genitalia. We concluded that two superficially similar species have been historically misidentified as one species. We had examined the types of *L. atricornis*, *L. gracilicornis*, *L. major* (the three known species of subgenus *Linda* with elytra and antennae all black), and most of the other species of this genus. After careful observation and dissection, we separate *L. subatricornis* n. sp. and herein describe it as new to science.

2. Materials and Methods

Types and other material studied are deposited in the following institutions or private collections.

CCH: Collection of Dr. Carolus Holzschuh, Villach, Austria.

CPS: Collection of Dr. Carlo Pesarini and Dr. Andrea Sabbadini, Milano, Italy.

HBU: Museum of Hebei University, Hebei, China.

IRSNB: Institut royal des Sciences naturelles de Belgique, Bruxelles, Belgique.

IZAS: Institute of Zoology, Chinese Academy of Sciences, Beijing, China.

MHNG: Muséum d'Histoire Naturelle de Genève, Switzerland.

MNHN: Muséum National d'Histoire Naturelle, Paris, France.

SYSU: Sun-Yatsen University, Guangzhou, China.

3. Results

3.1. Linda (Linda) atricornis Pic (Figures 1 and 2). *Linda atricornis* Pic, 1924 [3]: 19 (Jiangsu, Shanghai) (MNHN). *Linda atricornis*; Savio, 1929 [4]: 3; Gressitt, 1939: 126 [5]; 1940: 197 [6]; 1942 [7]: 10 (part); 1942: 41 [8]; 1947 [9]: 548 (part); Gressitt, 1951 [10]: 605 (part); Hua et. al, 1992 [11]: 54, 55, 170, 303; Hua, 2002 [12]: 213 (part).

Linda (Linda) atricornis; Löbl and Smetana, 2010 [2]: 293 (part).

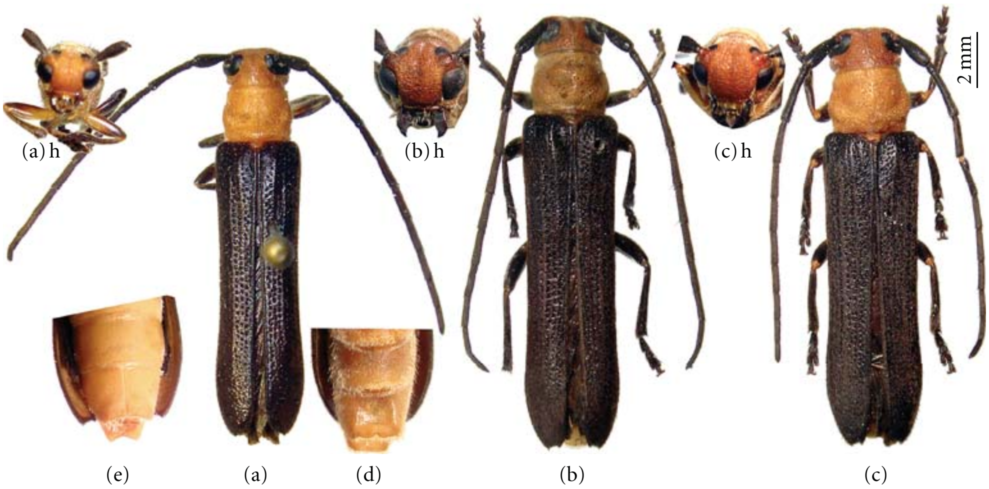

Figure 1: Habitus, *Linda atricornis* Pic, 1924. (a) Holotype, male, from Shanghai (Jiangsu). (b) Male, from Fujian. (c) Female, from Jiangsu. h: head, in frontal view. (d-e). Showing last visible sternite, not to scale. (d) Male. (e) Female. Scale 2 mm.

Redescription. Male (Figures 1(a) and 1(b)): length: 14.0–16.5 mm, humeral width: 3.0–3.6 mm. Female (Figure 1(c)): length: 14.2–17.5 mm, humeral width: 3.2–4.0 mm. Head (except eyes, labrum and mandibles), prothorax, scutellum, ventral surface of body, basal third of femora, extreme bases of tibiae and tarsal claws reddish testaceous; antennae, elytra, eyes, labrum, mandibles and most of legs black; pale portions covered with fine silvery pubescence and erect hairs; bases of elytra and undersurfaces of antennae with sparse erect hairs. Head densely and rugulose punctuate; vertex shallowly grooved; antennae shorter than body, about 5/6 (female) to 6/7 (male) of body length, antennomere ratio: male: 13 : 3 : 16 : 15 : 14 : 13 : 13 : 12 : 11 : 10 : 10; female: 14 : 3 : 16 : 15 : 14 : 13 : 12 : 11 : 10 : 9 : 9. Prothorax much broader than long, swollen above and behind middle of each side; scutellum declivitous, truncate. Elytron slightly emarginate apically, with sutural and outer angles slightly projected. Last visible sternite with a broad and deep groove and apex with a small nick in middle (male, Figure 1(d)) or with a thin line and apex smoothly emarginated (female, Figure 1(e)).

Male Terminalia (Figures 2(a)–2(d)). Tegmen length about 3.0 mm; lateral lobes not so stout, each about 0.5 mm long and 0.2 mm wide, mostly covered with moderate long setae, with one short but broad basal lobe furnished with short setae (in ventral view, Figure 2(d)); median lobe plus median struts slightly curved (Figure 2(b2)), a little longer than tegmen (7 : 6); the median struts slightly longer than half of the whole median lobe in length; dorsal plate slightly shorter than ventral plate; apex of ventral plate pointed (Figure 2(d)); median foramen slightly elongated; internal sac about twice as long as median lobe plus median struts, with 3 pairs of basal armature, and 2 pair of rods of endophallus; 2 longer rods each about 1.5 mm, about one-half of tegmen length, the shorter pair about 0.6 mm. The ratio of short pair to long pair always bigger than 1/3. Tergite VIII (Figure 2(a)) broader than long, apex truncated, rounded at side, with dense but short setae (hairs).

Female Genitalia (Figure 2(e)). Spermathecal capsule having a strongly sclerotized rounded apical lobe (with a very short stalk) and a not so sclerotized basal stalk, spermathecal duct not very longer than spermathecal capsule. Spermathecal gland extended from a strongly sclerotized broad ring, which attach to duct directly. Tignum shorter than abdomen. In our observation, tignum 6.5 mm for an adult with a 7.8 mm abdomen in ventral view.

Diagnosis. Femera mostly black, body not over 20 mm, these two characters easily separate it from *L. major* and *L. gracilicornis*.

Host (mixed with host of L. subatricornis). Cydonia sp. (ROSACEAE), *Juglans regia* Linnaeus (JUGLANDACEAE), *Malus* sp. (ROSACEAE), *Morus alba* Linnaeus (MORACEAE), *Populus davidiana* Dode (SALICACEAE), *Prunus armeniaca* Linnaeus (ROSACEAE), *Prunus mume* Siebold and Zuccarini (ROSACEAE), *Prunus persica* (Linnaeus) Batsch (ROSACEAE), *Prunus salicina* Lindley (ROSACEAE), *Rubus* sp. (ROSACEAE), and *Salix* sp. (SALICACEAE).

Remarks. The records from 17 provinces of China by Hua [12] or Löbl and Smetana [2] need confirmation based on specimens. The following provinces may misidentifications of *L. subatricornis*: Ningxia, Shaanxi; the northern provinces may not have this species: Inner Mongolia, Gansu, Hebei; the others may have this species but specimens are required to confirm it: Henan, Hubei, Hunan, Guizhou, Yunnan.

Distribution (Based on Specimens). China: Jiangsu, Zhejiang, Jiangxi, Fujian, Guangdong, Guangxi, Sichuan.

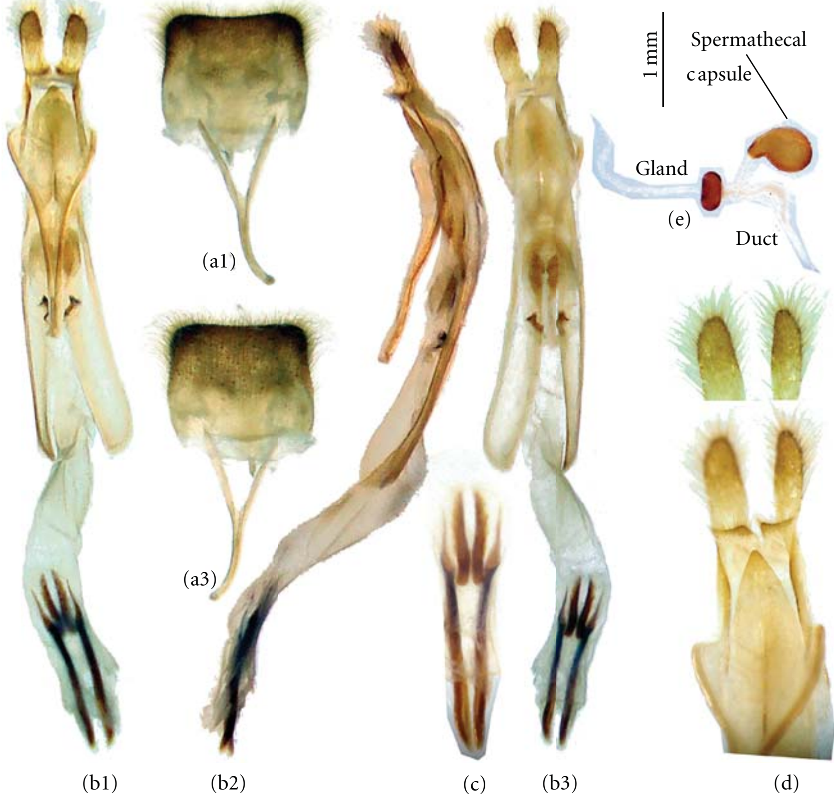

Figure 2: Genitalia of *Linda atricornis* Pic, 1924. (a) Tergite VIII and sternites VIII and IX. (b) Male genitalia. 1: ventral view; 2: lateral view; 3: dorsal view. Scale 1 mm. (c–e) not to scale. (c) Showing rods of endophallus. (d) Showing apex of ventral plate of median lobe and lateral lobes. (e) Spermatheca.

Type Specimens Examined. Type, male, Zi-ka-wei (MNHN, ex Coll. M. Pic).

Other Specimens Examined. Jiangsu: 1 female, Ihing, 1923. VII.16 (IZAS); 1 female, Shanghai, 1935.VII (IZAS); 1 female, Shanghai, 1939.VI.5, leg. O. Piel (IZAS).

Zhejiang: 1 female, T'ienmu Shan, 1936.VIII.1, leg. O. Piel (IZAS); 1 male 2 females, T'ienmu Shan, 1935.VIII.4 (IZAS); 1 female, Huangyan, 1955.VI.26 (IZAS); 1 female, Chekiang, Mokanshan, env. 50 k de Hangtcheou, 1925, leg. A. Pichon (MNHN); 2 males, Chusan, 1931.VI.12, leg. O. Piel (IZAS).

Jiangxi: Kiang-si, 1901, leg. C. L. Gonon (MNHN, ex Coll. R. Oberthür, 1952).

Fujian: 1 female, Chongan, Xingcun, Guadun, alt. 900–1100 m, 1963.VII.6, leg. ZHANG Youwei (IZAS); 1 female, same data but alt. 840–1160 m, 1960.VII.14, leg. ZHANG Yiran; 1 male, Chongan, Xingcun, Sangang, alt. 740 m, 1960. VII.30, leg. MA Chenglin (IZAS); 1 male, Dehua, Chengguan, alt. 510–550 m, 1960.VI.1, leg. PU Fuji (IZAS); 1 male, Dehua, Shangyong, Guifu, alt. 780–950 m, 1960.VI.18, leg. MA Chenglin (IZAS); 1 male, Fuzhou, Gushan, 1953.VI, leg. HUANG Jiabin (IZAS).

Guangxi: 4 males 2 females, Kouangsi, Region de Nanning, 1931 (MNHN, ex Coll. R. Oberthür, 1952); 9 males 4 females, same data but (IRSNB); 1 female, Prov. Kwangsi, Mts. Toyen-chan (MNHN, ex Coll. M. Pic); 1 female, Huangshahe, 1955.VIII.14 (IZAS).

Sichuan: 1 male, Pengshui, alt. 850 m, 1989.VII.11, leg. SUN Baowen (IZAS).

3.2. Linda (Linda) subatricornis n. sp. (Figures 3 and 4). Linda gracilicornis m. *tatsienlui* Breuning, 1954 [13]: 550 (Sichuan). (MHNG) infrasubspecies, nomen nudum.

Linda (s. str.) atricornis; Pu, 1992 [14]: 611 (misidentification).

Linda atricornis; Pic, 1935 [15]: 12 (misidentification); Gressitt, 1942 [7]: 10 (part); 1947 [9]: 548 (part); Gressitt, 1951 [10]: 605 (part); Wang and Chiang, 1988 [16]: 144 (misidentification); Hua, 2002 [12]: 213 (part).

Linda (Linda) atricornis; Löbl and Smetana, 2010 [2]: 293 [part].

Description. Male (Figures 3(a) and 3(b)), length: 13.5–16.0 mm, humeral width: 2.8–3.4 mm. Female (Figure 3(e)–3(g)), length: 15.4–18.5 mm, humeral width: 3.2–4.2 mm. Head (except eyes, antennal tubercles, labrum, and mandibles), prothorax, scutellum, ventral surface of body, basal thirds of femora, and tarsal claws reddish testaceous; antennae, antennal tubercles, elytra, eyes, labrum, mandibles, and most of legs black; pale portions covered with fine silvery pubescence and erect hairs; bases of elytra

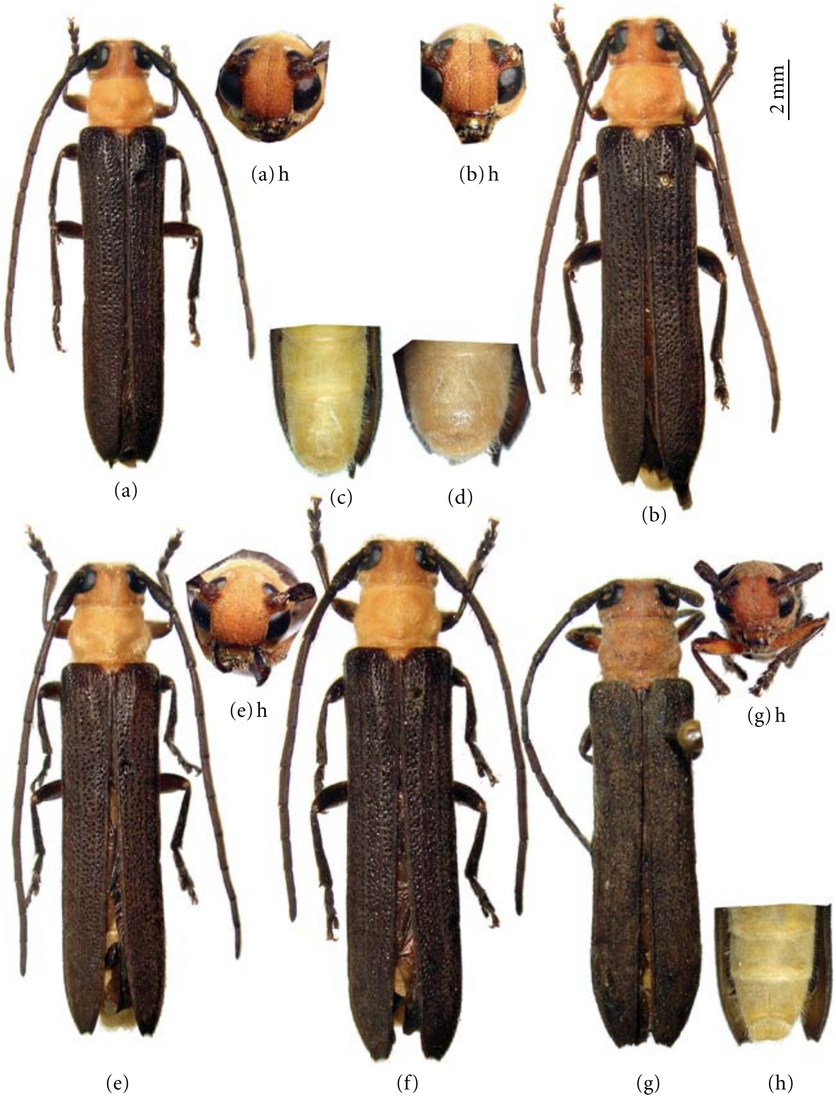

Figure 3: Habitus, *Linda subatricornis* n. sp. (a) Holotype, male, from Sichuan. (b) Paratype, male, from Beijing. (e–f) Paratype, female, from Sichuan. (g) Holotype of *Linda* (*Linda*) *gracilicornis* m. *tatsienlui* Breuning, 1954, female, from Sichuan. Scale 2 mm. h: head, in frontal view. (c-d) and (h) showing last visible sternite, not to scale. (c-d) Male. (h) Female.

and undersurfaces of antennae with sparse erect hairs. Head densely and rugulose punctuate; vertex shallowly grooved; antennae shorter than body, about 4/5 (female) to 9/10 (male) of body length, antennomere ratio: male: 13 : 2 : 16 : 14 : 13 : 13 : 12 : 11 : 10 : 9 : 10; female: 15 : 3 : 19 : 16 : 15 : 14 : 13 : 12 : 11 : 10 : 11. Prothorax much broader than long, swollen (three) above and behind middle of each side; scutellum declivitous, truncate. Elytron slightly emarginate apically, with sutural and outer angles slightly projected. Last visible sternite with a moderate broad and deep groove and apex with a small groove in middle (male, Figure 3(c) and 3(d)) or with a thin line and apex smoothly emarginated (female, Figure 3(h)).

Male Terminalia (Figures 4(a)–4(f)). Tegmen length about 3.0 mm; lateral lobes stout, each about 0.5 mm long and 0.25 mm wide, mostly covered with moderate long setae, with one short but broad basal lobe furnished with short setae (in ventral view, Figure 4(d)); median lobe plus median struts slightly curved (Figure 4(b2)), a little longer than tegmen (6 : 5); the median struts slightly longer than half of the whole median lobe in length; dorsal plate slightly shorter than ventral plate; apex of ventral plate narrowly rounded (Figure 4(c)); median foramen elongated; internal sac less than twice of median lobe plus median struts in length, with 3 pairs of basal armature, and 2 pairs of rods of endophallus; 2 longer rods each about 1.9 mm, longer than one-half of tegmen, the shorter pair about 0.6 mm. The ratio of short pair to long pair always smaller than 1/3. Tergite VIII (Figure 4(a)) broader than long, apex truncated, rounded at side, with dense but short setae (hairs).

Female Genitalia (Figures 4(g)–4(j)). Spermathecal capsule having a strongly sclerotized rounded apical lobe (with

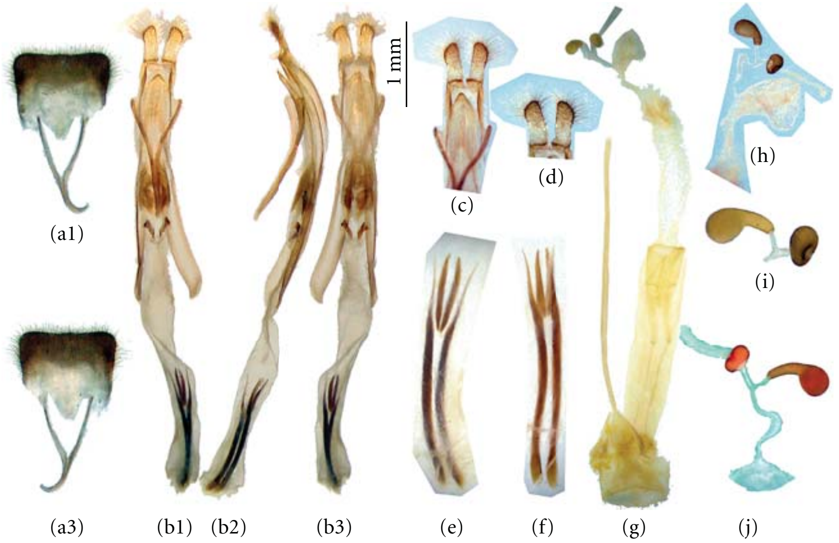

Figure 4: Genitalia of *Linda subatricornis* n. sp. (a) Tergite VIII and sternites VIII and IX. (b) Male genitalia. 1: ventral view; 2: lateral view; 3: dorsal view. Scale 1 mm. (c–j) Not to scale. (c-d) Showing apex of ventral plate of median lobe and lateral lobes. (e-f) Showing rods of endophallus. (e) From Sichuan. (f) From Beijing. (g–j) Female genitalia. (g-h) From Sichuan. (i) From Sichuan. (j) From Beijing.

a short to long stalk) and a not so sclerotized basal stalk, spermathecal duct not very longer than spermathecal capsule. Spermathecal gland extended from a strongly sclerotized broad ring, which attach to duct directly. Tignum shorter than abdomen. In our observation, tignum 6.8 mm for an adult with a 9.0 mm abdomen in ventral view.

Diagnosis. Differs from *L. atricornis* by antennal insertions black, extreme bases of tibiae black, groove of last visible sternite of male not so broad, last antennomere longer than tenth antennomere, rods of endophallus slender and the ratio of short pair to long pair smaller than 1/3, lateral lobes stouter, and so forth.

Differs from *L. major* and *L. gracilicornis* by antennal insertions black, femera mostly black, and body not over 20 mm. Differs from all the other species of subgenus *Linda* by antennae and elytra all black.

Etymology. Named after misidentification as *L. atricornis* in the collections.

Remarks. The female genitalia is difficult to separate species. In this species, the stalk attached to the strongly sclerotized rounded apical lobe is quite variable in length (Figure 4(h)–4(j)).

L. (*L.*) *gracilicornis* m. *tatsienlui* Breuning, 1954 [13] is a nomen nudum of this species, while *L.* (*L.*) *gracilicornis* m. *rufofemorata* Breuning, 1954 [13] should be a nomen nudum of *Linda femorata* (Chevrolat, 1852).

Host (mixed with host of L. atricornis). Cydonia sp. (ROSACEAE), *Juglans regia* Linnaeus (JUGLANDACEAE), *Malus* sp. (ROSACEAE), *Morus alba* Linnaeus (MORACEAE), *Populus davidiana* Dode (SALICACEAE), *Prunus armeniaca* Linnaeus (ROSACEAE), *Prunus mume* Siebold and Zuccarini (ROSACEAE), *Prunus persica* (Linnaeus) Batsch (ROSACEAE), *Prunus salicina* Lindley (ROSACEAE), *Rubus* sp. (ROSACEAE), *Salix* sp. (SALICACEAE).

Distribution. Sichuan, Fujian, Shaanxi, Hebei, Ningxia.

Type specimens examined. Holotype, male, Sichuan, Luding, Moxi, alt. 1600 m, 1983.VI.20, leg. CHAI Huaicheng (IZAS).

Paratypes. Sichuan: 13 males 9 females, Luding, Moxi, alt. 1500 m, 1983.VI.20, leg. ZHANG Xuezhong (IZAS); 1 male 4 females, same data but alt. 1600–1650 m, 1983.VI.18-19, leg. WANG Shuyong; 1 female, same data but 1982.IX.14, leg. WANG Shuyong; 10 males 7 females, Luding, Xinxing, alt. 1800–2100 m, 1983.VI.13–19, leg. WANG Shuyong, ZHANG Xuezhong, CHEN Yuanqing (IZAS); 1 male, Luding county, Moxi env., 1994.V.22–VI.10, leg. V. Beneš (CCH); 1 male, Abazhou, Nanping, Jiuzhaigou, alt. 2000 m, 1991.VI.8–13, leg. C. Holzschuh (CCH); 9 males, Emeishan, Baoguosi, 1957.V.5, leg. HUANG Keren (IZAS); 3 males, same data but 1957.V.12; 6 males 2 females, Emeishan, Baoguosi, alt. 550–750 m, 1957.V.3–VI.5, leg. HUANG Keren, ZHU Fuxing, LU Youcai (IZAS); 1 male, Emeishan, alt. 700 m, 1957.VI.1, leg. ZHU Fuxing (IZAS); 1 male, Emeishan, Qingyinge, alt. 800–1000 m, 1957.VI.11, leg. LU Youcai (IZAS); 4 females, Emeishan, 1955.VI.13–14, leg. HUANG Keren, JIN Gentao (IZAS); 1 female, Emeishan, alt. 1100–1800 m, 1955.VI.23, leg. GE Zhonglin (IZAS); 1 female, Mt. Emei, alt. 1050 m, 1990.VII.18, leg. L. & M. Bocák (CCH); 2 males, Wenchuan, Yingxiu, alt. 900 m, 1983.VIII.3, leg. WANG Shuyong (IZAS); 1 male, Jintang, 1943.V.9, leg. K. O. V. Lieu (IZAS); 1 female, Guanxian, Qingchengshan, alt. 700–1600 m, 1963.V.4, leg. ZHANG Xuezhong (IZAS); 1 female, Fengjiexian, 1980.VI.30, leg. QIAN Yuanzhi (IZAS);

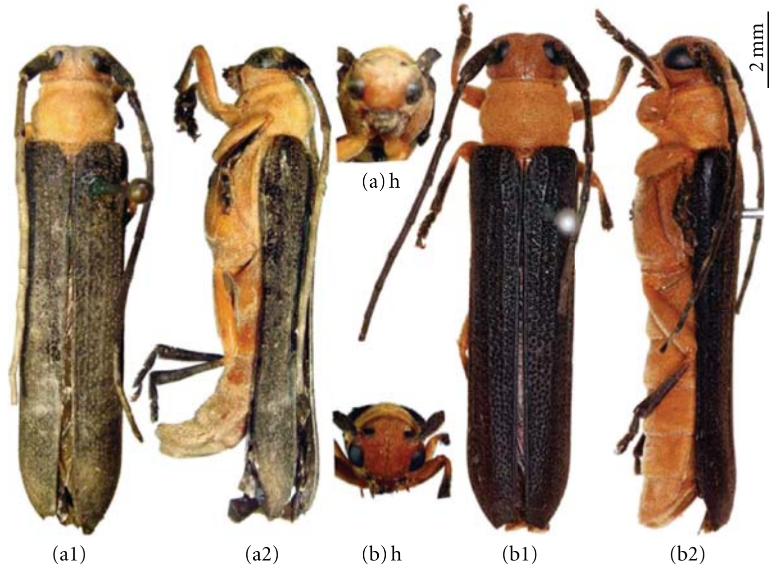

Figure 5: Habitus, holotype. (a) *Linda gracilicornis* Pic, 1907, male, from Yunnan. (b) *Linda major* Gressitt, 1942, female, from Anhui. 1. dorsal view. 2. lateral view. h: head, in frontal view. Scale 2 mm.

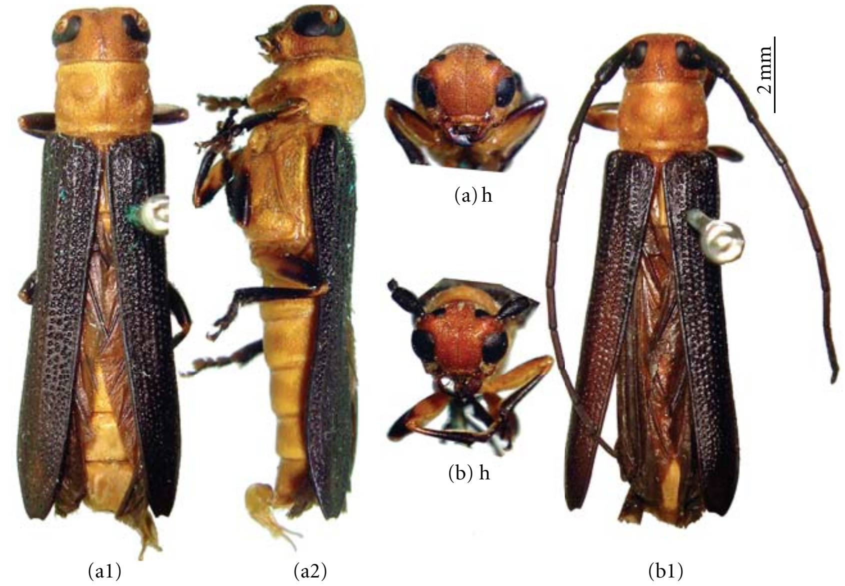

Figure 6: Habitus, *Oberea holatripennis* Breuning, 1982. (a) Holotype, female, from Beijing. (b) "Paratype," male, from Beijing, may be *Linda atricornis* Pic, 1924. 1: dorsal view; 2: lateral view; h: head, in frontal view. Scale 2 mm.

1 female, Yuechi, 1981.VIII.7, leg. LUO Dongming (IZAS); 1 female, Kangding, alt. 2500 m, 1983.VI.26, leg. WANG Shuyong (IZAS); 1 male, Kangding, 10 km North, alt. 2600 m, 1992.VII.9, leg. G. C. Bozano (CPS); 1 female, Xingou, 180 km S.W of Chengdou, alt. 1600 m, 1991.VII.16, leg. E. Giacomazzo (CPS); 2 females, Su-Tchuen, Siào-Lou, 1897 (MNHN, ex Coll. R. Oberthür, 1952); 2 females, Siào-Lou, 1901/1904, leg. Chasseurs du P. Dejéan (MNHN, ex Coll. R. Oberthür, 1952); 2 females, Siao-Lou-Lou-Chan, 1897, leg. Chasseurs Thibétains (MNHN, ex Coll. R. Oberthür, 1952); 1 male 1 female (holotype of *Linda gracilicornis* m. *tatsienlui* Breuning, 1954), Szetschuan, Tatsienlu (MHNG, ex Coll. S. Bruning, ex Coll. Reitter); 3 males 4 females, Su-Tchuen, 1903, leg. Chasseurs Indignes (MNHN, ex Coll. R. Oberthür, 1952).

Fujian: 1 male, Foochow (MHNG).

Shaanxi: 1 male, Qinlingshan, 6 km East of Xunyangba, alt. 1000–1300 m, 2000.V.23–VI.13, leg. C. Holzschuh (CCH); 1 male, Danfeng, NE env., alt. 900–1500 m, 1995.V.28–29, leg. L. & R. Businský (CCH); 1 male, Shaanxi (IZAS).

Hebei: 2 males, Beijing, Sanpu, 1964.VII.9, leg. LIAO Subai (IZAS); 1 female, Beijing, Sanpu, alt. 550 m, 1972.VII.4, leg. JIANG Shengqiao (IZAS); 1 male, Beijing,

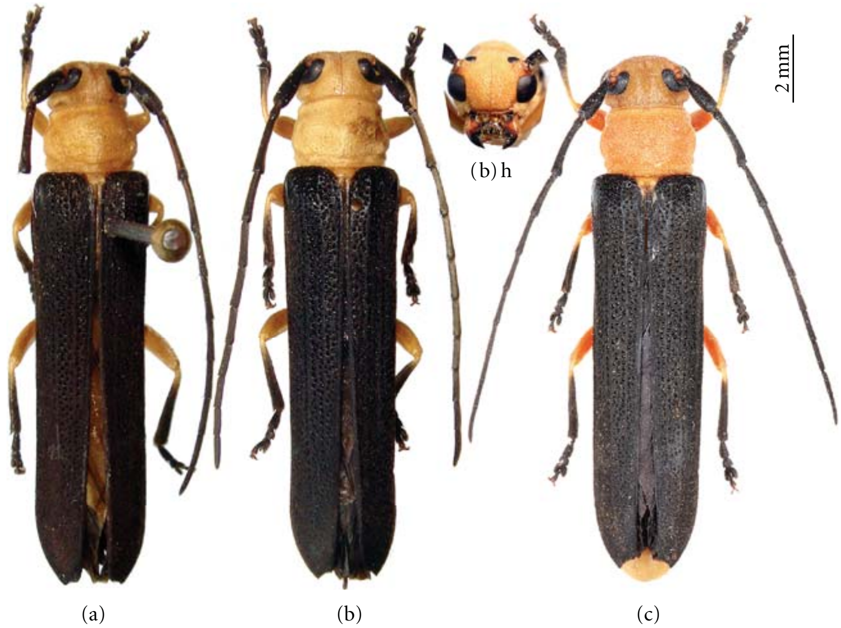

Figure 7: Habitus. (a-b) *Linda* cf. *gracilicornis*. (a) Male, from Sichuan. (b) Female, from Guangxi. h: head, in frontal view. (c) *Linda* cf. *major*, female, from Zhejiang. Scale 2 mm.

Shangfangshan, alt. 400 m, 1961.VII.17, leg. WANG Shuyong (IZAS); 1 female, Beijing, Shangfangshan, 1979.VII.25, leg. JIANG Shengqiao (IZAS).

Ningxia: 1 male, Jingyuan, Mt. Liupanshan, 1995.VI.14, leg. LIN924 group (HBU); 1 female, Jingyuan, 1981.VI.8 (IZAS); 1 male, Guyuan, 1981.VI.17 (IZAS).

4. Discussion

Including the new species described above, there are four species of subgenus *Linda* with elytra and antennae all black. They are *Linda atricornis*, *L. subatricornis*, *L. gracilicornis*, and *L. major*. *L. gracilicornis* Pic, 1907[17], was described based on one male from Yunnan. The holotype (Figure 5(a1), 5(a2), and 5(a)h deposited in MNHN) is in bad condition, with mud covering the punctures. It is very difficult to conclude if *L. major* Gressitt, 1942 [18] (Figure 5(b1), 5(b2), and 5(b)h deposited in SYSU) is a synonym of *L. gracilicornis* or a separate species. In the keys by Gressitt [7, 9, 10], *L. gracilicornis* "Elytra irregularly punctured; body slender; antennae relatively slender and as long as body," while *L. major* "Elytra subregularly punctured, body more or less stout; antennae relatively thick and shorter than body; elytra with round punctures; femora entirely testaceous and length over 20 mm," descriptions which did not well match with the types. Before enough material are available for further study, we consider them as two different species and *L. major* differs from *L. gracilicornis* by pronotum quite smooth, without accidented tubercles, elytral punctures denser, and more irregular. One male from Sichuan (Figure 7(a)) and one female from Guangxi (Figure 7(b) and 7(b)h) are identified as *L.* cf. *gracilicornis*, while one female from Zhejiang (Figure 7(c)) as *L.* cf. *major* according to above consideration. We wait for more material especially specimens from the type localities to make a better conclusion.

Based on the holotype (MNHN, ex Coll. J. Thomson, 1952, ex Musaeo ARM. DAVID, 1900), *Oberea holatripennis* Breuning, 1982 [19], from Beijing (Figure 6(a1), 6(a2), 6(a)h) is similar to *L. atricornis* but can be separated by the denser and irregular elytral punctures, and the pronotum with three visible swollen. The "paratype" (Figure 6(b1) and 6(b)h, determined by Breuning, deposited in MHNG, ex Musaeo Arm. David, 1900) is possibly a male of *L. atricornis*. More material and genitalia dissections are needed for further study.

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Research Article

On the Genus *Paragoniastes* Comellini, 1979, with Description of a New Species from Ilhéus, Brazil (Coleoptera, Staphylinidae, Pselaphinae)

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The types of the species of the *Goniacerine* genus of Pselaphinae *Paragoniastes* Comellini are revised. *Paragoniastes parki* Comellini, 1979, is synonymized with *P. besucheti* Comellini, 1979 (*P. besucheti* = *P. parki* syn. nov.), and *P. uesci* Cuccodoro & Kurbatov sp. nov. is described from the Brazilian state of Bahia. These taxa are described, illustrated, and keyed. Additional characters pertaining to the genus are given.

1. Introduction

Members of *Paragoniastes* are small predaceous rove beetles of the pselaphine tribe Goniacerini inhabiting the forest leaf litter. The genus was erected by Comellini to accommodate *Goniastes westwoodi* Raffray, 1890 from "Brésil" and three new species from the southern Brazilian states of Santa Catarina and Parana (*P. besucheti*, *P. parki*, and *P. raffrayi*).

In the frame of a survey of the pselaphine fauna of the Brazilian state of Bahia, we collected several *Paragoniastes* at the campus of Universidade Estadual de Santa Cruz, Ilhéus. Comparison of these specimens with the types of *Paragoniastes* housed in the Muséum d'histoire naturelle, Geneva, indicated not only that they were a new species but also that the holotype and unique specimen of *P. parki* is conspecific with the types of *P. besucheti*. The new species (*P. uesci*) is the first record of the genus in the Brazilian Nordeste.

The species are described and keyed, and their habitus is figured. We present drawings of the aedeagi of all the species of *Paragoniastes*, including that of *P. westwoodi*. We also mention some additional features pertaining to the genus, such as the system of meso- and metasternal foveae, and the conformation of the second visible abdominal sternite.

2. Material and Methods

All the specimens mentioned in this study (167 specimens) have been examined. These are housed in the Muséum d'histoire naturelle (MHNG), Geneva, Switzerland, in the Muséum National d'Histoire Naturelle (MNHN), Paris, France, and in the Universidade Estadual de Santa Cruz (UESC), Ilhéus, Brazil. In the future, the material deposited in UESC will be deposited in the Museu de Zoologia da Universidade de São Paulo (MZSP), Brazil.

The label data of the type of *P. westwoodi* are reproduced literally between " ", with additional information pertaining to labels, or localities between [ ], with | as a separator between each individual label.

Measurements are defined as follows: body length is measured from anterior outline of head (i.e., apical margin of labrum) to apex of abdomen; head width (HW) is the distance between outer outline of head just behind eyes;

head length (HL) is the medial distance between tip of frontoclypeus and occipital margin; pronotal length (PL) is the medial distance between anterior and posterior margins of pronotum; pronotal width (PW) is the maximal distance between lateral outline of pronotum; elytral length (EL) = elytral sutural length; elytral width (EW) is the maximal width of the elytra taken together. Antennal articles are measured in dorsal view, their length axially (without basal stalk), and their width at their maximal width.

The abdominal tergites and sternites are numbered according to Chandler [3] in Arabic (visible position) and Roman (morphological position) numerals; they are counted from tergite 1 (IV) and sternite 1 (III). Terminology of surface sculpturing follows Harris [4]. The aedeagi and other body parts illustrated here were mounted in Canada balsam on acetate slides and drawn using a drawing tube mounted on a compound microscope. The habitus figures are composites taken using a digital camera mounted onto a Leica MZ Apo dissecting microscope and processed using Automontage software.

3. Taxonomy

Key to the species of *Paragoniastes*

(1) (a) Center of pronotum areolate—*P. besucheti* Comellini.
    (b) Center of pronotum covered with longitudinal ridges slightly diverging anteriorly—2.
(2) (a) Elytra smooth, without marked discal striae—*P. westwoodi* Raffray.
    (b) Elytra scabriculate, with marked discal striae—3.
(3) (a) Elytra lacking humeral stria. Mesofemora bearing posteriorly a conspicuous subbasal tooth-like process—*P. raffrayi* Comellini.
    (b) Elytra with humeral stria. Mesofemora bearing posteriorly at most an obsolete subbasal tooth-like process—*P. uesci* sp. Nov.

3.1. Paragoniastes Comellini, 1979.

> *Paragoniastes* Comellini, 1979: 681; type species: *Paragoniastes raffrayi* Comellini (by original designation).

Additional Characters. Habitus as in Figures 1(a), 1(b), 1(c), 1(d), 2(a), and 2(b). Maxillary palpi as in Figure 3(e). Pronotum with shallow medial antebasal fovea and pair of well-marked lateral antebasal depressions (without true lateral antebasal foveae). Elytron with sutural and lateral striae on entire elytral length; usually present is internal discal stria and external discal stria, and occasionally humeral stria; when present, discal and humeral striae evanescent subapically, internal discal and humeral striae reaching basal margin, and external stria evanescent subbasally. Prosternum with pair of lateral procoxal foveae; medial carina absent. Mesosternum (Figure 3(b)) scabriculate, except prepectus smooth; the latter larger than mesosternal shield, medially carinate and laterally concave to allow accommodation of procoxae; pair of lateral foveae in connection with pair of promesocoxal foveae. Mesocoxal cavities separated. Metasternum (Figure 3(b)) scabriculate, markedly delimited from mesosternum; with pair of lateral mesocoxal foveae and medial metasternal fovea. Legs with second tarsomeres slightly shorter than third; single tarsal claw. Abdominal tergites each 1–5 bearing four macrosetae, tergites 1–3 each with medial ridge. Abdominal sternite 1 (Figure 3(d)) very short, visible only on mesal portion; sternite 2 (Figure 3(d)) bearing pair of deep transverse cavities densely covered by pubescence, each with basolateral and mediobasal foveae. Aedeagus with basal bulb of median lobe membranous and two symmetrical parameres.

Comments. Particularly notable in the genus is the structure of the lateral and promesocoxal foveae, which are unusually connected with each other, suggesting that the promesocoxal foveae might result from an extreme bifurcation of the lateral foveae. The membranous basal bulb of the aedeagus can easily collapse and its shape is thus not discriminant. Sexual dimorphism appears to affect only the medial area of the abdominal sternites. The presence of mesofemoral spines in both sexes is particularly notable, as it is usually a sexually dimorphic feature in Pselaphinae.

3.1.1. Paragoniastes raffrayi Comellini, 1979. See Figures 1(a), 1(b), and 3.

> *Paragoniastes raffrayi* Comellini, 1979: 682.

Material examined (holotype and 156 paratypes, all in MHNG): “Brésil, Santa Catarina, Nova Teutonia, F. Plaumann, vi.1972”, 1 macrophthalmus male (holotype), 13 microphthalmus males and 12 females; same data, but xii.1967, 3 microphthalmus males and 1 female; same data, but xii.1969, 1 female; same data, but i.1970, 2 females; same data, but ii.1970, 1 macrophthalmus male and 2 females; same data, but v.1970, 1 macrophthalmus male; same data, but iii.1972, 1 female; same data, but x.1972, 1 macrophthalmus male, 2 microphthalmus males and 2 females; same data, but xii.1972, 2 females; same data, but ii.1973, 1 macrophthalmus male and 1 female; same data, but xi.1974, 2 macrophthalmus males and 5 females; same data, but iv.1976, 21 macrophthalmus males, 34 microphthalmus males and 48 females.

Description. Body (Figures 1(a) and 1(b)) 1.75–1.80 mm long. Head 1.2-1.3 times longer that wide (without eyes). Antennae (holotype) with scape 1.1-1.2 times longer than pronotal width; 2nd article somewhat transverse; 3rd 1.7-1.8 times longer than wide, 2.6–2.8 times longer than 2nd and 1.4-1.5 times longer than 4th; 4th 1.1-1.2 times longer than wide; 5th 1.6 times longer than wide and about as long as 3rd. Pronotum as long as wide, covered with longitudinal ridges slightly diverging anteriorly, except posterior quarter scabriculate. Elytra scabriculate, without transverse carinulae. Elytral internal and external discal striae well marked; humeral stria absent, or at most obsolete on basal quarter.

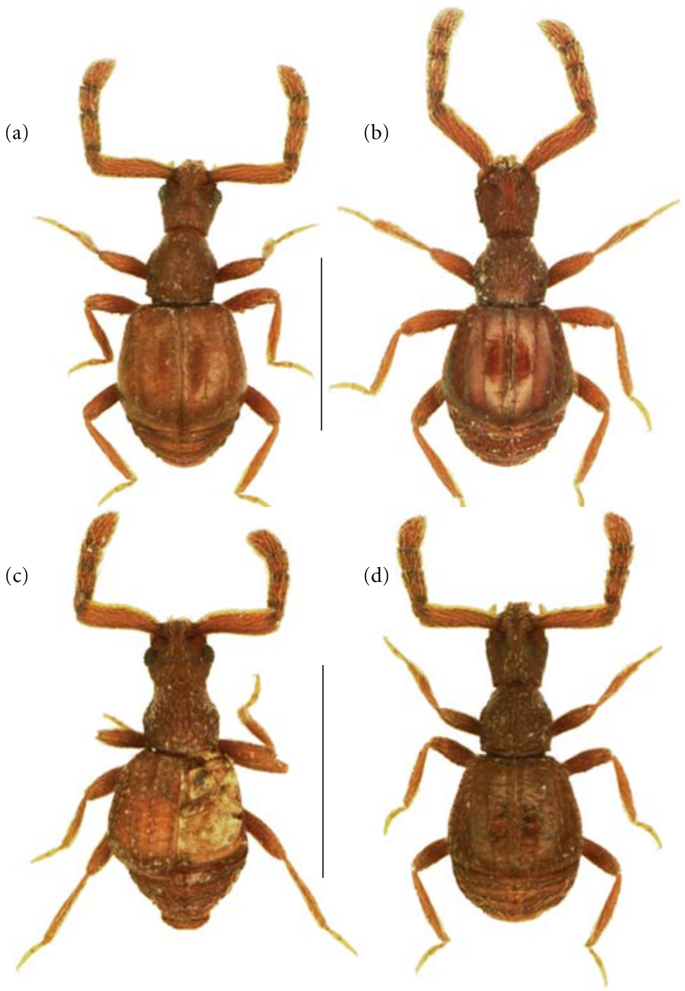

Figure 1: (a) and (b) *Paragoniastes raffrayi*, male, macrophthalmus (a) and microphthalmus (b). (c) and (d): *P. besucheti*, male, macrophthalmus ((c): holotype of *P. parki*) and microphthalmus ((d): holotype). Scale bars: 1 mm.

Presence of 13 setae arranged along internal discal striae; strial setae about as long as interval between them, and more than three times longer that interstrial setae, the latter almost indistinct. Mesofemora bearing posteriorly a conspicuous subbasal tooth-like process.

Measurements (holotype): HL = 0.43 mm; HW = 0.34 mm; PL = 0.40 mm; PW = 0.40 mm; EL = 0.71 mm; EW = 0.75 mm.

(i) Male. Eyes of macrophthalmus individuals with 28–30 facets, microphthalmus individuals with 5–8 facets. Abdominal sternites 2–5 medially depressed. Aedeagus (Figures 3(a) and 3(c)) 0.20–0.21 mm long.

(ii) Female. Eyes with 5 facets.

Distribution. *Paragoniastes raffrayi* is apparently restricted to the south Brazilian state of Santa Catarina, where it occurs in sympatry with *P. besucheti*.

Comments. Within the specimens examined, the sex ratio is 50%, and 35% of the males are macrophthalmus. *Paragoniastes raffrayi* is easily distinguished from its congeners by the presence of marked discal striae in combination with the almost completely evanescent humeral stria. The presence of mesofemoral spines in both sexes is particularly notable, as it is usually a sexually dimorphic feature in Pselaphinae.

3.1.2. *Paragoniastes besucheti* Comellini, 1979. See Figures 1(c), 1(d), 4(a)–4(d).

Paragoniastes besucheti Comellini, 1979: 686.

Paragoniastes parki Comellini, 1979: 685 **syn. nov**.

Material examined (6 specimens, all in MHNG): "Brésil, Santa Catarina, Nova Teutonia, F. Plaumann, xii.1976", 1 microphthalmus male (holotype of *P. besucheti*); same data, but xi.1976, 1 macrophthalmus male (holotype of *P. parki*) and 1 microphthalmus male (paratype of *P. besucheti*); "Brésil, [Parana], Rondon, <24°38′S; 54°07′E> F. Plaumann, iii.1965" 2 microphthalmus males and 1 female (paratypes of *P. besucheti*).

Description. Body (Figures 1(c) and 1(d)) 1.45–1.50 mm long. Head 1.2-1.3 times longer than wide (without eyes).

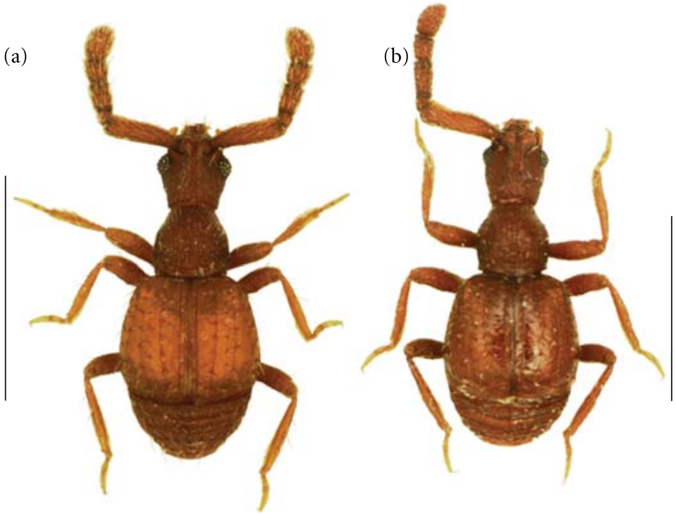

Figure 2: (a) *Paragoniastes uesci*, male, macrophthalmus (paratype). (b) *P. westwoodi*, male, macrophthalmus (holotype). Scale bars: 1 mm.

Antennae (holotype) with scape 1.1-1.2 times longer than pronotal width; 2nd article as long as wide; 3rd 1.6-1.7 times longer than wide, 1.8-1.9 times longer than 2nd and 1.3-1.4 times longer than 4th; 4th somewhat longer than wide; 5th 1.6-1.7 times longer than wide and 1.2-1.3 times longer than 3rd. Pronotum slightly wider than long, areolate, except anterior quarter covered with longitudinal ridges slightly diverging anteriorly. Elytra smooth, irregularly covered with transverse carinulae. Elytral internal and external discal striae well-marked; humeral stria present on more that three quarters of elytral length. Presence of 13 setae arranged along internal discal striae; strial setae about as long as interval between them, and about as long as interstrial setae. Mesofemora bearing posteriorly at most obsolete subbasal tooth-like process.

Measurements (holotype): HL = 0.37 mm; HW = 0.30 mm; PL = 0.32 mm; PW = 0.34 mm; EL = 0.54 mm; EW = 0.60 mm.

(i) Male. Eyes of macrophthalmus individuals with 34 facets, microphthalmus individuals with 3–5 facets. Abdominal sternites 2–4 medially depressed, 2-3 each with spinose medial process consisting of few agglomerated setae projecting from small medial tubercle, these tubercles not contiguous. Aedeagus (Figures 4(a), 4(b), 4(c) and 4(d)) 0.27-0.28 mm long.

(ii) Female. Eyes with 4-5 facets.

Distribution. *Paragoniastes besucheti* occurs in the south Brazilian states of Santa Catarina (in sympatry with *P. raffrayi*) and Paraná.

Comments. This species is the only member of the genus to possess an areolate pronotum. Close examination of the holotypes *P. besucheti* and *P. parki* indicated that the apparent differences between Comellini's aedeagal drawings of these two species result from misinterpretation of details and deformations of these structures on the microscope slides where the aedeagi were mounted.

3.1.3. Paragoniastes uesci Cuccodoro & Kurbatov **sp. nov**.

See Figures 2(a), 4(e), and 4(f).

Holotype (macrophthalmus male, in UESC): "Brazil, Bahia, Ilhéus, Universidade Estadual de Santa Cruz (UESC), 80 m <14° 39′S, 39° 10′W> L. Pereira de Oliveira, M. Santana Fonseca & G. Cuccodoro, 31.vii.2011, sifting leaf litter in the forest of the campus".

Paratypes (2): same data as holotype, 1 macrophthalmus male in MHNG and 1 female in UESC.

Description. Body (Figure 2(a)) 1.50 mm long. Head 1.2-1.3 times longer than wide (without eyes). Antennae (holotype) with scape as long as pronotal width; 2nd article as long as wide; 3rd 1.4-1.5 times longer than wide, 1.9-2.0 times longer than 2nd and 1.3-1.4 times longer than 4th; 4th as long as wide; 5th 1.3-1.4 times longer than wide and 1.2-1.3 times longer than 3rd. Pronotum slightly wider than long, entirely covered with longitudinal ridges slightly diverging anteriorly. Elytra scabriculate, irregularly covered with transverse carinulae. Elytral internal and external discal striae well-marked; humeral stria present on more that three quarters of elytral length. Presence of 10 setae arranged along internal discal striae; strial setae about as long as interval between them, and about two times longer than interstrial setae. Mesofemora bearing posteriorly at most an obsolete subbasal tooth-like process.

Measurements (holotype): HL = 0.36 mm; HW = 0.29 mm; PL = 0.32 mm; PW = 0.34 mm; EL = 0.57 mm; EW = 0.62 mm.

(i) Male. Eyes of macrophthalmus individuals with 30–32 facets (microphthalmus individuals unknown). Abdominal sternite 2 with small medioapical tubercle bearing short setae directed forward; sternites 3–5

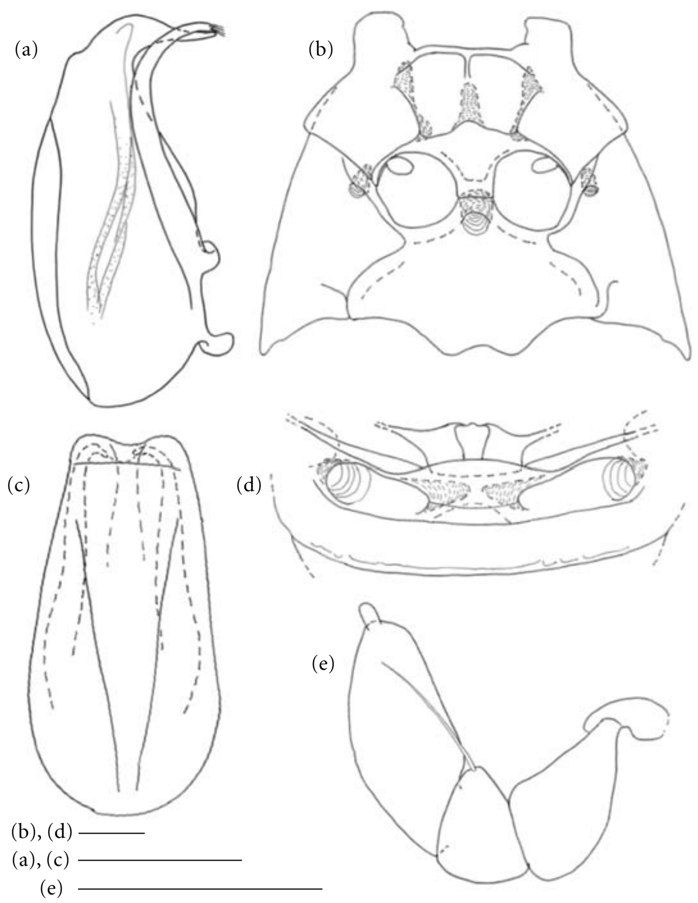

Figure 3: *Paragoniastes raffrayi*. (a) and (c): Aedeagus in lateral (a) and dorsal (c) views. (b) Mesosternum and metasternum, female, ventral view, pubescence omitted. (d): Medial area of abdominal sternites 1-2, female, ventral view, pubescence omitted. (e) Left maxillary palpus, female, dorsal view. Scale bars: 0.1 mm.

medially depressed. Aedeagus (Figures 4(e) and 4(f)) 0.25–0.26 mm long.

(ii) Female. Eyes with 1 facet.

Distribution. This species is known so far only from the state of Bahia and represents the first record of the genus in the Brazilian Nordeste.

Comments. Paragoniastes uesci is the only member of the genus with the pronotum entirely covered with longitudinal ridges slightly diverging anteriorly in combination with long and well-marked elytral humeral striae.

Etymology. The epithet *uesci* is an acronym for the Universidade Estadual de Santa Cruz, Ilhéus, campus where the new species was discovered.

3.1.4. Paragoniastes westwoodi (Raffray, 1890). See Figures 2(b), 4(g), and 4(h).

Goniastes westwoodi Raffray, 1890: 209.

Material examined (holotype, macrophthalmus male, in MNHN): "Brésil [handwritten on white rectangular label] | Muséum Paris, 1917/col. A. Raffray [typewritten on green rectangular label] | Type [typewritten on red rectangular label] | *G. westwoodi* [handwritten]/A. Raffray det. [typewritten on white rectangular label] | *Goniastes westw* [handwritten on green rectangular label] | *Goniastes westwoodi* Raffray type | *Paragoniastes westwoodi* (Raffray) type [handwritten on white rectangular label]".

Description. Body (Figure 2(b)) 1.80 mm long. Head 1.4-1.5 times longer than wide (without eyes). Antennae (holotype) with scape 1.1-1.2 times longer than pronotal width; 2nd article as long as wide; 3rd 1.7-1.8 times longer than wide, 1.9-2.0 times longer than 2nd and 1.4-1.5 times longer than 4th; 4th 1.1-1.2 times longer than wide; 5th 1.5-1.6 times longer than wide and 1.1-1.2 times as long as 3rd. Pronotum as long as wide, covered with longitudinal ridges slightly diverging anteriorly, except posterior quarter scabriculate. Elytra smooth, irregularly covered with transverse carinulae. Elytral internal and external discal striae evanescent; humeral stria absent, or at most obsolete on basal quarter. Presence of 13 setae arranged along internal discal striae; strial setae about as long as interval between them, and about as long as interstrial setae. Mesofemora bearing posteriorly at most an obsolete subbasal tooth-like process.

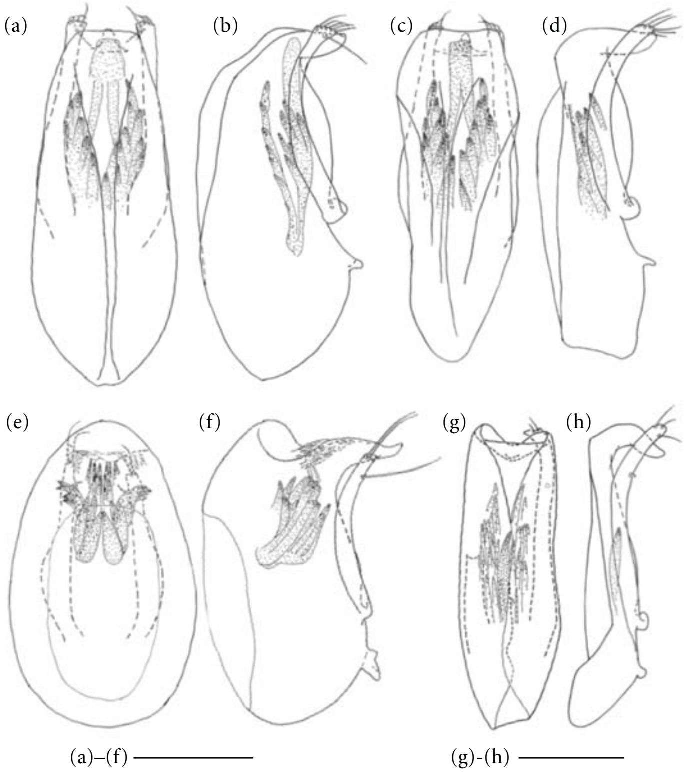

Figure 4: (a–b) *Paragoniastes besucheti* ((a) and (b) holotype; (c) and (d) holotype of *P. parki*). (e) and (f) *P. uesci* (holotype). (g) and (h) *P. westwoodi* (holotype, feft paramere broken). Aedeagus in dorsal (a, c, e, and g) and lateral (b, d, f, and h) views. Scale bars: 0.1 mm.

Measurements (holotype): HL = 0.49 mm; HW = 0.34 mm; PL = 0.41 mm; PW = 0.41 mm; EL = 0.72 mm; EW = 0.77 mm.

(i) Male. Eyes of macrophthalmus individual with 34 facets (microphthalmus individuals unknown). Abdominal sternites 2-3 not depressed, each with small medial tubercle bearing short setae directed backward, these tubercles contiguous; sternite 4 medially depressed. Aedeagus (Figures 4(g) and 4(h)) 0.35 mm long.

(ii) Female. Unknown.

Distribution. The only information available on the distribution of this species is that it comes from Brazil.

Comments. Paragoniastes westwoodi is the only member of the genus with evanescent discal striae.

Acknowledgments

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Research Article

Oriental *Hydrocyphon* (Coleoptera: Scirtidae: Scirtinae): Seven New Species from Indonesia, Thailand, Malaysia, and India

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Application Specific Instruction-set Processors (ASIPs) expose to the designer a large number of degrees of freedom. Accurate and rapid simulation tools are needed to explore the design space. To this aim, FPGA-based emulators have recently been proposed as an alternative to pure software cycle-accurate simulator. However, the advantages of on-hardware emulation are reduced by the overhead of the RTL synthesis process that needs to be run for each configuration to be emulated. The work presented in this paper aims at mitigating this overhead, exploiting a form of software-driven platform runtime reconfiguration. We present a complete emulation toolchain that, given a set of candidate ASIP configurations, identifies and builds an overdimensioned architecture capable of being reconfigured via software at runtime, emulating all the design space points under evaluation. The approach has been validated against two different case studies, a filtering kernel and an M-JPEG encoding kernel. Moreover, the presented emulation toolchain couples FPGA emulation with activity-based physical modeling to extract area and power/energy consumption figures. We show how the adoption of the presented toolchain reduces significantly the design space exploration time, while introducing an overhead lower than 10% for the FPGA resources and lower than 0.5% in terms of operating frequency.

1. Introduction

The genus *Hydrocyphon* Redtenbacher is represented by 100 species divided into 13 species groups from the Palaearctic and the Oriental Regions (see, e.g., [1, 2] and Tables 1 and 2). The larvae of this genus inhabit running water, for example, small rivers and streams, and the adults are frequently collected by sweeping around the larval habitat. The genus is well defined by certain characteristics (e.g., small body, deeply notched anterior margin of the mesosternum, well-developed parameres and parameroids), and has been comparatively well studied taxonomically [1, 2]. In the present paper, I describe seven new species from Indonesia, Thailand, Malaysia, and India. In addition, new combination and additional specimens examined are presented.

This is the twelfth part of my comprehensive study of "Scirtidae of the Oriental Region" [2–12].

2. Materials and Mandhods

This study was conducted based on the dried specimens preserved in the following public collections.

Ehime University Museum, Matsuyama (EUMJ).

Systematic Entomological Laboratory, Hokkaido University (SEHU).

Staatliches Museum für Naturkunde Stuttgart (SMNS).

The methodology was as shown in a previous study [2]. The photographs in Figure 1 were taken under a Leica MZ95 and produced by automontage software Combine ZM.

The abbreviations used in the present paper are as follows: PL: length of pronotum; PW: width of pronotum; EL: length of elytra; EW: width of elytra; TL: total length (PL plus EL). The average value is given in parentheses after the range.

3. Description of the New Species

3.1. *Hydrocyphon jogjaensis sp.n. (See Figures 1(a), 1(b), 2, and 11(a))*

Type Material. Holotype male (EUMJ): "Ngaglik, Yogyakarta 7°42′28.34′′S 110°24′45.34′′E Java, INDONESIA 28. II. 2010 H. Yoshitomi leg."

Paratype female (EUMJ): same data as for the holotype.

Table 1: The list of the species, distribution, ZooBank LSID, and species group of the genus *Hydrocyphon*. An Excel file version is also available at the following URL: https://sites.google.com/site/waterbeandlesofjapan/home/support-files-on-articles/Appendix 1.xls.

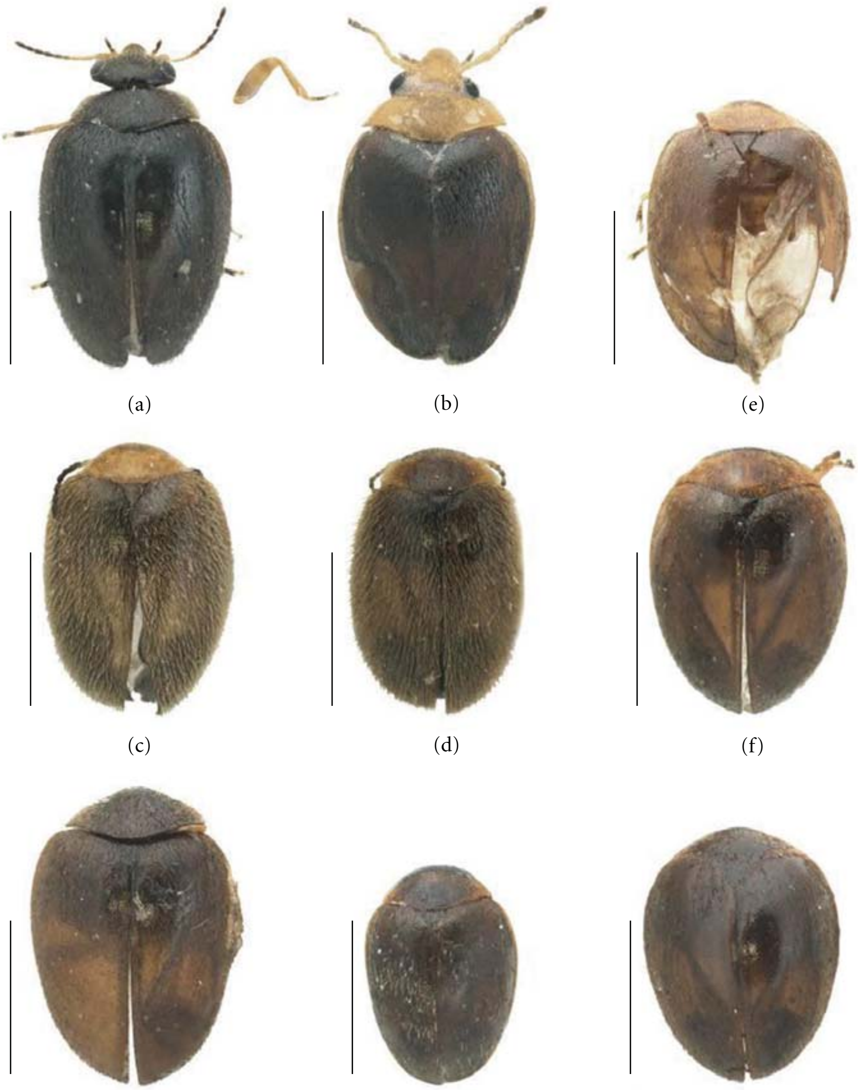
No.	Species	Description	Distribution	Zoobank LSID	Species group
1	*alticola*	(Klausnitzer, 1976)	Bhutan: Gogona, India	urn:lsid:zoobank.org:act:F8C20A8C-A3AA-4D0D-9B03-57FCA48F24A4	*kambaiticus*
2	*amaurus*	(Klausnitzer, 1980)	India	urn:lsid:zoobank.org:act:4536CA5C-FFAB-49A6-BE87-05601C01612C	*nyholmi*
3	*aritai*	Yoshitomi, 2001	Taiwan	urn:lsid:zoobank.org:act:578D5320-29EF-4ED3-9063-C682CD9F14D5	*kambaiticus*
4	*auratus*	Ruta, 2004	Vietnam	urn:lsid:zoobank.org:act:F2DF47E7-3368-4940-AA4E-3FABFAF260E2	*pallidicollis*
5	*australis*	Linder, 1864	France, Spain, Algeria, Italy, Sicily,	urn:lsid:zoobank.org:act:DC94FAD9-F097-4480-A753-70AD4B3D536D	*australis*
6	*baliensis*	Yoshitomi and Satô, 2005	Indonesia	urn:lsid:zoobank.org:act:DA3B36F3-6003-4C5E-B95D-7540D9CF956C	*pallidicollis*
7	*bhutanensis*	Klausnitzer, 1976	Bhutan: Tongsa, Nepal	urn:lsid:zoobank.org:act:55404EFA-F485-4608-80E1-2208B26A6B1D	*australis*
8	*bicolor*	Yoshitomi and Satô 2003	Laos	urn:lsid:zoobank.org:act:65E8EFE3-F292-4E78-A1AD-F9B209AE5A0F	*bicolor*
9	*bicornis*	Yoshitomi and Klausnitzer, 2003	China	urn:lsid:zoobank.org:act:642BDF4D-0977-4496-A971-12E2A9CE2CDB	*bicornis*
10	*bifidus*	Yoshitomi and Satô, 2005	China	urn:lsid:zoobank.org:act:B468746C-1769-4061-A792-271FC252C675	*kambaiticus*
11	*boukali*	Yoshitomi and Satô, 2005	India	urn:lsid:zoobank.org:act:033F7904-B6A4-4CA8-A92D-534263880C19	*pallidicollis*
12	*celatus*	Klausnitzer, 1980	India	urn:lsid:zoobank.org:act:3D4EC532-1502-408A-9466-2DF296B8D9C9	*deflexicollis*
13	*championi*	Reitter, 1903	Spain	urn:lsid:zoobank.org:act:0656F99B-8CBB-4EB2-8D84-91FCB0B899E0	*deflexicollis*
14	*chiangmaiensis*	Yoshitomi and Satô, 2005	Thailand	urn:lsid:zoobank.org:act:19450260-7983-471F-8586-A143F268FF29	*pallidicollis*
15	*consolatorius*	Klausnitzer, 1990	Iran	urn:lsid:zoobank.org:act:392F24BC-1E1A-454F-894B-90792E23A22A	*australis*
16	*deflexicollis*	(Müller, 1821)	Europe	urn:lsid:zoobank.org:act:40EFD794-C074-4959-9964-30D7BE6FA9D9	*deflexicollis*
17	*deformis*	Yoshitomi, in present study	India	urn:lsid:zoobank.org:act:110BF6C5-79A4-4E12-9BA6-6A563EA40B38	*pallidicollis*
18	*dentatus*	Yoshitomi and Satô 2003	Laos	urn:lsid:zoobank.org:act:F4E68B0F-5FA9-4906-8818-32800FEE837B	*dentatus*
19	*dispar*	Yoshitomi and Satô, 2005	Thailand	urn:lsid:zoobank.org:act:CEC2D61D-EFB7-4BAF-8C9A-D5AF14DBD1D8	*pallidicollis*
20	*doiinthanonensis*	Yoshitomi, in present study	Thailand	urn:lsid:zoobank.org:act:E8C8ED04-BD9D-4AE1-AFBC-5C6001B6D29D	*deflexicollis*
21	*dubius*	(Klausnitzer, 1980)	India	urn:lsid:zoobank.org:act:A74EFB59-0D2D-47F7-AD69-66735D85C5DF	*renati*
22	*dudgeoni*	Yoshitomi and Klausnitzer, 2003	China	urn:lsid:zoobank.org:act:DB9A3BAA-B439-4405-8205-E583C6DAC007	*pallidicollis*
23	*elongatus*	Nyholm, 1981	Burma	urn:lsid:zoobank.org:act:28F2AC64-C3BA-436B-8B9C-985BFB8DC50E	*renati*
24	*finitimus*	Nyholm, 1977	Turkey	urn:lsid:zoobank.org:act:8670FE8B-5559-4B6A-8283-7BF9DFBA5D48	*australis*
25	*forficulatus*	Nyholm, 1981	Burma	urn:lsid:zoobank.org:act:009E27DE-0576-497B-8D6B-11081577B50E	*deflexicollis*
26	*fulvescens*	Nyholm, 1977	Spain	urn:lsid:zoobank.org:act:F54C50AC-4EEB-45E8-A204-98DBB374FD33	*deflexicollis*
27	*fuscatus*	Klausnitzer, 1970	Albania: Kruma	urn:lsid:zoobank.org:act:3733A42A-4A10-4769-A161-DDE68F73CFFC	*deflexicollis*
28	*gereckei*	Hernando, Aguilera, Ribera 2004	Morocco: Oued Zloul	urn:lsid:zoobank.org:act:194ED02C-FAFF-420A-925B-B67CBF4C4FB3	*pallidicollis*
29	*graseri*	Klausnitzer, 2006	Nepal	urn:lsid:zoobank.org:act:74A0D485-1433-4B3E-B2FF-731B28A6E166	*renati*
30	*guangxiensis*	Yoshitomi and Klausnitzer, 2003	China	urn:lsid:zoobank.org:act:BD810938-3FB8-4413-BE0B-F54F6D009F50	*pallidicollis*
31	*hainanensis*	Yoshitomi and Klausnitzer, 2003	China	urn:lsid:zoobank.org:act:D9D2302B-A302-48FA-BD23-BC3186C5A2AB	*pallidicollis*
32	*hamiota*	Nyholm, 1972	Spain	urn:lsid:zoobank.org:act:25875BEA-7E78-4C3D-A3EB-42ACC0F155C0	*pallidicollis*
33	*hydrocyphonoides*	(Tournier, 1868)	S. Italy, Sicily, Tunisia, Algeria	urn:lsid:zoobank.org:act:6A2A9ECF-4168-4241-A581-DE5A66B0266C	*pallidicollis*
34	*illiesi*	Klausnitzer, 1991	Algeria	urn:lsid:zoobank.org:act:CB511D28-1FFF-4860-A340-A31D6E3B20C4	*deflexicollis*
35	*indonesianus*	Yoshitomi and Satô, 2005	Indonesia	urn:lsid:zoobank.org:act:615A6523-2FD4-42DF-9ED1-B293E09D81C4	*Pallidicollis*

Table 1: Continued.

No.	Species	Description	Distribution	Zoobank LSID	Species group
36	*interrogationis*	Klausnitzer, 1980	Pakistan	urn:lsid:zoobank.org:act:5022A70A-8612-4DEC-B192-22F337EC6611	*deflexicollis*
37	*iriomotensis*	Yoshitomi, 2001	Japan	urn:lsid:zoobank.org:pub:53354434-8857-4500-8FE1-C3E009029BE0	*renati*
38	*jaechi*	Yoshitomi and Klausnitzer, 2003	China	urn:lsid:zoobank.org:act:EA5536A7-A98F-492F-ABB4-95D97CFE2C0B	*renati*
39	*javanicus*	Yoshitomi and Satô, 2005	Indonesia	urn:lsid:zoobank.org:act:7CF900C5-A969-40D6-9BF5-D18368B9E90A	*pallidicollis*
40	*jogjaensis*	Yoshitomi, in present study	Indonesia	urn:lsid:zoobank.org:act:CC3549AD-2680-4182-93F0-2E9E7509D070	*pallidicollis*
41	*kachinensis*	Yoshitomi and Satô, 2005	Myanmar	urn:lsid:zoobank.org:act:F8E9C354-6DD9-4033-B740-55F0821EF452	*deflexicollis*
42	*kambaiticus*	Nyholm, 1981	Burma	urn:lsid:zoobank.org:act:8F1AA735-F07C-499A-A171-17E4EE8DB7D1	*kambaiticus*
43	*kaszabi*	Klausnitzer, 1980	Vietnam	urn:lsid:zoobank.org:act:26FD1BD2-E452-41FA-BE50-08EAA71D0BDE	*deflexicollis*
44	*keralaensis*	Yoshitomi and Satô, 2005	India	urn:lsid:zoobank.org:act:60A84554-498B-4344-B778-CE47153FE7AC	*pallidicollis*
45	*kinabalensis*	Yoshitomi and Satô, 2005	Malaysia	urn:lsid:zoobank.org:act:101D411A-4D72-45BB-A1FB-FBAA75B8C4BC	*kinabalensis*
46	*klapperichi*	Yoshitomi, in present study	Indonesia	urn:lsid:zoobank.org:act:82B11C88-DDBF-4B12-9D94-19F74AAAEB7A	*pallidicollis*
47	*klausnitzeri*	Yoshitomi and Satô, 2005	Thailand	urn:lsid:zoobank.org:act:776698B5-109A-4323-814D-FFEA49622164	*pallidicollis*
48	*kodadai*	Yoshitomi and Satô, 2005	Philippines	urn:lsid:zoobank.org:act:F53BFE82-CB92-45A8-938D-C22374CC3AE3	*pallidicollis*
49	*komareki*	Yoshitomi, 2008	China	urn:lsid:zoobank.org:act:1D12096D-BDBD-440E-A3D9-E86811FEADB0	*kambaiticus*
50	*kopanddaghensis*	Ruta, 2007	USSR	urn:lsid:zoobank.org:act:E63FC1A7-8AFD-400E-B075-200977093FD8	*australis*
51	*laandicolor*	Nyholm, 1967	Spain	urn:lsid:zoobank.org:act:6AAA825E-E1CA-4E78-BC55-6E86A24E7C93	*pallidicollis*
52	*laosensis*	Yoshitomi and Satô 2003	Laos	urn:lsid:zoobank.org:act:D03DCA00-B60A-4232-8D3F-6475A3AAC74B	*pallidicollis*
53	*lii*	Yoshitomi and Klausnitzer, 2003	China	urn:lsid:zoobank.org:act:3992B473-B847-4672-AD45-E20CF2E75EAB	*pallidicollis*
54	*lusonensis*	Yoshitomi and Satô, 2005	Philippines	urn:lsid:zoobank.org:act:96F9212D-8494-4ED6-A927-2AB719A2E22B	*deflexicollis*
55	*malaysianus*	Yoshitomi and Satô, 2005	Malaysia	urn:lsid:zoobank.org:act:432974B2-E5F0-47BD-BE78-4A6D212F1B27	*pallidicollis*
56	*manfredi*	Yoshitomi and Satô, 2005	Indonesia	urn:lsid:zoobank.org:act:197987B9-91AF-4953-AA96-0DF26ECB2501	*pallidicollis*
57	*masatakai*	Yoshitomi, 2008	China	urn:lsid:zoobank.org:act:2A22EF58-D756-45C2-A94B-859B23CAA9F0	*deflexicollis*
58	*minous*	Nyholm, 1967	Crete	urn:lsid:zoobank.org:act:2C9A3DD3-5A36-4DF3-8004-9C6A78816611	*australis*
59	*mirabilis*	Yoshitomi and Satô, 2005	China	urn:lsid:zoobank.org:act:E40910F4-028A-4D9C-8B6A-C1AD5E13D769	*mirabilis*
60	*nakanei*	Yoshitomi, 2001	Japan	urn:lsid:zoobank.org:act:5AF6E7DB-3A8D-4DB8-BB32-11CA423E45A2	*kambaiticus*
61	*narraensis*	Yoshitomi and Satô, 2005	Philippines	urn:lsid:zoobank.org:act:11147154-F37B-4CC9-ADFE-A28E1A7FEBAD	*pallidicollis*
62	*nepalensis*	Yoshitomi and Satô, 2005	Nepal	urn:lsid:zoobank.org:act:F1F7DEB9-B007-49E6-BD56-F137E101E723	*renati*
63	*novaki*	Nyholm, 1967	Italy, Yugoslavia, Albania, Greece	urn:lsid:zoobank.org:act:6ACC76F1-7F7F-4D71-B191-3D3C63E7FA62	*deflexicollis*
64	*nuristanicus*	Klausnitzer, 2004	Afghanistan	urn:lsid:zoobank.org:act:849E2F10-EE37-44C4-9D17-724ED68D23AB	*pallidicollis*
65	*nyholmi*	Yoshitomi and Satô, 2005	Nepal	urn:lsid:zoobank.org:act:23F104FD-61CB-480A-9252-A114A46E6558	*nyholmi*
66	*oblongulus*	Nyholm, 1967	Cyprus	urn:lsid:zoobank.org:act:0ACA5B94-7CAB-4ADC-956D-C308859596C6	*australis*
67	*ovatus*	Nyholm, 1967	Italy	urn:lsid:zoobank.org:act:9D3B8572-8ADC-4EEA-9D64-181977729C1A	*deflexicollis*
68	*palawanensis*	Yoshitomi and Satô, 2005	Philippines	urn:lsid:zoobank.org:act:9289FAC1-3398-47E3-A114-892DFEB4D90C	*pallidicollis*
69	*pallidicollis*	Raffray, 1873	Corsica, Sardinia, Algeria, Morocco	urn:lsid:zoobank.org:act:BF53220B-9EDE-4E40-A427-90798C2291E3	*pallidicollis*
70	*palniensis*	Yoshitomi and Satô, 2005	India	urn:lsid:zoobank.org:act:2C983331-0F6B-4EDA-9C7C-9A76CF8CF5D2	*pallidicollis*
71	*panensis*	Yoshitomi and Satô 2003	Laos	urn:lsid:zoobank.org:act:E148DD4D-5CAB-4494-9796-DC517B8AAE1B	*pallidicollis*
72	*pernigrans*	Nyholm, 1967	Spain	urn:lsid:zoobank.org:act:098642CD-DC7D-4229-A722-E29D5D13F671	*deflexicollis*
73	*proximus*	Nyholm, 1967	Italy	urn:lsid:zoobank.org:act:C56E7ACA-6D6D-4220-A2DE-5C68D4EFA124	*deflexicollis*

Table 1: Continued.

No.	Species	Description	Distribution	Zoobank LSID	Species group
74	*pulchellus*	Klausnitzer, 1980	Nepal	urn:lsid:zoobank.org:act:C7793CC3-C759-4CD8-B843-FF36EE5987A7	*deflexicollis*
75	*rectangulus*	Klausnitzer, 1991	Algeria	urn:lsid:zoobank.org:act:BE470234-B4AB-4860-AE6A-EF17BA092ABF	*pallidicollis*
76	*renati*	Nyholm, 1981	Burma	urn:lsid:zoobank.org:act:40BCCB76-42A7-4094-B4C4-50F686FB3107	*renati*
77	*rivulorum*	Nyholm, 1977	Turkey	urn:lsid:zoobank.org:act:3DA3840B-6AD2-4BAA-883B-848DF04E3E15	*deflexicollis*
78	*rufithorax*	(Gemminger, 1869)	India/Sri Lanka	urn:lsid:zoobank.org:act:4DD9F8CA-E157-4039-A17B-C3F4D6396F9A	*pallidicollis*
79	*sagaingensis*	Yoshitomi and Satô, 2005	Myanmar	urn:lsid:zoobank.org:act:3BD8291D-9F48-4348-BA10-E0EF70715AEF	*pallidicollis*
80	*sagittiger*	Yoshitomi, in present study	Indonesia	urn:lsid:zoobank.org:act:0D309EFD-80D3-456D-821F-4C011C5A6598	*pallidicollis*
81	*sakaii*	Yoshitomi and Satô 2003	Laos	urn:lsid:zoobank.org:act:4A679CAE-4C32-417A-B49B-8F1BF14B2167	*deflexicollis*
82	*sarawakensis*	Yoshitomi and Satô, 2005	Malaysia	urn:lsid:zoobank.org:act:07DDD979-4676-484E-A7F0-FBBE4B516A16	*pallidicollis*
83	*satoi*	Yoshitomi, 2001	Japan, Taiwan, Korea	urn:lsid:zoobank.org:pub:53354434-8857-4500-8FE1-C3E009029BE0	*renati*
84	*schoenmanni*	Yoshitomi and Klausnitzer, 2003	China	urn:lsid:zoobank.org:act:9DABE069-D895-4ACD-97DD-3EDF94BFBACF	*pallidicollis*
85	*segrex*	Nyholm, 1972	Turkey, Anatolia, Iran, Caspian Sea	urn:lsid:zoobank.org:act:1D970446-0F80-4967-B9FD-CF69C16C5C32	*australis*
86	*serratibasialis*	Yoshitomi, in present study	India	urn:lsid:zoobank.org:act:1E9F878B-737F-4B31-99B7-FA7663EFFF3C	*deflexicollis*
87	*sieberi*	(Klausnitzer, 2010)	India	urn:lsid:zoobank.org:act:88BA77DB-C779-46C1-BE0C-069DFC296A53	*pallidicollis*
88	*similis*	Ruta, 2004	Vietnam	urn:lsid:zoobank.org:act:C88A15E4-2F83-4DBD-B515-AD418A16CB01	*pallidicollis*
89	*sinicus*	Pic, 1934	China	urn:lsid:zoobank.org:act:77951535-1B3B-494F-AC41-EBD6A45B53F6	*kambaiticus*
90	*spinosus*	Yoshitomi and Satô, 2005	India	urn:lsid:zoobank.org:act:2408AC80-003B-41E4-8834-CEFEEB5CA987	*pallidicollis*
91	*steueri*	Klausnitzer, 2006	Nepal	urn:lsid:zoobank.org:act:93909299-F337-490A-9E8A-BF4C6D008FC9	*kambaiticus*
92	*stupendus*	Nyholm, 1981	Burma	urn:lsid:zoobank.org:act:46B80B2B-8178-4EB0-B3F3-7F0D4D15C2E1	*kambaiticus*
93	*subcelatus*	Yoshitomi and Satô, 2005	India	urn:lsid:zoobank.org:act:1115FB1D-1947-4199-92FA-755C4AEBA401	*deflexicollis*
94	*submalaysianus*	Yoshitomi and Satô, 2005	Malaysia	urn:lsid:zoobank.org:act:95BAB60F-C4C7-42F7-B756-0757C10827FD	*pallidicollis*
95	*subrotundus*	Yoshitomi and Satô, 2005	Thailand	urn:lsid:zoobank.org:act:45CDFA7B-AEDE-47B8-AE79-C7A055FD9ADD	*deflexicollis*
96	*subtrilobus*	Yoshitomi and Satô, 2005	Indonesia	urn:lsid:zoobank.org:act:4EB51544-94BF-4305-ABF4-E979ACC971AA	*pallidicollis*
97	*sumatrensis*	Yoshitomi and Satô, 2005	Indonesia	urn:lsid:zoobank.org:act:5CD0CE1E-AED2-481B-9FD2-CF5087C1299E	*pallidicollis*
98	*taiwanus*	Yoshitomi, 2001	Taiwan	urn:lsid:zoobank.org:act:3A5B6F9A-E586-4BC8-9FAF-9F6B749AFEAB	*renati*
99	*takizawai*	Yoshitomi, in present study	Malaysia	urn:lsid:zoobank.org:act:9BB8C12B-3602-491D-81F9-58C3FDC8831F	*pallidicollis*
100	*tamilensis*	Yoshitomi and Satô, 2005	India	urn:lsid:zoobank.org:act:9C01DBD6-2538-408C-98BA-C53BACFB6F42	*tamilensis*
101	*thailandicus*	Yoshitomi and Satô, 2005	Thailand	urn:lsid:zoobank.org:act:E584D03E-D879-49C5-BF37-3F7F6BD5A20F	*Renati*
102	*triforius*	Yoshitomi and Satô, 2005	Malaysia, Thailand	urn:lsid:zoobank.org:act:6DBD3361-AD78-430E-8B0E-662012550497	*renati*
103	*trilobus*	Yoshitomi and Satô, 2005	Thailand	urn:lsid:zoobank.org:act:DBBB00C2-5B88-45C0-A3AD-8A72C1D424E6	*pallidicollis*
104	*uenoi*	Yoshitomi and Klausnitzer, 2003	China	urn:lsid:zoobank.org:act:A48EC5C2-38F1-4CCC-A832-CEF4B14C09F4	*kambaiticus*
105	*vicinans*	Nyholm, 1972	Turkey, Israel	urn:lsid:zoobank.org:act:DB707751-48AB-4693-A863-8671E0DCFDCC	*australis*
106	*wakaharai*	Yoshitomi and Satô 2003	Laos	urn:lsid:zoobank.org:act:8A75EBCF-66F9-4E52-B555-A6F4A6155E79	*renati*
107	*wangi*	Yoshitomi, 2008	China	urn:lsid:zoobank.org:act:C64DD841-7071-4993-8F36-61CE488BA954	*kambaiticus*
108	*yoshitomii*	Klausnitzer 2002	Nepal	urn:lsid:zoobank.org:act:C690CF7A-9E10-4D03-8FD7-558279A45203	*yoshitomii*


(a) (b) (e)

(c) (d) (f)

(g) (h) (i)

Figure 1: Habitus of *Hydrocyphon* spp., holotypes, male (a, c, e–i) and paratypes, female (b, d). (a, b) *H. jogjaensis* sp.n.; (c, d) *H. takizawai* sp.n.; (e) *H. sagittiger* sp.n.; (f) *H. serratibasialis* sp.n.; (g) *H. doiinthanonensis* sp.n.; (h) *H. klapperichi* sp.n.; (i) *H. deformis* sp.n. Scale = 1.0 mm.

Table 2: The list of the species excluding from the genus *Hydrocyphon*. An Excel file version is also available at the following URL: https://sites.google.com/site/waterbeandlesofjapan/home/support-files-on-articles/Appendix 2.xls.

No.	Species	Description	Distribution	Zoobank LSID	Transferred
1	*Hydrocyphon atratus*	Motschulsky, 1863	Ceylon	urn:lsid:zoobank.org:act:7CE1145A-0783-4196-A201-B05397235AE5	*Cyphon*

Male Description. Body oval, well-convex dorsally, shiny, closely covered with short yellowish-white setae. Coloration of body blackish-brown, but antennal segments I–V, lateral part of pronotum and legs yellowish-brown.

Head moderate in size, lightly convex dorsally, finely punctuate, with straight front margin of clypeus; the distance between eyes about 1.9 times as long as the maximum diameter of an eye. Eyes moderate in size, prominent. Antennae short, slender, reaching at basal part of elytra. Pronotum punctuate as in head, lightly convex dorsally, lightly depressed ventrally in lateral parts; front margin almost straight; anterolateral corners obtuse; posterolateral corners right-angle; lateral and posterior margins gently arcuate; PW/PL 2.69. Scutellum small, equilateral-triangular, punctuate as in head. Elytra oval, convex dorsally, broadest at basal 1/3, punctuate as in head; humeral parts indistinct; EL/EW 1.22; EL/PL 4.80; EW/PW 1.47; TL/EW 1.47. Legs relatively long, slender.

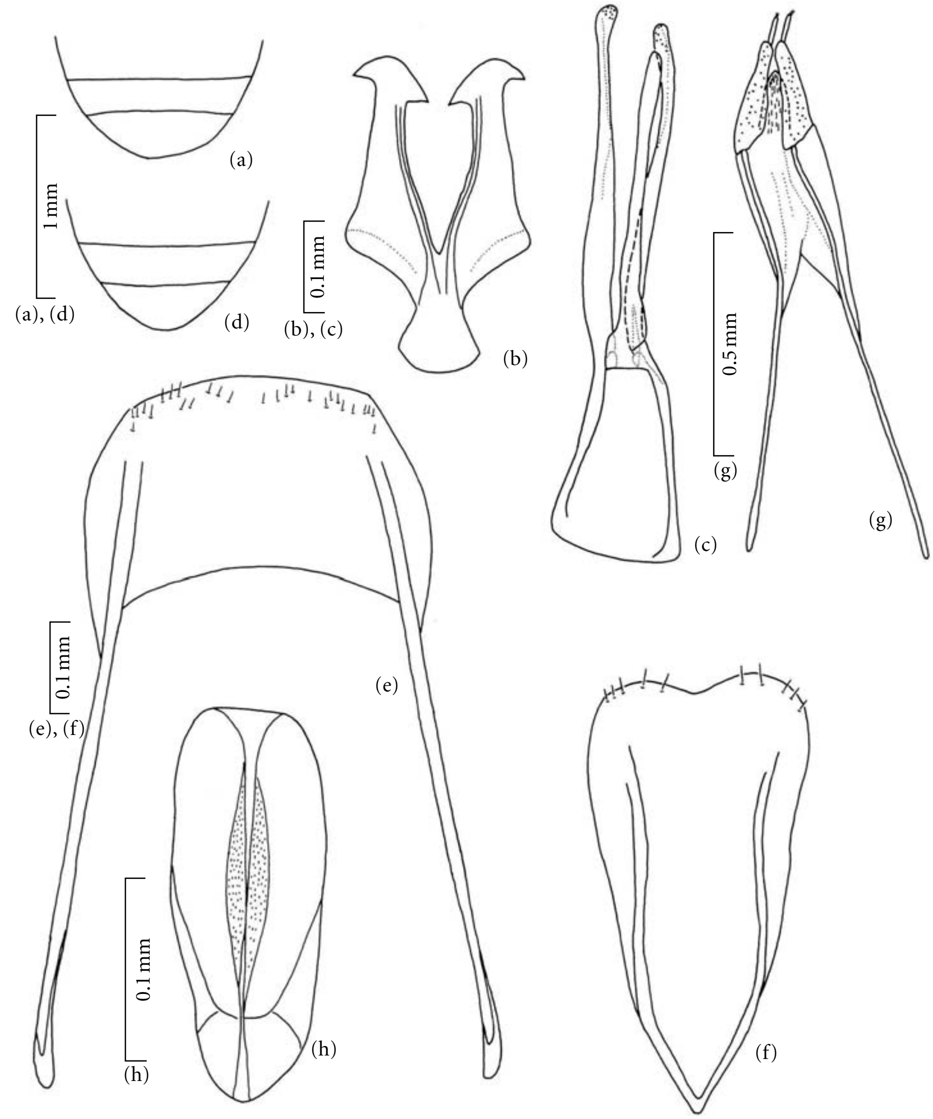


Figure 2: *Hydrocyphon jogjaensis* sp.n., holotype, male (a–c) and paratype, female (d–h). (a, d) Sternites V–VII; (b) tegmen; (c) penis; (e) tergite VIII; (f) sternite VIII; (g) ovipositor; (h) prehensor.

Caudal margin of sternite VII gently arcuate. Tergites VIII—IX moderately sclerotized, trapezoidal. Tegmen short, well sclerotized; proximal part short, fan-shaped, arcuate in basal margin; parameres stout, gently expanded laterally in basal parts, arrow-like shape in apical parts. Penis long, well sclerotized, asymmetrical, about 1.7 times as long as tegmen; pala subtrapezoidal, widest at base; parameroids long and slender, slightly asymmetrical, gently widened and punctuate in apical parts, obtuse at apices; trigonium with one long and slender projection, a little shorter than parameroids, obtuse at apex; median plate indistinct.

Female. Similar to male; pronotum yellow (probably teneral specimen); antennae relatively stout; PW/PL 2.55; EL/EW 1.23; EL/PL 4.25; EW/PW 1.35; TL/EW 1.52.

Caudal margin of sternite VII slightly pointed. Tergite VIII moderately sclerotized, trapezoidal, bearing short setae in caudal parts, with long apodemes; sternite VIII slightly sclerotized, oblong, bearing short setae along caudal margin. Ovipositor relatively short; relative length of stylus, coxite, and baculus ($n = 1$) as 1.0 : 4.0 : 15.3. Prehensor small, well sclerotized, oblong, bearing short spines in mesal part.

Measurements. Male ($n = 1$): TL 2.03 mm; PW 0.94 mm; PL 0.35 mm; EL 1.68 mm; EW 1.38 mm. Female ($n = 1$): TL 2.10 mm; PW 1.02 mm; PL 0.40 mm; EL 1.70 mm; EW 1.38 mm.

Remarks. The species belongs to the *pallidicollis* species group. It is similar to *H. trilobus* Yoshitomi and Satô and *H. subtrilobus* Yoshitomi and Satô with respect to the shape of

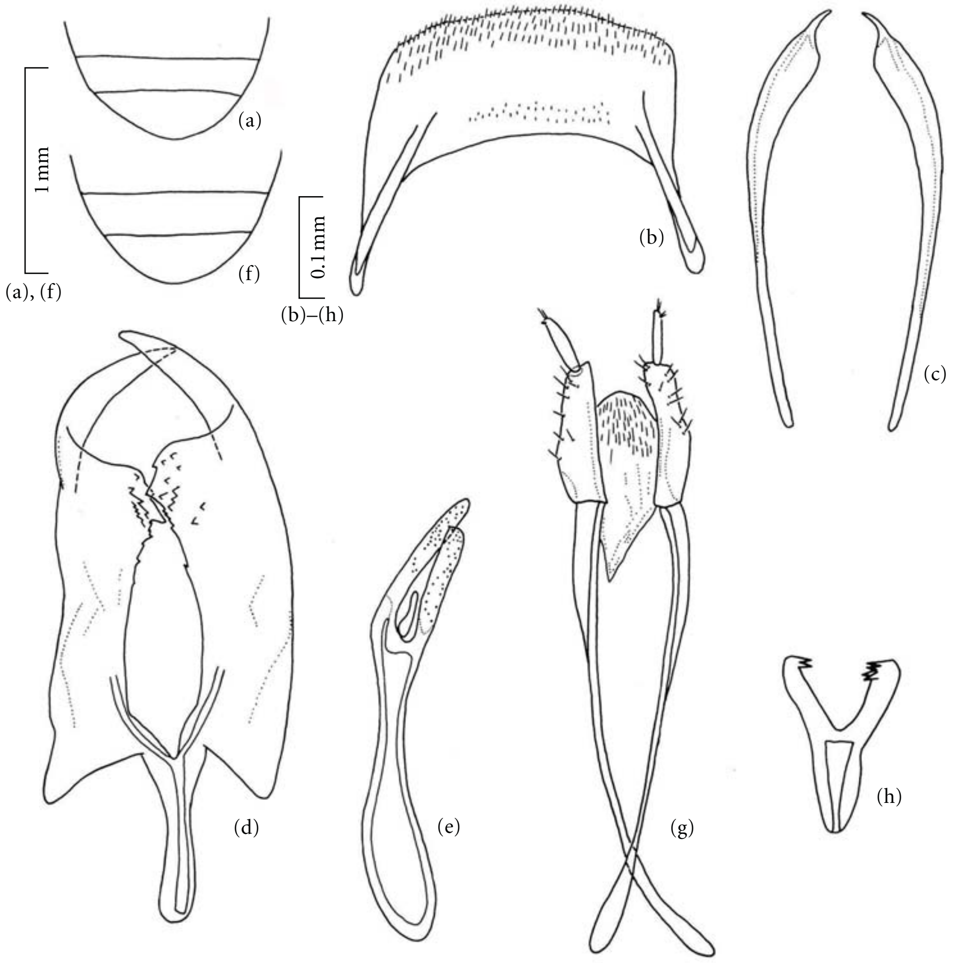


Figure 3: *Hydrocyphon takizawai* sp.n., holotype, male (a–e) and paratype, female (f–h). (a, f) Sternites V–VII; (b) tergite VIII; (c) sternite IX; (d) tegmen; (e) penis; (g) ovipositor; (h) prehensor.

the penis, but differs from them by the apices of the parameres which have an arrow-like shape.

Biological Notes. The type locality was a small river situated halfway up Mount Merapi (Figure 11(a)). The river was somewhat polluted by waste water flowing from cichlid fish farms.

Etymology. The species is named after the type locality.

3.2. Hydrocyphon takizawai sp.n. (See Figures 1(c), 1(d), 3, 4, 5, and 11(b))

Type Material. Holotype male (EUMJ): "Kinabalu Park, HQ Sabah, MALAYSIA 2–4. V. 2010 H. Yoshitomi leg."

Paratypes 2 females (EUMJ): same data as for the holotype.

Male Description. Body oval, well convex dorsally, shiny, closely covered with yellowish white short setae. Coloration of head, mouth parts, antennal segments I–IV, prothorax and legs yellowish-brown, but posterior part of head and tarsi infuscate; antennal segments V–XI, scutellum, elytra, meso- and metaventries, and abdominal segments brown.

Head moderate in size, slightly convex dorsally, finely punctuate; clypeus rather long, straight in front margin; the distance between eyes about 1.7 times as long as the maximum diameter of an eye. Eyes moderate in size, prominent. Antennae short, reaching about proximal 1/6 of elytra. Pronotum punctuate as in head, slightly convex dorsally, depressed ventrally in lateral parts; front margin straight; antero- and posterolateral corners obtuse; lateral and posterior margins gently arcuate; PW/PL 2.51. Scutellum small, equilateral-triangular. Elytra oval, convex dorsally, broadest at the middle; humeral parts gently projecting dorsally; EL/EW 1.41; EL/PL 4.83; EW/PW 1.36; TL/EW 1.70.

Caudal margin of sternite VII arcuate. Tergite VIII moderately sclerotized, transversal trapezoidal, bearing short spines in caudal part, with a pair of short apodemes. Sternite IX well sclerotized, consisting of a pair of hemisternites, with pointed at apices. Tegmen large, well sclerotized; proximal part short, subparallel-sided; parameres long, minutely serrate in apical 1/3 of inner parts, distinctly protruding

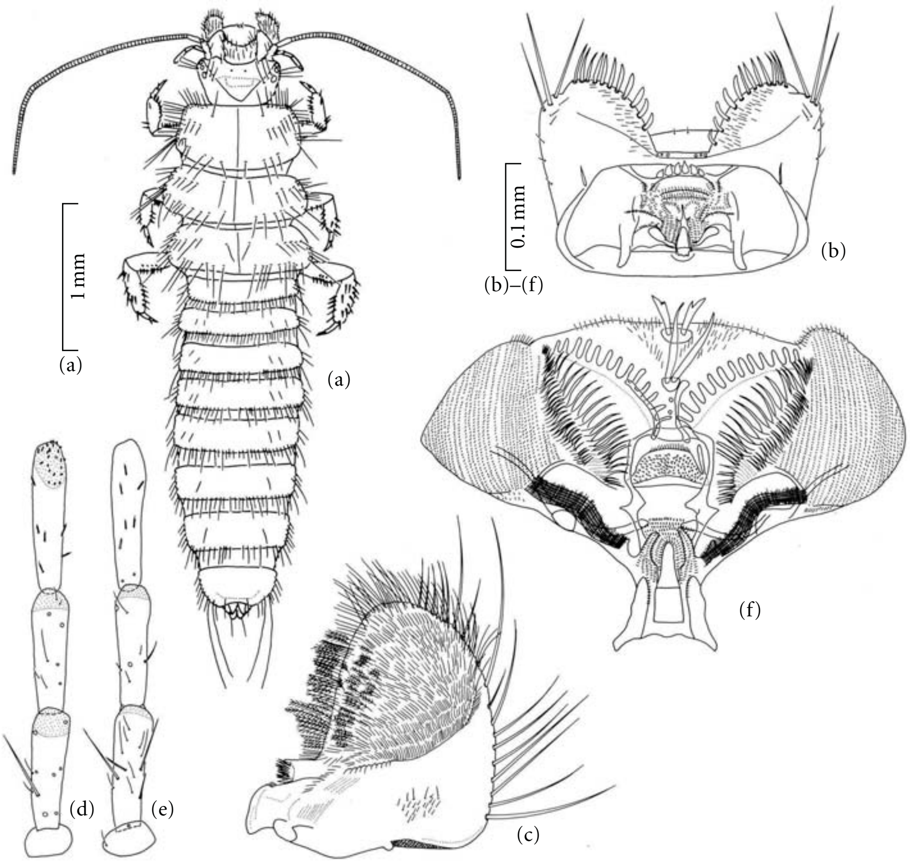


Figure 4: *Hydrocyphon takizawai* sp.n., larva. (a) Dorsal habitus; (b) labrum in ventral aspect; (c) mandible in ventral aspect; (d) maxillary palpus in ventral aspect; (e) ditto in dorsal aspect; (f) hypopharynx.

postero-interiorly in postero-lateral corners, projecting anteriorly in anterolateral corners. Penis asymmetrical, short, well sclerotized, about 0.8 times as long as tegmen; pala oblong, widest near base, tapered in proximal 2/3; parameroids short and almost straight, obtuse at apices, finely punctuate, left one long and slender, right one short and stout; trigonium consisting of a small lobe.

Female. Sexual dimorphism indistinct, but mesal part of pronotum infustate in paratype; PW/PL 2.24; EL/EW 1.42; EL/PL 4.59; EW/PW 1.45; TL/EW 1.73.

Caudal margin of sternite VII arcuate. Ovipositor relatively short; stylus with two pairs of apical setae; coxite bearing short spines; baculus without branch; relative length of stylus, coxite and baculus ($n = 1$) as 1.0 : 2.3 : 7.5. Prehensor small, slightly sclerotized, Y-shaped, bearing short spines in inner margins of apices.

Measurements. Male ($n = 1$): TL 2.04 mm; PW 0.88 mm; PL 0.35 mm; EL 1.69 mm; EW 1.20 mm. Female ($n = 1$): TL 2.07 mm; PW 0.83 mm; PL 0.37 mm; EL 1.70 mm; EW 1.20 mm.

Larvae. Body about 4.0 mm length in fully expanded specimens, subparallel-sided in thorax and abdomen which bearing short and long setae on lateral and posterior margins. Coloration of body right brown.

Head slightly protruding laterally, with three pairs of nonmelanized stemmata situated near anterolateral corners. Antennae relatively long, reaching at abdominal segment I; scape slightly curved posteriorly; flagellum 51–73 (64) segmented ($n = 4$). Labrum transverse, covered with long setae on dorsal surface; ventral lobes projecting anteriorly, with 12 pairs of stout and short setae on inner margins. Maxillary palpi long and slender; 1st segment covered sparsely with short and long setae on dorsal surface; 3rd rounded at apex, with widely apical sensory area; relative length of each segment ($n = 1$) as 1.0 : 1.0 : 1.3. Mandibles and hypopharynx typical for the genus. Thorax widest at posterior margin of mesothorax. Abdomen subparallel-sided, widest at segment V, then gently tapering posteriorly, bearing two (II–V) or one (VI–VII) pairs of short setae on lateral part. Tergite VIII trapezoidal, shallowly concave in posterior margin, with a pair of very long setae protruding from posterolateral corners. Sternite VIII semicircular, bearing long setae on lateral and posterior margins, two of those very long. Tergite IX semicircular, convex at apex, with a pair of long setae at apex, bearing pectinate short setae on lateral margin. Sternite IX transversal semicircular, with pectinate setae on posterior margin.

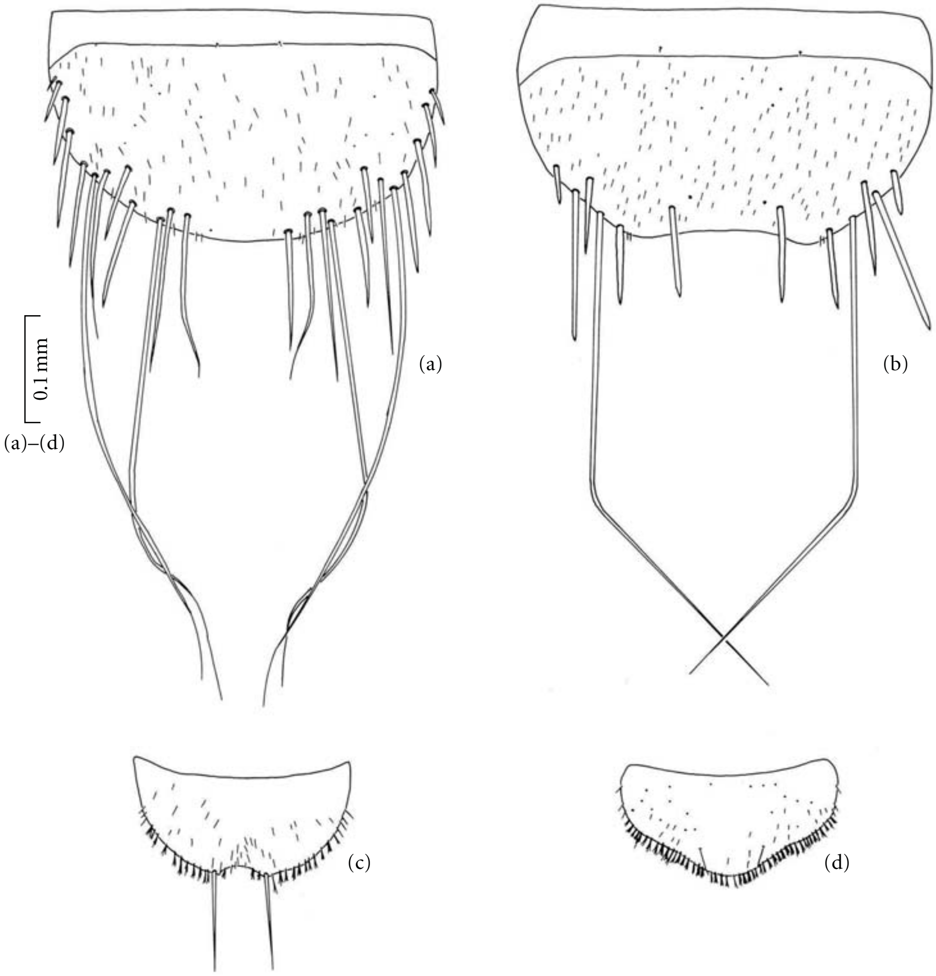


Figure 5: *Hydrocyphon takizawai* sp.n., larva. (a) Sternite VIII; (b) tergite VIII; (c) tergite IX; (d) sternite IX.

Measurements of Larvae (n = 3). TL 5.40–6.50 (5.80) mm; HW 0.80–0.90 (0.83) mm; PL 0.50–0.55 (0.52) mm; PW 1.05–1.20 (1.15) mm; TW 1.20–1.40 (1.33) mm.

Specimens Examined of Larvae. 29 exs. (mature larvae), Kinabalu Park, HQ Sabah, Malaysia, 2–4. V. 2010, H. Yoshitomi leg.; 5 exs. (mature larvae), Liwagu river, Kinabalu Park, HQ, Sabah, Malaysia, 28. II. 2009, H. Uno leg.

Remarks. The species belongs to the *pallidicollis* species group. Judging from the shape of the penis, it is similar to *H. palawanensis* Yoshitomi and Satô, *H. javanicus* Yoshitomi and Satô, *H. baliensis* Yoshitomi and Satô, *H. manfredi* Yoshitomi and Satô, and *H. sarawakensis* Yoshitomi and Satô, but differs from them by the shape of the parameres projecting posteriorly and serrate in the inner margin.

The larva of this species is distinguished from the three previously known species of the larvae in the genus (*H. deflexicollis* [13], *H. satoi* [14], *H.* sp. [4]) by the following characteristics: (1) segment III of maxillary palpi somewhat short (about 1.5 times as long as segment I in *H. deflexicollis* and *H. satoi*); (2) the short setae on the lateral and posterior margins of tergite IX and sternite IX pectinate (simple setae in *H. satoi* and *H.* sp.).

Biological Notes. The type locality was a small stream in the Kinabalu National Park (Figure 11(b)). The stream was clear, and many aquatic insects were collected with this species.

Etymology. The species is named after Dr. H. Takizawa.

3.3. Hydrocyphon sagittiger sp.n. (See Figures 1(e) and 6)

Type Material. Holotype male (EUMJ): "(Indonesia) West Sumatra Batipuh 26. XI. 1974 T. Kobayashi," "Egyptian kidney bean."

Holotype male (EUMJ): "(Indonesia) West Sumatra Batipuh 26. XI. 1974 T. Kobayashi," "Egyptian kidney bean."

Male Description. Body oval, well convex dorsally, shiny, closely covered with yellowish-white setae. Coloration of body blackish-brown, but anterior part of head, lateral parts of pronotum, antennal segments I–V, and legs right-brown.

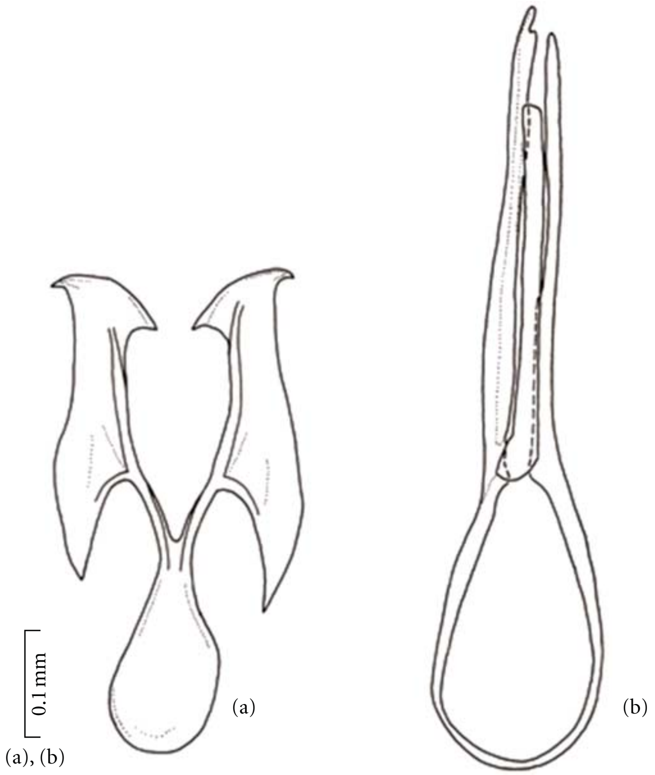


FIGURE 6: *Hydrocyphon sagittiger* sp.n., holotype, male. (a) Tegmen; (b) penis.

Head moderate in size, flat in dorsally, finely punctuate; clypeus short, straight in front margin; the distance between eyes about 2.1 times as long as the maximum diameter of an eye. Eyes moderate in size, prominent. Antennae short, reaching about proximal 1/8 of elytra. Pronotum punctuate as in head, slightly convex dorsally, depressed ventrally in lateral parts; front margin straight; antero- and postero-lateral corners obtuse; lateral and posterior margins gently arcuate; PW/PL 2.57. Scutellum small, equilateral-triangular. Elytra oval, strongly convex dorsally, broadest at the middle; humeral parts gently projecting dorsally; EL/EW 1.26; EL/PL 4.65; EW/PW 1.43; TL/EW 1.54.

Tegmen large, well sclerotized; proximal part long, peg-like; parameres wide, distinctly projecting anteriorly in antero-lateral corners, projecting subtriangularly in inner and outer corners of apices. Penis long, slightly asymmetrical, well sclerotized, about 1.5 times as long as tegmen; pala short, oblong, widest at proximal 1/3 of pala; parameroids very long, asymmetrical, almost straight, left one slightly longer than right one, excised at inner margin of left apex; trigonium consisting of a long lobe, straight, shorter than parameroids, obtuse at apex; median plate indistinct.

Female. Unknown.

Measurements. TL 2.09 mm; PW 0.95 mm; PL 0.37 mm; EL 1.72 mm; EW 1.36 mm.

Remarks. The species belongs to the *pallidicollis* species group, and is related to *H. jogjaensis* sp.n., *H. trilobus* Yoshitomi and M. Satô, 2005, and *H. subtrilobus* Yoshitomi and M. Satô, 2005. It differs from them by the following characteristics: inner corner of parameres projecting interiorly; left parameroid excised at apex; pala oblong.

Etemology. The species name refers to the shape of the apices of the tegmen.

3.4. Hydrocyphon serratibasialis sp.n. (See Figures 1(f) and 7)

Type Material. Holotype male (SEHU): “INDIA: KERALA Dhony Hills 180–450 m 7 DEC 1978 JAP-IND CO TR”.

Male Description. Body oval, well convex dorsally, shiny, closely covered with yellowish-white setae. Coloration of head, scutellum, elytra, and ventral surface of thorax and abdomen blackish-brown; pronotum, legs, and antennae yellowish-brown.

Head moderate in size, slightly convex dorsally, finely punctuate; clypeus short, straight in front margin; the distance between eyes about 2.2 times as long as the maximum diameter of an eye. Eyes moderate in size, prominent. Antennae rather stout. Pronotum punctuate as in head, lightly depressed ventrally in lateral parts; front and lateral margins straight; antero-lateral corners about 120°; postero-lateral corners right-angle; posterior margin gently arcuate; PW/PL

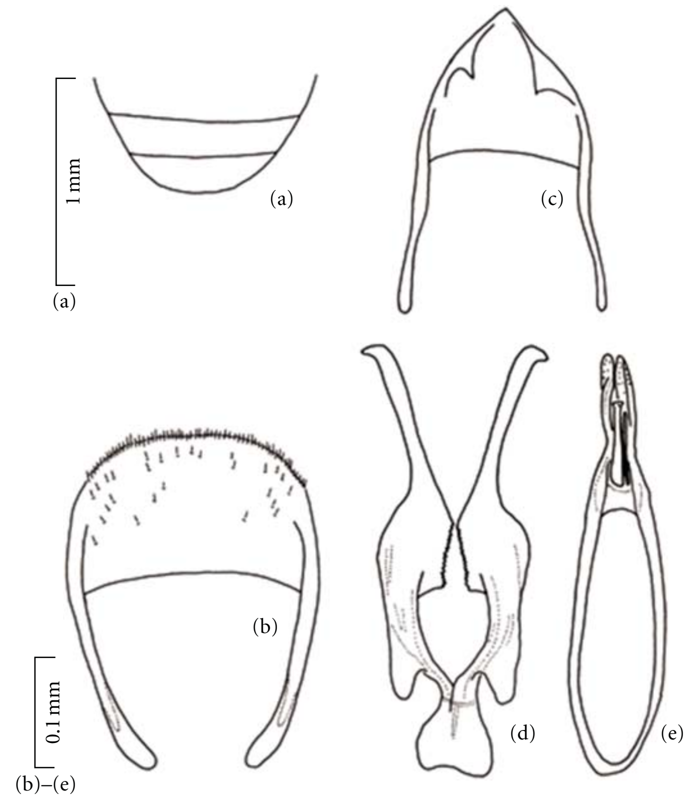


Figure 7: *Hydrocyphon serratibasialis* sp.n., holotype, male. (a) Sternites V–VII; (b) tergite VIII; (c) sternite IX; (d) tegmen; (e) penis.

2.78. Scutellum small, equilateral triangular. Elytra oval, strongly convex dorsally, broadest at the middle; humeral parts gently projecting dorsally; EL/EW 1.21; EL/PL 4.59; EW/PW 1.36; TL/EW 1.48.

Caudal margin of sternite VII gently arcuate. Tergite VIII moderately sclerotized, trapezoidal, bearing short spines along caudal margin, sparsely covered with short setae in caudal part, with a pair of slender apodemes, Sternites IX slightly sclerotized, upturned in postero-lateral parts, with a pair of long apodemes. Tegmen long, well sclerotized; proximal part short, expanded antero-laterally; parameres long, minutely serrate in mesal part of inner margin, distinctly protruding postero-laterally in apical parts, projecting anteriorly in antero-lateral corners. Penis asymmetrical, long, well sclerotized, about 0.9 times as long as tegmen; pala oblong, widest at basal 1/6; parameroids longer than trigonium, finely punctuate; trigonium consisting of two lobes, longer one forked, shorter one slender; median plate indistinct.

Female. Unknown.

Measurements. TL 2.07 mm; PW 1.03 mm; PL 0.37 mm; EL 1.70 mm; EW 1.40 mm.

Remarks. The species belongs to the *deflexicollis* species group, but is a distinct species having a characterized tegmen.

Etymology. The species name refers to the shape of the tegmen: "serrati−" = serrate + "basialis" = basal.

3.5. Hydrocyphon doiinthanonensis sp.n. (See Figures 1(g) and 8)

Type Material. Holotype male (EUMJ): "[North THAI] Maeo Khun klang 1350 m, Doi Inthanon 19. X. 1983 M. Sakai".

Male Description. Body oval, well convex dorsally, weakly shiny, closely covered with yellowish-white setae. Coloration of body brown, but antennae, apical part of femora, tibiae, and tarsi pale brown.

Head moderate in size, slightly convex dorsally, finely punctuate; clypeus short, straight in front margin; the distance between eyes about 2.3 times as long as the maximum diameter of an eye. Eyes moderate in size, prominent. Antennae rather stout. Pronotum punctuate as in head, slightly depressed ventrally in lateral parts; front and lateral margins straight; antero-lateral corners about 120°; postero-lateral corners almost right-angle; posterior margin gently arcuate; PW/PL 2.45. Scutellum small, equilateral triangular. Elytra oval, broadest at basal 1/4; humeral parts indistinctly projecting; EL/EW 1.28; EL/PL 4.63; EW/PW 1.48; TL/EW 1.55.

Caudal margin of sternite VII gently arcuate. Tergite VIII moderately sclerotized, trapezoidal, bearing short spines along caudal margin, sparsely covered with short setae in

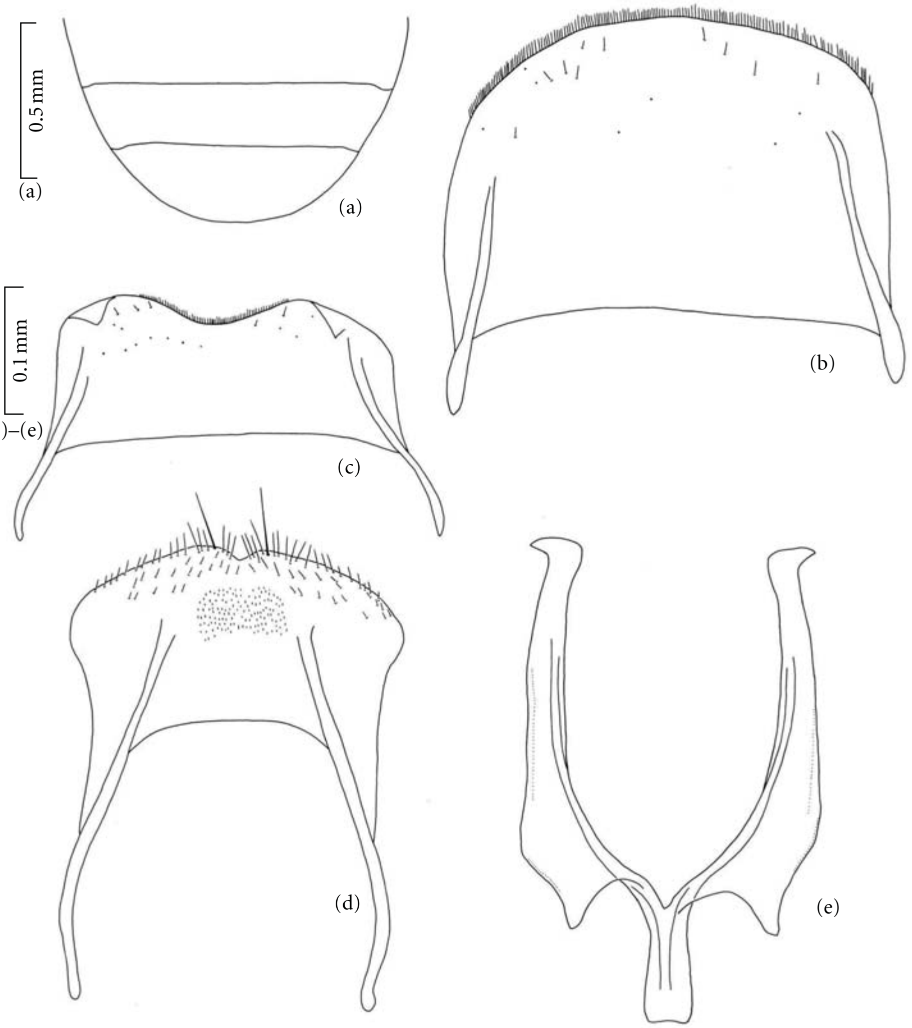


Figure 8: *Hydrocyphon doiinthanonensis* sp.n., holotype, male. (a) Sternites V–VII; (b) tergite VIII; (c) tergite IX; (d) sternite IX; (e) tegmen.

caudal part, with a pair of short apodemes. Sternite IX slightly sclerotized, bearing irregular setae in caudal part, with a pair of long apodemes. Tergite IX slightly sclerotized, trapezoidal, concave and bearing short spines in caudal margin, upturned in postero-lateral parts, bearing short setae in caudal part, with a pair of long apodemes. Tegmen large, well sclerotized; proximal part short, subparallel-sided; parameres long, projecting laterally in apices, projecting subtriangularly in anterior corners. Penis missing.

Female. Unknown.

Measurements. TL 2.25 mm; PW 0.98 mm; PL 0.40 mm; EL 1.85 mm; EW 1.45 mm.

Remarks. The species belongs to the *deflexicollis* species group. This species is distinguished from the previously known species by the concave posterior margin of the sternite and tergite IX and the shape of the parameres of the tegmen.

Etymology. The species is named after the type locality.

3.6. Hydrocyphon klapperichi sp.n. (See Figures 1(h) and 9)

Type Material. Holotype male (SMNS): “INDONESIEN: Sumatra, Prov. Aceh-Selatan, Babahrot 15–20. 8. 1983 leg. J. KLAPPERICH”.

Paratypes 2 female (SMNS): same data as for the holotype.

Male Description. Body oval, convex dorsally, shiny, closely covered with yellowish-white setae. Coloration of body blackish-brown, but lateral parts of pronotum and legs paler.

Head moderate in size, flat in dorsal surface, finely punctuate; clypeus short, straight in front margin; the distance between eyes about 2.2 times as long as the maximum diameter of an eye. Eyes relatively large, prominent. Pronotum punctuate as in head, slightly depressed ventrally in lateral parts; front and lateral margins straight; antero-lateral corners 120°; postero-lateral corners right-angle;

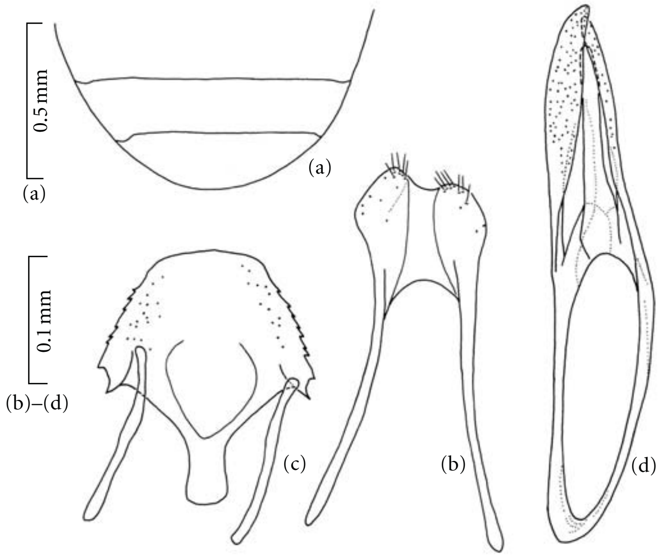


FIGURE 9: *Hydrocyphon klapperichi* sp.n., holotype, male. (a) Sternites V–VII; (b) tergite IX; (c) tegmen; (d) penis.

posterior margin gently arcuate; PW/PL 2.53. Scutellum relatively large, equilateral-triangular. Elytra oval, rather convex dorsally, broadest at basal 1/3; humeral part indistinctly projecting; EL/EW 1.20; EL/PL 4.27; EW/PW 1.41; TL/EW 1.48.

Caudal margin of sternite VII gently arcuate. Tergite VIII moderately sclerotized, trapezoidal, with a short apodemes. Tergite IX slightly sclerotized, bearing short setae in apical part, with a pair of long apodemes. Tegmen relatively large, moderately sclerotized; proximal part peg-like, short; parameres obscure, serrate at lateral margins, projecting and bifid in antero-lateral corners, punctuate; lateral projections very long, as long as parameres. Penis asymmetrical, long, well sclerotized, about 2.1 times as long as tegmen; pala oblong, gently tapered anteriorly; parameroids distinctly asymmetrical, closely punctuate, almost straight, left one wider and longer than right one; trigonium consisting of a long lobe, shorter than parameroids, obtuse at apex; median plate indistinct.

Female. Sexual dimorphism indistinct in external features, but body is somewhat larger; PW/PL 2.50–2.67 (2.58); EL/EW 1.46–1.47 (1.47); EL/PL 4.05–4.56 (4.30); EW/PW 1.10–1.17 (1.13); TL/EW 1.79–1.84 (1.81).

Measurements. Male ($n = 1$): TL 1.58 mm; PW 0.76 mm; PL 0.30 mm; EL 1.28 mm; EW 1.07 mm. Female ($n = 2$): TL 2.02 & 2.50 mm; PW 1.00 & 1.20 mm; PL 0.40 & 0.45 mm; EL 1.62 & 2.05 mm; EW 1.10 & 1.40 mm.

Remarks. The shape of the tegmen of this species is similar to that of the *mirabilis*, the *tamilensis*, the *kinabalensis*, and the *renati* species groups, but this species is easily distinguished from the latter by the serrate parameres and the shape of the penis. Judging from the shape of the penis (e.g., asymmetrical parameroids and single projection of trigonium), this species probably belongs to the *pallidicollis* species group.

Etymology. The species is named after Dr. J. Klapperich, who was the collector of the holotype.

3.7. Hydrocyphon deformis sp.n. (See Figures 1(i) and 10)

Type Material. Holotype male (SEHU): "INDIA: KERALA Dhony Hills 180–450 m 7 DEC 1978 JAP-IND CO TR."

Male Description. Body oval, well convex dorsally, strongly shiny, closely covered with yellowish-white setae. Coloration blackish-brown, but lateral parts of pronotum, mouth parts, antennae, and legs paler.

Head moderate in size, flat in dorsal surface, finely punctuate; clypeus relatively long, straight in front margin; the distance between eyes about 2.5 times as long as the maximum diameter of an eye. Eyes moderate in size, prominent. Antennae short and stout, reaching about proximal 1/3 of elytra. Pronotum strongly transverse, punctuate as in head, depressed ventrally in lateral parts; front and lateral margins straight; antero-lateral corners obtuse, postero-lateral corners almost right-angle; posterior margin arcuate; PW/PL 2.80. Scutellum relatively large, equilateral triangular. Elytra semicircular, well convex dorsally, broadest at basal 1/3; humeral parts slightly projecting dorsally; EL/EW 1.14; EL/PL 4.57; EW/PW 1.43; TL/EW 1.39. Legs relatively long.

Caudal margin of sternite VII gently arcuate. Tergite VIII moderately sclerotized, trapezoidal, bearing short setae and spines along caudal margin, with a pair of short apodemes. Tergite IX membranous, with a pair of long and slender apodemes. Sternite IX slightly sclerotized, oblong, bearing short setae in postero-lateral parts. Tegmen moderately sclerotized; proximal part peg-like, short; parameres short, obtuse at

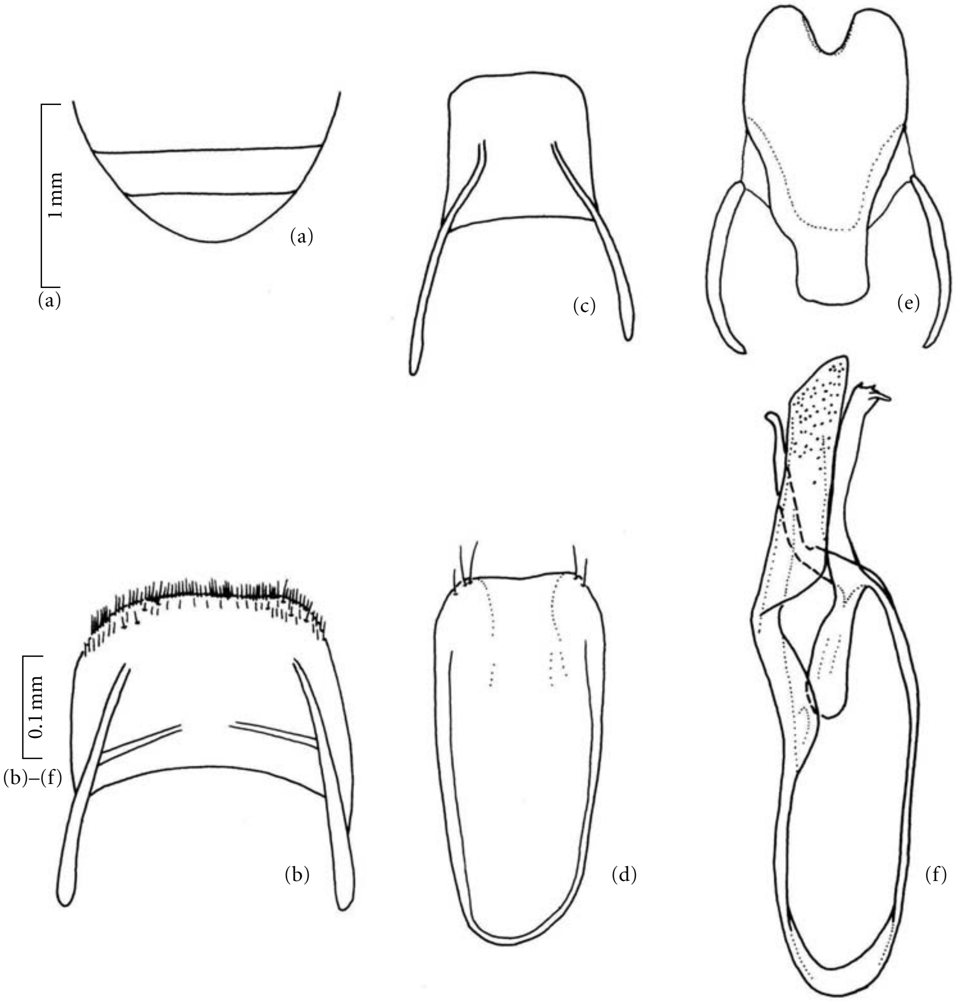
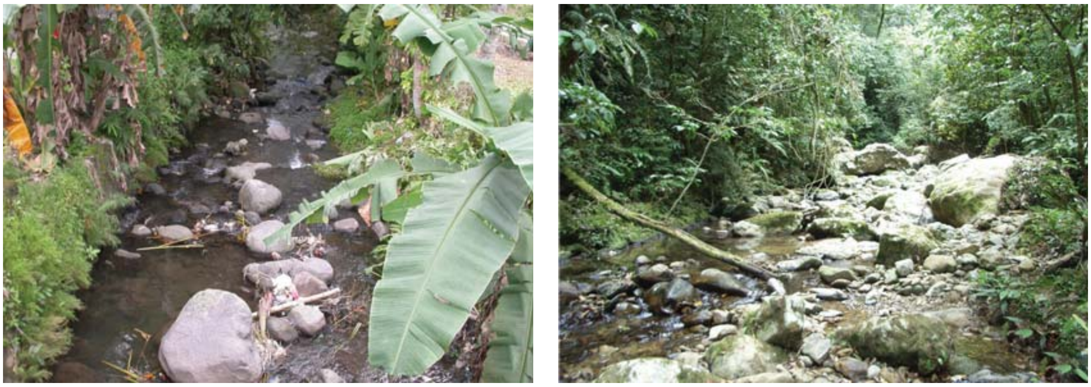


FIGURE 10: *Hydrocyphon deformis* sp.n., holotype, male. (a) Sternites V–VII; (b) tergite VIII; (c) tergite IX; (d) sternite IX; (e) tegmen; (f) penis.



(a)

(b)

FIGURE 11: Habitats of *Hydrocyphon* spp. (a) Ngaglik, Yogyakarta (type locality of *H. jogjaensis* sp.n., 28. II. 2010, photo by H. Yoshitomi); (b) Kinabalu Park, Sabah (type locality of *H. takizawai* sp.n., 4. V. 2010, photo by H. Yoshitomi).

apices; lateral projections long. Penis asymmetrical, long, about 2.1 times as long as tegmen; pala oblong, subparallel-sided, arcuate in caudal margin; parameroids distinctly asymmetrical, left one wide and closely punctuate, diagonal in apical margin, right one distinctly curved inwardly, slender, with rather pointed apex; trigonium a little shorter than parameroids, serrate at apex; median plate short.

Female. Unknown.

Measurements. TL 1.95 mm; PW 0.98 mm; PL 0.35 mm; EL 1.60 mm; EW 1.40 mm.

Remarks. Judging from the shape of the penis in having an asymmetrical trigonium projection, this species belongs to the *pallidicollis* species group; however, the shape of the tegmen of this species is similar to that of the *mirabilis*, the *tamilensis*, and the *renati* species groups. This species is also similar to *H. kinabalensis* Yoshitomi and Satô, 2005 [2] in the shape of the tegmen and the left parameroid of the penis, but differs from it by the presence of trigonium and plate-like sternite IX.

Etymology. The species name refers to the shape of the tegmen.

4. New Combination of the Species

4.1. *Hydrocyphon sieberi [15], Comb.n*

Remarks. Judging from the original description and figures [15], this species clearly belongs to the *pallidicollis* species group of the genus *Hydrocyphon*. It is closely similar to *H. guangxiensis* Yoshitomi and Klausnitzer, 2003 [1], known from China, and differs from it by the shape of the right parameroid which has small projections at the inner margin of the apex (lacking projection in *guangxiensis*).

Distribution. India.

5. Additional Specimens Examined

5.1. *Hydrocyphon sakaii Yoshitomi and Satô, 2003 [4]*

Additional Specimens Examined. 9 Males (EUMJ), "(LAOS) Ban Saleui Xam Neua 30-31. III. 2005 J. Yamasako leg."; 1 female (EUMJ), "Mt. Phu Pan, 1500–1800 m, N20′11E 104′01 Houaphan Prov. N. E. Laos 21–25. V. 2004 T. Mizusawa"; 1 male (EUMJ), "Phu Pan (Mt.) alt. 1500–1800 m N20°11′/E104°01′ Laos 25. IV-5. V. 2004′′; 2 males (EUMJ), ditto, but "16–19. V. 2004, M. Sato leg."

Distribution. Laos.

5.2. *Hydrocyphon wakaharai Yoshitomi and Satô, 2003 [4]*

Additional Specimens Examined. 1 Male (EUMJ), "[N. Laos] Phu-Pan Alt. ca. 1600–1750 m Xam Neua Pref. Houapan province 21. V. 2005 T. Kurihara leg."; 3 males (EUMJ), "N-VIETNAM: Tam Dao 21°28′N 105°38′E 19. 5.–13.6., 800–1000 m leg. Malicky 1995," genit. s. nos. HY 857, 878, 882; 1 male, "Mt. Phu Bia Saisombun Laos 21-III-2005 M. Sato leg."

Distribution. Laos, Vietnam.

5.3. *Hydrocyphon javanicus Yoshitomi and Satô, 2005 [2]*

Additional Specimens Examined. 3 Males and 3 females (EUMJ), "(Indonesia) Ciburum alt. 1,600 m Mt. Gede, Jawa Barat VII. 27. 1977 Shinji Nagai leg."; 1 male (EUMJ), ditto but "20. VII. 1997."

Distribution. Indonesia (Java Isl.).

5.4. *Hydrocyphon triforius Yoshitomi and Satô, 2005 [2]*

Additional Specimen Examined. 1 Male (EUMJ), "Ban A Chia 890 m Lai Chau N. Vietnam 8-V-1995 Y. Nishikawa", genit. s. no. HY 1098.

Distribution. Malaysia, Thailand, Vietnam (new record).

5.5. *Hydrocyphon tamilensis Yoshitomi and Satô, 2005 [2]*

Additional Specimens Examined. 2 Males & 1 female (SEHU), "INDIA: TAMIL N. Coonoor 1700–1900 m 29 NOV 1978 JAP-IND CO TR."

Distribution. India.

Acknowledgments

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Research Article

Splendid Hybrids: The Effects of a Tiger Beetle Hybrid Zone on Apparent Species Diversity

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Nonexpert citizen groups are being used to monitor species to track ecosystem changes; however, challenges remain for proper identification, especially among diverse groups such as beetles. Tiger beetles, *Cicindela* spp., have been used for biological diversity monitoring because of their diversity and the ease of recognition. The finding of an apparent hybrid zone among *Cicindela denverensis* Casey, *Cicindela limbalis* Klug, and *Cicindela splendida* Hentz in central Nebraska prompted a detailed study of the biogeography of this species group within Nebraska, a test of characteristics that could be used by citizen scientists, and limited breeding experiments. This study suggests that while *C. denverensis* appears to hybridize with both *C. limbalis* and *C. splendida* within the hybrid zone, all three species maintain their integrity across most of their ranges, largely occupy unique geographic regions, and at least *C. denverensis* and *C. splendida* cooccur in many areas with no evidence of hybridization. Evidence of hybridization between *C. limbalis* and *C. splendida* was found at only two sites. Furthermore, breeding experiments with virgin *C. splendida* and *C. denverensis* showed that they are capable of producing hybrid larvae in the laboratory. The presence of morphological intergrades serves as a cautionary note when using biological indicator species.

1. Introduction

Hybridization of distinct lineages has been recognized as an important area of evolutionary research since the time of Charles Darwin. Although much of the past research has been on plant hybridization, attention to animal species has been increasing and has become the subject of focused research by evolutionary biologists [1–4]. Unfortunately for the field of conservation biology, hybridization can be extremely problematic. Moreover, the challenge of hybridization to the conservation of unique species has increased as anthropomorphic changes to environment and globalization and introduction of exotic species have combined to increase interactions among species [5].

As global changes take place and loss of biodiversity is a growing concern, many research organizations have sought to increase biological monitoring by citizen groups. A growing number of examples exist for monitoring of aquatic ecosystems for pollution [6]. More recently, citizen scientist groups have successfully detected both invasive species [7] and rare native species such as the nine-spotted lady beetle, *Coccinella novemnotata* [8]. Citizen science programs have also been used to collect data over broad scales such as the case for determining monarch butterfly, *Danaus plexippus*, migration routes [9].

Despite these and many other benefits in the use of citizen scientists for ecosystem monitoring, many challenges remain, including training citizen scientists, coordinating monitoring programs, and ensuring the accuracy of identification (e.g., [6, 7]). Relatively, few citizen monitoring programs exist for terrestrial invertebrate diversity, likely as a result of the enormous diversity of terrestrial insects. Among groups that have been monitored, dragonflies, butterflies, and ladybird beetles have received the most attention. Another candidate group is the tiger beetles, Coleoptera: Carabidae: Cicindelinae.

The tiger beetles of North America have been studied thoroughly and are well known even to the subspecies level,

Table 1: Identification characters used to differentiate between species in the *Cicindela splendida* group.

Character	*C. denverensis*	*C. limbalis*	*C. splendida*
Dorsal head	Green to blue-green	Purple, red, or dull red	Green to blue
Margins of head	Green to blue-green	Green to blue	Green to blue
Dorsal pronotum	Green to blue-green	Purple, red, or dull red	Green to blue
Margins of pronotum	Green to blue-green	Green to blue	Green to blue
Elytra	Green to blue-green	Purple, red, or dull red	Red, purple, or (rarely) green
Margins of elytra	Green to blue-green	Green to blue	Green to blue
Proepisternum	Green to blue	Red to orange	Green to blue

Table 2: Locality information for specimens of *C. denverensis* examined.

Species	County	County total	Location	Location total
C. denverensis	Banner	2	Bull Canyon	2
	Buffalo	54	Amherst	8
			Cherry Creek	3
			Kearney	43
	Custer	13	Ansley	13
	Dawes	4	Chadron	2
			Crawford	2
	Dawson	103	Gothenburg	41
			Sumner	62
	Garfield	9	Burwell	9
	Kimball	1	Pine Bluffs	1
	Red Willow	1	McCook	1
	Scotts Bluff	14	Scottsbluff	14
	Sherman	90	E Loup City	3
			Hazard	77
			W Loup City	10
	Sioux	58	Crawford	6
			Harrison	52
	Valley	11	Arcadia	3
			Elyria	4
			Ord	4
	Total counties = 12		Total sites = 20	Total = 360

Table 3: Locality information for specimens of *C. limbalis* examined.

Species	County	County total	Location	Location total
C. limbalis	Buffalo	4	Cherry Creek	2
			Kearney	2
	Burt	1	Decatur	1
	Butler	10	Bellwood	10
	Cass	1	Murdock	1
	Douglas	8	Omaha	8
	Greeley	4	Scotia	2
			Wolbach	2
	Howard	28	St. Paul	28
	Lancaster	4	Lincoln	4
	Merrick	4	Palmer	4
	Nance	5	Fullerton	3
			Palmer	2
	Polk	1	Osceola	1
	Sarpy	4	Ashland	1
			Gretna	3
	Saunders	3	Otoe Creek	3
	Sherman	26	E Loup City	18
			Hazard	8
	Valley	2	Arcadia	1
			Davis Creek	1
	Washington	3	County Line Road	3
	Total counties = 15		Total sites = 22	Total = 108

although variation is considerable and the validity of many is still debated [11]. Because many tiger beetles are diurnally active predators, regionally diverse, and identified by color markings, they can potentially be useful for citizen groups as a biological indicator group [12]. Indeed, worldwide, tiger beetles have been used to predict species richness patterns in other taxa and have shown strong correlation with butterfly species richness [13–15]. In the United States, tiger beetle diversity varies by region, with the highest diversity found in the southwest and generally lower diversity found in the north [11]. The state of Nebraska has recorded 32 species of tiger beetles [16, 17]. Among the 93 Nebraska counties, as few as 0 and as many as 22 tiger beetle species have been recorded with the highest numbers recorded in areas with the most intensive sampling [16]. No pattern in number of species has been detected by latitude, ecoregion, or county size [16].

Among the tiger beetles occurring in Nebraska, one group, the *Cicindela splendida* group, remains controversial. The group consists of three named species, *Cicindela denverensis* Casey, *Cicindela limbalis* Klug, and *Cicindela splendida* Hentz, which are morphologically very similar and may only be readily separated by color. Schincariol and Freitag [18] determined that each of these three forms represented valid species and that they could be distinguished on the basis of elytral pattern, percent maculation, elytral color, and nonsensory setae number. These authors noted that the genitalia were very similar in all three of these forms. Interspecific copulation between species in this group has

Table 4: Locality information for specimens of *C. splendida* examined.

Species	County	County total	Location	Location total
C. splendida	Buffalo	70	Amherst	3
			Cherry Creek	7
			Kearney	57
			Pleasanton	3
	Butler	11	Bellwood	11
	Cass	4	Murdock	2
			Nebraska City	2
	Custer	6	Ansley	5
			Merna	1
	Dawson	37	Gothenburg	26
			Sumner	11
	Douglas	7	Omaha	7
	Franklin	28	Bloomington	27
			Naponee	1
	Gage	1	Virginia	1
	Garfield	2	Burwell	2
	Gosper	4	Elwood	4
	Greeley	6	Scotia	4
			Wolbach	2
	Harlan	9	Harlan Reservoir	1
			Oxford	5
			Ragan	3
	Howard	14	Ashton	4
			St. Paul	10
	Lancaster	22	Lincoln	19
			Spring Creek	3
	Merrick	3	Palmer	3
	Nance	1	Fullerton	1
	Phelps	14	S Holdrege	14
	Red Willow	4	McCook	4
	Saline	1	Crete	1
	Sarpy	2	Gretna	2
	Saunders	1	Otoe Creek	1
	Scotts Bluff	2	Scottsbluff	2
	Sherman	133	E Loup City	48
			Hazard	70
			W Loup City	15
	Sioux	8	Harrison	8
	Valley	7	Arcadia	3
			Davis Creek	1
			Ord	3
	Total counties = 25		Total sites = 41	Total = 397

Table 5: Colors used in character analyses (from [10]).

Code	Color definition
1	Very deep purplish red (257)
2	Deep red (13)
3	Grayish reddish orange (39)
4	Dark greenish yellow (103)
5	Deep yellowish green (132)
6	Deep bluish green (161)
7	Deep blue, royal blue (179)
8	Deep violet (208)

been reported in the literature [19], and, in Nebraska is frequently observed. However, these observations do not verify that these matings result in offspring or if offspring are viable. Moreover, Schincariol and Frietag [18] suggested that spermatophore ejection by the female allows these species to maintain their integrity.

During extensive sampling by the senior author, a number of apparent hybrids between *Cicindela denverensis* and *C. limbalis* were collected in central Nebraska from a zone extending north to south and about 30 km wide [17]. Apparent hybrids between two other species, *C. denverensis* and *C. splendida*, occur regularly across a zone in central Nebraska extending north to south and approximately 80 km wide.

In this study we conducted a morphological study of members of the *Cicindela splendida* group throughout Nebraska and did selective interspecific breeding experiments. In order to determine the occurrence of hybrids within this group, we tested the following hypotheses: (1) hybrids are most frequent in specific geographic areas, (2) the geographic areas in which hybrids are most frequent are related to the range and relative abundance of the species present, and (3) hybrid offspring would be produced in the laboratory using virgin males and females with interspecific pairings.

2. Materials and Methods

2.1. Character Analyses and Geographic Location. A total of 865 Nebraska specimens from this group were examined from the personal collections of Mathew Brust, Steve Spomer, and Paul Nabity (Tables 2, 3, 4). Individuals were identified to species based on the characters presented in Tables 1 and 6. Based on these characters, any specimen with a distinct blue to dark green margin on the head and pronotum was classified as *C. limbalis*, while any lacking this character but having the color of the head and pronotum differing from the color of the elytra or having distinct dark green to blue margins on the elytra was considered *C. splendida*. These designations were made to allow analyses with the null assumption of no hybridization.

Character analysis generally followed those of Schincariol and Frietag [18] and Schincariol [20]. One additional grade for color based on Kelly and Judd [10] was added to account for an unusual morph that was found at several locations. Thus, elytral color (1–8) and pronotal color (1–8) were scored for each specimen (Table 5) and analyzed in order to determine hybridization.

Maculation characters were also analyzed to test whether maculation could be used to differentiate these species, as suggested by Schincariol and Frietag [18]. The following

Table 6: Variations in color found in apparent hybrids between species in the *Cicindela splendida* group.

Character	*C. denverensis* × *limbalis*	*C. denverensis* × *splendida*	*C. limbalis* × *splendida*
Dorsal head	Orange-green, yellow-green, or green	Green to blue	Orange-green to red
Margins of head	Green-blue to blue	Green to blue	Orange-green to red, limited greenish
Dorsal pronotum	Orange-green, yellow-green, or green	Green to blue	Orange-green to red
Margins of pronotum	Green-blue to blue	Green to blue	Orange-green to red, limited greenish
Elytra	Orange-green, yellow-green, or green	Bronze, orange-green, yellow-green or green	Purple, red, or dull red
Margins of elytra	Green-blue to blue	Green to blue	Orange-green to red, limited greenish
Proepisternum	At least partially red or orange	Green to blue	At least partially red or orange

characters of maculation were graded: development of the humeral lunule (A–E), development of the middle band (A–E), development of the apical lunule (A–E), and overall development of maculation (A–E).

All characters were then analyzed across latitude and longitude using the PROC GLM procedure [21] with each one degree increment represented as a categorical variable (latitude = 1–3, longitude = 1–9). The results were checked for latitude by longitude interactions as well. When significant differences ($P < 0.05$) were found for a character by latitude or longitude, a post-hoc Tukey test was performed.

2.2. Hybridization in the Laboratory. Adults of *C. denverensis* and *C. splendida* emerge briefly in Fall after pupation, but do not mate until spring [11, 22]. Specimens for hybridization experiments were collected in October from the vicinity of Kearney, Nebraska. Three conspecific pairs consisting of a male *C. denverensis* and a female *C. splendida* were placed in individuals plastic aquaria (3.8 liter) with loess soil (approximately 70 cm deep) from collection sites. The aquaria were maintained at room temperature for about one week and were then placed into a refrigerator (approximately 6°C) for 8 weeks because a cool period is needed to trigger diapause and that this diapause is required for sexual maturity [22]. Aquaria were then placed at room temperature and adults were allowed to mate and oviposit. Resulting eggs and larvae were counted once the female in each aquarium had died. Adults and resulting larvae were fed apterous *Drosophila melanogaster* Meigen.

3. Results

3.1. Character Analyses and Geographic Location. Examination of more than 860 specimens belonging to the *C. splendida* group revealed considerable variation in elytral and pronotal color among the group (Figure 1, Table 6). For *C. denverensis*, no significant differences in elytral color were detected, while the elytral coloration for both *C. splendida* and *C. limbalis* differed significantly by longitude (Figures 4, 6, and 8). Elytral color converged for all three species between approximately 98° and 100° west longitude (Figures 10, 11, and 13).

Pronotal colors also varied by region with significant differences found for *C.denverensis*, *C. limbalis*, and *C. splendida* (Figures 5, 7, and 9). Among species, no latitude by longitude interactions were found. In the region between 98° and 100° west longitude, the pronotal color of *C. limbalis* became significantly more like that of *C. denverensis* (Figure 7).

Character analyses based on Schincariol and Freitag [18] revealed no consistent differences in elytral maculation that would allow the three species to be consistently distinguished. Significant differences in markings occurred for all species across their distribution for at least some of the elytral markings. For *C. denverensis*, differences in total maculation, humeral lunule, middle band, and apical lunule varied by longitude (Figure 1). For both *C. limbalis* and *C. splendida* significant differences were detected for the middle band and apical lunules but not for total maculation (Figures 2 and 3).

3.2. Hybridization in the Laboratory. The three females used in the hybridization experiments produced 66, 23, and 4 eggs respectively. Of these eggs, 39, 12, and 0 hatched, respectively. Attempts were made to rear the larvae to adulthood but all died before maturity as a result of mold infection.

4. Discussion

Pearson and Cassola [12] suggest that tiger beetles represent a well-characterized fauna that is suitable for use by non-experts as a biological indicator group. In Nebraska, the three species examined in this study display an apparent hybrid zone based on color and markings across the central region of the state. Field observations of interspecific pairings along with the small-scale laboratory breeding experiments reveal that hybridization is possible, although these results should be cautiously interpreted. To determine the extent of hybridization, molecular studies over a large region should be conducted as was accomplished for *Cicindela dorsalis* Say [23] and *C. splendida* and *C. limbalis* [24] Further studies of interbreeding and rearing conditions should also be

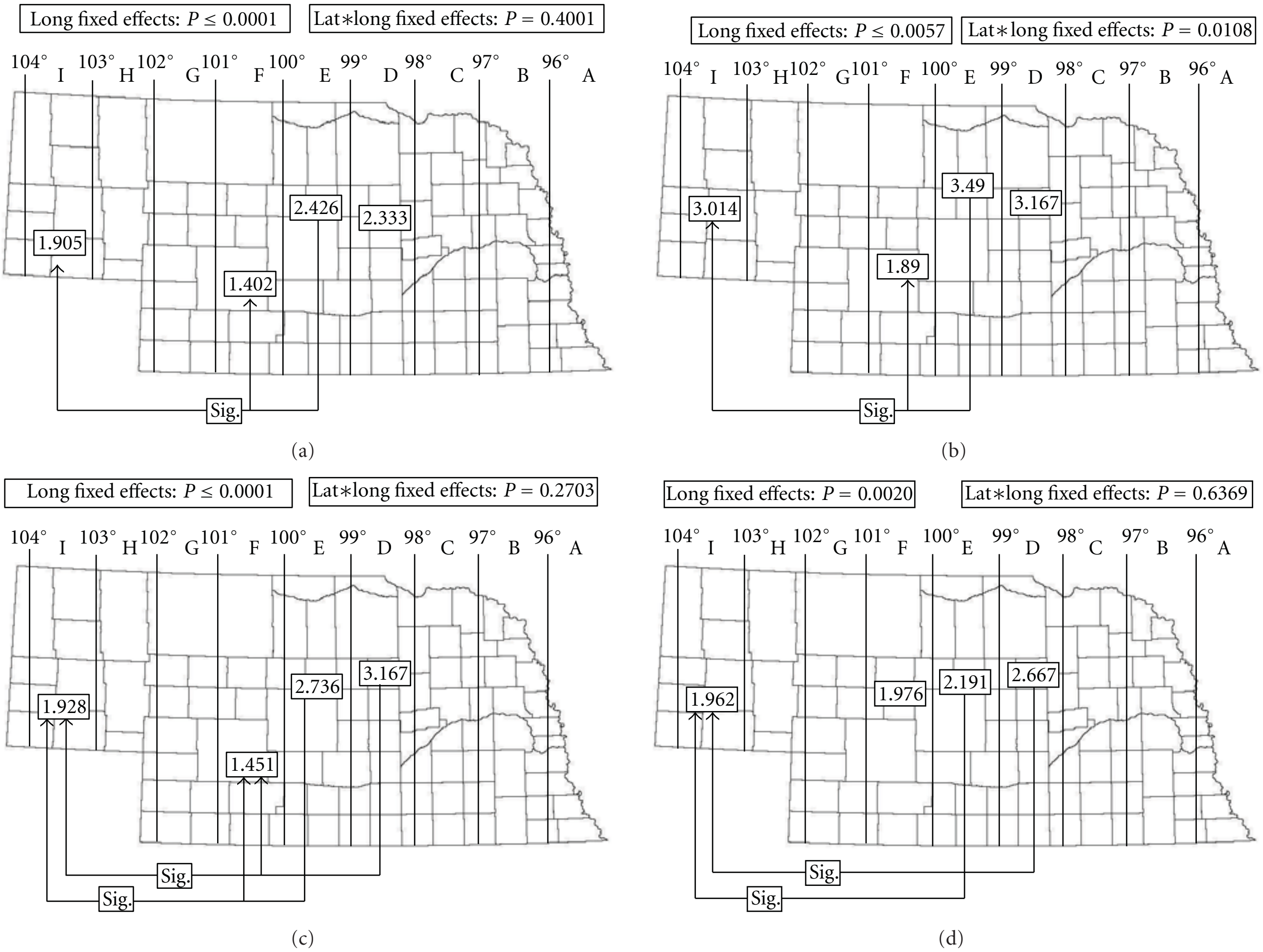

Long fixed effects: $P \leq 0.0001$
Lat∗long fixed effects: $P = 0.4001$
104° I 103° H 102° G 101° F 100° E 99° D 98° C 97° B 96° A
1.905
1.402
2.426
2.333
Sig.
(a)
Long fixed effects: $P \leq 0.0057$
Lat∗long fixed effects: $P = 0.0108$
104° I 103° H 102° G 101° F 100° E 99° D 98° C 97° B 96° A
3.014
1.89
3.49
3.167
Sig.
(b)
Long fixed effects: $P \leq 0.0001$
Lat∗long fixed effects: $P = 0.2703$
104° I 103° H 102° G 101° F 100° E 99° D 98° C 97° B 96° A
1.928
1.451
2.736
3.167
Sig.
Sig.
(c)
Long fixed effects: $P = 0.0020$
Lat∗long fixed effects: $P = 0.6369$
104° I 103° H 102° G 101° F 100° E 99° D 98° C 97° B 96° A
1.962
1.976
2.191
2.667
Sig.
Sig.
(d)

Figure 1: Elytral maculation character states for *C. denverensis* (adapted from [18]). (a) Total maculation, (b) humeral lunule, (c) middle band, and (d) apical lunule. Significant differences between longitudes shown by arrows and mean value presented in each longitudinal grouping. Fixed effects and effects of latitude by longitude interactions presented at top of each map.

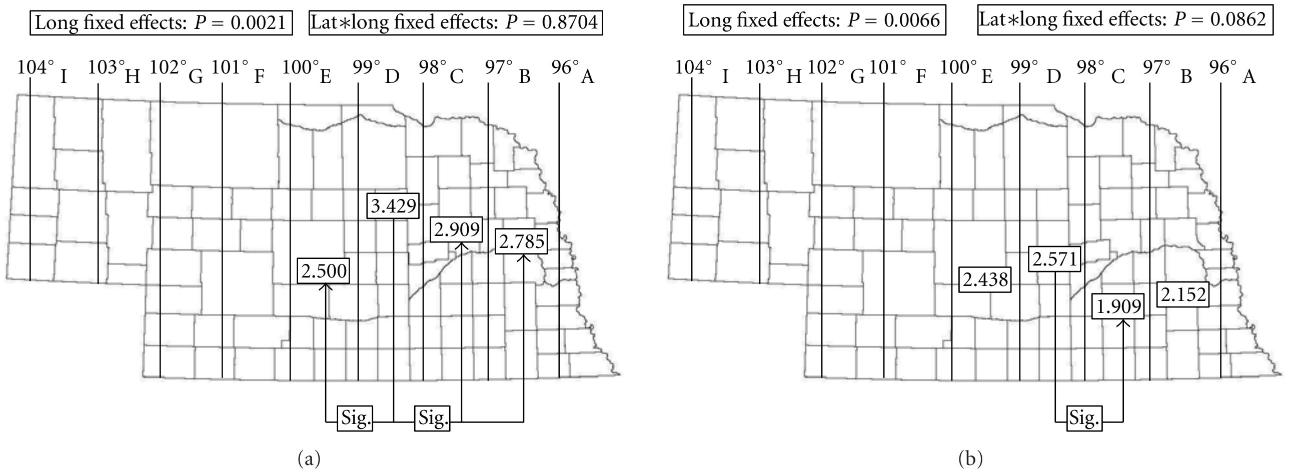
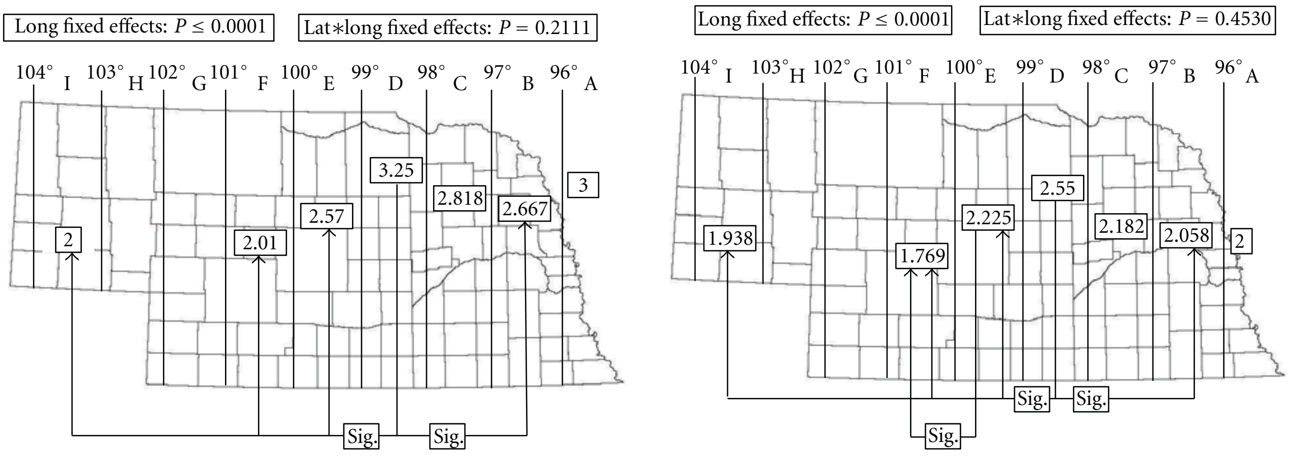

Long fixed effects: $P = 0.0021$
Lat∗long fixed effects: $P = 0.8704$
104° I 103° H 102° G 101° F 100° E 99° D 98° C 97° B 96° A
2.500
3.429
2.909
2.785
Sig.
Sig.
(a)
Long fixed effects: $P = 0.0066$
Lat∗long fixed effects: $P = 0.0862$
104° I 103° H 102° G 101° F 100° E 99° D 98° C 97° B 96° A
2.438
2.571
1.909
2.152
Sig.
(b)

Figure 2: Elytral maculation character states for *C. limbalis* (adapted from [18]). (a) Middle band and (b) apical lunule. Total maculation and humeral lunule not presented as no significant differences found. Significant differences between longitudes shown by arrows and mean value presented in each longitudinal grouping. Fixed effects and effects of latitude by longitude interactions presented at top of each map.


Long fixed effects: $P \leq 0.0001$
Lat∗long fixed effects: $P = 0.2111$
104° I 103° H 102° G 101° F 100° E 99° D 98° C 97° B 96° A
2
2.01
2.57
3.25
2.818
2.667
3
Sig.
Sig.
Long fixed effects: $P \leq 0.0001$
Lat∗long fixed effects: $P = 0.4530$
104° I 103° H 102° G 101° F 100° E 99° D 98° C 97° B 96° A
1.938
1.769
2.225
2.55
2.182
2.058
2
Sig.
Sig.
Sig.

(a) (b)

Figure 3: Elytral maculation character states for *C. splendida* (adapted from [18]). (a) Middle band and (b) apical lunule. Total maculation and humeral lunule not presented as no significant differences found. Significant differences between longitudes shown by arrows and mean value presented in each longitudinal grouping. Fixed effects and effects of latitude by longitude interactions presented at top of each map.

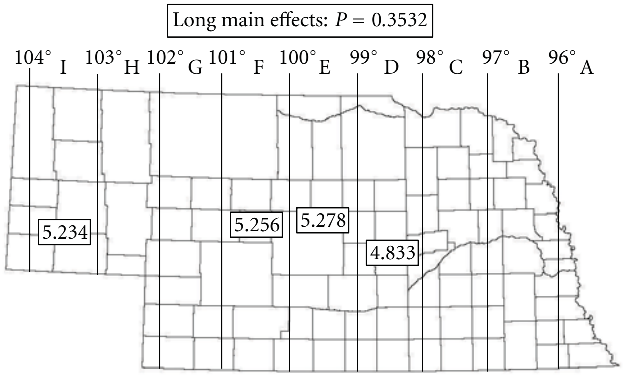

Long main effects: $P = 0.3532$
104° I 103° H 102° G 101° F 100° E 99° D 98° C 97° B 96° A
5.234
5.256
5.278
4.833

Figure 4: Mean elytral color by code for *Cicindela denverensis* by longitudinal region. Significant differences between longitudes shown by arrows and mean value presented in each longitudinal grouping. Fixed effects presented at top. No latitude by longitude interactions found.

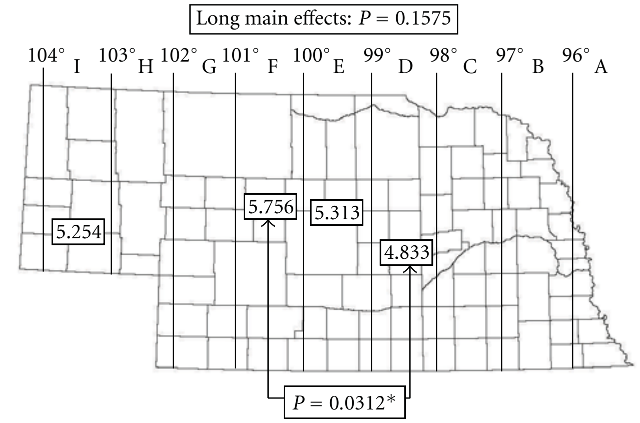
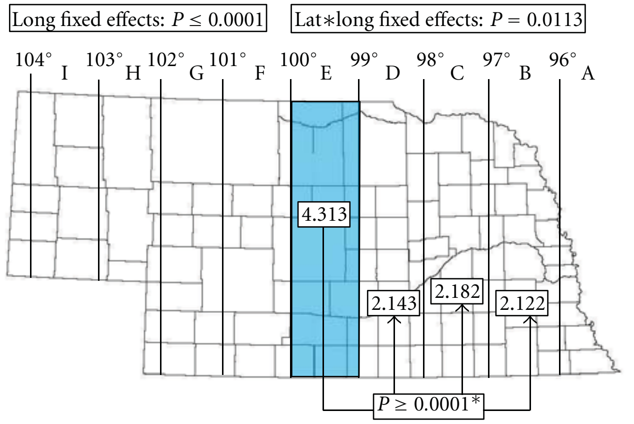

Long main effects: $P = 0.1575$
104° I 103° H 102° G 101° F 100° E 99° D 98° C 97° B 96° A
5.254
5.756
5.313
4.833
$P = 0.0312^*$

Figure 5: Mean pronotal color by code for *Cicindela denverensis* by longitudinal region. Significant differences between longitudes shown by arrows and mean value presented in each longitudinal grouping. Fixed effects presented at top. No latitude by longitude interactions found.

conducted because environmental conditions can influence adult coloration patterns [25]. Because Nebraska's tiger beetle fauna consists of 32 species and most counties have 8 or less [16], the inability to properly identify species or the presence of hybrids will affect estimates of biological diversity.

In Nebraska, the apparent hybrid zone affects parts of at least seven counties and approximately 20% of the state (Figure 12). The geographic and morphological analyses indicate a hybrid zone extending from central Custer and Dawson Counties east to the eastern third of Valley and Hall Counties (Figure 12). The termination of this hybrid zone to the north and south coincides with a general lack of suitable habitat as the Rainwater Basin occurs south of this area, and the Sand Hills occur to the north.

In the eastern half of the hybrid zone, all three species cooccur west at least to Kearney. Nearly all of the *C. limbalis* collected in this area exhibit at least a moderate greenish hue, suggesting hybridization with *C. denverensis*. Greenish *C. limbalis* have been recorded in other areas as well [26, 27], mostly where *C. denverensis* and *C. limbalis* cooccur. Across the entire zone, the majority (especially toward the east) exhibit coppery bronze to greenish elytra, which in some cases might suggest the "*ludoviciana*" [28] phenotype. However, the majority of these specimens have a green to bluish green pronotum, while in "*ludoviciana*" the pronotum is deep blue. Some specimens have variable amounts of coppery bronze on the anterior parts of the elytra, diffusing into green elsewhere. For the analyses, these were classified as coppery green, but, importantly, this phenotype has not been previously documented in *C. splendida* elsewhere in its range. Interestingly, specimens with features suggesting hybridization between *C. limbalis* and *C. splendida* were found only on the eastern edge of the hybrid zone.


Long fixed effects: P ≤ 0.0001
Lat∗long fixed effects: P = 0.0113
104°
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97°
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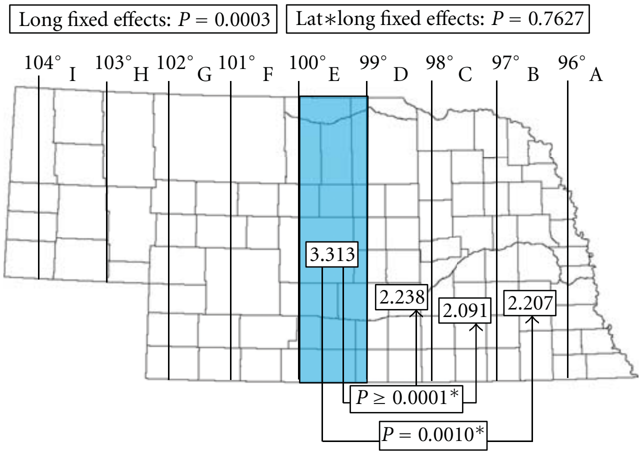
96°
A
4.313
2.143
2.182
2.122
P ≥ 0.0001*

Figure 6: Mean elytral color by code for *Cicindela limbalis* by longitudinal region. Significant differences between longitudes shown by arrows and mean value presented in each longitudinal grouping. Fixed effects presented at top. No latitude by longitude interactions found. Shaded area depicts longitudinal regions differing from all others.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Lat∗long fixed effects: P = 0.7627
104°
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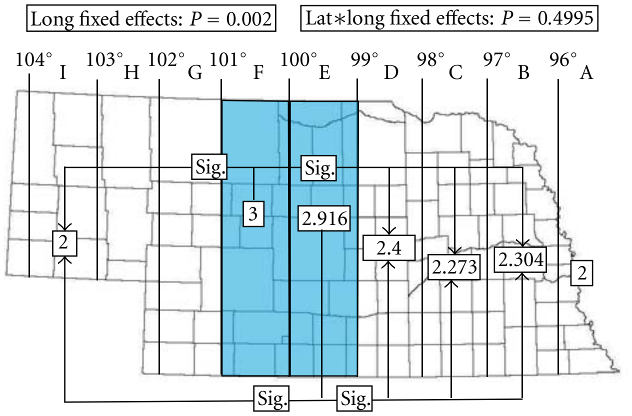
E
99°
D
98°
C
97°
B
96°
A
3.313
2.238
2.091
2.207
P ≥ 0.0001*
P = 0.0010*

Figure 7: Mean pronotal color by code for *Cicindela limbalis* by longitudinal region. Significant differences between longitudes shown by arrows and mean value presented in each longitudinal grouping. Fixed effects presented at top. No latitude by longitude interactions found. Shaded area depicts longitudinal regions differing from others.


Long fixed effects: P = 0.002
Lat∗long fixed effects: P = 0.4995
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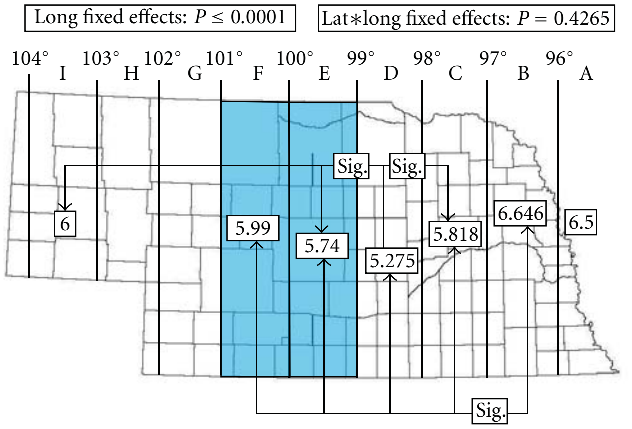
101°
F
100°
E
99°
D
98°
C
97°
B
96°
A
Sig.
Sig.
2
3
2.916
2.4
2.273
2.304
2
Sig.
Sig.

Figure 8: Mean elytral color by code for *Cicindela splendida* by longitudinal region. Significant differences between longitudes shown by arrows and mean value presented in each longitudinal grouping. Fixed effects presented at top. No latitude by longitude interactions found. Shaded area depicts longitudinal regions differing from others.


Long fixed effects: P ≤ 0.0001
Lat∗long fixed effects: P = 0.4265
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H
102°
G
101°
F
100°
E
99°
D
98°
C
97°
B
96°
A
Sig.
Sig.
6
5.99
5.74
5.275
5.818
6.646
6.5
Sig.

Figure 9: Mean pronotal color by code for *Cicindela splendida* by longitudinal region. Significant differences between longitudes shown by arrows and mean value presented in each longitudinal grouping. Fixed effects presented at top. No latitude by longitude interactions found. Shaded area depicts longitudinal regions differing from all others except each other.

Observed hybridization among members of the butterfly genus *Liminitis* in North America appears correlated to one species occurring at extremely low densities alongside a sister species that is more numerous. Under such conditions, a male of the rare species may choose to mate with a female of the more common species if he is unable to find a mate of his own species [29]. However, Wirtz [30] concluded in a review of the literature that females are the choosier sex and that in most cases females of rare species will mate with males of more common species as a last resort. Although genetic analysis is needed to determine the direction of crossing in these species in Nebraska, rarity of individuals of a species appears to contribute to interbreeding at least for *C. limbalis*.

Rarity of individuals does not appear to explain intergrades between *C. denverensis* and *C. splendida* which often and widely co-occur in Nebraska and elsewhere with little evidence of interbreeding. It does seem possible that hybridization between *C. denverensis* and *C. limbalis* could lead to a cascade of hybridization events perhaps causing hybrid offspring to interbreed with any of the three species, resulting in offspring of a broad range of phenotypes. Elsewhere, *C. denverensis* and *C. limbalis* may hybridize where they cooccur, but they are mostly geographically separated, potentially as a result of differing moisture preferences. In Colorado, Kippenhan [31] reported few locations where both species had been collected. It appears that *C. limbalis* dominates sites with a long and stable history. For example, although the steep loess bluffs in Fremont County Iowa just across the Missouri River from Nebraska City present habitat suitable for *C. limbalis* and *C. splendida* and are within the range

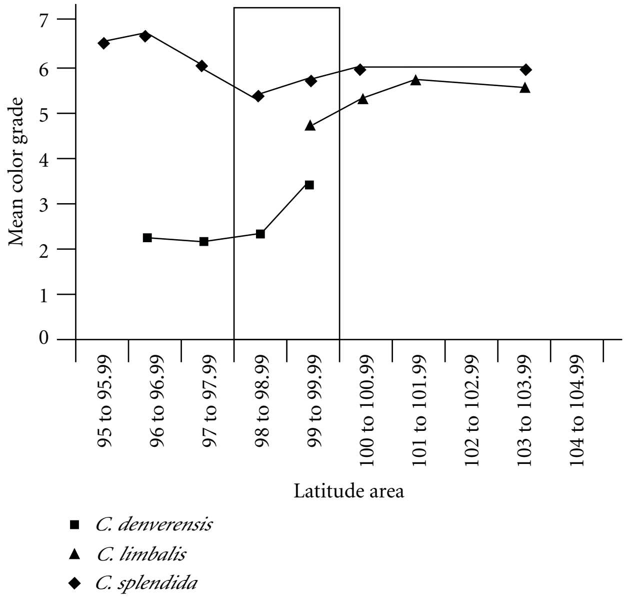

Figure 10: Graph of mean pronotal color by code for *C. denverensis*, *C. limbalis*, and *C. splendida* by longitude. Region of character convergence for *C. denverensis* and *C. splendida* depicted by rectangle.

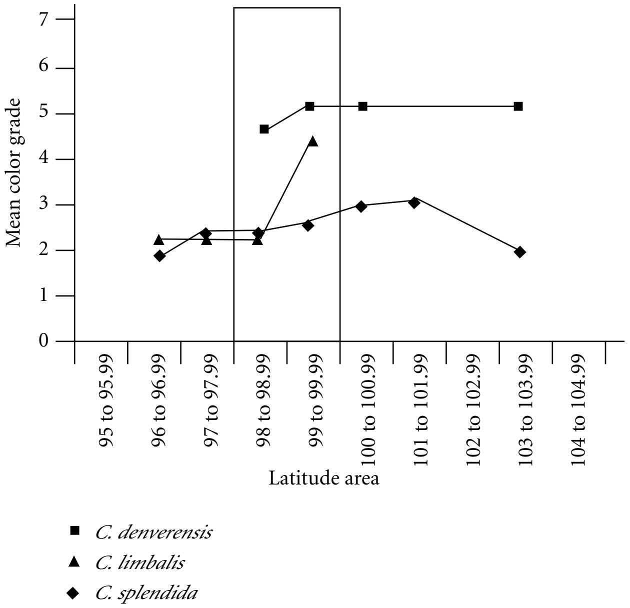

Figure 11: Graph of mean elytral color by code for *C. denverensis*, *C. limbalis*, and *C. splendida* by longitude. Region of character convergence for *C. denverensis* and *C. splendida* depicted by rectangle.

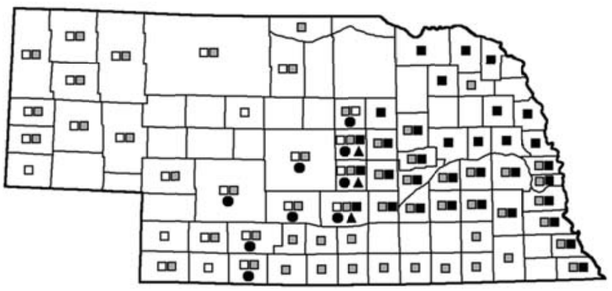

Figure 12: Approximate delineation of hybrid zones in the *Cicindela splendida* group in Nebraska. The occurrence of *C. denverensis* is depicted by an empty square, *C. splendida* by a gray square, and *C. limbalis* by a black square. A black circle indicates presence of *C. denverensis* × *splendida* hybrids, and a black triangle indicates the presence of *C. limbalis* × *denverensis* hybrids.

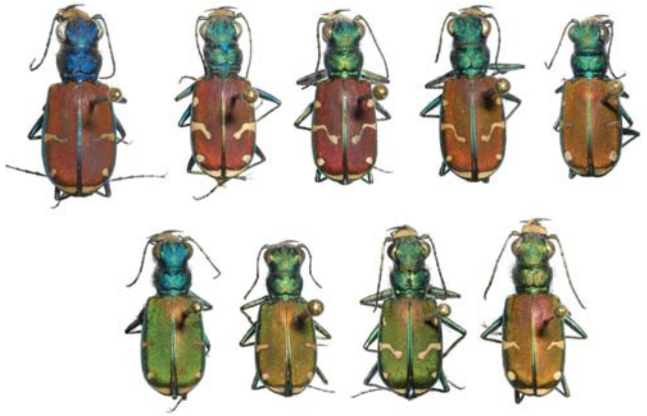

Figure 13: Series of *Cicindela splendida* showing variation in maculation and color. Top row: Largely pure *C. splendida*, bottom row: Hybrid *C. denverensis* × *splendida*.

of both species, *C. limbalis* is common while *C. splendida* is rare there. Thus, disturbance, either from natural causes or anthropomorphic changes, may also influence the hybrid zone.

It is also unknown if the location of the hybrid zone is stable over time. Dasmahapatra et al. [32] found that a hybrid zone in the lepidopteran genus *Anartia* had moved significantly in Central America over a twenty-year period. Future collection in the hybrid zone in Nebraska should reveal if the zone is spatially stable. It is also unknown if the hybrids suffer from reduced fitness as has been found in some other studies [33–35].

Finally, it is unknown whether assortative mating [36, 37], female choice, or male choice are the major factors influencing the hybrid zone. Male tiger beetles will attempt to mate with nearly any other tiger beetle of similar size [38]. Thus, it may be that females make the final choice in determining if the spermatophore is suitable. It is also possible that the dispersal ability of each of these three species may also play a role in cooccurrence [39]. Carter [40] suggested that *C. limbalis* did not colonize new sites as rapidly as *C. splendida*, and this matches our own observations.

Mitochondrial studies used to distinguish between closely related species are sometimes of limited value. For example, Schmidt and Sperling [3] suggested that rare hybridization between tiger moth species in the genus *Grammia* might explain why their mtDNA tree appeared to follow geographic distribution rather than previously supported phylogeny. The authors also suggested that while mtDNA analyses can be misleading for distinguishing closely related species, these tools are an excellent tool for detecting hybridization [3]. Of particular interest in such cases is why such mitochondrial lineages are passed on and proliferate. Perhaps such phenomena support the hybrid vigor hypothesis.

Mitochondrial DNA evidence suggests that all three species in the *C. splendida* group may represent a single variable species [22, 24]. However, ecological preferences and the complex phenotypic interrelationships between these forms suggests otherwise. Even the concept of subspecies does not apply as this would suggest that across much of the United States, two subspecies occur sympatrically without interbreeding. The remaining explanations are (1) unique phenotypes within a single species which affect coloration, mating preference, and habitat associations, perhaps as a result of differing selection pressures, (2) ecological species, or (3) a ring species phenomenon.

If the first explanation is correct, it would suggest a group in the process of speciation. Indeed, if phenotype affects mating preference, this would largely keep each of these forms distinct. It is apparent that while males will attempt to mate with females of any of these three forms, many observed matings between forms resulted in rejection of the spermatophore [20]. Both the second and third explanations suggest overlaps in habitat preference, but differences in optimal habitat. If these tiger beetles qualify as a ring species, the geographic pattern of phenotypes suggests that *C. denverensis* would form the middle of the ring, and *C. limbalis* and *C. splendida* the two ends. If this phylogenetic relationship is true, it would differ from the hypothesis presented by Schincariol and Freitag [18], who suggested that *C. limbalis* is most representative of the ancestral form, that *C. denverensis* represents an early split, and that *C. splendida* represents a later split from a *C. limbalis* type ancestor.

While the biological species concept suggests that the occurrence of any hybrids represents incomplete speciation [41, 42], the fact that these three tiger beetle species maintain their integrity over most of their range suggests that they “function” as individual species in most areas. For now, based on morphological and mating studies, it appears that *C. denverensis* is phylogenetically closer to both *C. limbalis* and *C. splendida* than these two species are to each other. Perhaps more sensitive genetic studies may reveal the true phylogenetic relationships among these three species. This study is an example of the difficulty in applying species concepts for closely related species that differ in a small number of characters and hybridize in at least limited areas.

This study shows the complexity of species definitions, especially based on color morphologies. Across much of their ranges, these forms function as distinct species; however, the observed hybrid zone in central Nebraska causes the validity of this conclusion to be questioned. Our findings have important implications for conservation and for monitoring biological diversity. Based on the frequency of hybridization in this group of species in Nebraska all three species could be lumped into a single species. Alternatively, if only morphology is used, hybrids could be viewed as different species, leading to the possibility of four or five species being present. Doing rapid biodiversity assessment in central Nebraska using tiger beetles could result in either underestimating or overestimating tiger beetle diversity or both if citizen scientists were used for these surveys [6].

In Nebraska, the Salt Creek tiger beetle, *Cicindela nevadica lincolniana* Casey, is a federally endangered subspecies of the much more widely distributed *C. nevadica* [43]. Thus, the designations of subspecies based on phenotypes can have important consequences for conservation as well. Our findings of morphological variation and hybridization among multiple species suggests that tiger beetle taxonomy based on morphological characters alone should be cautiously interpreted and that additional research using molecular and behavioral techniques is warranted. Because tiger beetles are among the most charismatic and well-studied beetle groups, it is likely that similar or even greater problems will be encountered for other beetle groups that are potential indicators of ecosystem changes.

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Research Article

Mesozoic Coleopteran Faunas from Argentina: Geological Context, Diversity, Taphonomic Observations, and Comparison with Other Fossil Insect Records

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The order Coleoptera is the most diversified group of the Class Insecta and is the largest group of the Animal Kingdom. This contribution reviews the Mesozoic insects and especially the coleopteran records from Argentina, based on bibliographical and unpublished materials (86 described species, 526 collected specimens). The material came from different geological units from the late Middle Triassic to the Late Triassic (Bermejo, Cuyo, and Malargüe basins) to the Middle-Late Jurassic and Early Cretaceous (Deseado Massif, Cañadón Asfalto, and San Luís Basin). The coleopteran record is composed of 29 described species with 262 collected specimens (isolated elytra) mainly represented by Triassic species and only four specimens recorded in Jurassic units, all of them currently unpublished. These fossil coleopterans provide fundamental information about the evolution of insects in the Southern Hemisphere and confirm the Triassic Argentinean insect deposits to be among the most important in the world.

1. Introduction

Continental invertebrate communities from the Mesozoic Era are represented principally by two phyla: Arthropoda and Mollusca. Arthropods constitute the most abundant and diverse fossil record in lacustrine sequences of Argentina with "conchostracans", insects and ostracods as most representative groups. The mollusks, represented by bivalve and occasionally gastropods, have a low diversity and restricted distribution [1].

The recent insects constitute the richest class in terms of species diversity with estimates ranging from 3 to 50 million species [2–5]. There are 1275 families of known insects in the fossil record and 967 presently existing, of which 70% are also known as fossils [6, 7]. The data from insect families/genera indicate that the group's growth model follows an exponential curve of evolution, contrary to the occurrence of new orders, which declines [8, 9].

The Coleoptera represents the most diverse order within the Class Insecta, both taxonomically and ecologically. In addition, it is the most specious group in the Animal Kingdom with the number of described modern species exceeding 350,000, representing about 40% of the known insect fauna [10]. This diversity is probably related to certain features that allow adult living in restricted niches while retaining their ability to fly [10].

The systematics and phylogeny of fossil beetles is a very complex area, with old and new proposals and numerous publications which surprisingly still not clear this question. Most studies use different methodologies and are based mainly on adult morphology and rarely use immature stages of ontogenetic development. The basis of modern classification of fossil coleopterans was initially established by Crowson [10, 11].

The oldest record of the Coleoptera is from Paleozoic Era: Lower Permian deposits of the Wellington Formation from Oklahoma and Kansas (USA) [12, 13], Obora (Czech Republic) [14, 15], and the Chekarda, Ural region (Russia) [16].

Mesozoic beetles were much more common and diverse than Paleozoic; geological information shows that beetles have had a dominant record among the group of insects since early Jurassic [16]. However, further information still remains elusive as the Mesozoic beetles are less informative, because of isolate elytra, than those of the Paleozoic [11]. The Mesozoic associations consisted mainly of xylophagous forms and larval stages have been found in numerous localities, except in the Upper Cretaceous.

The aim of this paper is to present the record of abundance and diversity of Mesozoic beetles in Argentina. The information comes from a bibliographic compilation and study of materials found in recent paleontological expeditions conducted by our research group. At the same time, we provide information on the geology and age of localities where the material was collected and present a comparison with other Mesozoic groups of insects collected in Argentina and contemporary faunas from southern Hemisphere. Finally, it provides paleobiogeographic information and highlights the importance of beetles in understanding the evolution of the Mesozoic after the Permian Triassic extinction event.

Our analyses are based primarily on elytra in the Upper Triassic localities from Argentina, which provide a significant amount of information for Coleoptera and allow to have a vision of the composition of the assemblages about 252 million years ago. Cretaceous and Jurassic materials are mentioned in passing, and their study is in progress.

2. Previous Coleopteran Records

Contributions involving the study of beetles in Mesozoic continental sediments of Argentina began with Frenguelli [17, 18], who observed small curculionid elytra in shale samples from southwestern Mendoza Province (stratigraphical levels unknown) and other beetle elytra originating from various levels of the Ischigualasto Formation (probably levels of the Los Rastros Formation *sensu* Stipanicic and Bonaparte [19]), San Juan Province. Fossa-Mancini [20] reported the presence of galleries related to burrowing larvae of certain xylophagous beetles in silicified trunks from Upper Cretaceous of Patagonia. Feruglio [21] mentioned fossil beetles represented by silicified incomplete elytra, thorax, and abdomen remains, probably attributable to the Elateridae, from Laguna del Molino locality (Gran Bajo de San Julián) in Santa Cruz Province.

Subsequently, Genise [22] presented a description of different ichnofossils found in fossil trunks and fructifications from the Upper Cretaceous of Rio Negro Province, assigned to the probable activity of beetle and termites larvae. Further, Genise and Hazeldine [23] described insect traces in fossil wood from La Matilde Formation from the Jurassic Petrified Forest of Jaramillo in Santa Cruz Province, probably assigned to the activity of buprestid larvae. Martins-Neto and Gallego [24] disclosed the remains of beetles consisting of isolated elytra and body parts from the La Matilde Formation of Gran Bajo de San Julián in Santa Cruz Province, assigned mainly to Caraboidea. Gallego et al. [25] reported a second discovery of fossil beetles as *Argentinocupes* and *Argentinosyne* and other insects as blattids and hemipterans in the Bermejo Basin in San Juan Province and the first record from the upper Los Rastros Formation of this basin.

The Order Coleoptera was also treated in the works of Martins-Neto et al. [26–30] and Martins-Neto and Gallego [31], where new Triassic species assigned to Permosynidae, Schizocoleidae, Cupedidae, and Elateridae were described. The material (elytra) was collected in Ischichuca and Los Rastros Formations of the Bermejo Basin in La Rioja Province and Potrerillos and Cacheuta Formations, Cuyo Basin of Mendoza Province. Monferran et al. [32] mention a new locality with a record of Coleoptera: Estancia Fossati, Puesto Almada Member of the Cañadón Asfalto Formation, from Middle to Late Jurassic in age. Brauckmann et al. [33] describe two elytra of Permosynidae (*Ademosyne rosenfeldi* and *Ademosyne llantenesensis*) from the Llantenes Formation of the Malargüe Basin, Mendoza Province.

3. Material and Methods

Triassic specimens from the Cuyo Basin originate from three areas: (a) south of Cerro Cacheuta at the Puesto Miguez, Quebrada del Durazno and Agua de las Avispas localities, in Potrerillos and Cacheuta strata of the Upper Triassic; (b) north of Cerro Bayo at the Quebrada del Cerro de las Cabras locality in Cerro de las Cabras Formation of the Middle Triassic and Quebrada del Puente locality in Potrerillos Formation of the lower Upper Triassic; and (c) southeast of Cerro de los Colorados, Paramillos de Uspallata, in strata of the Cacheuta Formation (Upper Triassic).

In the Malargüe Basin, material was collected from the upper portion of the Llantenes section of the Llantenes Formation (Late Triassic). In the Bermejo Basin, the fossil insects come from Río Gualo, Picos Gemelos, Agua Escondida, Quebrada de Ischichuca Chica, and Chañares localities, belonging to Los Rastros (early Late Triassic) and Ischichuca (late Middle Triassic to early Upper Triassic) Formations, in La Rioja Province.

Jurassic beetles were collected from the La Matilde Formation of Middle to Late Jurassic ages, Laguna del Molino locality (Gran Bajo de San Julián), and from Estancia El Malacara locality (Bahía Laura), from Santa Cruz Province, and the Cañadón Asfalto Formation, of Middle to Late Jurassic age, from the Estancia Fossati locality, Chubut Province.

Cretaceous insects originate from the Anfiteatro de Ticó Formation, of the Baqueró Group, Bajo Grande, in Santa Cruz Province and La Cantera Formation, Gigante Group, in San Luis Province; both are from the Early Cretaceous.

It is important to emphasize that material collected in the Potrerillos-Cacheuta sequences during an expedition occurring in April of 2010 was included in our analyses, as was Jurassic specimens from Chubut Province, collected during a 2009 fieldtrip and material from the Los Rastros Formation. This newly discovered material is important for understanding beetle evolution in Argentina during the Mesozoic because it is so diverse, abundant and well preserved.

The material cited in the literature is deposited in the paleontological collection with the acronyms PULR-I (Invertebrate Paleontological Collection, Universidad Nacional de La Rioja, La Rioja Province), CTES-PZ (Paleozoological Collection of the Universidad Nacional del Nordeste, Corrientes Province), MCNAM (Museo de Ciencias Naturales y Antropológicas "J. C. Moyano", Mendoza Province), MHIN-UNSL-GEO (Museo de Historia Natural de la Universidad Nacional de San Luis, San Luis Province), MLP (Museo de La Plata, Invertebrate Paleontology, La Plata, Buenos Aires Province), and CORD-PZ (Palaeozoological Collection, Universidad Nacional de Cordoba, Córdoba Province).

4. Mesozoic Insects Record from Argentina

Most information on the Mesozoic insect faunas of Argentina comes from the Triassic Period and, above all, from collections made of the Los Rastros Formation, Bermejo Basin (La Rioja), and Potrerillos Formation, Cuyo Basin (Mendoza). Both provide 90% of the total abundance of insects in Argentina, constituting one of the most important records of continental life developed in Gondwana. In addition, these basins are known for their large extent, exceptional outcrops, well-developed stratigraphy columns, and the wealth of their taphofloras and vertebrate faunas.

In Argentina, knowledge of Mesozoic insects has made remarkable strides in recent years, having as a background the works published by Wieland [34, 35], Tillyard [36], and Cabrera [37] on fossil insects from the Cacheuta and Potrerillos Formations of early Late Triassic age. So far, 86 species in 27 families of 12 orders have been described from 526 samples collected (Figure 1). The fossil insect fauna of Argentina comes from stratigraphic levels of continental sediments assigned to the interval between the upper Middle Triassic to Lower Cretaceous.

4.1. Insects Records and Geological Context (Figure 2)

4.1.1. Triassic. The fossiliferous potential of the Argentina Triassic units is highly significant, based on 510 collected specimens and 81 described species. The insect record comes from Los Rastros and Ischichuca Formations of the Bermejo basin, La Rioja Province; Potrerillos, Cacheuta, and Cerro de las Cabras Formations of the Cuyo Basin, Mendoza Province; and Llantenes Formation of the Malargüe Basin, southern Mendoza Province (Figure 3).

The Cuyo Basin of central western Argentina is composed of thick sedimentary sequences from the Middle to Late Triassic that constitute the Uspallata Group. These units have a rich and well-known *Dicroidium* flora, as well as a microflora and a fauna as invertebrates, fishes, and tetrapods that are interpreted as dwelling in fluvial-lacustrine systems. Insect collections of coleopterans, blattids, hemipterans, miomopterans, orthopterans, trichopterans, plecopterans, odonatans, and grylloblattids originate from the Cerro de las Cabras Formation (1 specimen), the Potrerillos Formation (21 described species and 229 collected specimens), and the Cacheuta Formation (2 described species and 27 specimens) [29, 30, 39–43].

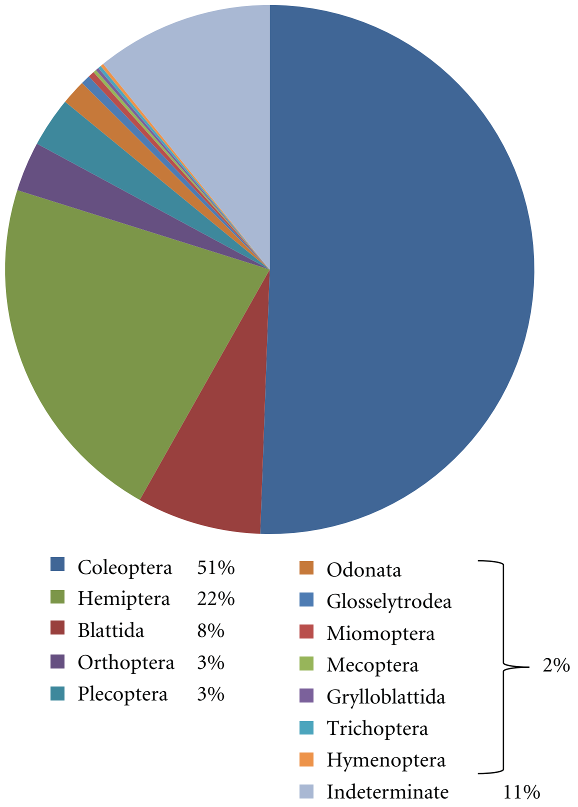

Figure 1: Pie-chart showing the abundance of Mesozoic specimens from Argentina, grouped by taxonomic order.

The Malargüe Basin of southern Mendoza Province includes Choiyoi volcanic and overlain siliciclastic deposits. The insect fauna was collected from the upper section of the Llantenes Formation (Late Triassic) which is built of two coarsening-upward cycles reflecting a deltaic progradation of a fluvial into a lacustrine environment (lower part), succeeded by repeated progradation into a floodplain dominated environment (upper part; with insects, conchostracans, fish, and plants remains). The insect remains includes 2 coleopteran elytra and 1 mecopteran, and these news finds represent the youngest Triassic occurrence from Argentina and South American and the second youngest record from the Southern Hemisphere [33].

The Los Rastros and Ischichuca Formations (Agua de la Peña Group, La Rioja Province) are part of the Bermejo Basin (Ischigualasto-Villa Unión Basin), an extant basin located in northwestern Argentina. The Los Rastros Formation of early Late Triassic age is a lacustrine-deltaic that consists of several sedimentary cycles of black shales, siltstones, and sandstones. The succession is characterized by record of five plants, four invertebrates, and four vertebrates taphofacies [44]. The

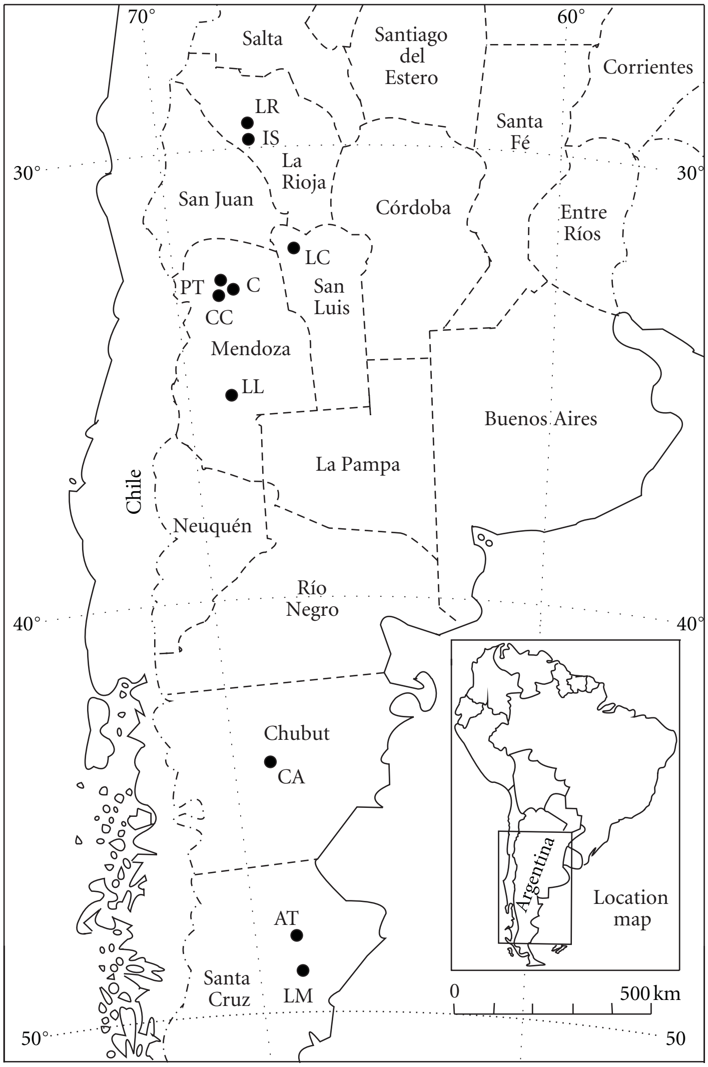
70°
60°
Salta
Santiago del Estero
Corrientes
LR
IS
La Rioja
Santa Fé
30°
30°
San Juan
Córdoba
Entre Ríos
LC
PT
C
CC
San Luis
Mendoza
LL
Buenos Aires
La Pampa
Chile
Neuquén
Río Negro
40°
40°
Chubut
CA
Argentina
Location map
AT
Santa Cruz
LM
0
500 km
50°
50

FIGURE 2: Map showing the Mesozoic units and outcrop areas approximately that carried the insect fauna mentioned in the text. AT: Anfiteatro de Ticó; C: Cacheuta; CA: Cañadón Asfalto; CC: Cerro de las Cabras; IS: Ischichuca; LC: La Cantera; LL: Llantenes; LM: La Matilde; LR: Los Rastros; PT: Potrerillos. (modified from Zavattieri [38]).

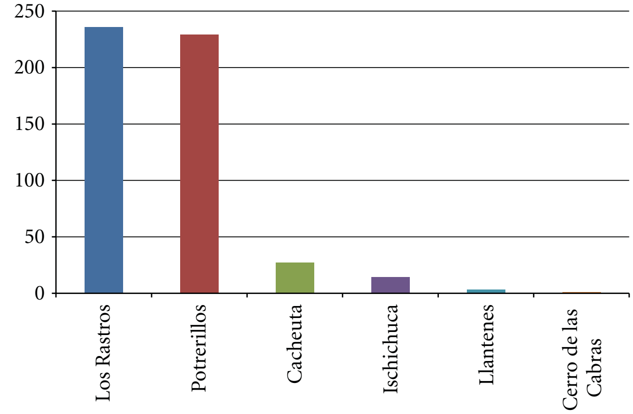

FIGURE 3: Bar graphic showing the abundance of specimens in different Triassic insect localities. (Note: included new undescribed materials).

insect record consists of 44 described insect species and 236 collected specimens and is comprised of coleopteran elytra and wing remains of blattids, plecopterans, miomopterans, orthopterans, glosselytrodeans, and odonatans [26, 27, 42, 45]

The Ischichuca Formation of late Middle Triassic to early Late Triassic age is predominantly composed of shallow and deep lacustrine facies with progradational deltaic successions, that starts to begin as fanglomerates with sandstones and tuffitic intercalations and continue with dark carbonaceous shales alternating with sandstones and pelitic and tuffaceous strata. In particular, insect remains are found in dark brown to olive-green claystones with abundant plant remains from the middle to the basal upper parts of the unit. This interval is interpreted as a shallow, partly saline lake (interpreted from a perennial playa lake association facies, L2 from Melchor [46, 47]) that ranges to a deep freshwater lake (deep freshwater lake association facies, L3 from Melchor [46, 47]).

The fossil insects, consisting of 11 described species and 14 specimens, comprise a blattid wing and coleopteran elytra [31, 41, 48].

4.1.2. Jurassic. The Jurassic insect fauna from Argentina (Table 1) is less well known than the Triassic one and also sparse when compared with other Jurassic localities of the world. This rarity is due to its restricted geographic distribution and low species diversity and is related to the low number of collections and studied localities.

The first contribution probably was made by Frenguelli [18] who mentioned and illustrated the "rare insect remains" of a paleohemipteran wing from the "Estancia El Malacara" from Bahia Laura, in Santa Cruz, Province. In the 1990s one of us (O. F. Gallego) and Rafael Martins-Neto restudied this specimen and concluded that it is probably not an insect. Later, Feruglio [21] reported the presence of silicified coleopteran remains from the Laguna del Molino locality of Gran Bajo de San Julian, in Santa Cruz Province) associated with plant fragments, bones, freshwater mollusks, and conchostracans. These previous contributions of insect reports were summarized by Gallego and Martins-Neto [49], Martins Neto and Gallego [24, 50], Monferran et al. [32, 51, 52], Genise et al. [53], and Gallego et al. [54] who reported new finds of insects from the orders Coleoptera (elytra and body remains), Heteroptera (fragmentary wings), and Trichoptera (wing fragments and larval cases) from both the La Matilde Formation of the Laguna del Molino locality and Cañadón Asfalto Formation of Cerro Cóndor and Estancia Fossati localities. These records are associated also with gastropods, bivalve mollusks, ostracods, conchostracans, plants, and fish remains. Other Jurassic insect records such as the presence of chironomid head capsules and larval cases [55] and mecopterans (Bittacidae) with complete bodies [56] come from Gan Gan locality of the Cañadón Asfalto Formation in Chubut Province.

The Jurassic fossil insect record from Argentina comes from two geological units: the La Matilde Formation (late Middle Jurassic, Santa Cruz Province) and the Cañadón Asfalto Formation of late Middle Jurassic to Late Jurassic age, Chubut Province.

The late Middle Jurassic La Matilde Formation comprises a volcaniclastic sequence that bears silicified woods (ferns and gymnosperms), a taphoflora, invertebrates, and tetrapods. The sediments show a typical continental sequence of a low-energy fluvial system, with lentic water bodies under reduced conditions within a floodplain environment, influenced by intensive volcanism evidenced by pyroclastic deposits [57].

The Cañadón Asfalto Formation of Middle-Late Jurassic age is a thick sedimentary sequence with volcanic intercalations that constitutes one of the most important nonmarine Jurassic records from South America. Two members can be distinguished, the lower one, the Las Chacritas Member, and the upper one, the Puesto Almada Member [58]. The insects were found in the upper member and particularly from the locality "Estancia La Sin Rumbo", an assemblage of "conchostracans", ostracods, bivalves, and caddisfly cases. This assemblage occurs in the upper part of a volcaniclastic lacustrine sequence consisting of yellowish tuffs and tuffites providing evidence of dry climatic conditions. The assemblage recorded from the Estancia Fossati locality came from three levels consisting of shales, limestones, and tuffs, associated with invertebrates such as bivalve mollusks, insect larval cases, ostracods, conchostracans), and fish scales. The Estancia Fossati faunas represent low-energy shallow freshwater environments within associated plant communities [54, 59].

Table 1: Mesozoic insects from Argentina.

Order	Triassic	Jurassic	Cretaceous	Mesozoic insects
Coleoptera	262	4		266
Hemiptera	104	4	6	114
Blattida	39		1	40
Orthoptera	16			16
Plecoptera	16			16
Odonata	7		1	8
Glosselytrodea	3			3
Miomoptera	2			2
Mecoptera	1			1
Grylloblattidae	1			1
Trichoptera	1			1
Hymenoptera	1			1
Indeterminate	57			57
Total	510	8	8	526

4.1.3. Cretaceous. The poorly known Cretaceous insect fauna from Argentina is reflected by only 5 described species and only 8 collected specimens (Table 1). These records are based on two Early Cretaceous localities: (a) the Anfiteatro de Ticó Formation, Bajo Grande locality, from Santa Cruz Province, and (b) the La Cantera Formation, Gigante Group of San Luis Province.

The Anfiteatro de Ticó Formation of the Baqueró Group is composed by siliciclastic and volcaniclastic deposits [60] and the insect fauna consisting of the species *Blattulopsis popovi* (Blattida) [61] and *Argentinopetala archangelskyi* (Odonata) [62].

The La Cantera Formation of the Gigante Group exhibits greenish to gray limonite and arcilite with intercalated red sandstones and shales in the upper part [63]. The La Cantera assemblage is composed of a large, diverse association of ostracods, insects (hemipterans, orthopterans, coleopterans and caddisfly larval cases), palynomorphs, plants (leaves and sphenopsid reproductive organs), and fish fragments [64]. The paleontological content of this deposit suggests a lakeshore environment, attributable to fish taxonomic diversity and the poor state of terrestrial insect preservation [65]. Insect remains comprise of *Canteronecta irajae* (Naucoroidea) [66], *Rhomboidella popovi* (Corixidae) [67] and

Notonecta mazzoniae (Notonectidae) [65]. Also are recorded the poorly preserved orders Coleoptera, Orthoptera, and Trichoptera.

5. Mesozoic Coleopterans from Argentina

The analysis of Coleoptera used information obtained from previous publications [21, 26–33, 39, 44]. Information was also obtained from observation of new unpublished material collected in the 2010 expedition to the Potrerillos Formation of Mendoza Province (Figures 4(f)–4(r)), Los Rastros Formation of La Rioja Province, and Jurassic specimens from Cañadón Asfalto Formation of Chubut Province.

Coleoptera is the most abundant and exhibits the greatest speciosity among Mesozoic insect fauna in Argentina. Until now, 262 specimens have been collected, covering about 50% of the total abundance, and 29 species have been described, including the permosynids *Ademosyne umutu*, *A. llantenesensis*, *A. rosenfeldi*, *A. arcucciae* (Figure 4(c)), *A. punctuada* (Figure 4(d)), *A. elongatus*, *A. hexacostata*, *Ademosyne sp. 1*, *Ischichucasyne cladocosta*, *and Delpuentesyne menendezi*; the schizocoleids *Argentinosyne ischichucaensis*, *A. duraznoensis*, *A. bonapartei*, *A. frengüelli* (Figure 4(b)), *A. rugosa*, *A. gualoensis*, *A. gonaldiae*, *and A. losrastrosensis*, Gen. et sp. indet. 1 and 2; the elaterids *Babuskaya elaterata, Gemelina triangularis, Cardiosyne obesa, C. elegans*; the cupedids *Argentinocupes sara, A. pulcher* (Figure 4(a))*, and A. abdalai* (Figure 4(e)); and two specimens Gen. et sp. indet. 1 tentatively assigned to the Permosynidae family and other specimen (Gen. et sp. indet. 1) of uncertain position.

From an analyses of the information about Triassic beetles, the best represented groups are families Permosynidae (135 specimens) and Schizocoleidae (40 specimens); the genera *Ademosyne* (129 specimens) and *Argentinosyne* (39 specimens); and the species *Ademosyne arcucciae* (96 specimens, Figure 4(c)) and *Ademosyne hexacostata* (17 specimens). Ninety-eight percent of the specimens collected are from Triassic continental sedimentary rocks, from Los Rastros Formation (69% of total abundance), Potrerillos Formation (17%), Cacheuta Formation (9%), Ischichuca Formation (4%), and Llantenes and Cerro de las Cabras Formations (2%).

The beetles preserved in the various localities appear as complete impressions, body part impressions (abdomens, thoraces), and as elytra moulds that can be complete or fragmentary, articulated or disarticulated, smooth or striated, with or without ornamentation.

Two analyses were performed. The first considered the state of preservation, namely, if the specimen was complete/incomplete, and its degree of articulation/disarticulation. For these measurements, there were used 262 elytra distributed in the different formations as shown in Table 2.

The beetle fauna consists mainly in complete and disarticulated elytra (211 samples; Figures 4(a), 4(d), 4(f)–4(l), 4(n)–4(r)), followed by incomplete and disarticulated elytra (41 samples), complete and articulated elytra (8 samples; Figures 4(b), 4(c), 4(e), 4(m)), and last incomplete and articulated elytra (2 samples) (Figure 5).

In the second analysis, elytra ornamentation was observed, used in describing the following specimens: 187 specimens from Bermejo Basin and 8 from Malargüe and Cuyo Basins. Accordingly, the ornamented elytra may be smooth (46 specimens; Figures 4(b), 4(f), 4(g), 4(k), 4(l), 4(m), 4(o), 4(q) and 4(r)) or striated (149 specimens; Figures 4(a), 4(c)–4(e), 4(h), 4(i), 4(n), 4(p)). In the case of striated elytra, costae may be smooth (91%; Figure 4(c)), punctuate (6%; Figure 4(d)), or granular (3%). The number of costae can vary between 6 and 11. Elytra also can have ornamentation (173 specimens) or lack it (22 specimens). Lastly, the ornamentation can be granular (93%; Figure 4(c)), rough (5%), with rows of cells (1%, Figure 4(a)), or striated (1%).

5.1. Jurassic and Cretaceous Coleopterans. Knowledge of beetles and of insects in general from Jurassic and Cretaceous sediments in Argentina is poorly developed. This absence of data highlights the importance of exploring for new fossil insect localities. In general, beetles are only referenced in works that treat other insect groups. By contrast, information generated for the Jurassic and the Cretaceous of Argentina will allow its characterization and comparison with other continental faunas from the Southern Hemisphere (South America, Antarctica, and Australia), currently which are better known. Therefore, owing to the scarce information about the Jurassic and Cretaceous specimens, the present study has considered only examination of Triassic material.

6. Comments on Other Insect Orders from Argentina (Figure 6)

Hemiptera is the second most common order in the Mesozoic from Argentina with 114 specimens collected (22%) and 19 described species from various assemblages. The material is preserved as impressions of wings (fore or hind, complete or fragmentary, with or without a clavus) and occasionally complete bodies of insects, most attributable to the families Dysmorphoptilidae (9 specimens) and Scytinopteridae (7 specimens); the genus *Gallegomorphoptila* (9 specimens); and the specie *Gallegomorphoptila acostai* (5 specimens). For the rest of the Mesozoic, there are only three recorded Cretaceous species: *Canteronecta irajae* (Naucoroidea) [66], *Rhomboidella popovi* (Corixidae) [67], and *Notonecta mazzoniae* (Notonectidae) [65].

The Blattida (cockroaches) includes 40 specimens (7%) and 18 species, preserved as disarticulated tegmina (complete or fragmentary, with missing or not the clavus). The assemblage is dominated by the families Mancusoblattidae and Mesoblattinidae (10 specimens) and by the genus *Hermosablatta* (9 specimens). The species *Samaroblatta corrientesina*, *S. gualoensis*, *Hermosablatta crassatella*, *H. pectinata*, *Lariojablatta neiffi*, and *Condorblatta lutzae* are the most abundant taxa. The *Blattulopsis popovi* species occurs in the Lower Cretaceous of Santa Cruz Province [61].

The rest of the orders, Plecoptera and Orthoptera with 16 specimens, Odonata with 8 specimens, Glosselytrodea with

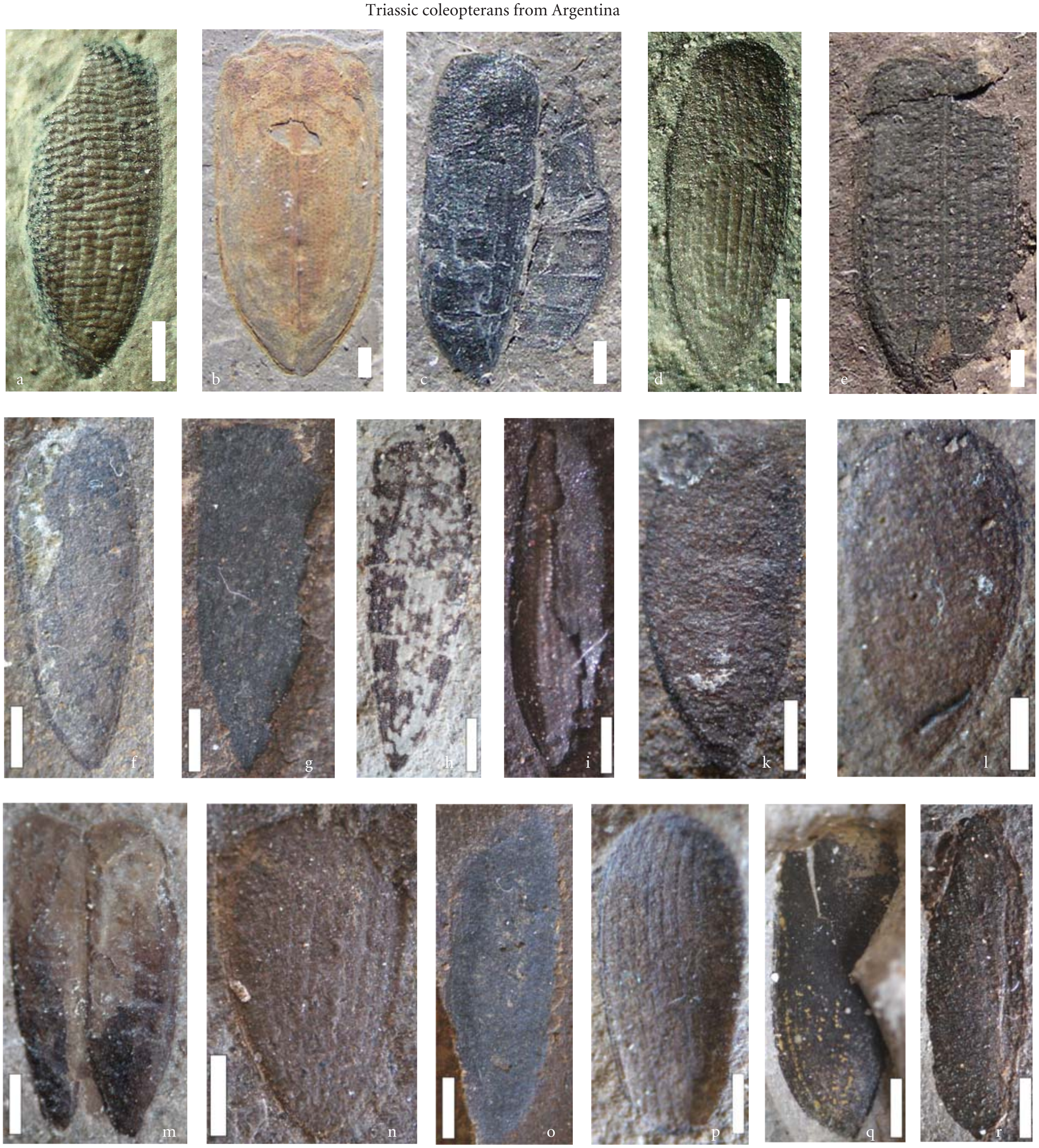

Figure 4: Examples of fossil insects collected from Triassic insects localities, Western Argentina, illustrating the stage of preservation seen in the localities. (a) *Argentinocupes pulcher*, complete desarticulated elytron. (b) *Argentinosyne frengüelli*, complete articulated elytron. (c) *Ademosyne arcucciae*, complete articulated elytron and thoracic and abdominal elements. (d) *Ademosyne punctuada*, complete desarticulated elytron. (e) *Argentinocupes abdalai*, complete articulated elytron (Martins-Neto et al., [27]); (f)–(r) Indet. material: complete articulated elytron (m) and complete desarticulated elytron ((f)–(l), (n)–(r)). Ornamentation of elytra: smooth ((b), (f), (g), (k), (l), (m), (o), (q), (r)) and striate ((a), (c)–(e), (h), (i), (n), (p)). Scale bar: 1mm.

Table 2: State of preservation of the Triassic beetles.

Elytron	Formation					
	Los Rastros	Ischichuca	Potrerillos	Cacheuta	L1antenes	Cerro de las Cabras
Articulated complete	5	0	3	0	0	0
Articulated incomplete	2	0	0	0	0	0
Disarticulated complete	140	5	41	24	1	0
Disarticulated incomplete	33	5	0	1	1	1
Total	180	10	44	25	2	1

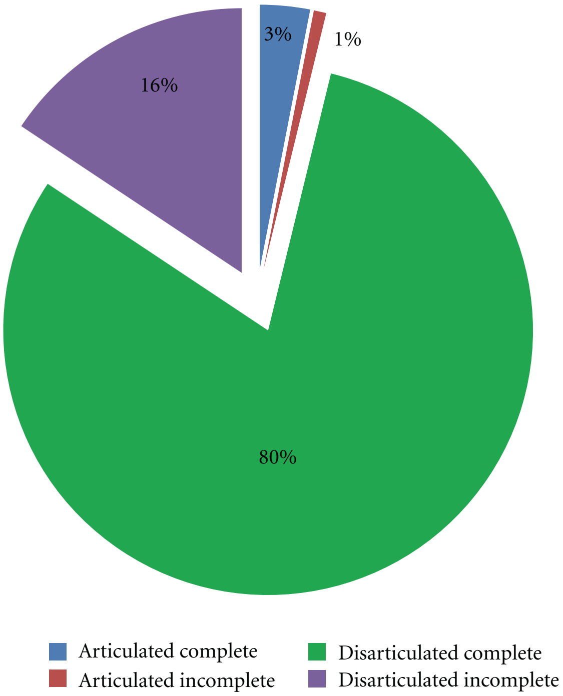

Figure 5: Pie-chart showing the relative percentage of specimens from beetle locality collection, grouped by element type.

3, Miomoptera with 2 specimens, and Mecoptera, Grylloblattida, Trichoptera, and Hymenoptera, all with one specimen each, constitute 9% of the total abundance of the material collected. Each one is represented by a handful of impressions of fore wings and complete or fragmentary bodies of nymphs such as head and abdominal sclerites.

The label "indeterminate" record (57 specimens, 11%) includes the complete, incomplete or poorly preserved elements, in which we were unable to be confidently assigned to any particular order or alternatively it is under study. They are represented by impressions of wings (complete or fragmentary), some elements associated with abdominal sclerites and partial bodies.

7. Paleoecology and Taphonomy

Given the aforementioned information on the Mesozoic insect fauna of Argentina, the material recovered includes disarticulated and fragmentary specimens (evidence of postmortem transportation) approximately 97.5% of which comprises mostly impressions of wings of hemipterans and blattids, elytra of beetles, and other isolated body parts (thoraces, abdomens, appendages) of the Orthoptera, Mecoptera, Grylloblattida, Plecoptera, Trichoptera, Miomoptera, Odonatoptera, Hymenoptera, and Glosselytrodea, in addition to head capsules and dwelling tubes of chironomid and caddisfly cases.

From an ecologic perspective, the analysis of this fauna shows that most groups have a subaerial or terrestrial habit as adults, and some are represented by immature (often aquatic forms), such as the Plecoptera, Grylloblattida, Hemiptera, and Odonata. This implies that the fossil record is biased by the absence of other autochthonous aquatic forms, the causes of which required future inquiry.

8. Final Considerations

Fossil beetles are one of the most interesting objects in paleontological and stratigraphical research, but they remain poorly understood, as they are difficult to study [68]. The great diversity and abundance that occurs in the fossil record is probably correlated with the composition of the elytra and its shape preservation, appearing as elytra moulds that can be articulated or disarticulated.

In South America, the previous literature reviews and the analyses of unpublished material indicate the relevance of Triassic and Tertiary beetle records. Nevertheless, the group is poorly studied and the intensity of collections is low. Therefore, it requires much more skilled work.

The record (Table 3) is restricted to continental sediments from Brazil, Argentina, Chile, and Peru. In Brazil, 17 species were discovered and come from Early to Middle Permian ages of Irati Formation [69, 70] and from Middle to Late Triassic ages of the Santa María Formation [71] of the Paraná Basin, from the Early Cretaceous age of Santana Formation in Araripe Basin [72–75], from the Oligocene age of Tremembé Formation in Taubate Basin and Fonseca Formation in Fonseca Basin [76–78]. In Argentina the order is restricted to Late Triassic age with twenty nine species described [25–31, 33] from Los Rastros and Ischichuca Formations in La Rioja Province, the Cortaderita Formation in San Juan Province, the Potrerillos, Cacheuta, Cerro de las Cabras, and Llantenes Formation in Mendoza Province; Middle to Late Jurassic ages of the La Matilde Formation in Santa Cruz Province and the Cañadón Asfalto Formation in Chubut Province [21, 24, 32]; Middle to Late Eocene

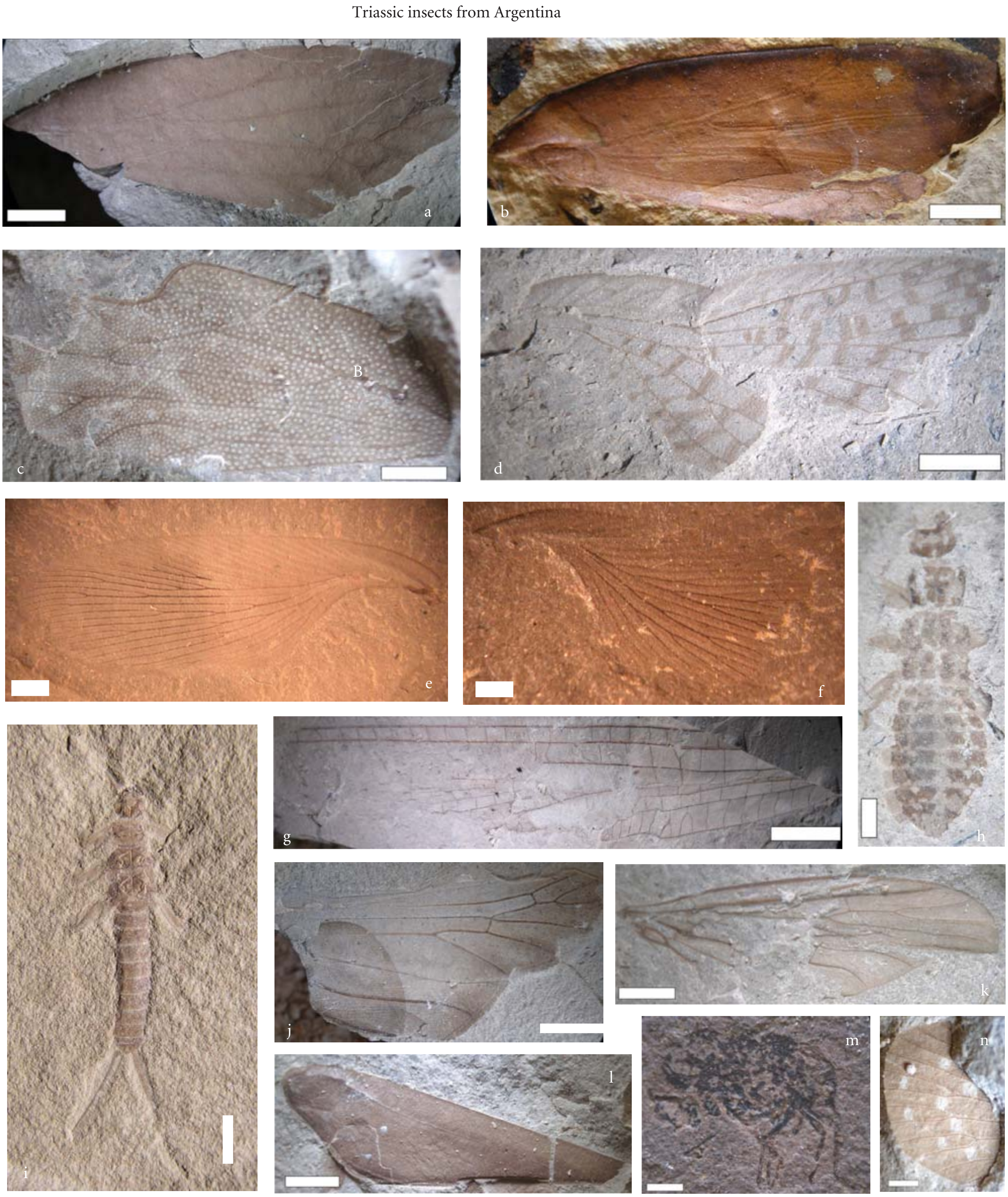

Figure 6: Examples of fossil insects collected from Triassic insects localities, Western Argentina, illustrating its diversity and the stage of preservation (impression) seen in the localities. (a), band (c): Hemiptera. (d): Orthoptera. (e): *Mancusoblatta pulchellaand* (f): *Hermosablatta crassatella* (Blattida), Martins-Neto et al., [45]. (g) and (h): Odonata adult and nymph, respectively. (i): *Platyperla marquati* (nymph), Gallego et al., [54]. (j), (k), (m), and (n): indet. material. l: Hemiptera clavus. Scale bar: 1 mm (except (a), (b), (c), and (i): 2 mm).

Table 3: Main stratigraphic units and beetle localities in South America.

Erathern Era	System Period	Series Epod	Argentina	Brazil	Chile	Perú
Cenozoic	Quaternary	Holocene				
		Pleistocene				Talara
	Neogene	Pliocene				
		Miocene	Palo Pintado Formation			
	Paleogene	Oligocene	Ventana Formation	Tremembé and Fonseca Formation		
		Eocene	Lumbrera Formation Maíz Gordo Formation			
		Paleocene				
Mesozoic	Cretaceous	Upper			Dorothea Formation	
		Lower		Santana Formation		
	Jurassic	Upper	La Matilde Formation Cañadón Asfalto Formation			
		Middle				
		Lower				
	Triassic	Upper	Cacheuta Formation Llantenes Formation Potrerillos Formation Cortaderita Formation Los Rastros Formation Cerro de las Cabras Formation Ischichuca Formation	Santa María Formation	Santa Juana Formation	
		Middle				
		Lower				
Paleozoic	Permian	Upper				
		Middle		Iratí Formation		
		Lower				

ages from the Lumbrera Formation (Grupo Salta) in Salta Province [79]; Late Paleocene to Early Eocene age from the Maíz Gordo Formation in Jujuy Province with thirty one species [80–84]; Eocene to Early Oligocene age from the Ventana Formation in Neuquen Province [85]; and Late Miocene age from the Palo Pintado Formation in Salta Province [86]. In Chile the material just comes from Santa Juana Formation of Late Triassic age with one species [87] and Dorothea Formation of Upper Cretaceous [88]. In Peru the specimens come from the Pleistocene tar-seeps of Talara in Piura Province [89].

The Mesozoic localities in Argentina, typically from the Triassic Period, have proven to be rich in fossil insect material; recent findings of insects in the strata of the Cuyo Basin have increased the number of taxa represented to currently 510 specimens. This makes Argentina one of the most important paleoentomologic regions not only in South America but also generally in the Southern Hemisphere, such as the Triassic sequences already known from Australia and South Africa.

All the knowledge provides invaluable information about the composition of the Mesozoic biota, essential for understanding biological processes that different groups of organisms experienced after the great Permian extinction event about 252 million years ago. In addition, time series of taxa within lineages could provide data for understanding beetles evolution.

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Research Article

Effect of Population Density on Timing of Oviposition and Brood Size Reduction in the Burying Beetle *Nicrophorus pustulatus* Herschel (Coleoptera: Silphidae)

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Burying beetles (*Nicrophorus* spp.) bury small carcasses to feed their larvae. Carcasses are a limited, high-quality resource and contests over carcasses become more frequent with increasing population density. Successful beetles kill eggs and larvae present on carcass. In response, females should accelerate oviposition, while offspring development should increase to minimize mortality. Both value of a carcass and frequency of contests decrease as larvae develop. If overproduction of offspring is an insurance against high mortality, females should reduce brood size as carcass value declines. Testing our predictions, we reared female burying beetles, *Nicrophorus pustulatus*, at high and low densities and compared oviposition and brood reduction. High-density females delayed oviposition, suggesting that high population density imposes nutritional and/or physiological stress. Females responded to the physiological constraints and the potentially high mortality rates of eggs and newly hatched larvae by lengthening oviposition period and changing brood reduction rate.

1. Introduction

In response to environmental conditions, females can adjust their reproductive phenotype by modifying the number and size of offspring [1–6]. As population density increases, females often produce fewer and larger offspring [1, 3–8]. This is commonly observed when females compete for access to resources, and increased population density leads to more frequent and intense contests over these resources [1, 3, 6]. Adjustment of offspring number and, to a smaller degree, adjustment of offspring size, can occur during every stage of reproduction [9–11]. Females can regulate the number and size of eggs they produce [2, 12] and actively destroy eggs [11]. In animals with extensive parental care, reduction of offspring number can also occur after hatching or birth of offspring [9, 10]. Typically, females cause the death of an offspring indirectly by feeding the most competitive offspring preferentially and/or ignoring aggressive fatal interactions among competing siblings [10]. In some species, filial infanticide has been observed as the means of brood reduction [9, 10].

Burying beetles (*Nicrophorus* spp.) provide extensive parental care and are known to adjust brood size primarily through filial infanticide [13–15]. Burying beetles use small dead vertebrates (e.g., mice and birds) as food resource for their offspring. Severe contests over the ephemeral resources are usually won by the larger beetles. A carcass, once discovered by a pair of burying beetles or a single female, is quickly buried. While burying the carcass, the beetles remove fur or feathers, deposit oral and anal secretions, and work the carcass into a ball. Within 12 to 48 h after discovery of the carcass, the female lays eggs in the soil surrounding the carcass [13–15]. Clutch size is substantially larger than the number of larvae dispersing [14]. Females lay larger clutches when carcass size increases [16]. After the larvae have hatched, they crawl to the carcass where they are fed by the parents with regurgitated carcass for about three days. Afterwards, the larvae feed by themselves. The male generally deserts the brood when the larvae are feeding by themselves, while the female stays with the larvae until the larvae have consumed the carcass and are dispersing to pupate in the soil [13–15].

Despite being buried, the carcass can still be discovered by other burying beetles [14, 15, 17]. Successful intruders kill all eggs and larvae encountered [18]. Takeovers occur most frequently between burial of the carcass and hatching of the larvae [19]. Thereafter, takeovers quickly become less likely because the resource value of the carcass decreases as it is consumed, and levels off at very low values when the larvae are about two-to three-days old. [18]. Assuming that overproduction of eggs is an insurance against high mortality rates of eggs and newly hatched larvae [20], we predict that female burying beetles will start adjusting brood size through infanticide after the larvae started hatching and both the value of the carcass and the likelihood of takeovers are declining rapidly, and finish adjusting brood size when the two- to three-day-old larvae have reduced the value of the carcass for other burying beetles. Changes in population density may influence the temporal pattern of offspring number reduction. With higher population density, we expect that intrusions become more likely and with it the possibility of partial infanticide in a failed take-over attempt. To be able to compensate potential loss of eggs and larvae, high-density females should therefore wait longer to reduce their broods.

Based on life history theory [21], we assume that a high probability of takeovers exerts strong selection on minimizing the duration of the developmental stage(s) with very high offspring mortality rates. At high population density, females should thus accelerate oviposition as well as egg and larval development, which can be influenced by females though transmission of nongenetic developmental resources [22, 23]. Consequently, larvae would hatch earlier and develop faster, thus consuming the carcass sooner causing a steeper decline in the value of the carcass. The final brood size of high-density females, however, should be lower than that of low-density females. To maximize fitness, females experiencing high population density with frequent contests over resources won by large individuals, should produce fewer, but larger, offspring than low-density females [24].

To test our hypotheses, we reared female *N. pustulatus* at high- and low densities and compared timing of oviposition and brood reduction as well as offspring development and size between high- and low-density females. In particular, we addressed the following questions: (1) do high-density females lay eggs earlier than low-density females?, (2) do eggs and larvae of high-density females develop more rapidly?, (3) do high-density females adjust brood size later than low-density females, and (4) will they have fewer, but larger, larvae dispersing?

2. Material and Methods

Burying beetles *N. pustulatus* used in this study, came from a laboratory colony established in 2002 with 92 pairs of beetles caught in the Research Forest of Berea College, KY, USA. The beetles of this laboratory colony are kept individually in containers (15 cm × 10 cm × 5 cm) filled 2 cm deep with humid peat at a 15L:9D photoperiod and 22 ± 1°C. The beetles are fed a pea-sized amount of canned cat food (Science Diet, Hill's Pet Nutrition Inc., Topeka, KS, USA) twice weekly.

This study was carried out in summer 2006. To investigate the effect of population density on timing of oviposition, offspring development, and brood size reduction as well as offspring size, we assigned newly emerged females from the laboratory colony at random to a high-density (i.e., four females per container) or a low-density treatment (i.e., one female per container). Container size (15 cm × 10 cm × 5 cm) was the same for both treatments. All containers were filled 2 cm deep with moist peat. Twice weekly, we placed a pea-sized amount of canned cat food (Science Diet, Hill's Pet Nutrition Inc., Topeka, KS, USA) in all containers with low-density females, while containers with high-density females received four times the amount of canned cat food.

When the females were between 28 and 58 days old and sexually mature, the females were assigned at random to offspring developmental stages at which the trial would be terminated. At termination, offspring number and size as well as time elapsed to reach the particular developmental stage were determined. The offspring developmental stages included: $E_{1.5}$ (first laid eggs were 1.5 days old), $L_{0.5}$, L_1, $L_{1.5}$, L_2, $L_{2.5}$, L_3 (oldest larvae were 0.5, 1, 1.5, 2, 2.5, or 3 days old), and $L_{\text{dispersal}}$ (larvae had left the brood cavity and were dispersing). Females assigned to the different offspring developmental stages and density treatments did not differ in age (developmental stage of offspring: $F_{7,281} = 1.84$, $P = 0.08$; female density treatment: $F_{1,281} = 2.40$, $P = 0.12$). Once assigned to an offspring developmental stage, females were transferred to a new container (15 cm × 10 cm × 5 cm) filled 2 cm deep with moist peat and mated with a randomly chosen, nonsibling male from the colony. A total of 320 matings were set up: 20 matings for each combination of female density and offspring developmental stage. The following day, the male was removed, and a previously frozen mouse (mean ± SD: 23.7 ± 2.0 g; range: 18.0 to 27.9 g) was placed into the container with the female. Average mouse weight did not differ between high- and low-density females ($F_{1,281} = 0.18$, $P = 0.67$) and between the different developmental stages ($F_{7,281} = 0.38$, $P = 0.91$). To simulate conditions underground, containers with mice were kept in a dark room at 22 ± 1°C. Containers were checked twice daily for eggs and newly hatched larvae. When the offspring had reached the designated developmental stage, the container was searched for eggs, and the larvae were removed from the carcass. For all developmental stages, eggs were counted, but egg size was only determined for the stage $E_{1.5}$. From each container of stage $E_{1.5}$, the length of a subsample of 10 eggs was measured using a dissecting scope with an ocular micrometer. The larvae of each container were counted, and the brood weight was measured. Larval weight for each brood was calculated as brood weight divided by the number of larvae. Offspring number for each brood was determined as the sum of all eggs and all larvae found in a container. Time elapsed until oviposition was determined as the number of half-days between the time when the mouse was added to the container (i.e., earliest time when females could discover the carcass), and the first observation of eggs in the container. Egg development was defined as number of half-days between the first observation of eggs and the first observation of newly hatched larvae. Larval development was calculated as number of half-days between the first observation of newly hatched larvae and the point in time when larvae were dispersing.

2.1. Statistical Analysis. Time elapsed until oviposition, egg development, and larval development were analyzed with the GENMOD procedure in SAS (SAS Institute Inc., Cary, NC, USA). Poisson distribution and the log function were chosen as error distribution and link function, respectively. Female density was the main effect in the model and mouse weight was included as covariate. Offspring number and number of eggs and larvae were also analyzed with the GENMOD procedure using Poisson distribution and the log function as error distribution and link function, respectively. We included in the models as main factors female density and developmental stage of offspring and the interaction of the two main effects. Mouse weight was used as covariate. Egg length and larval weight were analyzed with the GLM procedure in SAS. The model for egg length included female density as main effect and mouse weight as covariate. The model for larval weight contained as main factors female density and developmental stage of offspring and the interaction of the two main effects. Mouse weight was used as covariate. Larval mass was natural log-transformed before analysis to achieve normality. Not all of the original 340 matings were successful; therefore, only 297 broods were included in the data analyses.

3. Results

3.1. Duration of Development. High-density females began to lay eggs later than low-density females ($\chi^2 = 6.57$, d.f. = 1, $N = 297$, $P = 0.01$; Figure 1). The duration of egg and larval development did not differ between high- and low-density females (eggs: $\chi^2 = 0.02$, d.f. = 1, $N = 260$, and $P = 0.89$; larvae: $\chi^2 = 0.17$, d.f. = 1, $N = 40$, and $P = 0.68$; Figure 1). Mouse weight had neither an effect on the start of oviposition ($\chi^2 = 0.05$, d.f. = 1, $P = 0.83$) nor on the duration of egg or larval development (eggs: $\chi^2 = 0.15$, d.f. = 1, and $P = 0.70$; larvae: $\chi^2 = 0.01$, d.f. = 1, and $P = 0.93$).

3.2. Number of Offspring. The total number of offspring was significantly affected by the developmental stage of the offspring ($\chi^2 = 1778.18$, d.f. = 7, $N = 297$, and $P < 0.0001$). Offspring number was largest at the egg stage and declined continuously until the first-hatched larvae were two days old. From then on, offspring number changed little (Figure 2(a)). Both high-density and low-density females showed the same pattern in the change of offspring number in relation to the developmental stage of the offspring (interaction between density and developmental stage: $\chi^2 = 11.47$, d.f. = 7, $N = 297$, and $P = 0.12$). However, high-density females had significantly fewer offspring than low-density females ($\chi^2 = 10.47$, d.f. = 1, $N = 297$, and $P = 0.001$; Figure 2(a)). Offspring number increased as mouse weight increased ($\chi^2 = 24.15$, d.f. = 1, $N = 297$, $P < 0.0001$, and slope = 0.03).

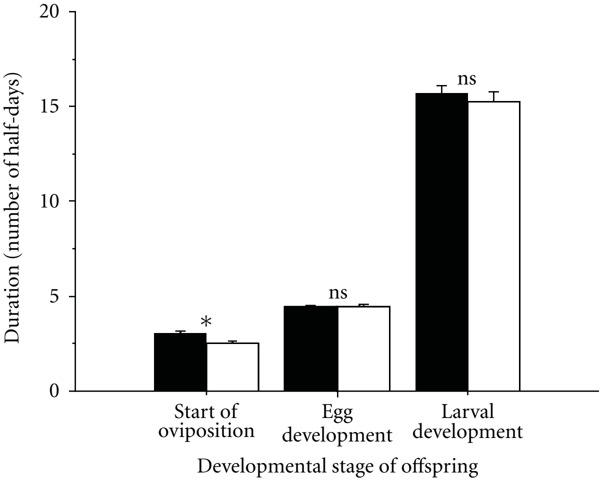
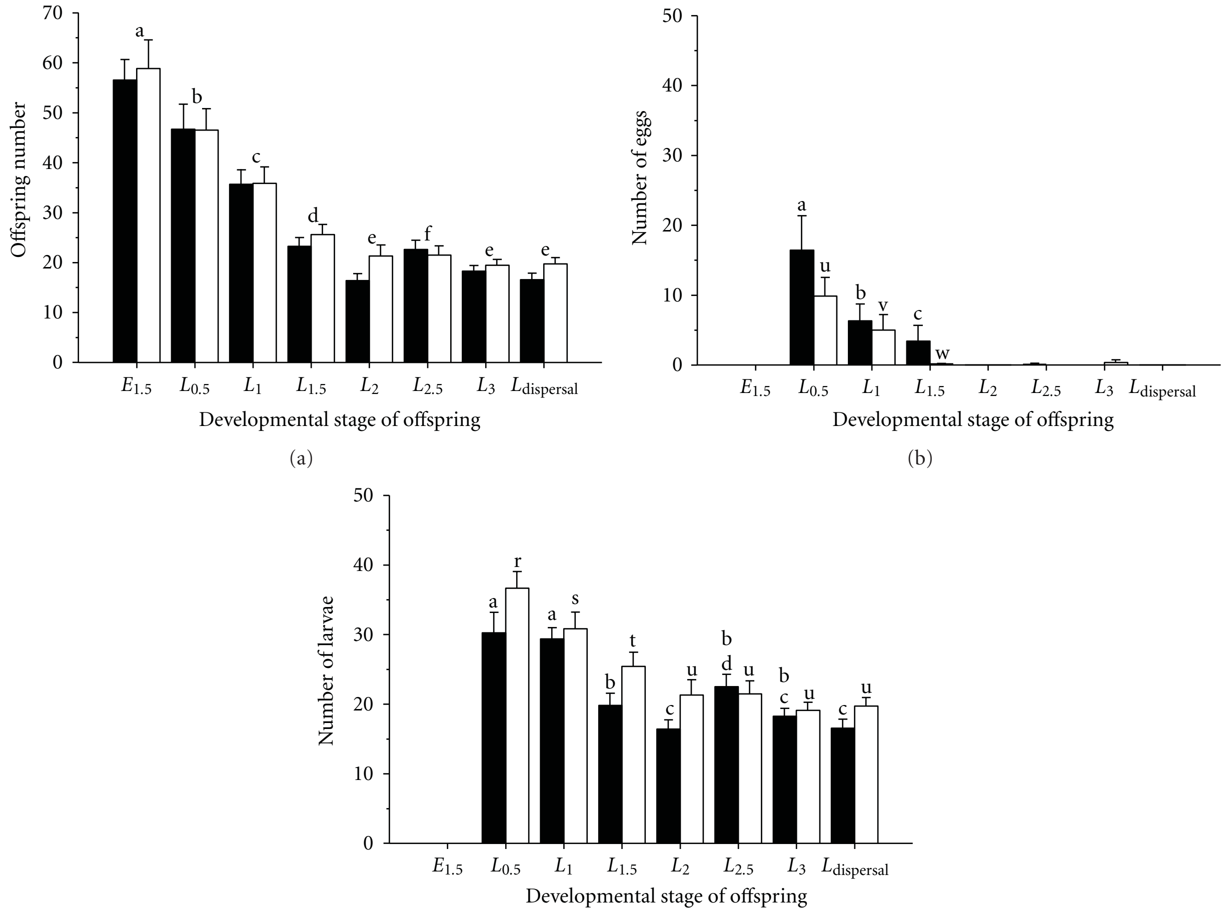


Figure 1: Effect of density on time elapsed until the first egg was laid (i.e., start of oviposition) as well as egg and larval development. Black bars: high-density females; white bars: low-density females. Means and SE are shown. $^*P = 0.01$; ns: not significant.

The number of eggs present after the first larvae had hatched, depended on the developmental stage of the offspring ($\chi^2 = 335.70$, d.f. = 3, $N = 220$, and $P < 0.0001$; Figure 2(b)). However, in broods of high-density females, there were more eggs present and for a longer time than in broods of low-density females (Density: $\chi^2 = 7.08$, d.f. = 1, $N = 220$, and $P = 0.01$; interaction between density and developmental stage: $\chi^2 = 25.47$, d.f. = 3, $N = 220$, and $P < 0.0001$). The number of eggs present increased as mouse weight increased ($\chi^2 = 29.03$, d.f. = 1, $N = 220$, $P < 0.0001$, and slope = 0.09).

The number of larvae present on the carcass after the first larvae had hatched was significantly affected by the age of the larvae ($\chi^2 = 354.89$, d.f. = 6, $N = 260$, and $P < 0.0001$; Figure 2(c)). The pattern of change in larvae number with larval age differed between high- and low-density females (interaction between density and developmental stage: $\chi^2 = 14.82$, d.f. = 6, $N = 260$, and $P = 0.02$). On carcass of low-density females, the decline in larvae number began earlier, but leveled off at the same time as the number of larvae on carcass of high-density females (Figure 2(c)). Overall, high-density females had fewer larvae than low-density females ($\chi^2 = 24.46$, d.f. = 1, $N = 260$, and $P < 0.0001$). The number of larvae increased with increasing mouse weight ($\chi^2 = 10.37$, d.f. = 1, $N = 260$, $P = 0.001$, and slope = 0.02).

3.3. Offspring Size. Egg size (measured as egg length) did not differ between high- and low-density females ($F_{1,33} = 0.69$ and $P = 0.41$; Figure 3(a)). The size of larvae (measured as larval weight) changed significantly with larval age ($F_{6,245} = 224.45$, and $P < 0.0001$; Figure 3(b)). But the pattern of the larval weight increase with larval age did not differ between



(a)

(b)

(c)

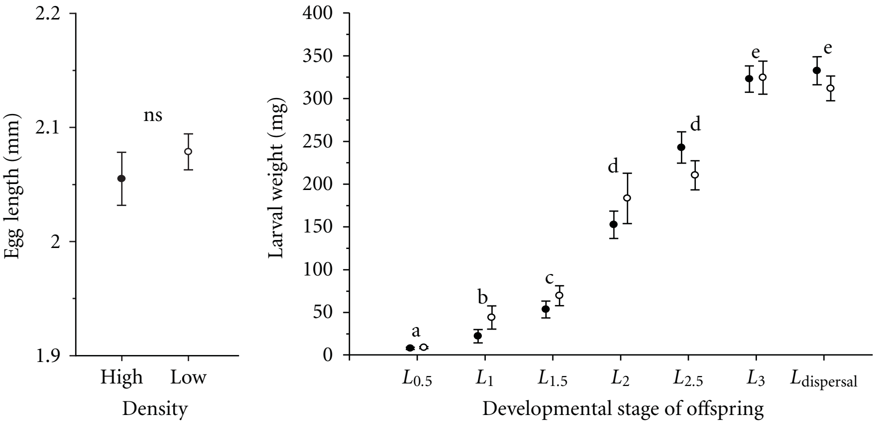
Figure 2: Total number of offspring (a), number of eggs present after first larvae had hatched (b), and number of larvae (c) in relation to developmental stage of offspring for high-density (black bars) and low-density females (white bars). $E_{1.5}$: first laid eggs were 1.5 days old; $L_{0.5}$, L_1, $L_{1.5}$, L_2, $L_{2.5}$, and L_3: oldest larvae were 0.5, 1, 1.5, 2, 2.5, or 3 days old; $L_{\text{dispersal}}$: larvae had left the brood cavity and were dispersing. Different letters above bars in (a) indicate difference at $P < 0.05$ between developmental stages for both female densities combined, while different letters above bars in (b) and (c) indicate difference at $P < 0.05$ between developmental stages within each female density treatment. Means and SE are shown.

high- and low-density females (interaction between density and developmental stage: $F_{6,245} = 1.96$ and $P = 0.07$). Larval weight did not differ between high- and low-density females ($F_{1,245} = 2.98$, $P = 0.09$). Mouse weight affected neither egg size ($F_{1,33} < 0.01$, $P = 0.95$) nor larval weight ($F_{1,245} = 0.03$, $P = 0.86$).

4. Discussion

As predicted, high-density females had overall fewer offspring (i.e., eggs and larvae) and, in particular, fewer larvae than low-density females, corroborating other studies on burying beetles [1, 6]. However, contrary to our prediction and Creighton's [1] study on *N. orbicollis*, offspring of high-density females were not larger than offspring of low-density females. This may be due to weaker selection on larval mass in *N. pustulatus* compared to *N. orbicollis*, as we have suggested previously [6]. Although *N. pustulatus* accept mice readily for reproduction in the laboratory and show the same behaviors as other burying beetles, a host shift to snake eggs has been observed [25, 26]. A clutch of snake eggs may provide breeding opportunities for more than one pair of beetles therefore reducing intensity of selection on body size.

Our experiment showed that the number of larvae on carcass of high-density females started to decline later than on carcass of low-density females, even though the number of eggs was declining for both high- and low-density females (Figures 2(b) and 2(c)). This suggests that high-density females began brood reduction at the same time as low-density females, but differed in the rate of brood reduction. After initiation of brood reduction, high-density females seemed to reduce their broods at a slower rate than low-density females, but soon after reduced their broods much faster (Figure 2(c)). Consequently, both high- and low-density females reached their final brood size when the larvae were two days old (Figure 2(c)). This pattern of brood reduction may be a bet-hedging strategy allowing high-density females, in case a failed takeover with partial



(a)

(b)

Figure 3: Egg size (measured as egg length) (a) and larval weight in relation to developmental stage of offspring (i.e., larval age) (b) for high (filled circles) and low-density females (open circles). $L_{0.5}$, L_1, $L_{1.5}$, L_2, $L_{2.5}$, and L_3: oldest larvae were 0.5, 1, 1.5, 2, 2.5, or 3 days old; $L_{\text{dispersal}}$: larvae had left the brood cavity and were dispersing. Different letters indicate difference at $P < 0.05$ between developmental stages for both female densities combined. Means and SE are shown.

infanticide occurs, to compensate the loss of offspring, while maximizing larval growth. Reducing the number of larvae as fast as possible may be beneficial because a larger brood size early in development can negatively affect the final size of larvae (Chris Effken and Claudia Rauter, unpublished data).

High-density females began to oviposit later and had more eggs and for a longer time than low-density females suggesting that high-density females oviposit not only later, but also over a longer time period and that larvae hatch more asynchronously. This oviposition pattern may have been caused by lower body condition of high-density females. Even though high-density females received the same amount of food per individual, behavioral interactions among high-density females may have lead to higher energy use and unequal access to food causing nutritional stress and lower body condition. In a similar experiment [6] where we maintained 1, 2, 4, or 6 female beetles in a container, we found that body weight of small females within high-density containers, decreased as density increased indicating nutritional stress. In the burying beetle *N. orbicollis* nutritional stress in form of low quality diet leads to slower ovarian development and delayed oviposition as well as to fewer and smaller eggs [27]. An alternative, but not mutually exclusive, physiological explanation for the delayed and extended oviposition by high-density females may be physiological stress in form of elevated stress hormone levels. Competitive interactions among conspecifics can cause physiological stress in form of elevated levels of stress hormones [28–32], which can adversely affect reproduction [32, 33].

Later oviposition and a longer oviposition period at high population density, may not only be the consequence of physiological stress, but also be an adaptive response to high population density with increased competition for carcass. Delayed oviposition of high-density females can have fitness benefits, if after an unsuccessful take-over attempt the losing, subdominant female stays close and lays eggs, usually earlier than the successfully defending, dominant female, near the carcass [34, 35]. Typically, the larvae of the subdominant female hatch earlier and are killed by the dominant female [35]. Larvae present on the carcass, before the female's own larvae are hatching, are killed by the female because burying beetles use temporal cues to distinguish between their own and unrelated larvae [35, 36]. Eggert and Müller [35] suggested that delayed oviposition by the dominant female in nontolerant breeding associations allows the dominant female to better discriminate between related and unrelated larvae, and preferentially kill unrelated larvae, thus increasing its fitness.

At high-density, an extended oviposition period and thus more asynchronous hatching of larvae may also be an adaptation to unpredictable and variable survival of eggs and newly hatched larvae when the take-over risk is high. Assuming that the value of a carcass decreases with decreasing carcass size similarly to the decrease in value observed in carcasses that are consumed by developing larvae [18], our results show the same patterns as findings in *N. vespilloides* [37]. With increasing carcass size, female *N. vespilloides* lay eggs in larger intervals resulting in more asynchronous hatching of larvae [37].

Our data suggest that high population density imposes nutritional and/or physiological stress on females causing delayed oviposition. However, females respond to the physiological constraints and the potentially high mortality rates of eggs and newly hatched larvae by lengthening the oviposition period and changing the brood reduction rate.

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Research Article

Taxonomic Studies on the Genus *Athesapeuta* (Coleoptera: Curculionidae: Baridinae) from India with Description of Three New Species

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Six species of genus *Athesapeuta* Faust (Coleoptera: Curculionidae: Baridinae) from India and the adjacent countries are included, of which three new species, namley *meghalayensis* sp. nov., *richardi* sp. nov., and *spinulatus* sp. nov., are described. An annotated checklist of known species along with their synonymy and distribution is given. Descriptions are supplemented with details of genitalia and elytral vestiture; a key to the species studied is provided.

1. Introduction

Athesapeuta belongs to the tribe Madarini which at present is considered under the subfamily Baridinae of the family Curculionidae [1]. The majority of its species are known from the Oriental region, of which eight are from India. Except for the studies by Faust [2, 3] and Marshall [4], taxonomic studies on the Indian fauna are inadequate, scattered, and need to be updated and supplemented with descriptions of genitalia, morphometrics, and terminology. In particular, elytral vestiture and female genitalia which are emerging as important characters have not been included in descriptive work. The present study addresses these gaps for the currently recognized species and adds three new species.

2. Materials and Methods

Voucher specimens and type material are deposited with the National Pusa Collection (NPC) of the Division of Entomology, Indian Agricultural Research Institute, New Delhi. Materials from the National Pusa Collection (NPC) of the Division of Entomology, Indian Agricultural Research Institute, New Delhi; Forest Research Institute (FRI), Dehra Dun; Zoological survey of India (ZSI), Kolkotta; Panjab University (PU), Chandigarh, were included. Paratypes were studied for all the species described, and they are compared with original literature and photographs. All taxonomic characters, except the elytral vestiture and genitalia, were studied in intact specimens. Specimens were processed following Supare et al. [5], and genitalia and elytral vestiture were studied following Ramamurthy and Ghai [6]. The terminology of Supare et al. [5], Thompson [7], Poorani and Ramamurthy [8], O′Brien and Pakaluk [9], Wanat [10], and Davis [11] was followed for the description of female and male genitalia. General taxonomic characters and genitalia were studied with Leica M205FA stereozoom microscope, and elytral vestiture was studied with Leica DM1000 phase contrast microscope. Photographs were captured using the software Leica application Suite ver. 2.8.2 on a Leica DFC290 camera. Illustrations were made using a drawing tube fitted with a camera lucida and scales of magnification provided in the illustrations. Total length given in the descriptions is excluding rostrum, and the standard length is from anterior margin of pronotum to end of pygidium. The measurements given in the descriptions are mean and standard error except for new species, wherein measurements are of holotype.

3. Genus: *Athesapeuta* Faust

Athesapeuta Faust 1894 [2]. Type species: *Baridius subsignatus* Motschulsky, 1866 [12] = *Athesapeuta motschulskyi* Voss, 1958 [13]; gender: Female. (By "Original Designation").

Description. General colour shiny black; body rhomboidal, subcylindrical, or oblongovate, much longer than broad. Head not separated from rostrum by a deep transverse incision, frons narrower than base of rostrum, 1.5–4x as broad as long. Rostrum long, cylindrical, straight at least in basal half, separated from head by a sharp sulcus between eyes; more or less curved with mandibles adducent type, dentate internally or conical with straight cutting edge, 0.79–0.98x as long as head and pronotum combined (Figure 1(a)); eyes 1.28–2.12x as wide as long. Antennae inserted at 1.1–1.8x of length from base of rostrum; scape 6.25–10x as long as broad; funicle widening distally; seventh funicle not fitting closely to club (Figure 1(b)). Prothorax generally shallowly bisinuate at posterior dorsal margin, anterior dorsal margin truncate, not produced over head when viewed in profile, as broad as to 1.28x as broad as long. Scutellum generally trapezoidal, as broad as to 1.5x as broad as long. Elytra oblong or ovate, smooth; 0.5–0.92x as wide as prothorax; separately arcuate at base; deeply striate, striae 10 complete; separately rounded at apices, with narrow membrane and paler fringe along apical margin, without any trace of subapical calli (Figure 1(c)). Legs with femora clavate (Figure 1(d)), unarmed, not sulcate beneath; tibiae with longitudinally confluent punctures, sulcate, not tuberculate (Figure 1(e)); claws almost parallel to each other, more or less divergent, narrowly separated (Figure 1(f)). Sternum with a furrow on prosternum; procoxa 1.53–2.5x as wide as intercoxal process; hind margin of prosternum produced in middle; mesosternum transversely impressed, intercoxal process 0.9–1.25x as broad as a coxa; metasternum elongate, 1.5–1.66x as long as mesocoxa; pro-, meso-, and metasterna not interrupted in continuity by mesosternum (Figure 1(g)). Abdomen with first and second ventrites not connate; pygidium fully or broadly exposed, directed posterioventrally, visible dorsally, without transverse carina along hind margin of elytra in general; lateral part of fifth ventrite visible dorsally on each side of pygidium. Female genitalia with spermatheca more or less sclerotised at distal arm, distal arm shorter than proximal arm; spiculum ventrale 1.5–2.5x as long as basal plate; apical end with hairs. Male genitalia with median lobe moderately sclerotised; apophyses 0.25–1.06x as long as median lobe.

4. Annotated Checklist

Faust [2]described this genus, and Voss [13] synonymised *Baridius subsignatus* with *Athesapeuta motschulskyi*, the type species. The majority of species were described before 1950. Faust contributed the most with nine species, followed by Marshall (7), Voss (6), Bohemann and Hustache (3 each); Motschulsky (2), and Fabricius, Gerstaecker and Zimmerman (1 each). This genus is predominantly Oriental (18 species) Thirteen are Afrotropical, and five occur in the Palaearctic.

Checklist: Type species: Baridius subsignatus Motschulskyi, 1866 [12] = *Athesapeuta motschulskyi* Voss, 1958 [13]. *(For more details see Table 1)* .

5. Description of Species

5.1. Athesapeuta cyperi Marshall, 1928 [17]. (Figures 2(a), 3(a), 4(a), 5(a), 6(a), 6(b), 8(a), 9(a), 10(a), 11(a), 11(d), 12(a), 13(a)–13(c), 14(a), 14(b), 15(a)–15(d) and 16(a)–16(c)).

Description. Colour shiny black; antennae, tibiae, elytra on lateral and apical margins reddish brown. Head bare, finely alutaceous with sparse punctures, 1.5x as broad as long; eyes 1.28x as wide as long. Rostrum 0.79x as long as head and pronotum combined, strongly curved, smooth, with four rows of small punctures in addition to punctate area above scrobes, sparse vestiture on each side of base, at middle 0.77x as broad as at apex, 0.76x as broad as at base (Figures 2(a) and 3(a)). Antennae inserted at 1.57x of length from base of rostrum; scape slender, long, 9.13x as long as broad, impunctate; funicle with first segment 3.5x as long as second and third combined, third 0.5x as long as broad, segments three to seven slightly transverse, subequal in length and breadth; funicle 1.18x as long as club (Figure 4(a)). Prothorax 1.03x as broad as long, sides subparallel from base to middle, anterior margin truncate, behind apex with tubular constriction, posterior margin shallowly bisinuate, at middle 1.91x as broad as at apex, and 0.98x as broad as at base; dorsum gently convex longitudinally, set with close shallow separated punctures, with a broad impunctate median stripe, punctures on pleurae larger and subreticulate (Figure 5(a)). Scutellum bare, trapezoidal, with two low longitudinal costae, 1.09x as broad as long. Elytra oblong ovate, separately rounded at apex, at middle 1.14x as broad as at apex, 1.13x as broad as at base; striae deep, indefinitely punctate, not diminishing at apex, striae 10 complete; intervals flat, 3x as broad as a striae, with a row of large shallow punctures, each with minute recumbent scale, vestiture small on intervals one to five, larger, scale-like on outer intervals (Figure 8(a)); elytral vestiture whitish, rod-shaped, tapering and pointed at base, blunt at apex, surface with striations reaching apex (Figure 9(a)). Legs coarsely punctate, each puncture containing a grey elongate vestiture; tibia sulcate; profemur with a fringe of long vestiture on ventral surface (Figure 6(a)), 1.12x as long as mesofemur, 1.09x as long as metafemur. Protibia 1.53x and 1.25x as long as meso- and metatibia, respectively, with a sharp tooth on inner edge at about middle in males (Figure 6(b)), females without it. First tarsal segment 1.1x as long as broad, 1.1x as long as second, 0.83x as long as and 0.71x as broad as third, third 1.16x as broad as long, fourth 3x as long as broad. Prosternum with deep transverse sulcus behind apex, base with raised fovea. Procoxa 1.87x as broad as its intercoxal process; mesosternum plate-like, depressed at base, raised at apex, intercoxal process 0.96x as broad as mesocoxa; metasternum depressed in middle with longitudinal impressed line, intercoxal process 1.33x as broad as metacoxa. Venter black, strongly punctate, each with broad vestiture; anterior margin of first ventrite broadly and shallowly ogival, posterior margin straight, 2.92x as broad as long, 1.74x as long as second, second 5x as broad as long, 0.65x as long as three and four combined, ventrites third and fourth subequal in length, five 2.18x as broad as long,

Table 1

Sl No	Species	Distribution
(1)	*affinis* Faust, 1898 [3]	India
(2)	*amoena* Voss, 1958 [13]	China
(3)	*armata* Hustache, 1932 [14]	Madagascar
(4)	*atronuda* Marshall, 1941 [15]	Uganda
(5)	*aurantiaca* Faust, 1894 [2]	Myanmar
(6)	*bengalica* Faust, 1894 [2]	India
(7)	*chinensis* Faust, 1894 [2]	China
(8)	*conradti* Hustache, 1932 [16]	Cameroun
(9)	*cyperi* Marshall, 1928 [17]	Philippines
(10)	*dodonis* (Marshall) = *Baris dodonis* Marshall, 1936 [18]; Pajni and Kohli, 1990 [19]	Uganda
(11)	*famula* (Fabricius) = *Curculio famula* Fabricius, 1798 [20]; Hustache, 1938 [21] = *Rhynchaenus famula* (Fabricius, 1798 [20]); Fabricius, 1801 [22]; Hustache, 1938 [21] = *Baridius famula* (Fabricius, 1798 [20]); Boheman in Schoenherr, 1836 [23]; Hustache, 1938 [21] = *centrodentatus* Desbrochers des Loges, 1891 [24]; Hustache, 1938 [21]	India
(12)	*flavicornis* (Boheman in Schoenherr) = *Baridius flavicornis* Boheman in Schoenherr, 1836 [23]; Hustache, 1938 [21]	USA
(13)	*gyrosicollis* Marshall, 1948 [25]	Southern Shan States
(14)	*immaculata* Faust, 1898 [3]	India
(15)	*latifasciata* Voss, 1958 [13]	China
(16)	*lineolatofasciata* (Motschulsky) = *Baridius lineolatofasciata* Motschulsky, 1866 [12]; Faust, 1894 [2]	India
(17)	*madugodana* Voss, 1957 [26]	Sri Lanka
(18)	*meghalayensis* sp. nov.	India
(19)	*motschulskyi* Voss, 1958 [13]	China
(20)	*oryzae* Marshall, 1916 [4]	India
(21)	*pinguis* Faust, 1894 [2]	Myanmar
(22)	*politirostris* Voss, 1962 [27]	Congo
(23)	*richardi* sp. nov.	India
(24)	*sculptilis* Gerstaecker, 1871 [27] = *scutellaris* Faust, 1896: 145	Africa
(25)	*secura* Faust, 1894 [2]	Myanmar
(26)	*semirubra* (Hustache) = *Titanobaris semirubra* Hustache, 1935 [28]; Marshall, 1941 [15]	Angola
(27)	*soror* Faust, 1898 [3]	India
(28)	*spinulatus* sp. nov.	India
(29)	*subcalva* Marshall, 1941 [15]	Uganda
(30)	*subsignatus* (Boheman in Schoenherr) = *Baridius subsignatus* Boheman in Schoenherr, 1836 [23]; Faust, 1894 [2]	Africa
(31)	*subsignata* (Motschulsky) not Boheman = *Baridius subsignata* Motschulsky, 1866 [12]; Faust, 1894 [2]	India
(32)	*sculpticollis* Voss, 1958 [13]	China
(33)	*ulvae* Zimmerman, 1942 [29]	Guam
(34)	*varicolor* Marshall, 1941 [15]	Uganda
(35)	*versicolor* (Boheman) = *Baridius versicolor* Boheman, 1859 [30] = *Baris versicolor* (Boheman, 1859 [30]); Hustache, 1938 [21]; Pajni and Kohli, 1990 [19]	Indonesia
(36)	*vinculata* Faust, 1894 [2]	Myanmar

posterior margin truncate; pygidium distinctly punctate with fringes of vestiture, exposed on ventral side, with an arch-shaped marking at middle in males (marking being the junction of tergites VII and VIII), females without it, 1.03x as broad as long (Figure 10(a)).

Female Genitalia. Spermatheca not sclerotised, distal arm as long and as broad as proximal arm, angle between proximal and distal arms not acute, nodulus small, ramus flat, cornu blunt (Figures 11(a) and 14(a)). Spiculum ventrale with shaft elongate, 1.5x as long as basal plate, basal plate 5x as long as

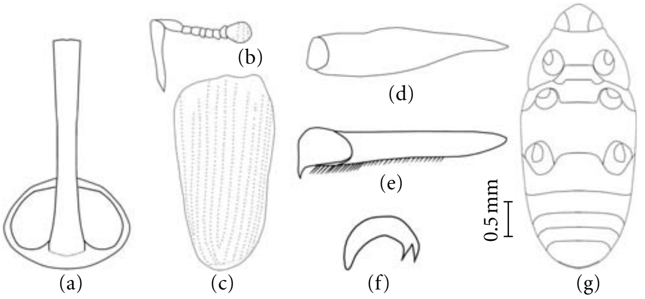

Figure 1: *Athesapeuta*. Genus characters: (a), rostrum, dorsal view; (b), antennae; (c), elytron, dorsal view; (d), femur, lateral view; (e), tibiae; (f), tarsal claw; (g), habitus; ventral view.

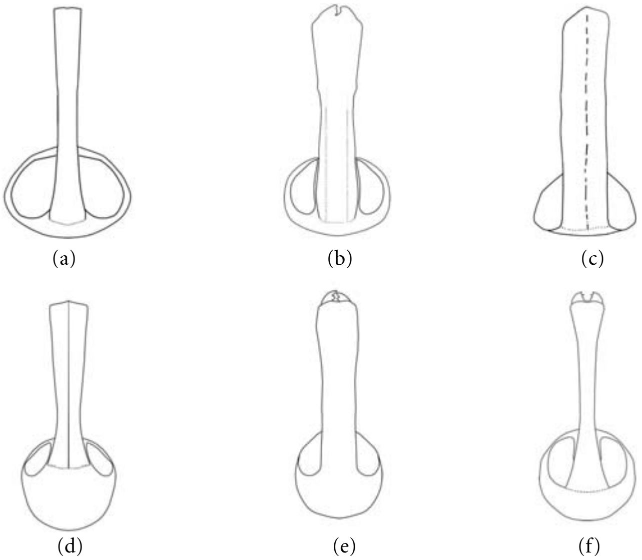

Figure 2: Rostrum, dorsal view: (a), *A. cyperi*; (b), *A. immaculata*; (c), *A. meghalayensis* sp. nov.; (d), *A. oryzae*; (e), *A. richardi* sp. nov.; (f), *A. spinulatus* sp. nov.

broad, apical end truncate, without hairs (Figures 11(d) and 14(b)).

Male Genitalia. Apophyses 0.25x as long as median lobe, 0.25x as long as spiculum gastrale; median lobe moderately sclerotised, parallel sided from base to behind middle, apex sinusoidal, at middle 1.42x as broad as at apex and as broad as at base (Figures 13(a)–13(c) and 15(a)–15(d)). Spiculum gastrale uniformly thick, curved at apex, 11.6x as long as broad (Figures 12(a) and 15(d)).

Measurements. Total length: 3.25 ± 0.23 mm; standard length: 3–3.30 mm; breadth: 1.36 ± 0.07 mm.

Material Examined. India: 6♂♂, Maharashtra: Phaltan, feeding on nut sedge, x.1999, Coll. Nimbkar; 4♂♂, 3 ♀♀, West Bengal: Kolkata, 11.i.2011, Coll. Ramasubramanian, larvae boring on *Cyperus rotundus*.

Distribution. India: Maharashtra; West Bengal. Philippines: Los Banos. Hawaii: Honolulu, Ohau.

5.2. Athesapeuta immaculata Faust, 1898 [3]. (Figures 2(b), 3(b), 4(b), 5(b), 8(b), 9(b), 9(c), and 16(d)–16(f)).

Description. Colour shiny black. Head with sparse punctures, 3.2x as broad as long; eyes 1.38x as wide as long. Rostrum 0.98x as long as head and pronotum combined, strongly curved, with two dorsal carinae from base to antennal insertion and then fading out, at middle 0.85x as broad as at apex, 0.92x as broad as at base (Figures 2(b) and 3(b)). Antennae inserted at 1.8x of length from base of rostrum; scape brown, impunctate, 8.33x as long as broad; funicle with first segment 1.16x as long as second and third combined, third as long as broad, segments three to seven slightly transverse and subequal in length and breadth; funicle 1.81x as long as club; club ovate (Figure 4(b)). Prothorax as broad as long, with granular punctures, anterior margin truncate, behind the apex without tubular constriction, posterior margin shallowly bisinuate, at middle 1.78x as broad as at apex, and 0.75x as broad as at base; dorsum gently convex longitudinally, set with shallow regular punctures, confluent in curves, without any median line. Scutellum raised, trapezoidal, sparsely punctate, as long as

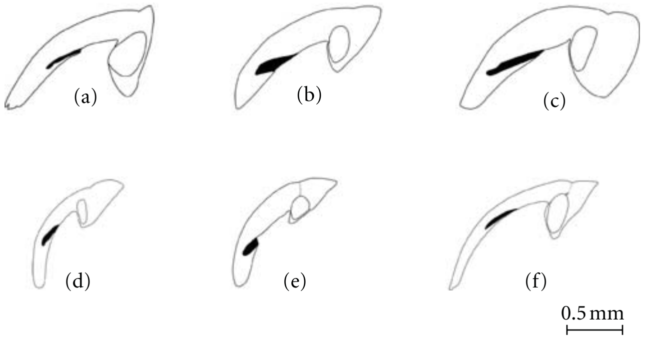

Figure 3: Rostrum, lateral view: (a), *A. cyperi*; (b), *A. immaculata*; (c), *A. meghalayensis* sp. nov.; (d), *A. oryzae*; (e), *A. richardi* sp. nov.; (f), *A. spinulatus* sp. nov.

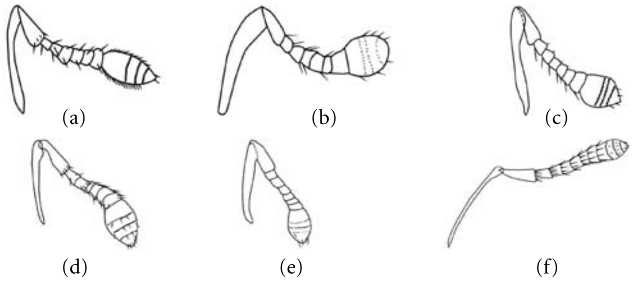

Figure 4: Antenna: (a), *A. cyperi*; (b), *A. immaculata*; (c), *A. meghalayensis* sp. nov.; (d), *A. oryzae*; (e), *A. richardi* sp. nov.; (f), *A. spinulatus* sp. nov.

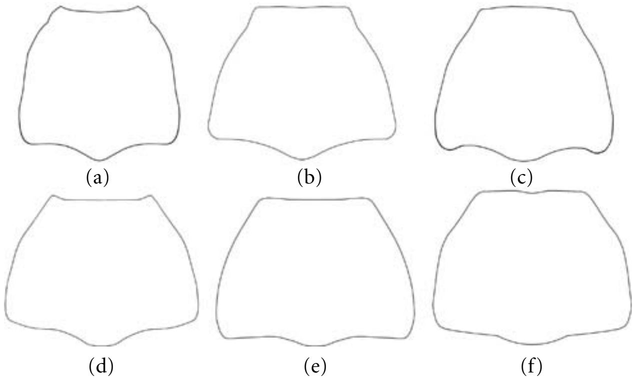

Figure 5: Prothorax, dorsal view: (a), *A. cyperi*; (b), *A. immaculata*; (c), *A. meghalayensis* sp. nov.; (d), *A. oryzae*; (e), *A. richardi* sp. nov.; (f), *A. spinulatus* sp. nov.

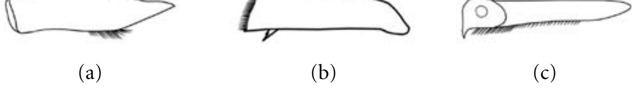

Figure 6: (a, b) Profemur and metatibia: *A. cyperi*; (c) Protibia: *A. oryzae*.

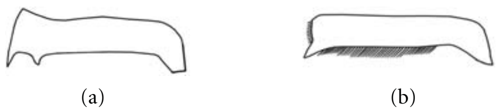

Figure 7: Protibia and metatibia: *A. richardi* sp. nov.

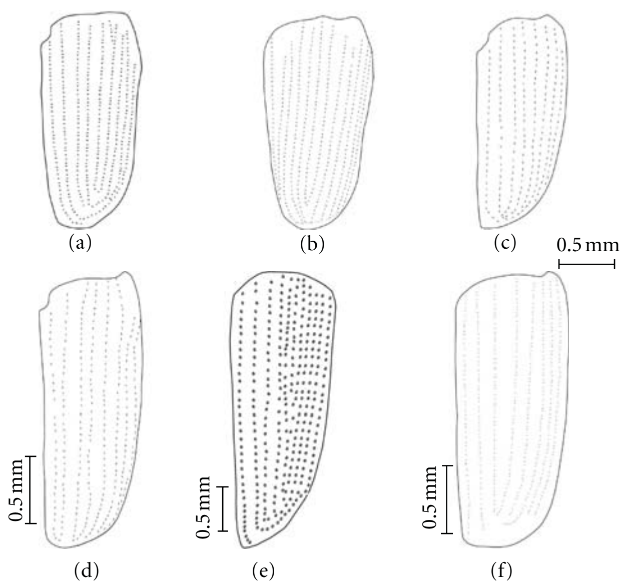

Figure 8: Elytron, dorsal view: (a), *A. cyperi*; (b), *A. immaculata*; (c), *A. meghalayensis* sp. nov.; (d), *A. oryzae*; (e), *A. richardi* sp. nov.; (f), *A. spinulatus* sp. nov.

broad (Figure 5(b)). Elytra oblong ovate, separately rounded at apex, without any posterior calli, at middle 1.32x as broad as at apex, 1.22x as broad as at base; striae shallow, with distant catenulate punctures which do not encroach on intervals, striae 10 complete; intervals flat, 1.5x as broad as a striae, with a row of transverse punctures, each containing a minute recumbent scale (Figure 8(b)). Elytral vestiture predominantly of two types; either yellowish white, base tapering and apex concave, surface with granular serrations (Figure 9(b)), or brownish yellow, both ends broader and surface with dense granular serrations (Figure 9(c)). Legs coarsely punctate, each puncture containing grey vestiture; tibia sulcate; profemur 0.90x as long as mesofemur, 0.83x as long as metafemur. Protibia 1.2x and 0.93x as long as meso- and metatibia, respectively. First tarsal segment 1.66x as long as broad, 1.66x as long as second, as long as and 0.54x as broad as third, third 0.90x as broad as long, fourth 4x as long as broad. Prosternum with deep transverse sulcus behind apex, base of prosternum with raised fovea, with definite punctures all over sternum and each punctures with a grey recumbent vestiture. Procoxa 2.5x as broad as intercoxal process; mesosternum plate like, depressed at base and apex, intercoxal process 1.25x as broad as mesocoxa; metasternum depressed in middle with longitudinal impressed line, intercoxal process 1.08x as broad as metacoxa.

Measurements. Total length: 5.4–5.5 mm; standard length: 4.7 mm; breadth: 2.2–2.4 mm.

Material Examined. 2 specimens, location unknown, from Nagasilla grass (abdomen damaged), 12.vii.1985, Coll. unknown.

Distribution. India.

5.3. Athesapeuta meghalayensis sp. nov. (Figures 2(c), 3(c), 4(c), 5(c), 8(c), 9(d), 9(e), 10(b), 12(b), 13(d)–13(g), 15(e)–15(i), and 16(g)–16(i)).

Diagnosis. It is closely related to *A. richardi* sp. nov., but differs in prothorax with broad stripe of yellow vestitures (white in *A. richardi* sp. nov.), with a smooth median line (absent in *A. richardi* sp. nov.); posterior end of tibia does not carry sharp tooth (present in *A. richardi* sp. nov.), metatibia lateroventrally without fringes of grey hairs (present in *A. richardi* sp. nov.).

Description. Colour black. Head with close regular punctures, 4x as broad as long; eyes ventrally placed, 2.12x as wide as long. Rostrum 0.9x as long as head and pronotum combined, strongly curved, gradually widening, irregularly punctate, each punctures with yellow vestiture, more prominent in basal region, irregular punctures become reticulate and rugose beyond antennal insertion, with a median smooth impunctate line, almost parallel sided, without any subbasal dilation (Figures 2(c) and 3(c)), at middle 1.09x as broad as at apex, 0.92x as broad as at base. Antennae inserted at 1.22x of length from base of rostrum; scape robust, 6.25x as long as broad, almost impunctate; funicle with first segment 1.2x as long as second and third combined, segments second to seven carry sharp spines all over surface, third as long as broad, segments three to seven transverse and subequal in length and breadth; funicle 1.46x as long as club; club ovate (Figure 4(c)). Prothorax 1.03x as broad as long, with granular punctures, with a stripe of yellow vestiture on lateral aspect just behind the anterior margin on both sides, which is continuous with scaling of lower surface, tubular constriction at apex, sides gently rounded, posterior margin shallowly bisinuate, at middle 1.8x as broad as at apex, 0.95x as broad as at base, dorsum gently convex

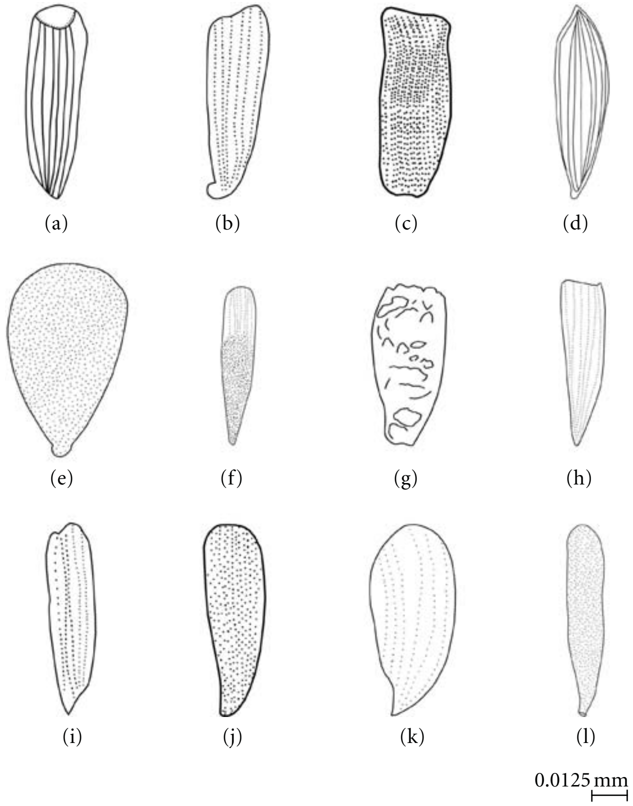

Figure 9: Elytral vestiture: (a), *A. cyperi*; (b)-(c), *A. immaculata*; (d)-(e), *A. meghalayensis* sp. nov.; (f)-(g), *A. oryzae*; (h)–(j), *A. richardi* sp. nov.; (k)-(l), *A. spinulatus* sp. nov.

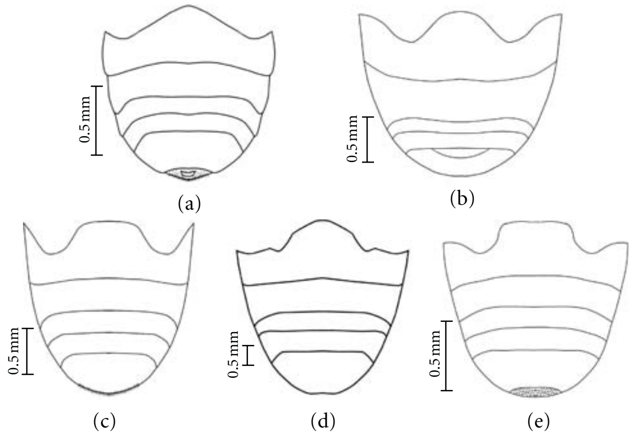

Figure 10: Venter: (a), *A. cyperi*; (b), *A. meghalayensis* sp. nov.; (c), *A.oryzae*; (d), *A. richardi* sp. nov.; (e), *A. spinulatus* sp. nov.

longitudinally, set with close granular punctures, confluent in curves, with smooth median line, patches of yellowish white vestiture just above posterior margin of prothorax (Figure 5(c)). Scutellum strongly transverse, square shaped, not punctate, without median impression, as broad as long. Elytra ovate, without deep subapical impressions, without posterior calli, apices rounded, at middle 1.14x as broad as at apex, 1.33x as broad as at base; striae shallow, with distant separate punctures which do not encroach on intervals, striae 10 complete; intervals 4x as broad as a striae, with a row of catenulate transverse punctures, each containing a minute black recumbent scale, lateral margin smooth at apex, interval five with a patch of yellowish vestiture on basal end, middle of elytra with larger patch of vestiture on interval 4 and 5 (Figure 8(c)); elytral vestiture either brownish yellow, with basal end blunt, apex pointed with lines on surfaces reaching apex (Figure 9(d), or greyish white, pear shaped, broad at apex with granular striations on surface (Figure 9(e)). Legs coarsely punctate, each puncture containing a grey vestiture; tibiae sulcate; profemur 1.31x

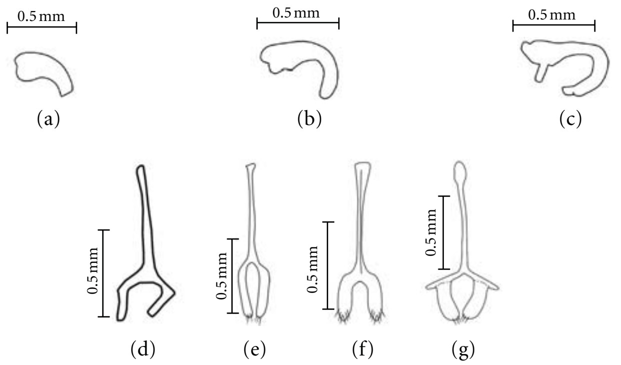

Figure 11: Female genitalia, spermatheca, and spiculum ventrale: (a), (d), *A. cyperi*; (b), (e), *A. oryzae*; (c), (f), *A. richardi* sp. nov.; (g), *A. spinulatus* sp. nov.

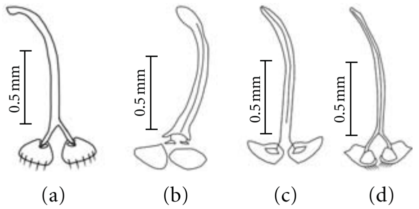

Figure 12: Male genitalia, spiculum gastrale: (a), *A. cyperi*; (b), *A. meghalayensis* sp. nov.; (c), *A. richardi* sp. nov.; (d), *A. spinulatus* sp. nov.

as long as mesofemur, 0.92x as long as metafemur. Protibia 0.97x as long as mesotibia, and as long as metatibia. First tarsal segment as long as broad, 1.25x as long as second, 0.83x as long as third, third 1.66x as broad as long, fourth 2.75x as long as broad. Prosternum with deep transverse sulcus behind apex, base of prosternum with raised fovea. Procoxa 1.98x as broad as intercoxal process; mesosternum plate like, depressed at base and apex, intercoxal process 1.03x as broad as mesocoxa; metasternum shallowly depressed at middle with impressed longitudinal line, intercoxal process 2.01x as broad as metacoxa. Venter black, clothed with broad greyish yellow vestiture, strongly punctate, each with broad white scale, anterior margin of first ventrite ogival, posterior margin subtruncate, 2.7x as broad as long, 1.66x as long as second, second 4.22x as broad as long, 1.12x as long as three and four combined, third and fourth subequal in length, ventrite five 3.12x as broad as long, posterior margin rounded; pygidium distinctly punctate, broadly exposed with long hairs from each puncture, 1.53x as broad as long (Figure 10(b)).

Male Genitalia. Apophyses 1.06x as long as median lobe, 0.7x as long as spiculum gastrale, 1.84x as long as tegmen; median lobe moderately sclerotised, parallel sided from base to behind middle, apex truncate, at middle 1.25x as broad as at apex, and 1.42x as broad at base (Figures 13(d)–13(f) and 15(e)–15(g)). Tegmen 1.9x as long as manubrium, 1.58x as long as parameroid lobe; manubrium flat (Figures 13(g) and 15(i)). Spiculum gastrale uniformly thick, curved at apex, with a median line, 12.5x as long as broad (Figures 12(b) and 15(h)).

Measurements of Holotype. Total length: 4.20 mm; standard length: 3.80 mm; breadth: 1.78 mm.

Material Examined. Holotype ♂, India: Meghalaya: Tura, date and coll. unknown, from wild plant (latitude: 25° 30′ N; Longitude: 90° 16′ E). Paratypes (2♂♂): 1♂, India: Meghalaya: Ambashi, from wild plant, 26.v.1988, Coll. D. Kumar; 1♂, Assam: Nagora, from wild plants, 23.v.1988, Coll. Baljinder.

Distribution. India: Meghaya; Assam.

Etymology. The specific epithet refers to the type locality.

5.4. *Athesapeuta oryzae* Marshall, 1916 [4]. (Figures 2(d), 3(d), 4(d), 5(d), 6(c), 8(d), 9(f), 9(g), 10(c), 11(b), 11(e), 14(c), 14(d)).

Description. Colour shiny black. Head convex, finely shagreened with regular close puncture, 1.75x as broad as long; eyes 1.47x as wide as long. Rostrum 0.92x as long as head and pronotum combined, strongly curved, without any subbasal dilation, with coarse punctures especially at sides, and with an impunctate median carinae, at middle 0.83x as broad as at apex, 0.67x as broad as at base (Figures 2(d) and 3(d)). Antennae black, with whorls of stout yellowish vestiture, inserted at 1.1x of length from base of rostrum; scape slender, 10x as long as broad; funicle with first segment 1.42x as long as second and third combined, third as long as broad, segments three to seven transverse and subequal in length and breadth; funicle 1.85x as long as

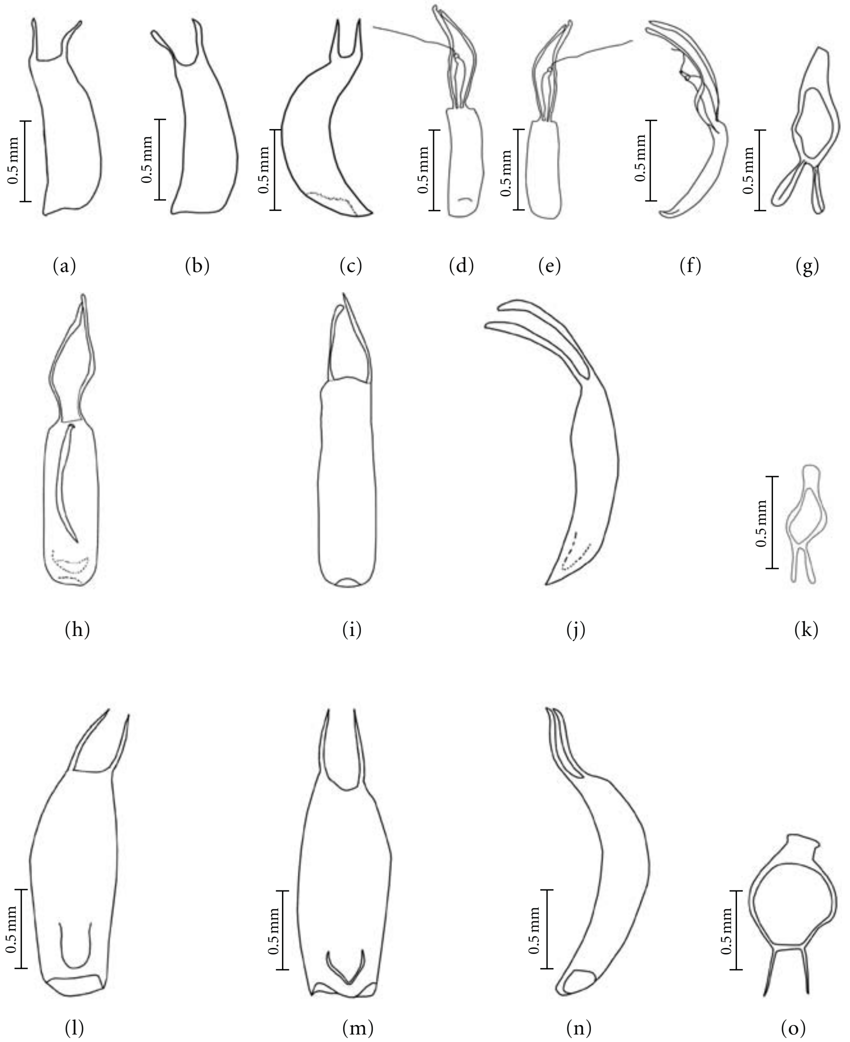

Figure 13: Median lobe (dorsal, ventral, and lateral view) and tegmen: (a)–(c), *A. cyperi*; (d)–(g), *A. meghalayensis* sp. nov.; (h)–(k), *A. richardi* sp. nov.; (l)–(o), *A. spinulatus* sp. nov.

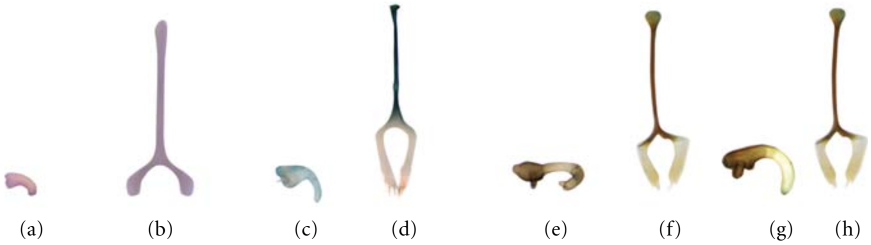

Figure 14: Female genitalia, spermatheca, and spiculum ventrale: (a), (b), *A. cyperi*; (c), (d), *A. oryzae*; (e), (f), *A. richardi* sp. nov.; (g), (h), *A. spinulatus* sp. nov.

club; club with whorls of vestiture (Figure 4(d)). Prothorax 1.19x as broad as long, anterior margin truncate, posterior margin bisinuate, at middle 1.9x as broad as at apex, 0.97x as broad as at base, with broad lateral stripe, which is continuous with scaling of lower surface, interrupted about middle by a small bare kidney-shaped spot, with shallow constriction at apex, gently rounded at sides, set with close coarse punctures, with an abbreviated impunctate median line (Figure 5(d)). Scutellum strongly transverse, coarsely punctate, with narrow vestiture, 1.2x as broad as long. Elytra oblong, separately rounded at apex, at middle 1.21x as broad as at apex, 1.21x as broad as at base, with a large basal patch consisting of lines of vestiture on intervals three to eight, those on fifth and sixth longest, those on four, three,

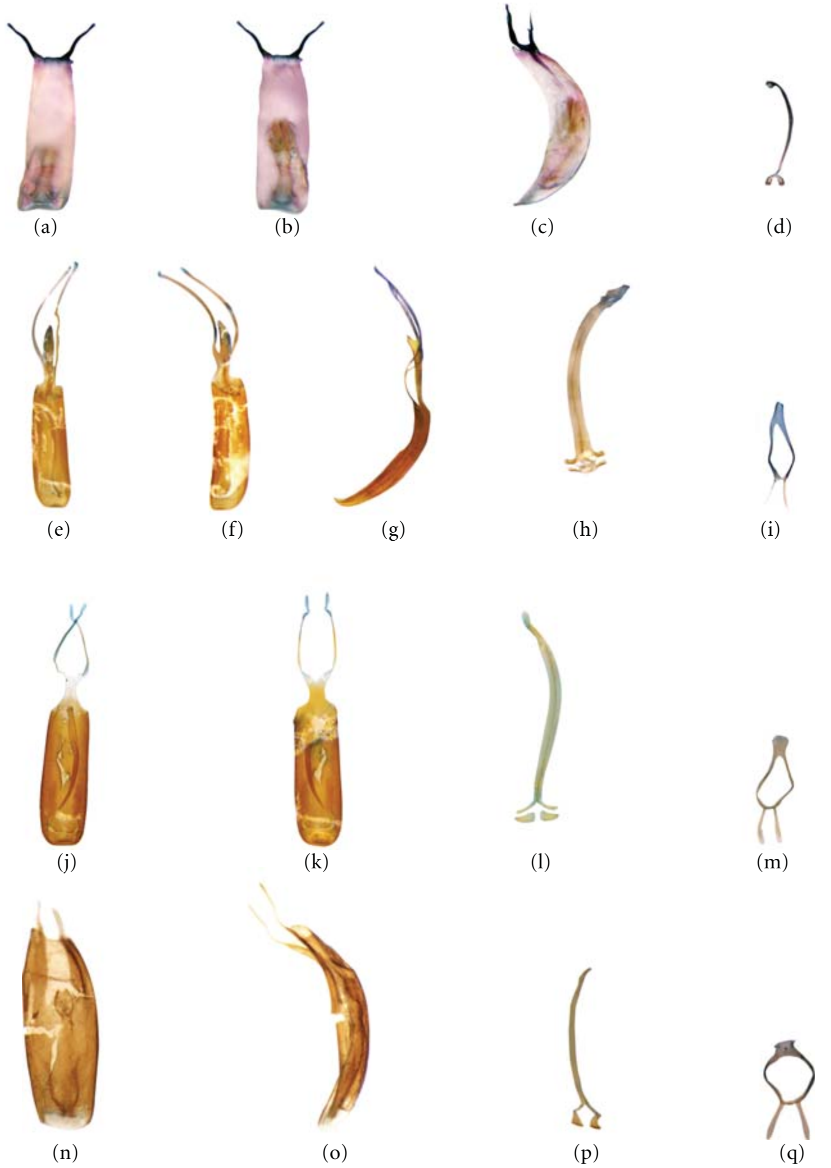

Figure 15: Male genitalia, median lobe (ventral, dorsal, and lateral view), spiculum gastrale, and tegmen: (a)–(d), *A. cyperi*; (e)–(i), *A. meghalayensis* sp. nov.; (j)–(m), *A. richardi* sp. nov.; (n)–(q), *A. spinulatus* sp. nov.

seven, and eight diminishing in the order given, whole patch covering about one-third of elytra and leaving shoulder bare, close behind it another large irregular patch, lines of which intervals 2, 3, 4, 8, and 9 are short, with 5, 6, and 7 being longer, that on 5 longest, with a "v"-shaped apical patch extending from interval 3 to 8; striae deep, striae 10 complete; intervals almost plane, 4x as broad as a striae (Figure 8(d)); elytral vestiture elongate, and with sparse punctures on it (Figure 9(f)), or with irregular patches on it (Figure 9(g)). Legs with densely clothed yellowish white vestiture; all tibiae at apical end on sides with fringes of hairs (Figure 6(c)); profemur 1.13x as long as mesofemur, 1.09x as long as metafemur. Protibia 1.25x and 1.12x as long as meso and metatibia respectively. First tarsal segment 0.85x as long as broad, 0.75x as long as second, 0.46x as long as and 0.53x as broad as third, third as long as broad, fourth 3.75x as long as broad. Prosternum with deep transverse sulcus behind apex, whole lower surface densely scaled, base of prosternum with fovea. Procoxa 2.42x as broad as intercoxal process; mesosternum plate like, raised at apex, intercoxal process 0.9x as broad as mesocoxa; metasternum flat without median line, vestiture closer on meta-episternum, intercoxal process 2x as broad as metacoxa. Venter black, strongly punctate, each with broad scale, anterior margin of first ventrite ogival, posterior margin shallowly straight, 3.73x as broad as long, 1.03x as long as second, second 4.5x as broad as long, 0.92x as long as three and four combined, third and fourth subequal in length, ventrite five 5x as broad as long, posterior margin rounded; pygidium indistinctly punctate, exposed on dorsal side, 1.33x as broad as long (Figure 10(c)).

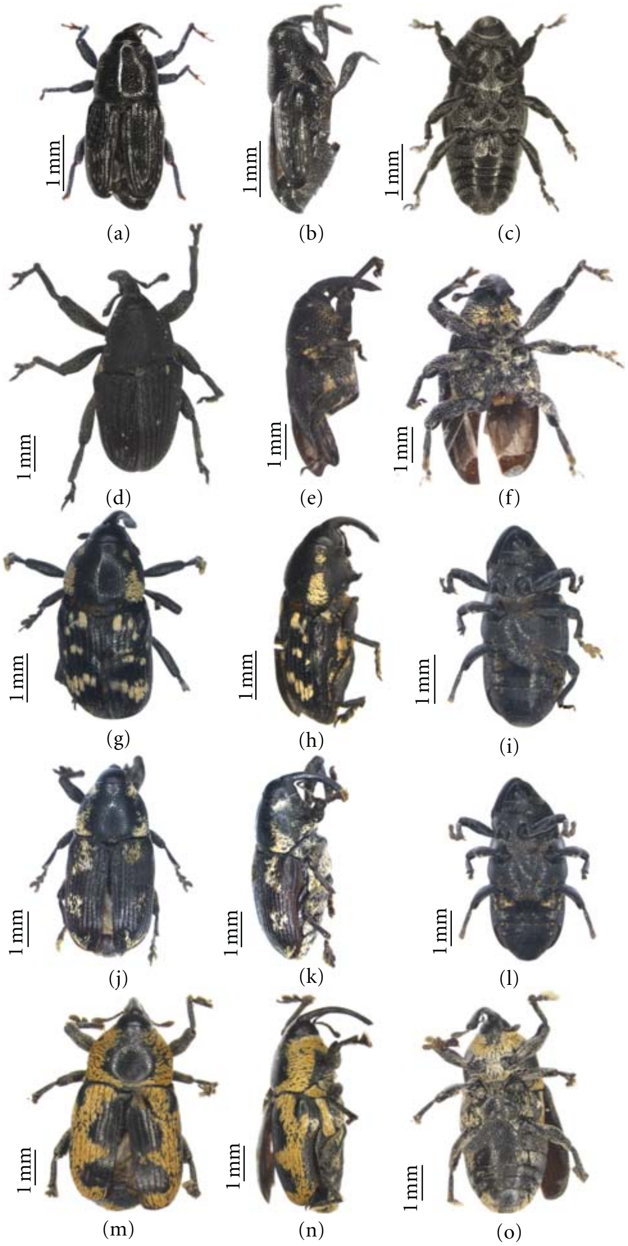

Figure 16: Habitus: dorsal, lateral, and ventral view: (a)–(c), *A. cyperi*; (d)–(f), *A. immaculata*; (g)–(i), *A. meghalayensis* sp. nov.; (j)–(l), *A. richardi* sp. nov.; (m)–(o), *A. spinulatus* sp. nov.

Female Genitalia. Spermatheca not sclerotised, distal arm as long as and as broad as proximal arm, angle between proximal and distal arms not acute, nodulus small, ramus flat, cornu slightly bent, not pointed (Figures 11(b) and 14(c)). Spiculum ventrale with shaft elongate, 1.84x as long as basal plate, basal plate 4.75x as long as broad, apical end rounded with hairs (Figures 11(e) and 14(d)).

Measurements. Total length: 5.38 mm; standard length: 4.90 mm; breadth: 2.28 mm.

Material Examined. 1 ♀, Coimbatore, Coll. and host unknown (written as Paratype and determined by G. A. K. Marshall).

Distribution. India: Tamil Nadu; Andhra Pradesh.

5.5. *Athesapeuta richardi* sp. nov. (Figures 2(e), 3(e), 4(e), 5(e), 7(a), 7(b), 8(e), 9(h)–9(j), 10(d), 11(c), 11(f), 12(c), 13(h)–13(k), 14(e), 14(f), 15(j)–15(m), and 16(j)–16(l)).

Diagnosis. This species is closely allied to *Athesapeuta oryzae* but differs in vestiture which is greyish white as compared to yellowish in *A. oryzae*; rostrum which is without impunctate median line, whereas in *A. oryzae* it is with impunctate median line; tibia with a sharp tooth just above mucro on lateral side and this is absent in *A. oryzae*, and female with cornu of spermatheca having finger-like projection in this species.

Description. Colour shiny blackish yellow. Head with close punctures, 1.76x as broad as long; eyes ventrally placed, 1.53x as wide as long. Rostrum 0.80x as long as head and pronotum combined, strongly curved, gradually widening, punctate, without median line, almost parallel sided, without any subbasal dilation, at middle 0.92x as broad as at apex, 0.63x as broad as at base (Figures 2(e) and 3(e)). Antennae inserted at 1.68x of length from base of rostrum; scape robust, 8.45x as long as broad, almost impunctate; funicle with first segment 0.76x as long as second and third combined, third subequal in length and breadth, segments three to seven transverse, subequal in length and breadth; funicle 2.56x as long as club; club ovate (Figure 4(e)). Prothorax 1.17x as broad as long, with granular punctures, with a broad lateral stripe of white vestiture, which is continuous with scaling of lower surface, interrupted about middle by a small bare kidney-shaped spot, apex with tubular constriction, sides gently rounded, anterior margin truncate, posterior margin shallowly bisinuate; at middle 1.80x as broad as at apex, 0.96x as broad as at base; dorsum gently convex longitudinally, set with close granular punctures, not confluent in curves, without any smooth median line (Figure 5(e)). Scutellum strongly transverse, square shaped, not punctate, without median impression, 1.5x as broad as long. Elytra ovate, without deep subapical impressions and posterior calli absent, apices rounded; at middle 1.62x as broad as at apex, 1.63x as broad as at base; striae shallow, with distant separate punctures which do not encroach on intervals, striae 10 complete; intervals 5.45x as broad as striae, with a row of irregular transverse punctures, each containing a minute vestiture, lateral margin smooth at apex (Figure 8(e)); elytral vestiture either white, with granular serrations reaching apex (Figure 9(h)), or brown with serrated granular ridges on it (Figure 9(i)), or surface without regular serrations but with scattered punctures (Figure 9(j)). Legs coarsely punctate, each puncture containing a grey vestiture; tibiae sulcate, posterior end of all tibiae just before mucro carries sharp tooth (Figure 7(a)); metatibia with fringes of grey hairs on lateroventral side (Figure 7(b)); profemur 1.06x as long as mesofemur, 0.93x as long as metafemur. Protibia 1.19x and 1.07x as long as meso- and metatibia, respectively. First tarsal segment 1.35x as long as broad, 0.98x as long as second, 0.76x as long as third, third 1.24x as broad as long, fourth 4.80x as long as broad. Prosternum with deep transverse sulcus behind apex, base with raised fovea. Procoxa 1.98x as broad as intercoxal process; mesosternum plate like, depressed at base and apex, intercoxal process 1.03x as broad as mesocoxa; metasternum shallowly depressed in middle with longitudinal line, intercoxal process 2.01x as broad as metacoxa. Venter black, strongly punctate, each with broad white vestiture, anterior margin of first ventrite ogival, posterior margin subtruncate, 3.09x as broad as long, 1.64x as long as second, second 4.70x as broad as long, 0.89x as long as three and four combined, third and fourth subequal in length, ventrite five 3.48x as broad as long, posterior margin rounded; pygidium with indistinct punctures, 1.53x as broad as long (Figure 10(d)).

Female Genitalia. Spermatheca more sclerotised at proximal arm, distal arm 0.93x as long as proximal arm, angle between proximal and distal arms acute, nodulus small, tapering towards apex, ramus tubular and long, cornu strongly pointed and with a finger-like projection (Figures 11(c) and 14(e)). Spiculum ventrale with shaft elongate, 2x as long as basal plate, basal plate 3x as long as broad, apical end pointed with hairs (Figures 11(f) and 14(f)).

Male Genitalia. Apophyses 1.06x as long as median lobe, 0.7x as long as spiculum gastrale, 1.84x as long as tegmen; median lobe moderately sclerotised, parallel sided from base to behind middle, apex truncate, at middle 1.25x as broad as at apex, and 1.42x as broad as at base (Figures 13(h)–13(k), 15(j) and 15(k)). Tegmen 1.9x as long as manubrium, 1.58x as long as parameroid lobe, manubrium flat (Figures 13(k) and 15(m)). Spiculum gastrale uniformly thick, curved at apex, with a median line, 12.5x as long as broad (Figures 12(c) and 15(l)).

Measurements of Holotype. Total length: 5.42 mm; standard length: 5.20 mm; breadth: 2.30 mm.

Material Examined. Holotype ♂: India: Meghalaya: Tura, Date and Coll. unknown, from wild plant (latitude: 25° 30′ N; longitude: ° 16′ E). Paratypes: 16 specimens (2 ♂♂, 1 ♀): India: 2 ♂, Meghalaya: Tura, date and coll. unknown, from wild plant; 1 ♀, 12 specimens, Andhra Pradesh: Patancheru, 18.ix.1985, Coll. M.Haq, from agricultural plants.

Distribution. India: Meghalaya; Andhra Pradesh.

Etymology. The name is derived from and in recognition of Dr. Richard Thompson for his contribution towards baridine weevils.

5.6. *Athesapeuta spinulatus* sp. nov. (Figures 2(f), 3(f), 4(f), 5(f), 8(f), 9(k), 9(l), 10(e), 11(g), 12(d), 13(l)–13(o), 14(g), 14(h), 15(n)–15(q), and 16(m)–16(o))

Diagnosis. This species is closely related to *A. immaculata* but differs in its larger size, with dense vestitures all over body; antennae with all funicular segments with four rows of spines; elytra ovate (oblong ovate in *immaculata*).

Description. Colour black. Head with shallow sparse punctures, 1.8x as broad as long; eyes ventrally placed, 1.48x as wide as long. Rostrum 0.91x as long as head and pronotum combined, curved, gradually widening, broadest at apex, closely punctate at basal side on lateral aspect, each puncture with yellow vestiture, dorsal surface smooth, at middle 0.75x as broad as apex, 0.66x as broad as at base, without any subbasal dilation (Figures 2(f) and 3(f)). Antennae inserted at 1.36x of length from base of rostrum; scape slender, long, 8.75x as long as broad, almost impunctate; funicle with first segment 1.1x as long as second and third combined, third 1.3x as long as broad, segments three to seven transverse and subequal in length and breadth; funicle 2.53x as long as club, all segments carry sharp four rows of spines on its surface, seventh funicular segment broadest, which is not in continuous with club; club ovate (Figure 4(f)). Prothorax 1.28x as broad as long, with granular punctures at centre, a stripe of yellow vestiture on lateral aspect on both sides, which is continuous with scaling of lower surface, without tubular constriction at apex, broadly rounded at sides, anterior margin truncate, posterior margin shallowly bisinuate, at middle 2.22x as broad as at apex, 1.03x as broad as at base, dorsum gently convex longitudinally without any median line (Figure 5(f)). Scutellum strongly transverse, not punctate, without median impression, 1.5x as broad as long. Elytra ovate, without subapical impressions and without posterior calli, apices rounded, at middle 1.2x as broad as at apex, 1.5x as broad as at base; striae shallow, with distant separate punctures which do not encroach on intervals, striae 10 complete; intervals 6x as broad as a striae, with a row of catenulate transverse punctures, each containing a minute black recumbent vestiture, lateral margin smooth at apex, a patch of yellowish vestiture starts from interval 2 which continues up to 10 (Figure 8(f)); elytral vestiture yellow, either with broad parallel striae running from base to apex (Figure 9(k)), or with granular striations on surface (Figure 9(l)). Legs coarsely punctate, each puncture containing a grey vestiture; tibiae sulcate; profemur as long as mesofemur, 0.8x as long as metafemur. Protibia 1.25x and 1.1x as long as meso- and metatibia, respectively. First tarsal segment 1.66x as long as broad, 1.5x as long as second, and as long as third, third 1.66x as broad as long, fourth 6.66x as long as broad. Prosternum with deep transverse sulcus behind apex, base of prosternum with raised fovea. Procoxa 1.53x as broad as intercoxal process; mesosternum plate like, depressed at base and apex, intercoxal process 1.1x as broad as mesocoxa; metasternum shallowly depressed in middle with impressed longitudinal line, intercoxal process 2.05x as broad as metacoxa. Venter black, clothed with broad greyish yellow vestiture, strongly punctate and each with broad vestiture, anterior margin of first ventrite sinusoidal, posterior margin subtruncate, 2.91x as broad as long, 1.72x as long as second, second 4.75x as broad as long, 0.75x as long as three and four combined, third and fourth subequal in length, ventrite five 2.48x as broad as long, its posterior margin rounded; pygidium distinctly punctate, broadly exposed with long hairs from each puncture, 1.02x as broad as long (Figure 10(e)).

Female Genitalia. Spermatheca more sclerotised at proximal arm, distal arm as long as proximal arm, angle between proximal and distal arms acute, nodulus small, tapering towards apex, ramus small, cornu bent and blunt at apex (Figure 14(g)). Spiculum ventrale with shaft elongate, 2.5x as long as basal plate, basal plate 2.85x as long as broad, apical end truncate with hairs, basal end with a lateral projection (Figures 11(g) and 14(h)).

Male Genitalia. Apophyses 0.44x as long as median lobe, 0.4x as long as spiculum gastrale, as long as tegmen; median lobe moderately sclerotised, parallel sided from base to behind middle, apex truncate, at middle 1.9x as broad as at apex, 1.35x as broad at base (Figures 13(l)–13(n), 15(n), and 15(o)). Tegmen 2.85x as long as manubrium, 2x as long as parameroid lobe, manubrium short and flat (Figures 13(o) and 15(q)). Spiculum gastrale uniformly thick, not curved at apex, with a median line, 16.66x as long as broad (Figures 12(d) and 15(p)).

Measurements of Holotype. Total length: 5.69 mm; standard length: 5.30 mm; breadth: 2.33 mm;

Material Examined. Holotype ♂: India: Haryana: Jind, 9.vii.1986, coll. unknown, from grass (latitude: 29° 48′ N; longitude: 78° 26′ E). Paratypes (2 ♂♂, 2 ♀♀): India: 1 ♀, Haryana: Jind, 9.vii.1986, coll. unknown, from grass; 2 ♂♂, Andhra Pradesh: Patancheru, 18.ix.1985, coll. unknown, from wild aquatic plant; 1 ♀ (no data).

Distribution. India: Haryana; Andhra Pradesh.

Etymology. The specific name is given after the funicular segments which have sharp spines in four rows.

6. Key to the Indian Species of *Athesapeuta*

(1) (a) Rostrum without carinae—2.

(b) Rostrum with carinae—3.

(2) (a) Rostrum with four rows of small punctures; prothorax with a broad impunctate median stripe; profemur with fringes of long vestiture on ventral surface (Figure 6(a)); protibia with sharp tooth on inner edge at about middle in males (Figure 6(b)), females without it—*cyperi*.

(b) Rostrum without four rows of small punctures; prothorax without a broad stripe; profemur without fringes of long vestiture on ventral surface; protibia without tooth in males or females—4.

(3) (a) Tibiae at apical end on sides with fringes of hairs (Figure 6(c)); elytra oblong, with large basal patch consisting of lines of vestiture on intervals three to eight, those on fifth and sixth longest, those on four, three, seven, and eight diminishing in the order given, whole patch covering about one third of the elytra and leaving the shoulder bare, close behind it another large irregular patch, lines of which intervals 2, 3, 4, 8, and 9 are short, and 5, 6 and 7 being longer, that on 5 longest, with a "v"-shaped apical patch extending from interval 3 to 8—*oryzae*.

(b) Tibiae without fringes of hairs; elytra not oblong but ovate or oblongovate—5.

(4) (a) Funicular segments without spines; posterior end of all tibiae carries sharp tooth (Figure 7(a)), metatibia with fringes of grey hairs on lateroventrally (Figure 7(b)); apex of prothorax with tubular constriction, granular punctures all over, sides gently

rounded; apophyses 1.06x as long as median lobe; spiculum gastrale 12.5x as long as broad—*richardi*, sp. nov.

(b) Funicular segments with spines (Figure 4(f)); posterior end of all tibia does not carry sharp tooth; apex of prothorax without tubular constriction, granular punctures only at centre, sides broadly rounded; apophyses 0.44x as long as median lobe; spiculum gastrale 16.66x as long as broad—*spinulatus*, sp. nov.

(5) (a) Prothorax with broad lateral stripe, with smooth median line; elytra ovate (Figure 8(c)); elytal vestiture either brownish yellow, with basal end blunt, apex pointed with lines on surfaces reaching apex (Figure 9(d)), or greyish white, pear shaped, broad at the apex with granular striations on the surface (Figure 9(e))—*meghalayensis*, sp. nov.

(b) Prothorax without broad lateral stripe, without median line; elytra oblongovate (Figure 8(b)); elytral vestiture either yellowish white, base tapering, apex concave, surface with granular serrations (Figure 9(b)), or brownish yellow, both ends broader, surface with dense granular serrations (Figure 9(c))—*immaculata*.

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Research Article

Laboratory Rearing of *Laricobius nigrinus* (Coleoptera: Derodontidae): A Predator of the Hemlock Woolly Adelgid (Hemiptera: Adelgidae)

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Coleopteran species are biological control agents of numerous invasive pests. *Laricobius nigrinus* (Coleoptera: Derodontidae), a predaceous, univoltine species, spends the summer aestivating but is active for the rest of the year. *Laricobius nigrinus* possesses many essential attributes for effective biological control of the hemlock woolly adelgid (Hemiptera: Adelgidae). The predator must be reared in large numbers for field releases. We describe some of the studies that led to the successful procedures currently used for mass rearing *L. nigrinus*.

1. Introduction

Laricobius nigrinus Fender (Coleoptera: Derodontidae) is a potential biological control agent of hemlock woolly adelgid (HWA), *Adelges tsugae* Annand (Hemiptera: Adelgidae), an exotic pest that attacks and kills hemlock trees (*Tsuga canadensis* L. (Carr.) and *T. caroliniana* Engelm.) in the eastern United States. Since its first release in 2003 [1], *L. nigrinus* has been established in the plant hardiness zones 7a, 6b, and 6a in the USA [2].

The ability to mass rear a biological control agent is fundamental in any classical biological control program. Delays in the program are often related to difficulties in laboratory rearing [3]. Biological control of HWA was hindered by poor success in mass rearing promising predators. Efficient rearing methods for producing large numbers of *L. nigrinus* is critical for it to be a viable biological control agent of HWA. HWA infests eastern hemlock in over 40% of its geographic range [4] and continues to spread [5], causing extensive damage and mortality of *Tsuga* spp. in the eastern USA [6, 7].

Laboratory rearing of *L. nigrinus* at Virginia Tech was initially constrained by high mortality rates and a lack of knowledge as to which life stages incur significant mortality because *L. nigrinus* has an obscure and complicated lifecycle. Additionally, adults have no observable sexual dimorphism [8]. They emerge from the soil in the fall and feed on developing HWA nymphs throughout the winter [9]. In early spring, eggs are laid in adelgid ovisacs where the larvae hatch and develop through four instars feeding on HWA eggs [10]. Mature larvae drop to the soil, each forms a pupal cell, pupates, and enters aestivation as an adult for the summer.

1.1. The Sequential Development of a Rearing Procedure from 2000 to 2010. Initially, adults of predators were reared on HWA-infested twigs maintained in flat-bottom Plexiglass oviposition cages containing a layer of peat moss. Fourth instars, upon reaching maturity, dropped down to the layer of peat moss on the floor of the cage, where they pupated and developed into adults.

Two primary objectives that resulted from our initial rearing efforts were to

(i) Identify the life stages of *L. nigrinus* that incur high developmental mortality in the laboratory,

(ii) Improve production at each life stage where survival was low.

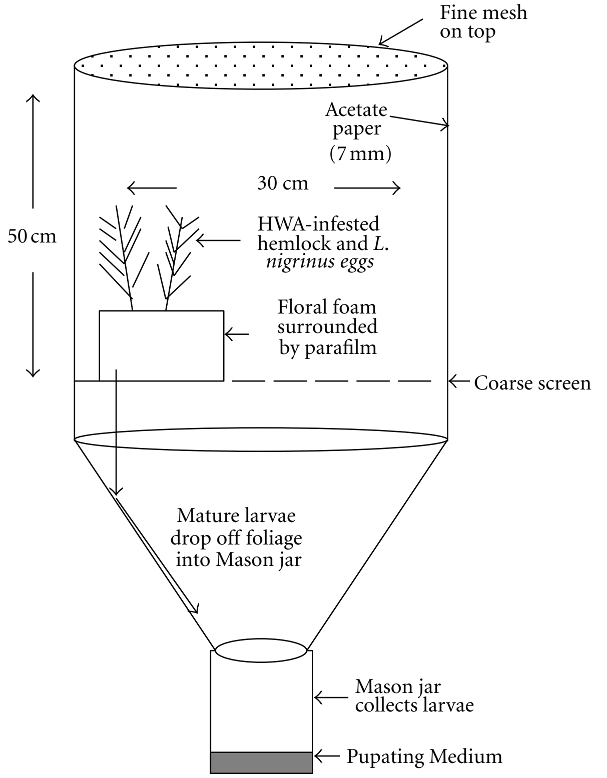

Fine mesh on top
Acetate paper (7 mm)
30 cm
50 cm
HWA-infested hemlock and *L. nigrinus eggs*
Floral foam surrounded by parafilm
Coarse screen
Mature larvae drop off foliage into Mason jar
Mason jar collects larvae
Pupating Medium

Figure 1: Diagram of the form and function of the *Laricobius nigrinus* larval rearing cage.

2. Methods and Materials

Studies were partitioned into two periods. Efforts focused on determining/enhancing survival of each life stage from 2000 to 2004. Emphasis on improving production numbers of *L. nigrinus* was the focus from 2005 to 2010.

2.1. Determining the Survival of Each Life Stage: 2000–2004. A rearing cage was designed to intercept mature larvae as they dropped from the hemlock foliage (Figure 1). The top section of the cage is an open-ended 30 cm diameter Dura-Lar acetate cylinder (0.018 cm thick) (Grafix, Cleveland, OH) with the top end covered with PeCap polyester mesh (0.14 mm^2) (Sefar America Inc., Kansas City, MO). The base of the cage is a nonswirl galvanized steel funnel (McMaster-Carr Co., Atlanta, GA) with a top diameter of 30 cm in which the acetate cylinder is placed. Hardware cloth ($5 \times 5\,\text{mm}^2$ mesh size) is cut to fit the inside of the funnels. The hardware cloth is placed inside the funnel base as a coarse screen, resting where the funnel constricts. HWA-infested hemlock twigs with *L. nigrinus* eggs are inserted into water-saturated (Oasis Deluxe) floral foam blocks ($8 \times 10 \times 3\,\text{cm}^3$ bricks). Each block is wrapped in Parafilm M (Fisher Scientific, Hampton NH) to retain moisture and prevent prepupae in search of a pupation site from entering the blocks.

Floral foam blocks containing hemlock branches with *L. nigrinus* eggs are placed on the hardware cloth within the funnel cages. When eggs hatch, the larvae develop in the hemlock foliage, feeding on HWA eggs within the funnel cages. Mature larvae drop from the foliage and accumulate in the (Kerr) 8 oz. mason jars (Jarden Home Brands, Muncie, IN) attached beneath the funnel. The jars are spray-painted black and the covers cut open. The jar cover rims are attached to the bottom of the funnels with glue; this way mason jars can be easily removed and reattached. Two teaspoons of steam-sterilized peat moss and 2 pieces of moistened filter paper (Whatman No. 1: 35 mm diam.) are placed in the mason jars three weeks after adding *L. nigrinus* eggs to the cage. Larvae having ingested a sufficient amount of HWA eggs drop from the hemlock foliage in search of a pupation site. The Mason jars are checked daily and mature larvae counted.

Funnel cages were set up in custom-built racks and held in concrete block rooms maintained at 13° ± 2°C with fluorescent lights on timers programmed to provide a photoperiod that mimicked natural conditions (increasing day length from 12 to 14 h light over spring). A detailed description of the recommended rearing procedures during these months is provided by Lamb et al. [11].

2.2. Enhancing Survival for Each Life Stage

2.2.1. Feeding Phase: Adults and Developing Larvae from October to June

(1) Adult Survival at 4° and 13°C after Emergence from Aestivation. Early emergence of adults had been a persistent issue, because of limited available HWA prey, resulting in high mortality. Techniques to minimize early emergence of aestivating *L. nigrinus* adults were developed by Lamb et al. [12], but some beetles continue to emerge before HWA break aestivation, making survival of early emerging beetles a continuing issue.

In 2002, in anticipation of early predator emergence, eggs laid by sistentes were stored in a refrigerator at 4°C from April to August. As adults emerged from aestivation before HWA, 70 of them were transferred to feeding containers with hemlock branches infested with aestivating HWA nymphs and one cold-stored branch with developing progredientes. This was not an ideal number of preys for the 70 adult beetles, but additional food was not available for the early emerging adults. There was sufficient HWA prey for only eight containers. All adults that emerged early (before HWA break aestivation) were fed every 12 days throughout September with HWA. One half of the containers was stored in environmental chambers at 4°C 12 : 12 (L : D) h, and the other half in a cold room at 13°C 12 : 12 (L : D) h. The number of adults surviving at each feeding period was recorded.

(2) Adult Sex Ratio within the Colony. Feeding containers were randomly selected from the colony, and individual adults were placed in separate 384 mL, clear plastic containers with a heavily HWA-infested hemlock twig (20–30 cm total linear length). After three days, each twig was examined for eggs with a microscope. Adults that did not lay eggs were

returned to separate containers with fresh host material and reexamined after another three days. Adults that still did not oviposit were considered to be males or unfertile females. In March and April 2003, adults from 21 containers were dissected and sexed ($n = 699$).

(3) Effect of Predator Egg Density on Survivorship to Mature Larvae. A randomized block design experiment was set up with five densities of *L. nigrinus* eggs (10, 20, 30, 40, or 50) in funnel cages with an adequate amount of HWA prey. The five densities formed a block and two blocks were arranged in 10 funnel cages. There were two replicates with a total of 20 cages. The number of larvae that reached maturity and dropped to the mason jar below each funnel was counted. A one-way analysis of variance was used to determine whether egg density influenced larval survival ($n = 20$).

(4) Survival of "Immature" Larvae. Immature larvae frequently drop early into collecting jars. They are usually smaller, darker in color, and less mobile than mature larvae and often still have white wool attached to their dorsal side. These larvae will search for prey if transferred back to a hemlock branch rather than drop off the branch as mature ones do. Immature larvae found in mason jars throughout the spring of 2002 and 2003 were transferred to fresh hemlock branches with HWA ovisacs and placed in one of nine "immature" funnel cages. The number of these larvae reaching maturity from each funnel cage was recorded and the overall survival rate calculated.

The mature larvae collected from the "immature" funnel cages were transferred to corresponding soil containers. The number of adults emerging from these containers was recorded and the emergence rate of adults that had left the hemlock foliage prematurely as immature larvae was calculated.

2.2.2. Nonfeeding Phase. In nature, mature larvae burrow into the soil immediately upon dropping from the hemlock foliage. In the lab, these larvae are transferred from the mason jars below the funnel cages to containers for pupation, eclosion, and adult aestivation. These containers have two layers of filter paper as a base lining to prevent pooling when the pupation medium is moistened with methyl paraben solution (0.42 g/250 mL distilled water) throughout the summer to inhibit fungal growth. To each pupation container is added at least 5 cm of pupating medium consisting of an equal mixture of peat moss (Premiere Horticulture Inc., Quakertown, PA), sphagnum moss (Mosser Lee Long Fiber, Westsel Inc., Harrisonburg, VA), and sand (Quikrete Play Sand, The Quikrete Product Line, Atlanta, GA). Peat moss is sifted through hardware cloth ($3 \times 3\,\text{mm}^2$), and sphagnum moss is ground in an industrial blender and sifted through hardware cloth. The mixture is moistened and steam-sterilized twice for 12 h, separated by 24 h at room temperature, and placed in plastic pupation containers that have at least one polyester mesh-covered hole for ventilation. Larvae burrow into the pupation medium, create a cell within the soil, assume a c-shaped position in the cell, and develop into pupae in approximately 14 days.

Pupation containers are kept at 15° ± 2°C and 12 : 12 (L : D) photoperiod for optimal pupal development [13]. Each container is maintained at ~30% saturation, receiving ~5–8 squirts of methyl paraben solution weekly. Pupation lasts approximately 14 days, and the newly eclosed adults remain under the soil surface, in aestival diapause, throughout the summer. This new generation of adults begins emerging from the soil in early fall. The pupation containers are checked daily for emerging adults over a period of several months (August–December). Emerging adults are transferred to adult containers.

(1) Prepupal Survival, Pupal Sex Ratio, and Adult Emergence from Aestivation. As sex cannot be determined in the adult stage because genitalia retract into the body after eclosion [14], sex ratio of progeny was obtained by microscopic examination of the external genitalia characters of pupae [15]. In spring 2003, six soil containers were randomly selected from the colony three weeks after larvae entered the soil. Using a paintbrush, the soil was sifted and each pupa sexed and transferred to a corresponding male or female container with fresh pupation medium. These containers were maintained with the rest of the colony in the cold room (15° ± 2°C and 14 : 10 (L : D) photoperiod, watered weekly) throughout the summer. Adults emerging from each container in the fall were recorded daily.

In spring 2004, 28 soil containers were selected to assess pupal survival and sex ratio. During this year, there was considerably more mold development in soil containers than in previous years even though the same methods were used for preparing the pupation medium. Soil containers with high levels of mold (present on entire surface and throughout the pupation medium), medium (present on entire surface only) and low mold contamination (present on less than half the surface) were selected three weeks after larvae had entered the soil. For each container, the soil was sifted through and surviving pupae sexed and transferred to corresponding male and female containers with fresh pupation medium. These containers were maintained with the rest of the colony at 15° ± 2°C, 12 : 12 (L : D) photoperiod until adult eclosion, 19° ± 2°C, 16 : 8 (L : D) h until 27 September 2004, and then decreased to 13° ± 2°C, 10 : 14 (L : D) photoperiod until emergence from the pupation medium [12]. Each container was watered weekly throughout the study period and the adults emerging from each container in the fall were recorded.

(2) Effect of Abiotic Factors on Pupal Survival/Adult Emergence from Aestivation

(a) Soil Moisture and Disturbance. Ten mature larvae were placed in each of 48 clear polystyrene containers (950 mL) that has an 8 cm diameter ventilation hole in the lid and 2 layers of filter paper moistened with methyl paraben solution. Pupation medium was added to a height of 2 cm

in each container (3 : 2 mixture of potting soil : peat moss) (Miracle-Gro, Scotts Company, Marysville, OH) maintained at one of three moisture levels (% saturation): high (35–45%), medium (20–25%), or low (5–10%). A Lincoln soil moisture meter (Forestry Suppliers Inc., Jackson, MS) was used to measure the relative soil moisture level in control containers (set up at same time with no larvae added) every other day throughout the study. Moisture levels were maintained by adding the same amount of water to test containers as the control containers.

For each moisture level, half of the containers was randomly selected and disturbed by sifting the pupation medium and counting the number of live individuals. The soil was sifted twice, three and six weeks following larval entry into the soil to determine survival to the pupal stage. Surviving individuals were put back in the soil in the container, and the total number of adults emerging from each container in the fall was recorded daily. This experiment was set up as a randomized complete block design with larval cohort serving as blocks (8). The effects of moisture and disturbance on pupal survival and adult emergence were determined with a 2-factor ANOVA using proc glm in SAS; means were separated using Fishers LSD ($n = 48$).

(b) Pupation Medium and Moisture Level. Four types of media were maintained at three moisture levels (12 treatments) during *L. nigrinus* pupation and aestivation to determine optimal conditions for survivorship at the pupal and adult stages. The experiment was set up as a generalized randomized block design with eight replicates in each of four blocks with larval cohorts serving as the blocks. The four soil types varied in concentrations of ground sphagnum moss, peat moss, and sand (3 : 0 : 1, 2 : 1 : 1, 1 : 2 : 1, 0 : 3 : 1 (sphagnum : peat : sand)).

For each block, 96 plastic containers (384 mL) with ventilated lids (5 cm diam.) were set up with two layers of moistened filter paper and 4 cm of pupation medium. Five mature larvae were added to each container. This process was repeated four times with a total of 20 genetically diverse larvae in each container. A third of each soil type was maintained at one of the following moisture levels: 30, 45, and 60% saturation. A control container representing each of the 12 treatment combinations was set up at the same time as each block. Moisture level of control containers was measured each week using a Lincoln soil moisture meter. The same amount of methyl paraben solution (same weight) was added to each treatment and control container.

To estimate survival through pupation, one container from each treatment was randomly selected eight weeks after larval entry into the soil and the media were scooped out of containers and combed thoroughly for recently eclosed adults under the microscope. Survivorship and approximate depth were recorded for each recovered adult. Total number of adults and time of emergence for each container were recorded daily from July 22 to December 11, 2002. The proportion of adults emerging and average time of emergence were compared across treatments using a 2-factor ANOVA in SAS, and means were separated using Fishers LSD ($P < 0.05$).

(c) Optimal Density per Pupation Container. Three levels of larval density per container were tested using a generalized randomized block design with larval cohort serving as the block. For each block, 13 plastic containers (950 mL low density polyethylene) were set up with 5 cm of pupation medium (2 : 2 : 1 sphagnum : peat : sand) and two layers of filter paper. Four replicates of five, 10, or 15 mature larvae were added to each container; the 13th container served as a moisture control. This was repeated six times to produce densities of 120, 240, and 360 individuals/larval density. In all, five blocks were set up on five consecutive days in May. Pupation medium was maintained weekly at 45% saturation by monitoring and manipulating the moisture level of the control containers using the Lincoln soil moisture meter and distilled water. Equal volumes of methyl paraben solution were added to the test containers as in the control containers using a balance scale. Adult emergence and duration of aestivation were determined for each container and compared across treatments using a 1-way ANOVA in SAS ($P < 0.05$).

(d) Assessing Importance of Sterilized Soil. Sterilized soil showed increasing levels of mold in 2004 and 2005. An experiment to test the effects of soil type (soil mix versus forest soil) and sterilization using an autoclave was initiated in May 2006. Thirty mature *L. niginus* larvae were placed in each square container with either soil mix or forest soil that had been autoclaved or left unsterilized. Five containers were used for each treatment ($n = 5$). Soil moisture was maintained at 20% to avoid excessive mold. Containers were kept at 15°C until beetles had entered the adult stage and then maintained at 19°C. Percentage emergence data were analyzed as a one-way CRD ANOVA using SAS with arcsine-square root transformation to stabilize variances.

2.3. Rearing Procedures to Increase Production Numbers of L. nigrinus: 2005–2010. The experimental studies from 2000 to 2004 improved rearing practices for 2005 to 2010. This included selection of the appropriate temperature, sex ratio, predator egg density, size of rearing containers, pupation medium, density of number of beetles, and abiotic factors that influence survival to adult emergence. Inclusion of field-collected beetles in the founding colony is important. Beginning in 2005, collections of adults from field populations in western USA were carried out annually and included into the rearing colony to provide hybrid vigor.

3. Results

3.1. Survival of Each Life Stage: 2000–2004. The numbers of larvae that reached maturity, pupating, and aestivating adults emerging were obtained for the first time in 2001. The colony began with 350 field-collected adults that produced 7,500 larvae, of which 69% pupated. Adults in aestivation and immediately following emergence from aestivation suffered high mortality, with 200 adults surviving in the fall (Table 1). In 2002, 1,000 field-collected adults added to the founding colony improved larval production that was much higher

Table 1: Survival of *Laricobius nigrinus* (Coleoptera: Derodontidae) at different life stages from laboratory rearing efforts between 2000 and 2004.

Life stage	Total number of individuals and mortality rate (%) per life stage				
	2000	2001	2002	2003	2004
Reproductive adults (starting colony)	200[F]	350[F]	100[L] 1,000[F]	3,000[L]	7,000[L] 660[F]
Mature larvae drop from foliage	N/A	7,500 (28%)	37000 (~30%)	30,000 (30+%)	27,000 (30+%)
Pupae	N/A	5,175 (31%)	25,900 (27%)	12,300 (41%)	7,000[Mf] + 2,200 [M] (43%), (94%)
Adults emerging from aestivation	30 (85%)	1,867 (36%)	21,000 (19%)	13,000 (6%)	8,000 (13%)
Adults surviving as HWA breaks aestivation in October	8 (74%)	200 (89%)	3,700 (83%)	12,000 (8%)	8,000 (0%)

[F] Adults collected in the field from western hemlock trees in Victoria, British Columbia.
[L] Adults reared in the laboratory at Virginia Tech.
[Mf] In soil containers that were free of mold contamination.
[M] In soil containers contaminated with mold.

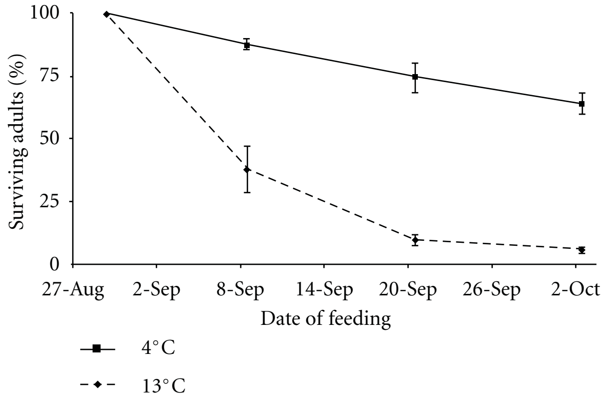


Figure 2: Mean percentage (±S.E.) of the original adults surviving at each feeding period maintained at 13° and 4°C in the weeks following emergence in 2003.

than in 2003 where only lab-reared beetles were used. Pupal survival was greater as well in 2002 compared with 2003 and 2004. Considerable pupal mortality in 2004 was likely attributed to contamination of the soil by mold.

3.2. Developing Rearing Procedures for Each Life Stage

3.2.1. Feeding Phase

(1) Adult Survival at 4° and 13°C after Emergence from Aestivation. Adult survival was higher and more consistent following emergence from aestivation when held at 4°C than at 13°C (Figure 2). Mortality rate was high during the first 12 days when over 60% of adults stored at 13°C and 13% at 4°C died. On October 2, after three feedings, 5.7% of the adults held at 13°C and 64.2% of those at 4°C were still alive.

(2) Adult Sex Ratio within the Colony. Of the 699 adults sexed, 458 were ovipositing females. Mean female-to-male ratio ($\overline{X}$ ± S.D.) within a container during the peak oviposition period in March/April was 1.91 (±0.18) : 1.

(3) Effect of Predator Egg Density on Larval Survival. Density of eggs per funnel cage (up to 50 individuals per cage) did not affect larval survival ($F_{4,19} = 0.90$, $P = 0.490$). Mean percentage of eggs ($\overline{X}$ ± S.D.) that hatched and newly eclosed first instar reaching larval maturity was 73.7 ± 15.4%, indicating that higher densities of eggs can be used in funnels to maximize larval production.

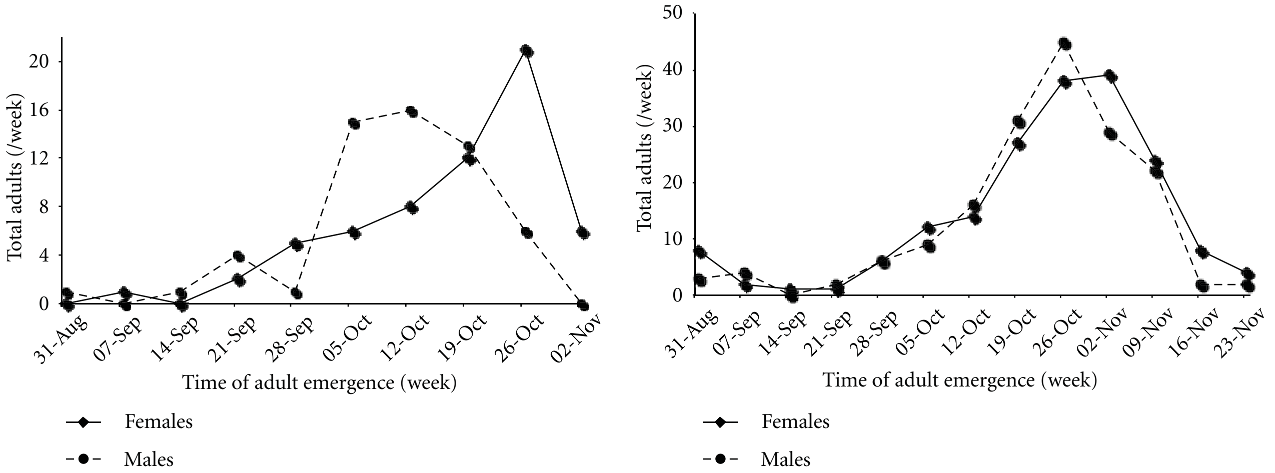
(4) Survival of "Immature" Larvae. There were 6,116 immature larvae recovered in mason jars throughout the spring of 2002. Of these, 3,486 (57%) reached maturity after being transferred back to funnel cages with prey and completed larval development.

In the spring of 2003, 8,002 immature larvae were collected from the mason jars and transferred back to funnel cages to complete development. Of these, 3,905 larvae (48.8%) completed development, entering the soil for pupation and aestivation. In fall, 1,843 of these individuals emerged from aestivation, representing 23% of immature larvae reaching adulthood.

3.2.2. Nonfeeding Phase

(1) Prepupal Survival, Pupal Sex Ratio, and Timing of Adult Emergence from Aestivation. In fall 2003, the mean percentage of larvae ($\overline{X}$ ± S.D.) that developed into pupae per soil container was 58.7 ± 18.2%. Of the 183 pupae sexed, 95 were female and 88 were male, with sex ratio ($\overline{X}$ ± S.D.) of 1.08 : 1 ± 0.51 : 1 F : M per adult container. Females and males had similar survival throughout aestivation (64.2 and 64.7%, resp.); however, males emerged earlier than females (Figure 3(a)).

In 2004, of the 933 pupae sexed, 515 were female and 418 were male. Ratio of female to male pupae per container was 1.19 : 1 ± 0.74 : 1. Survival of males through aestivation (40.9%) was higher than females (35.7%), but the time at which they emerged was better synchronized than the previous year (Figure 3(b)). Different emergence between years is attributed to a change in storage conditions; in 2004, adults were maintained at a higher temperature (19°C) throughout the summer, based on findings reported in [12].



(a) (b)

FIGURE 3: Weekly total number of female (solid line) and male (broken line) adults emerging from aestivation in fall 2003 (a) and 2004 (b). Note difference in scale: 2003 from 238 pupae sexed; 2004 from 933 pupae sexed.

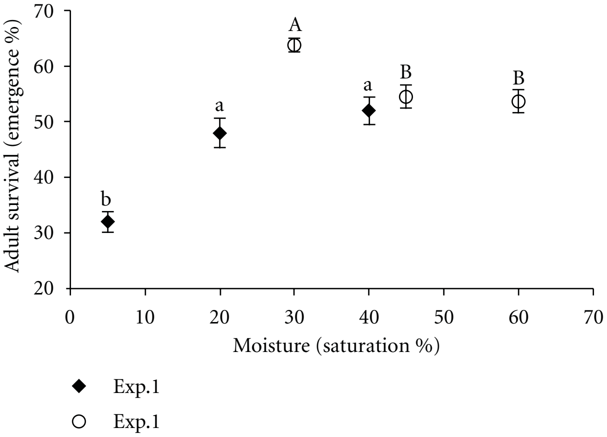


FIGURE 4: Mean percentage (±S.E.) of adults emerging when maintained at 5, 20, and 40% soil saturation in Exp. 1 and at 30, 45, and 60% soil saturation in Exp. 2. Means with different lower case letters are significantly different (Exp. 1, $n = 24$) and means with different upper case letters are significantly different (Exp. 2, $n = 372$).

(2) Effect of Abiotic Factors on Pupal Survival/Adult Emergence from Aestivation

(a) Soil Moisture and Disturbance on Pupal Survival. Moisture level did not affect the development of pupae ($F_{(1,\,23)} = 0.03$, $P = 0.9709$). The mean percentage of larvae ($\overline{X}$ ± S.E.) developing into pupae at all moisture levels was 69.1 ± 2.3%. However, the number of adults emerging from aestivation in the fall was affected by moisture level ($F_{(2,\,47)} = 6.02$, $P = 0.0050$) and by disturbance of pupae ($F_{(1,\,47)} = 4.08$, $P < 0.0498$). More adults emerged from containers with 40% or 20% moisture than at 5% (Figure 4). Disturbance of the soil to recover individuals lowered adult emergence by 10%. Mean emergence ($\overline{X}$ ± S.E.) of undisturbed pupae emerging as adults was 49 ± 2.3% compared with 39 ± 3.0% for disturbed pupae.

(b) Pupation Medium and Moisture Level. Mean pupal survival was 72.1 ± 3.5% ($\overline{X}$ ± S.E., $n = 20$). It was not affected by the composition of medium ($F_{(2,\,6)} = 1.19$, $P = 0.348$) nor the moisture level within the range of 30–60% ($F_{(3,\,6)} = 0.329$, $P = 0.804$). However, the number of adults emerging from aestivation was lower for individuals held in pure peat moss than those in pure sphagnum moss or the 1 : 2 sphagnum:peat mixture (Figure 5). The latter mixture had the highest mean emergence (61.5±0.8%) and the lowest was from containers with pure peat moss (52.8 ± 1.1%). Soil moisture did affect the percentage of adults emerging from aestivation (Figure 4, 2nd experiment). Beetles stored in soil with 30% moisture level emerged in greater numbers than those held at higher moisture levels.

The timing of emergence from diapause was not affected by soil type ($F_{(3,\,284)} = 0.30$, $P = 0.822$). Adults remained in the ground for 123.9 ± 0.25 days. Moisture level affected time of emergence as adults in soil maintained at 30 or 45% saturation emerged before those held at 60% saturation ($F_{(11,\,284)} = 22.51$, $P < 0.0001$). However, mean duration of aestivation of adults stored at 60% moisture level (126.4 ± 0.24) was only four days longer than the duration at lower moisture levels (122.6 ± 0.25).

(c) Optimal Density per Pupation Container. The density of adults per container (30, 60, and 90) did not influence the percentage of adults emerging from aestivation (32.2 ± 4.57%) ($F_{(4,53)} = 1.73$, $P = 0.1865$) or the duration of aestivation (142.7 ± 0.6 days) ($F_{(4,\,53)} = 0.02$, $P = 0.9836$).

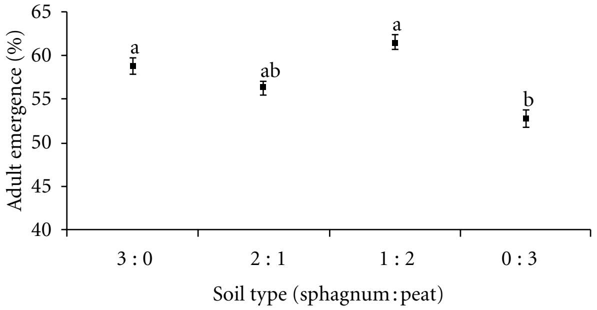


Figure 5: Mean percentage (±S.E.) of adults emerging from aestivation when stored in four different ratios of sphagnum : peat during pupation and aestivation. Means with different letters are statistically different ($F_{(3,\,284)} = 4.10$, $P = 0.0072$).

(d) Assessing Importance of Sterilized Soil. In fall of 2006 containers with unsterilized soil mix had higher mean emergence than the other treatments (67.3 ± 4%) (Table 2). Emergence from containers with unsterilized forest soil exceeded all those containers with autoclaved soil (39.3 ± 3%).

3.3. Rearing Procedures to Increase Production Numbers of L. nigrinus: 2005–2010. Production of *L. nigrinus* adults that successfully emerged after aestivation improved after 2006 (Table 3). In 2005 and 2006, the numbers of larvae produced dropped from about 31,000 in 2002–2004 (Table 2) to 17,042 (Table 3). Emerging adults dropped more drastically from 10,000 (Table 2) to 2,725 beetles (Table 3). Mold contamination of the soil medium was likely the main cause. The sterilizing study helped us determine that sterilizing the soil contributed to the onset of mold problems. By not sterilizing the soil, the mold problems disappeared. It was likely that beneficial microorganisms were being removed during sterilization allowing saprophytic fungi to flourish, but we did not investigate this further.

Beginning in 2007, we finally reached a stable point in rearing production. From 2007 to 2010, we produced an average of 19,036 adults per year (Table 3). There is still considerable variation from year to year due to environmental conditions. In 2009 our production dropped, due mostly to severe winter kill of HWA from low temperatures in February that year throughout the mid-Atlantic states, resulting in poor food availability for developing larvae. In contrast, HWA populations recovered in 2010. Food availability was adequate, and we obtained a remarkably high level of emergence, where 71% of the larvae survived pupation and aestivation.

4. Discussion

The creation of the funnel cage contributed greatly to the assessment of survivorship as well as providing a functional way to rear larvae. *L. nigrinus* larvae developed within the funnel without additional maintenance, such as adding foliage or searching for lost larvae under the microscope, as in previous years. Collection of *L. nigrinus* prepupae dropping from the foliage enabled us to determine egg and larval survival and allowed for the calculation of survivorship through *L. nigrinus* pupation and aestivation. A third benefit of using this cage is that, during spring, there is a consistent accessible supply of *L. nigrinus* larvae dropping from the foliage, allowing experiments to be set up with less effort and coordination since larvae do not have to be reared individually.

There are some challenges to using the funnel cages. Mature larvae must be transferred within 36 h of dropping, before they create a pupal cell and become immobile. Checking the jars daily for mature larvae is labor intensive and costly. Without adequate prey within the funnel cages, large numbers of immature larvae that end up in the jars must be transferred back to hemlock branches containing HWA eggs for them to survive. Also, high numbers of immature larvae increase the time required to check the funnels jars.

Survival is low when adults emerge from aestivation before October. Maintaining them at 4°C increases their survival. Since aestivating 1st instar HWA sistentes are not suitable food for postemergence adult beetles, HWA eggs laid by sistentes in the previous spring should be stored at 4°C throughout the summer to provide early emerging adults with developing progredientes nymphs. The increase in larval production in 2002 is attributed to the almost exclusive use of field beetles for the starting colony. These were larger and apparently oviposited more eggs than lab-reared adults. Sex ratio of pupae in mold-free soil was close to 1 : 1. Emergence of males occurred earlier than females when held at constant 15° ± 2°C, 14 : 10 (L : D) photoperiod. This may explain the high female : male ratio observed in ovipositing females. When maintained at a high temperature (19°C) after adult eclosion and lowered to 13° ± 2°C, 12 : 12 (L : D) photoperiod in late September to stimulate emergence, the emergence period of adults was much shorter than the emergence period of adults the previous year [12].

Moisture level and soil type influence the number of adults that emerge. Emergence is the highest at moisture levels of 30–40% saturation although a wide range of moisture levels in the soil is tolerated. This is advantageous because precipitation varies from year to year. Adults have the highest emergence from a mixture of sphagnum and peat mosses. Although these factors affect emergence, larval cohort often accounts for much of the variation observed in emergence, suggesting there are unexplored factors involved.

The time at which larvae enter the soil ranges over a period of 12–15 weeks and appears to influence the number of adults emerging from aestivation. The same pattern is observed each year in the colony; larvae maturing early in the spring have a higher rate of adult emergence than those maturing later in the year. This pattern may be explained by variation in nutritional value of HWA over a season. Larvae maturing early in the season feed on eggs laid by sistentes and those maturing later in the season feed on eggs laid by progredientes. By late spring, two generations of HWA have fed on the hemlock branches, which may be depleted in resources, possibly affecting the nutrition of HWA. The

Table 2: Total number of *Laricobius nigrinus* larvae, emerged adults, and percent emergence in each treatment.

Container	Soil type	Autoclaved	*n*	Total larvae	Emerged adults	% Emergence ± SE[A]
Square	Mix	Yes	5	150	30	20.0 ± 4% c
Square	Mix	No	5	150	101	67.3 ± 4% a
Square	Forest	Yes	5	150	5	3.3 ± 1% d
Square	Forest	No	5	150	59	39.3 ± 3% b

[A] Means followed by a different letter are significantly different from one another (Tukeys, $P \leq 0.05$).

Table 3: The number of *Laricobius nigrinus* in each life stage at the Virginia Tech Insectary from 2005 to 2010.

Year	Founding colony (reproductive adults)	Mature larvae	Adult : larvae	Emerging adults (after aestivation)
2005	713[a]	19,593	1 : 27	3,430
2006	1,067[a]	14,492	1 : 14	2,019
2007	1,231[b]	40,978	1 : 33	15,294
2008	1,200[a]	46,028	1 : 38	20,601
2009	1,030[a] + 200[c]	32,382	1 : 26	13,283
2010	870[a] + 200[c]	38,690	1 : 36	27,504

[a] Wild-caught adults from Washington, USA.
[b] Wild-caught adults from Washington, USA and Kentland Farm, Virginia, USA.
[c] Adult F_1 generation reared in the insectary at Virginia Tech.

nutritive chemical composition of host plants can affect the quality of phytophagous hosts and has been known to affect their predators [16, 17], particularly *S. tsugae* [18], a predator of HWA.

The experiments conducted between 2000 and 2004 led toward more consistent production of larvae and lower mortality at each individual stage. Consequently, the number of beetles produced is now more predictable and appears to be mostly a function of food quality (prey). In general, rearing beetle predators on a natural diet is enormously difficult when prey is not consistently available [19]. Artificial diets have been developed and tested but usually result in significantly greater mortality during development of the predator [20, 21]. Artificial diets can be used to augment predator rearing [22] and are currently being investigated for *L. nigrinus* [23], but much work is still needed. Therefore we continue to rely on the collection of preys from abundant populations on healthy host trees.

Developing a reliable mass rearing procedure was a critical objective addressed by these experiments. The results from the experiments carried out from 2000 to 2004 led to a detailed description of *L. nigrinus* rearing procedures documented in [11]. These have lead to procedures being followed by rearing labs at Virginia Tech, Clemson University, and the University of Tennessee. The success in colony rearing to date has resulted in the release of more than 100,000 adult *L. nigrinus* at 267 locations in 13 states in the eastern USA [24]. The techniques developed here are also applicable to rearing other *Laricobius* species being considered for release [25, 26].

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Research Article

A System for Harvesting Eggs from the Pink-Spotted Lady Beetle

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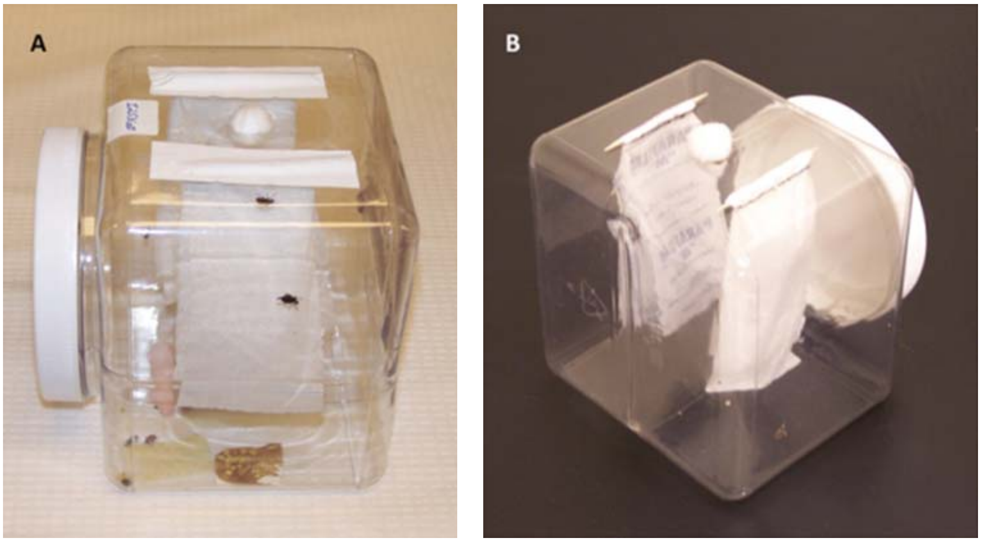
We describe a system for harvesting eggs from a predatory insect, the pink-spotted lady beetle, *Coleomegilla maculata* De Geer (Coleoptera: Coccinellidae). Adult beetles placed in square, transparent containers that included oviposition substrates hanging from the top of the cage deposited eggs on the materials provided. We harvested eggs from these substrates in quantities sufficient for either destructive sampling or synchronous development of larvae. We evaluated effects of crowding inside cages; effects of a chemical attractant on oviposition behavior; egg cannibalism. Females preferred a textured surface rather than a smooth, waxy one for laying eggs. Crowding inhibited oviposition of beetles. Presence of a chemical attractant (methyl salicylate) did not significantly improve oviposition. This paper describes an inexpensive system for harvesting eggs from *C. maculata*. Refinement of this system should improve oviposition and reduce cannibalism.

1. Introduction

The pink-spotted lady beetle, *Coleomegilla maculata* De Geer (Coleoptera: Coccinellidae), is a generalist predator native to North America [1]. In nature, it feeds on a wide range of soft-bodied insects and mites, and plant products (e.g., pollen, bean leaf tissue [2]) in managed and unmanaged landscapes [3–6]. In its native range, *C. maculata* does not overwinter in houses or become a nuisance pest. *C. maculata* is amenable to rearing, which makes it a good prospect for commercial production and genetics studies. Although several diets for *C. maculata* have been described for specific purposes [2, 7–13] and used in risk assessment studies [14], there is no single-standardized artificial food available for this species. Furthermore, cage systems for rearing *C. maculata* on a large scale are virtually unknown.

Our goal in this study was to develop a technique to harvest eggs of this predatory species in quantities sufficient for RNA/DNA extraction or large-scale production of insects for release studies or augmentative biological control. The oviposition behavior of *C. maculata* in a natural environment is well studied [6]. Females prefer ovipositing on plants with epidermal hairs (trichomes) on leaf surfaces rather than plants with smooth surfaces, devoid of trichomes [15, 16]. In laboratory conditions, individual females oviposit on smooth surfaces such as containers of food or water [7] or on the smooth surface of a Petri dish or other enclosures [8]. Removal of these eggs from the dish surface is possible but time consuming and often damages the eggs, resulting in poor-quality samples for downstream processes such as nucleic acid extractions (personal observation). Thus, one goal for an artificial system was to identify an oviposition substrate that could encourage oviposition and facilitate successful harvesting of eggs. Methyl salicylate (MeSA) is a common herbivore-induced plant volatile that attracts beneficial insects in many crops [17, 18]. It has been shown to attract at least one lady beetle [19]; however, the potential to stimulate or promote oviposition is unknown.

Cannibalism often occurs in the field and in the laboratory [8], which suggests that this behavior is an adaptive strategy for this species [20]. Thus, eggs are a challenge to obtain because both larvae and adults cannibalize them when reared under crowded conditions (personal observation). An additional goal of the research was to identify conditions that decreased cannibalism. We describe a cage system for harvesting lady beetle eggs for ecological and genetics experiments.



(a) (b)

Figure 1: Enclosures for collecting *Coleomegilla maculata* eggs: cage for adults including food, water, insects, and hanging oviposition substrates (a) and cage for performing oviposition substrate choice assay (b).

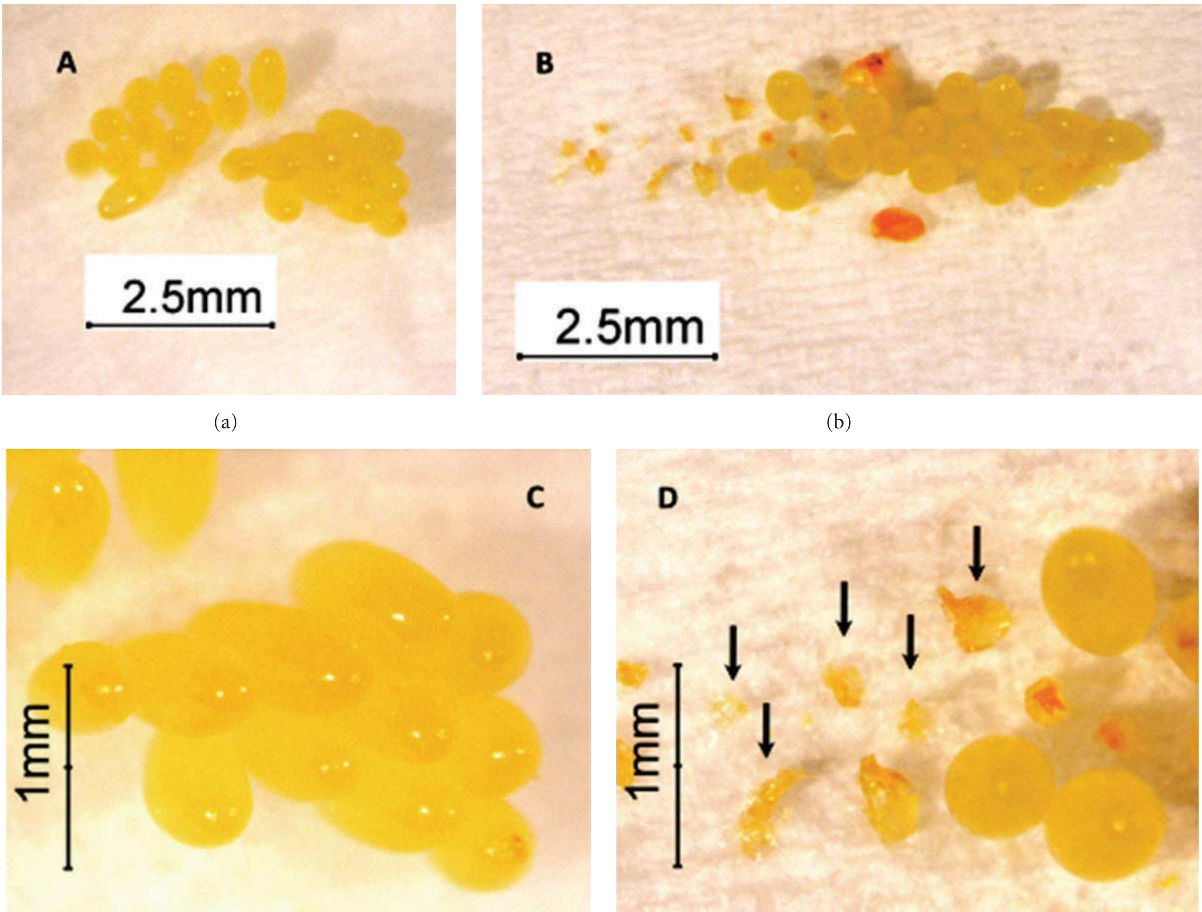
2. Materials and Methods

2.1. Insects. We established a laboratory colony of *C. maculata* from adults in continuous culture from Beltsville, Maryland, USA and Brookings, South Dakota, USA. Predators were cultured initially in standard disposable Petri dishes (100 × 15 mm), until populations demanded larger containers. Adult and larval stages were fed a combination of foods including bee pollen (Y. S. Organic Bee Farms, Sheridan, IL, USA), Brewer's yeast, honey, powdered sugar, and *Daphnia* (Hikari Bio-Pure, Hayward, CA, USA), and live eggs of plant bugs (*Lygus lineolaris* Palisot de Beauvois or *Lygus hesperus* Knight) in excess. We provided a free source of water on cotton balls in microcentrifuge tubes (1.5 mL capped with cotton) at the base of each cage. We maintained insects in an environmental chamber with a 16L: 8D lighting schedule at 23°C and 55%RH during the day and 20.5°C and 52%RH during the night.

2.2. Cages. Adult cages were clear plastic containers made by Rez-Tech Company (Kent, OH, USA). The dimensions of the cage were 14 cm × 14 cm × 15 cm, capacity 2,000 mL. We cut two slits (10 cm long) and a single 2 cm (diam) hole into one side of the container; oviposition substrates were in the slits (Figures 1(a), 1(b)), at a hanging position. Oviposition substrates were at the top of the cages, at some distance from food sources (at the base of the cage). Our hypothesis was that females would be less likely to cannibalize conspecific eggs if they deposited them on substrates that offered some seclusion away from food sources. To harvest eggs, we simply removed oviposition substrates, examined them, and replaced them with new ones. Females deposited their eggs in distinct masses (batches) on these substrates. We plugged the 2 cm diameter hole with cotton when not transferring beetles into cages.

2.3. Influence of Substrate Type on Oviposition. Materials tested as oviposition substrates were Kimwipes, providing a textured surface, and the paper backing of Parafilm strips, providing a smooth surface. We used five subsample cages, containing between 25 and 50 adults of similar age, at least 1 wk after adults eclosed. Male-to-female sex ratio was approximately 1 : 1 in all cages. We replaced insects that escaped or died. Each cage contained a set of textured and a set of smooth oviposition substrates of equal surface area (Figure 1(b)) and thus provided equal choice for oviposition. Each set consisted of eight individual sheets. We collected eggs each day (Monday–Friday) for 17 total collection dates. We recorded number of egg masses, number of eggs intact, and those cannibalized. Cannibalized eggs had remnants of eggs left on the substrate (Figure 2). Each day of egg collection represented a single sample, and the five cages provided replication of the treatments per day. We recorded number of egg masses and total eggs produced in each cage. We used total eggs collected from oviposition substrates per day for data analysis.

2.4. Influence of Crowding on Oviposition and Incidence of Cannibalism. To determine the influence of crowding on oviposition, we manipulated the number of adults in cages. Adult specimens were reproductively active (10–25 days old) and placed in cages as follows. In the first test, cages contained 100 females with 100 males, 50 females with 50 males, 25 females with 25 males, or 10 females with 10 males for a total of 4 cages. In the second test, a single cage contained 20 females with 20 males, 20 females without males, 10 females with 10 males, or 10 females without males for a total of 4 cages. Because of the large number of males and females manipulated in this experiment (in 8 total cages), it was not practical to use the cage as the source of replication. Using Kimwipes tissue as oviposition substrate



(a) (b)

(c) (d)

Figure 2: Intact (noncannibalized) eggs of *C. maculata* in a mass (a, c) and cannibalized eggs in a mass (b, d). Arrows indicate remnants of cannibalized eggs on substrate (d).

based on results from the study described above, we collected eggs from oviposition substrates from each of these cages for 10 consecutive days. Two sets of hanging oviposition substrate were used in each cage (see Figure 1(a)). We recorded number of egg masses and total eggs produced in each cage. We also estimated number of eggs cannibalized as previously described. We used total eggs collected from oviposition substrates per day for data analysis.

2.5. Influence of an Attractant on Oviposition. An arena was constructed from clear plastic containers of two sizes, the size mentioned above and a central chamber approximately twice as large, 14 cm × 14 cm × 30 cm, capacity 4000 mL, connected by clear plastic tubes. We provided food and water in the central chamber, while an oviposition substrate was in each of the two lateral chambers (Figure 3). We released 40 male and 40 female adults of reproductive age into the central chamber. Just prior to attachment of the oviposition substrate in one of the lateral chambers, we pipetted 2 μL of pure methyl salicylate (Alfa Aesar, Ward Hill, MA, USA) onto the central upper portion of the set of substrate leaflets. This treatment produced a human-detectable scent. We counted insects directly after chamber attachment and again at 4, 20, 24, and 28 hr, and a final count and egg collection at 44 hr. We determined gender of beetles in both chambers after 44 hr. To control for the possibility of methyl salicylate incorporation into the plastic, containers were washed thoroughly after each experiment with warm soapy water, then rinsed twice with bleach, and then rinsed with 70% ethanol followed by another rinse with distilled water. The containers were then allowed to dry in sunlight for 24 hours. We replicated the experiment five times with new insects used in each replicate. Each replicate was set up in a glasshouse at noon, with the same arena orientation to avoid any influence of daylight on movement of insects into lateral chambers. After final assembly and initial tally of insect movement (within 2 min), the arena was relocated to an environmental chamber as described above. We used the total number of insects in control or test lateral chambers and the number of intact eggs on oviposition substrates at the end of the experiment for data analysis.

2.6. Data Analysis. Experiments were set up following a completely randomized design. We used the paired *t*-test

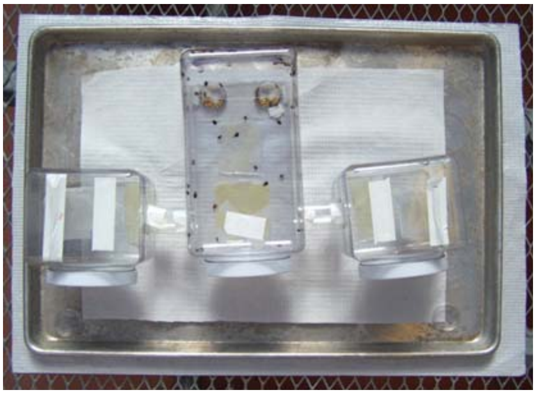


Figure 3: Three-chambered arena designed to measure beetle movement and oviposition in response to odors from methyl salicylate (MeSA). Beetles are visible in the central chamber.

to evaluate substrate type (textured versus smooth) on oviposition and cannibalism. We used one-way repeated measures analysis of variance (RM-ANOVA) using the Holm-Sidak method to evaluate the significance of crowding on oviposition and cannibalism. We also used a paired *t*-test to evaluate the influence of an attractant on insect movement and oviposition. Absolute data were square root transformed prior to analysis. Means were significantly different if $P < 0.05$. We used SigmaStat 3.0.1 (Systat Software Inc., Richmond, CA, USA) for data analysis. All data presented herein represent nontransformed values.

3. Results

3.1. Influence of Substrate Type on Oviposition. Over the course of the experiment, we collected 120 and 79 egg masses from textured and smooth substrates, respectively. The mean ± SEM number of eggs on textured and smooth substrates per cage per day was 19.0 ± 2.9 and 12.1 ± 2.5 eggs, respectively. Significantly more eggs were on textured substrate ($t = 2.2$, df = 16, $P = 0.046$).

We observed cannibalism of eggs on both oviposition substrates. The mean ± SEM number of eggs cannibalized on textured and smooth substrates per cage per day was not significant (3.7 ± 0.9 and 3.0 ± 0.8 eggs, resp.) ($t = 1.0$, df = 15, $P = 0.32$).

3.2. Influence of Crowding on Oviposition and Incidence of Cannibalism. We found that crowding of *C. maculata* adults (with males) in cages affected oviposition and egg cannibalism (Table 1). More eggs were laid by 10 females (with males) than by 50 or 100 females (with males) in experimental cages per day ($F = 6.6$, df = 3, 27; $P = 0.002$). The number of eggs laid by 25 females (with males) did not differ from other treatments. More eggs were eaten (cannibalized) by adults in cages with 25 females (with males) than with 100 females (with males). Cannibalism of eggs in the other treatments, 10 females (with males) and 50 females (with males), did not reveal any significant differences from the other treatments. Crowding of *C. maculata* females (with or without males) at adult densities tested did not significantly affect oviposition rate or egg cannibalism (Table 2). No differences were detected between treatments consisting of 10 females with or without males and 20 females with or without males ($F = 0.16$, df = 3, 27; $P = 0.9$).

Table 1: Effects of crowding (of *C. maculata* females with males) on the mean ± SEM number of eggs laid and cannibalized per cage per day.

Treatment	Eggs laid	Eggs cannibalized
10 females + males	24.3 ± 5.8^{a}	2.6 ± 1.3^{ab}
25 females + males	10.4 ± 4.5^{ab}	3.2 ± 1.4^{a}
50 females + males	6.5 ± 3.9^{b}	0.6 ± 0.6^{ab}
100 females + males	1.7 ± 1.6^{b}	0^{b}

Means ± SEM followed by a different letter in a column are significantly different ($P < 0.05$).

Table 2: Effects of crowding (of *C. maculata* females with or without males) on the mean ± SEM number of eggs laid and cannibalized per cage per day.

Treatment	Eggs laid	Eggs cannibalized
10 females + males	9.2 ± 2.8^{a}	3.3 ± 1.6^{a}
10 females	10.3 ± 5.1^{a}	0.1 ± 0.1^{a}
20 females + males	9.7 ± 3.4^{a}	3.1 ± 2.0^{a}
20 females	9.3 ± 4.3^{a}	0.5 ± 0.5^{a}

Means ± SEM followed by the same letter in a column are not significantly different ($P > 0.05$).

3.3. Influence of Attractant on Insect Movement and Oviposition. The attractant (methyl salicylate) affected the number of *C. maculata* adults moving in the arena (Table 3). More insects were in the test chamber than in the control chamber ($t = 3.3$, df = 5, $P = 0.02$). Males and females were moving into both chambers, as illustrated in Figure 4. The number of eggs laid in test and control chambers did not differ significantly ($t = 0.47$, df = 4, $P = 0.66$).

4. Discussion

The results described indicate that an easily accessible and inexpensive material can serve as an oviposition substrate for high volumes of egg collection. The use of hanging strips of tissue with a moderately textured surface provides a means of collecting multiple egg masses from groups of insects, while allowing assortive mating within the population. The hanging substrate provided refugia for resting, mating, and oviposition and was relatively easy to handle. Yellow or orange egg masses were easily visible on the white substrate and could be clipped from the leaves and transferred to another container or harvested for downstream processing.

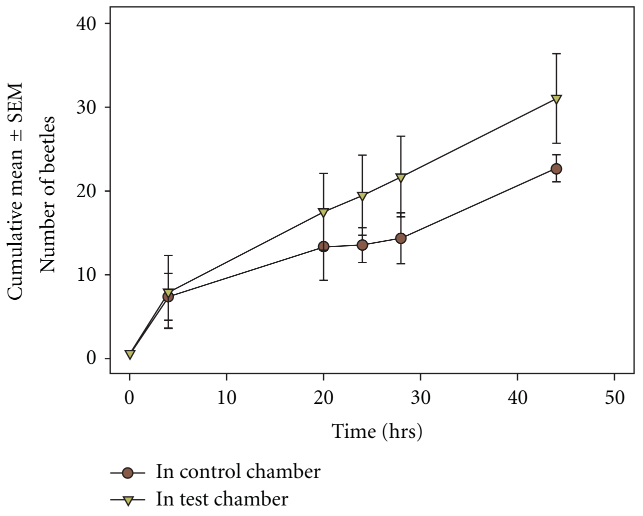


Figure 4: Cumulative mean ± SEM number of beetles moving into control or test (MeSA-treated) lateral chamber of arena over time.

Table 3: Effects of attractant (methyl salicylate) on the mean ± SEM number of *C. maculata* adults and eggs in control and test chambers of arena.

Treatment	Adults per arena	Gender	Eggs laid
Control chamber	11.8 ± 3.0^{a}	11 males: 12 females	55.2 ± 27.1^{a}
Test chamber	16.3 ± 4.4^{b}	12 males: 19 females	58.2 ± 26.0^{a}

Means ± SEM followed by a different letter in a column are significantly different ($P < 0.05$).

We describe an oviposition choice assay for laboratory culture. *C. maculata* prefers to oviposit on leaves with trichomes rather than on smooth leaves in the field [16]. Another study of this predator utilized sulfite paper as an oviposition substrate [11]. Our study indicates that in laboratory culture, a smooth paper substrate is also less preferable. More egg masses and total eggs were deposited on the textured surface compared to the smooth surface. Repeated mating may be important for cultures of *C. maculata*, and mating in this species has been shown to be density dependent and enhanced by male isolation [21]. Our crowding experiments suggest that high population densities of *C. maculata* restrict mating and oviposition. While some density of insects below 50/2000 mL was shown to be more optimal for egg production than 100 or 200/2000 mL, our tests do not identify a precise number or sex ratio per container to maximize egg production or minimize cannibalism. Additional replicated experiments may clarify this. It should be noted that the decrease in egg production in cages containing 100 or 200 individuals could represent cannibalism that was not detected. While every effort was made to visualize remnants of eggs that would indicate cannibalized egg masses, it is possible that eggs produced in those cages were completely consumed. Further research will help clarify this.

Regrettably, we did not extend our study to assess the influence of oviposition substrate and predator density on hatch rate and fertility of eggs. An examination of these life-history parameters is important to developing a mass rearing system, and we should consider them in a followup study.

Egg cannibalism by *C. maculata* is common in the laboratory and in the field [22, 23]. Neonates of this species feed on conspecific eggs and apparently benefit from this behavior [23]. Some studies on this species suggest that larvae should be provided individual containment to curb cannibalism [8, 11]. Because neonates remain on their egg mass for a period of time (24–48 hr) after hatch, daily or other periodic collection of eggs will reduce egg plus neonate interactions as well as egg plus adult interactions. Our results did not identify a density or sex ratio that reduced cannibalism. Cannibalism was not reduced significantly on either of the substrates tested. It has been suggested that pubescence on leaves may interfere with foraging, thus plants with glandular trichomes "provide ovipositional refuges from cannibalism" [16]. Additional surfaces such as fabrics may provide oviposition substrates that reduce such losses. Our collections were daily, and research has shown that oviposition by *C. maculata* is periodic, with eggs laid primarily afternoon and before midnight [16]. Egg collection might be optimized by either varying timing or frequency of collections or by restricting substrate availability. The cage system described here is ideal for these experiments.

We attempted to answer the question of how to stimulate oviposition from groups of insects using the arena method and oviposition substrates. Ovipositional stimulants have been tested for this species. For example, extracts from wood [24] and extracts of *Juniperus virginiana* L. plants [25] were found to stimulate oviposition from individual females. Methyl salicylate is not an oviposition stimulant for *C. maculata* based on our preliminary results. Future research should evaluate a variety of other compounds at a range of concentrations.

Results presented here demonstrate a system that produces substantial quantities of insect biomass at defined stages of development. The cage system described yielded the desired quantity of eggs; a set of six cages with ten females per cage can supply 100–200 eggs per day on a continuous basis (results not shown). RNA extraction for gene expression requires specimens at identical developmental stage. Eggs and newly hatched larvae are the smallest stage of these insects, at roughly 200 μg per individual. Because standard extraction kits utilize tissue samples 20–40 mg, a minimum quantity of 100 individuals per extraction sample is necessary. Furthermore, because it is more convenient to process samples of at least two at a time, a minimum quantity of 200 individuals is desirable. Researchers need to be able to culture candidate species in large quantities for large-scale genetic sequencing or on small scales for individual pair mating and selective inbreeding. Facilitated by studies described here, we have collected nucleic acids from every life stage of *C. maculata* and selected isofemale strains. Further refinement of this system should improve oviposition and reduce cannibalism. Then we can potentially scale up this system for commercial mass rearing.

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Research Article

Limited Transmission of the Ectoparasitic Fungus *Hesperomyces virescens* between Lady Beetles

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The ectoparasitic fungus *Hesperomyces virescens* Thaxter (Ascomycota: Laboulbeniales) commonly infects the invasive lady beetle *Harmonia axyridis* (Pallas) and several other aphidophagous lady beetles in North America and Europe. We tested the hypothesis that bodily contact between adults of different lady beetle species supports horizontal transmission of *H. virescens*. We used laboratory assays to determine whether *H. axyridis* or *Olla v-nigrum* (Mulsant) harboring *H. virescens* (i.e., source beetles) transmit the fungus to noninfected target beetles *H. axyridis*, *O. v-nigrum*, *Coccinella septempunctata* L., *Coleomegilla maculata* (De Geer), or *Hippodamia convergens* Guerin-Meneville. Results indicate that intraspecific transmission (i.e., for the source beetles *H. axyridis* and *O. v-nigrum*) was common but interspecific transmission (i.e., from source *H. axyridis* or *O. v-nigrum* to target species) was low. Interspecific transmission occurred at low rates from *H. axyridis* to both *C. septempunctata* and *O. v-nigrum* and from *O. v-nigrum* to both *C. septempunctata* and *H. convergens*. Based upon our laboratory assays of forced pairings/groupings of source and target beetles, we predict that horizontal transmission of *H. virescens* between species of aphidophagous coccinellids is possible but likely rare.

1. Introduction

Laboulbeniales (Ascomycota) are ectoparasitic fungi and nearly all 2,000 described species are obligate parasites that grow on the integument of living arthropods, mostly insects, and usually on the adult stage [1, 2]. Within the ten insect orders that contain host species, about 80% of these parasitic fungi are on beetles (Coleoptera) [2]. Of particular interest are Laboulbeniales that infect Coccinellidae (Coleoptera). Four species of *Hesperomyces* (*H. chilomenis*, *H. coccinelloides*, *H. hyperaspidis*, and *H. virescens*) attack entomophagous Coccinellidae [1, 3, 4]. Of these species, *H. virescens* Thaxter infects more coccinellid species than the other three species [4]. Negative impacts of parasitism by *H. virescens* on lady beetle populations are not well defined, but Kamburov et al. [5] found that infected *Chilocorus bipustulatus* L. adults suffered premature mortality.

Known coccinellid hosts of *H. virescens* include *Adalia bipunctata* (L.), *Brachiacantha quadripunctata* Melsheimer, *Chilocorus stigma* (Say), *Chilocorus bipustulatus* (L.), *Eriopis connexa* Germar, *Cycloneda munda* (Say), *Cycloneda sanguinea* (L.), *Coccinula crotchi* (Lewis), *Coccinula sinensis* Weise, *Coccinella septempunctata* L., *Hippodamia convergens* Guerin-Meneville, *Harmonia axyridis* (Pallas), *Olla v-nigrum* (Mulsant), and *Psyllobora vigintimaculata* (Say) [1, 3–9]. Although *H. virescens* may occur on these species, it may not occur on some other coccinellid species found within the same habitat at the same time. For example, Harwood et al. [8] sampled lady beetles using Malaise traps and recorded *H. virescens* from *B. quadripunctata*, *C. munda*, *H. axyridis*, and *P. vigintimaculata*. The fungus was not on *Coleomegilla maculata* (De Geer) or *Hyperaspis signata* (Olivier) in those samples. Riddick and Cottrell [10] found *H. virescens* infecting *H. axyridis*, *H. convergens*, and *O. v-nigrum* when beetles were collected using sweep nets. At the same time, *H. virescens* was not on *C. septempunctata*, *C. maculata*, *C. munda*, *Scymnus loewii* Mulsant, or *S. socer* LeConte. Harwood et al. [8] and Riddick and Cottrell [10] reported that the exotic *H. axyridis* had the highest percentage of infected individuals (82.3 and 50.1%, resp.) among the species sampled. Additionally Riddick and Cottrell [10] reported that *H. virescens* infected 33.1% of *O. v-nigrum* adults but only 4.7% of other species of adult lady beetles.

Horizontal transmission between adult Coccinellidae is via direct contact, usually during copulation but also within overwintering aggregations [11–15]. Indirect transmission of Laboulbeniales, that is, beetles infected from ascospores discharged onto a substrate is not likely [2].

Our goal was to use laboratory assays to test the hypothesis that bodily contact between different species of lady beetles provides an avenue for horizontal transmission of *H. virescens*. We paired infected beetles (i.e., source beetles) with noninfected beetles (i.e., target beetles) for varying times to determine whether transmission, within or between species, occurred. Additionally, we examined whether transmission within species occurred via indirect transmission under laboratory conditions.

2. Materials and Methods

2.1. Insects. We used adult beetles from laboratory colonies or field collections in experiments. We established laboratory colonies of *H. axyridis*, *C. maculata*, and *O. v-nigrum* from individual beetles collected at the USDA, ARS, Southeastern Fruit and Tree Nut Research Laboratory, Byron, GA, USA. Colonies were maintained on a diet of pecan aphids (Hemiptera: Aphididae), frozen *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs, and a meat-based diet (Beneficial Insectary, Redding, CA, USA) similarly as described by Cottrell [16]. Adult *H. convergens* and *C. septempunctata* were field-collected and maintained on a similar diet for 2-3 wk before use in experiments. Holding field-collected beetles in the laboratory before using them in assays permitted time to detect any infected individuals.

2.2. Ectoparasitic Fungus. We initiated separate colonies of *H. virescens*-infected *H. axyridis* and *O. v-nigrum* by collecting infected beetles from the field, confirming infection on beetles using a stereomicroscope, and maintaining beetles in the laboratory. We housed groups of infected beetles in containers (19 × 13.5 × 9 cm) and provided food and water as previously described. We perpetuated each of the infected colonies by the periodic addition of noninfected, laboratory-reared adult *H. axyridis* or *O. v-nigrum*. These infected beetles served as *H. virescens* source beetles used in fungus transmission studies. We did not consider beetle age or whether the beetle was field-collected or laboratory-reared when used in assays. Rather, we ascertained that mature thalli were present on the source beetles used in experiments and placed source and target beetles together, in the first transmission experiment (see below), with regard to thalli density on source beetles. Furthermore, we did not determine sex of beetles in order to reduce handling and potential spore dispersal prior to experimentation.

2.3. Transmission Studies

2.3.1. Seven-Day Exposure Experiment. We conducted this experiment using two separate trials, one in January and another in April 2010. For each trial, source *H. axyridis* and *O. v-nigrum* were tested separately against laboratory reared, target *H. axyridis*, *O. v-nigrum*, and *C. maculata*. Before trials began, source beetles were observed using a stereomicroscope, mature thalli were counted and each beetle was designated with high (≥24), moderate (14 to 23), or low (≤13) thalli density (range = 35). In both trials, we placed a source beetle (i.e., *H. axyridis* or *O. v-nigrum*) in a 9 cm diameter Petri dish with a target beetle (i.e., *H. axyridis*, *O. v-nigrum*, or *C. maculata*). Each trial consisted of three replicates of all possible source-target species combinations at a 1 : 1 ratio of source : target. Thus, we used five target beetles of one species per source species in each replicate for a total of 15 targets per trial and 30 targets for the experiment.

In the first trial, *O. v-nigrum* source beetles with similar thalli densities were lacking; therefore, not all five target beetles of each species (i.e., *H. axyridis*, *O. v-nigrum*, and *C. maculata*) were matched with *O. v-nigrum* source beetles harboring similar densities of thalli. However, similar treatments between target species within each replicate were achieved by using three targets each paired with a low thalli density source *O. v-nigrum*, a fourth target paired with a moderate thalli density source beetle and a fifth target paired with a high thalli density source beetle. Additionally during the first trial, all *H. axyridis* source beetles had low thalli densities thus two source beetles were placed with each target beetle (ratio of source : target = 2 : 1) to insure that an outcome showing a lack of transmission was not solely due to low thalli density of a single source beetle.

In the second trial, thalli density varied on both source species but source and targets were paired such that each of the five targets in each replicate was exposed to source beetles with similar thalli density. In both trials, Petri dishes containing source and target beetles were housed in an environmental chamber (25 ± 1°C and 14 : 10 [L : D]h) for seven days and provided food and water. After seven days, we removed source beetle(s) but kept the target beetle in that same Petri dish for one month. During this time, we examined target beetles three times per week under a stereomicroscope for development of mature (or at least nearly mature and identifiable) *H. virescens* thalli. We documented any target beetles containing at least one mature thallus and placed them into 70% ethanol for later confirmation of *H. virescens*.

2.3.2. Tumbled Beetles Experiment. In the previous experiment, pairs of same sex or interspecific source and target beetles may have hindered beetle interaction and affected *H. virescens* transmission. Additionally, thalli distribution on source beetles and the behavior of source beetles (e.g., not attempting to mate) could hinder *H. virescens* transmission. This experiment attempted to negate these factors by forcing interaction between the source and target. Again, *H. axyridis* and *O. v-nigrum* were the source beetles. The target species were *C. septempunctata*, *C. maculata, H. axyridis*, *H. convergens*, and *O. v-nigrum*. We paired source and target beetles in 2-dram vials and then placed the vials on an automated roller (Nostalgia Electrics HRD-565 Hot Dog Roller, Nostalgia Products Group, LLC, http://www.nostalgiaelectrics.com/), as used in insecticide assays to coat the inner walls of vials, and rolled them at 2.6 revolutions/min for 1 h. Paired beetles within the rolling vial were active and tumbling and thus became entwined while attempting to remain upright and maintain their footing on the rotating glass vial (TEC, personal observations). After being tumbled, we placed target beetles into Petri dishes and maintained them in an environmental chamber with food and water for one month. We observed beetles three times per week under a stereomicroscope to detect any mature thallus, documented its presence and preserved infected beetles in 70% ethanol.

2.3.3. Extended Exposure Experiment. We attempted to facilitate transmission of *H. virescens* between source and target beetles by keeping them in close contact for an extended period. We grouped a single source beetle with three individuals of the same target species in a Petri dish and replicated four times ($n = 12$ target beetles per species). This was done using *H. axyridis* and *O. v-nigrum* source beetles with the target species *C. septempunctata*, *C. maculata*, *H. axyridis*, *H. convergens*, and *O. v-nigrum*. These beetles remained together for 6 wk and were fed, watered and observed for development of *H. virescens* thalli. When source and target beetles were of the same species, we easily differentiated the single source beetle and the targets by presence/absence of *H. virescens* and later by thalli density when we detected the first mature *H. virescens* thallus on a target beetle.

2.3.4. Substrate Borne Transmission Experiment. Temporal and spatial overlap of coccinellids in the field might allow for substrate borne transmission as potential hosts contact *H. virescens* ascospores. We placed two source *H. axyridis* adults in a Petri dish with food and water then removed them after 24 hr. Density of thalli on source beetles varied from low to high. Immediately following removal of the source beetles, five target *H. axyridis* beetles were added to the same dish and maintained in that dish, with food and water, for 7 d. This procedure was replicated using five dishes ($n = 10$ source and 25 target *H. axyridis*). After 7 d, we transferred the target beetles into a clean dish (and thereafter on an as-needed basis) and maintained them with food and water for the next three weeks. We used the same experimental design to examine substrate borne transmission when source *H. axyridis* remained in the Petri dish for 120 h. Additionally, we examined the potential of substrate-borne transmission of *H. virescens* by source *O. v-nigrum* to target *O. v-nigrum* using the same experimental design; source beetles were exposed to Petri dishes for 24 and 120 h. For both target species, we examined individual beetles three times per week, during weeks 2–4 of the study, for mature thalli using a stereomicroscope.

Table 1: Previously noninfected target lady beetles infected with the ectoparasitic fungus *H. virescens* when exposed in a Petri dish for 7 d to already infected source *H. axyridis* or *O. v-nigrum* lady beetles.

Source beetle	Target beetle	Proportion infected (±SE)*
H. axyridis	*C. maculata*	0 b
H. axyridis	*H. axyridis*	0.46 ± 0.11 a
H. axyridis	*O. v-nigrum*	0 b
O. v-nigrum	*C. maculata*	0 b
O. v-nigrum	*H. axyridis*	0 b
O. v-nigrum	*O. v-nigrum*	0.87 ± 0.06 a

*Within each source beetle, different letters following the proportion infected indicate a significant difference ($P < 0.05$) between target beetles.

2.4. Statistical Analysis. Within source species, we combined data from both trials of the seven-day exposure experiment. For both the seven-day exposure and tumbled beetles experiments, we used the nonparametric Kruskal-Wallis analysis of variance by ranks to analyze the proportions of infected beetles. We did this separately for when *H. axyridis* or *O. v-nigrum* was the source beetle. We analyzed proportions to take into consideration that some target beetles died before it was conceivable that successful transmission could have been identified and thus these individuals were not included in analyses. When a significant difference between species was found for the proportions infected, the Tukey-Kramer Honestly Significant Difference multiple comparison was used [17, 18].

3. Results

3.1. Seven-Day Exposure Experiment. When *H. axyridis* was the source, the only target species found with mature thalli was *H. axyridis*, and the proportion of infected beetles was significantly higher than no infection observed for *C. maculata* or *O. v-nigrum* ($\chi^2_{0.05,2} = 10.59$; $P = 0.0050$) (Table 1). From the January 2010 trial when source *H. axyridis* had different thalli density, 25, 50, and 25% of the newly infected target beetles had been housed with source beetles rated with low, moderate, and high thalli densities, respectively. Additionally, the average time (±SE) to detect mature thalli on target *H. axyridis* when exposed to source *H. axyridis* was 25.8 ± 1.5 d.

When source *O. v-nigrum* beetles were paired with target *H. axyridis*, *C. maculata*, or *O. v-nigrum,* the only target observed with mature thalli was *O. v-nigrum* and the proportion of infected beetles was significantly greater than for either *C. maculata* or *H. axyridis* ($\chi^2_{0.05,2} = 11.74$; $P = 0.0028$) (Table 1). From both trials, infection of target beetles exposed to *O. v-nigrum* source beetles with low, moderate and high thalli densities was 45, 30, and 25%, respectively. The average time (±SE) to detect mature thalli on target *O. v-nigrum* when exposed to source *O. v-nigrum* was 15.0 ± 0.4 d.

3.2. Tumbled Beetles Experiment. When we paired source *H. axyridis* in a glass vial with the target beetles *C. maculata*, *C. septempunctata*, *H. axyridis*, *H. convergens*, or *O. v-nigrum*

Table 2: Previously noninfected target lady beetles infected with the ectoparasitic fungus *H. virescens* when a pair of target and source lady beetles were placed in a vial and tumbled on a vial roller for 1 h. Source and target beetles were then separated and target beetles observed for mature thalli over the next month.

Source beetle	Target beetle	Proportion infected (±SE)*
H. axyridis	*C. septempunctata*	0.11 ± 0.06 b
H. axyridis	*C. maculata*	0 b
H. axyridis	*H. axyridis*	0.52 ± 0.11 a
H. axyridis	*H. convergens*	0 b
H. axyridis	*O. v-nigrum*	0 b
O. v-nigrum	*C. septempunctata*	0.14 ± 0.10 b
O. v-nigrum	*C. maculata*	0 b
O. v-nigrum	*H. axyridis*	0 b
O. v-nigrum	*H. convergens*	0 b
O. v-nigrum	*O. v-nigrum*	0.61 ± 0.11 a

*Within each source beetle, different letters following the proportion infected indicates a significant difference ($P < 0.05$) between target beetles.

Table 3: Average days (±SE) that *H. virescens* source lady beetles and target lady beetles (that were not infected during the experiment) survived when one source lady beetle and three target lady beetles were housed together in Petri dishes for 44 days.

Source beetle	Target beetle	Average days (± SE) source survived	Average days (± SE) noninfected target survived
H. axyridis	*C. septempunctata*	21 ± 8	39 ± 4
H. axyridis	*C. maculata*	32 ± 7	38 ± 3
H. axyridis	*H. axyridis*	N/A[a]	9 ± 4[b]
H. axyridis	*H. convergens*	23 ± 2	30 ± 5
H. axyridis	*O. v-nigrum*	29 ± 9	16 ± 5
O. v-nigrum	*C. septempunctata*	20 ± 2	44 ± 0
O. v-nigrum	*C. maculata*	20 ± 7	33 ± 5
O. v-nigrum	*H. axyridis*	17 ± 4	34 ± 4
O. v-nigrum	*H. convergens*	22 ± 7	35 ± 4
O. v-nigrum	*O. v-nigrum*	5[c]	7 ± 1[d]

[a] All source beetles either survived longer than target beetles or past the time when a mature thallus was detected on infected target beetles.

[b] Two beetles survived for 5 or 13 d. A mature thallus was detected on all other target beetles between 15 and 21 d.

[c] One beetle survived only 5 days and others either survived longer than target beetles or survived past the time when infection was detected on target beetles.

[d] Most beetles (75%) died before mature thalli were likely to have been observed. Two of three that survived longer than 7 d were observed infected.

for 1 h and observed the targets for the next month, the only targets observed with mature thalli were *H. axyridis* and *C. septempunctata*. However, horizontal transmission of *H. virescens* was significantly higher between beetles of the same, rather than different, species ($\chi^2_{0.05,4} = 10.28$; $P = 0.0360$) (Table 2). The average number of days before we observed mature thalli on *H. axyridis* and *C. septempunctata* targets was 17.9 ± 0.9 and 21.0 ± 0.0 d, respectively. When *O. v-nigrum* was the source, only *O. v-nigrum* and *C. septempunctata* targets were infected. Transmission of *H. virescens* to *O. v-nigrum* was significantly higher between individuals of the same species than between different species ($\chi^2_{0.05,4} = 10.28$; $P = 0.0360$) (Table 2). As an interesting note, the nonparametric statistics for the analysis of variance by ranks was identical for when *H. axyridis* or *O. v-nigrum* was the source. The average number of days before we observed mature thalli on *O. v-nigrum* and *C. septempunctata* targets was 14.5 ± 0.33 and 22.5 ± 1.5 d, respectively.

3.3. Extended Exposure. Not all beetles (source or target) survived to the end of this experiment. When *H. axyridis* and *O. v-nigrum* source beetles were housed with *H. axyridis* and *O. v-nigrum* target beetles, respectively, most source beetles either survived longer than respective target beetles or past the average time required, as reported in the prior experiments, to detect a mature *H. virescens* thallus on a target beetle (Table 3). As such, insufficient time to transmit the fungus was not of concern. We found a mature thallus on most *H. axyridis* targets (i.e., 83%) after 19 ± 1 d of contact with *H. axyridis* source beetles. Two noninfected target *H. axyridis* did not survive this long and likely died before a thallus could mature and be recorded (Table 3). Average survival time of other noninfected target species, except *O. v-nigrum*, exposed to source *H. axyridis* was longer than 19 d (Table 3). We did not find mature thalli on *C. septempunctata*, *C. maculata*, and *H. convergens* after confinement with source *H. axyridis*. Only one *O. v-nigrum* target, confined with a source *H. axyridis*, harbored a mature thallus at 21 d (a longer period of thallus development than previously noted for target *O. v-nigrum* infected by source *O. v-nigrum*). Three other target *O. v-nigrum* surviving longer than 21 d with the source *H. axyridis* beetles were not infected.

We found two target *O. v-nigrum* infected after 13 ± 0 d when housed with source *O. v-nigrum* and these two infected targets represent 100% of available *O. v-nigrum*. The other *O. v-nigrum* targets (i.e., 83%) did not survive long enough for *H. virescens* thalli to mature (Table 3). The only other target found infected by source *O. v-nigrum* was *H. convergens*. A mature thallus was found on three (i.e., 30%) *H. convergens* after 17 ± 2 d (excluding two beetles that survived for only 13 d). Noninfected targets (i.e., *C. septempunctata*, *C. maculata*, and *H. axyridis*) survived longer than the time required before detection of a mature thallus on target *O. v-nigrum* or *H. convergens* (Table 3).

3.4. Substrate-Borne Transmission. We found no evidence of substrate-borne transmission of *H. virescens* (as suggested by a mature thallus on a target beetle) between adults of the same species, for either *H. axyridis* or *O. v-nigrum*. Mortality of target *H. axyridis* (mean ± SE) that remained alive for at least 15 d was 8 ± 8 and 12 ± 8% whether source beetles were exposed to Petri dishes for 24 or 120 h, respectively; similarly, mortality of target *O. v-nigrum* was and 8 ± 5 and 16 ± 10%, respectively.

4. Discussion

Transmission of *H. virescens* from a source to a target beetle was successful when a mature (or nearly mature) *H. virescens* thallus was on the target beetle [19]. In the absence of a mature thallus on a target beetle, we made no observations regarding whether ascospores transferred from source to target beetles. As such, we do not comment on whether absence of transmission resulted from transferred ascospores that germinated on the target beetle but failed to develop to maturity. Many physical, chemical, and biological factors affect adhesion of fungal spores to surfaces with subsequent attachment to and germination [20], any or all of which may have affected successful transmission in this study.

Direct transmission of Laboulbeniales, generally via sexual contact and within overwintering aggregations, is likely the primary mode of dispersal within the Coccinellidae [6, 11, 12, 14]. Overall, substrate-borne transmission of Laboulbeniales is rare considering that the ascospore is short lived [2]. We did not attempt to group beetles to determine evidence of transmission via sexual contact. Rather, our study grouped beetles in situations unlikely to occur naturally but very likely to result in considerable contact between source and target beetles. Under these conditions, we observed transmission (as denoted by target beetles with mature *H. virescens* thalli) between source and target beetles when both occupied the same container at the same time. In contrast, we did not observe transmission when target beetles occupied a container after removal of source beetles. Results presented here support the hypothesis that direct bodily contact between coccinellid hosts is necessary for transmission of *H. virescens* [2] and other Laboulbeniales [19, 21].

Transmission of *H. virescens* between coccinellids of the same species was more common than between different species in this study. Note that the tumbling experiment forced all paired source and target beetles, regardless of sex or species, to make considerable contact and allowed for horizontal transmission of *H. virescens* spores. Despite this, transmission between coccinellids of the same species still dominated. In the tumbling experiment, we only documented transmission between different species with target *C. septempunctata* exposed to *O. v-nigrum* or *H. axyridis* source beetles. Tumbling that led to the discharge of spores (onto the glass vial), which were transmitted to hosts is unlikely but cannot be ruled out from the results provided here. In addition to *C. septempunctata*, the only other instances of transmission between species was with target *H. convergens* exposed to source *O. v-nigrum* and target *O. v-nigrum* exposed to source *H. axyridis*.

In general, a higher rate of successful pathogen transmission within the same species is not surprising. Even though some entomopathogenic fungi such as *Beauveria bassiana* (Balsamo) and *Metarhizium anisopliae* (Metschnikoff) can infect a broad range of host insect species, isolates within each of these species can have a high degree of host specificity [22]. In fact, Cottrell and Shapiro-Ilan [23] found relatively high host specificity of *B. bassiana* isolates from source *O. v-nigrum* when tested against target *O. v-nigrum* and target *H. axyridis*. A similar scenario appears likely for *H. virescens* infecting different species of Coccinellidae. Isolates/strains of *H. virescens* may exist under field conditions and only infect closely related Coccinellidae or even a single species. At present, isolates/strains of *H. virescens* are unknown. Perhaps *H. virescens* that has had several passes on the same species is more virulent toward that species than to other species, similarly as Steinhaus [24] described increasing virulence of an entomopathogen.

In this study, the time required to detect a mature thallus on a target was less when the source and target were both *O. v-nigrum* than when the source and target were both *H. axyridis*. The separation in time to detect mature thalli on these two species is not explained simply by our schedule of observing specimens for mature thalli three times per week as opposed to daily. Even when we paired source and targets for one hour or seven days, detection of mature thalli occurred earlier when transmission was from *O. v-nigrum* to *O. v-nigrum* than from *H. axyridis* to *H. axyridis*. If there are no *H. virescens* isolates/strains infecting *O. v-nigrum* and *H. axyridis*, this time differential could be explained by nutritional quality of the two hosts and/or that more time is required for the ascospore to germinate and for the “foot” and haustoria to develop on *H. axyridis*. Although *H. virescens* may be pathogenic to numerous species of Coccinellidae, virulence against some species may be attenuated depending upon the number of passes it has gone through on other species, for example, *H. axyridis*. This could also explain why more time was required to detect a mature thallus on target *C. septempunctata*, infected from a source *O. v-nigrum*, than for *O. v-nigrum* intraspecific transmission.

As stated previously, our assay conditions used forced pairings/groupings not likely to occur in the field, yet we documented limited transmission between species. Direct contact between coccinellids of different species can occur in the field as promiscuous males attempt to mate with females of other coccinellid species [25, 26] (EWR, personal observation). Copulation (or mating attempts) between different coccinellid species has been observed in field cage tests among the phytophagous lady beetles *Henosepilachna yasutomii* Katakura and *H. niponica* Lewis [27]. Additionally, copulation attempts by a male *C. maculata* with an unidentified beetle species (Coleoptera: Cleridae) has been observed in the field (TEC, personal observation). Although we did not attempt to document copulation when source and target lady beetles were in Petri dishes for 7 d, we only observed transmission of *H. virescens* within the same host species. When source and target beetles were confined for an extended interval, mortality of source and target beetles was problematic in some groupings. Nonetheless, the results were similar as previously observed, that is, transmission of fungus occurred within the same host species with the exception of one target *O. v-nigrum* becoming infected from source *H. axyridis* and three *H. convergens* becoming infected from source *O. v-nigrum*. Infection through contact with substrate borne ascospores is rare among Laboulbeniales because the ascospore is short lived [2]. We did not find evidence of substrate-borne transmission between coccinellids in this study.

It is not clear why some coccinellid species sampled by Harwood et al. [8] and Riddick and Cottrell [10] had a relatively low prevalence of *H. virescens* infection. Their sampling methods, species abundance, or host specificity of *H. virescens* could have been influential. Further transmission studies on other species of Coccinellidae could provide insight regarding host specificity of *H. virescens*.

Interestingly, *H. virescens* represents one of the first parasites to infect *H. axyridis* in North America. Another parasite found on *H. axyridis* in North America is the ectoparasitic podapolipid mite *Coccipolipus hippodamiae* (McDaniel and Morrill) [28]. After *H. axyridis* established and quickly dispersed across North America, natural enemies may now be adapting to it. What impact *H. virescens* has on the dynamics of any coccinellid population is yet to be determined given that it is reported to cause anywhere from little impact to premature mortality [5, 6, 29].

In conclusion, it is likely that high host specificity and an apparent need for substantial periods of close contact between potential hosts will limit transmission of *H. virescens* by *H. axyridis* and *O. v-nigrum* with other coccinellids in the field [8, 10].

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Research Article

Taxonomic Position of the Oriental Species of *Mesosa* (*Mesosa*) (Coleoptera, Cerambycidae, Lamiinae, Mesosini)

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Twelve oriental mesosine species which had been belonged to the nominotypical subgenus of the genus *Mesosa* Latreille, 1829, are transferred to the subgenus *Dissosira* Pascoe, 1865 of the genus *Agelasta* Newman, 1842, as follows: *A. (D.) perplexa* (Pascoe, 1858); *A. (D.) columba* (Pascoe, 1859); *A. (D.) rufa* (Breuning, 1935); *A. (D.) catenatoides* Yamasako and N. Ohbayashi, nom. nov. [replacement name for *A. (D.) laosensis* (Breuning, 1935) already occupied by Pic (1925)]; *A. (D.) gardneri* (Breuning, 1938); *A. (D.) nigropunctata* (Breuning, 1938); *A. (D.) konoi* (Hayashi, 1956); *A. (D.) yonaguni* (Hayashi, 1962); *A. (D.) nigrostictica* (Breuning, 1967); *A. (D.) siamana* (Breuning, 1974); *A. (D.) praelongipes* (Kusama and Irie, 1976); *A. (D.) kumei* (Takakuwa, 1991).

1. Introduction

The genus *Mesosa* was erected by Latreille [1] on the basis of two Palearctic species, *Cerambyx curculionoides* Linnaeus, 1761 and *Lamia nebulosa* Fabricius, 1781, of which the former was designated as the type species of the genus. Later, Breuning [2] divided the genus *Mesosa* into six subgenera as follows: *Mesosa* (*Mesosa*) Latreille 1829; *M.* (*Aphelocnemia* [sic]) Stephens, 1831; *M.* (*Saimia*) Pascoe, 1866; *M.* (*Anthlyboscila*) Thomson, 1868; *M.* (*Perimesosa*) Breuning, 1939; *M.* (*Metamesosa*) Breuning, 1939. Recently, Yamasako and Ohbayashi [3] erected a new subgenus, *M.* (*Lissomesosa*), and they also [4] synonymized *M.* (*Anthlyboscila*) with *Agelasta* (*Dissosira*) Pascoe, 1865. As a result, the genus *Mesosa* now consists of six subgenera and includes more than 80 species.

Among the subgenera, *Mesosa* (*Mesosa*) is the nominotypical subgenus and comprised of 19 species until now. However, as a result of our close examination of the external and male genital features, they are considered to be polyphyletic and could be separated into some species groups. Of those, one group, including the name-bearing type *M. curculionoides* (Linnaeus, 1761), is the true nominotypical species group and is distributed throughout the Palearctic region. On the other hand, the 12 oriental species are a different phyletic line from the Palearctic species group and are closely related to the subgenus *Dissosira* Pascoe, 1865, of the genus *Agelasta* Newman, 1842.

As we have already pointed out in a previous paper (Yamasako and Ohbayashi [4]), the hitherto distinguishable feature between *Mesosa* and *Agelasta*, the rounded or truncated prosternal process in lateral view, is not a suitable characteristic, and many species in the genus *Mesosa* have been confused with the genus *Agelasta* because of this problem. According to other external features and the endophallic structures, the 12 species distributed in the Oriental region should be transferred to *Agelasta* (*Dissosira*) in spite of their rounded prosternal process. Therefore, we herein propose them to be transferred from *Mesosa* (*Mesosa*) to *Agelasta* (*Dissosira*). This is the ninth part of our studies on the Asian Mesosini.

2. Materials and Methods

This study was conducted based on the dried specimens preserved in the following public collections, our private collections, and also the collections of friends.

BMNH: The Natural History Museum, London, UK.

EUMJ: Ehime University Museum, Matsuyama, Japan.

MNHN: Muséum National d'Histoire Naturelle, Paris, France.

ZSM: Zoologische Staatssammlung München, Munich, Germany.

The verbatim label data indicated by double quotation marks ("") are given for the type materials, and the line breaks of the label are indicated by a slash (/).

The observational method and the corresponding terms of endophallus should be referred to Yamasako and Ohbayashi [5].

3. Systematics

Agelasta (Dissosira) Pascoe, 1865

Type species. *Agelasta catenata* Pascoe, 1862 (Figures 1(d)–1(f), 2(c)-2(d), 3(a)-3(b), 6(a), 7(a)–7(d), and 9(a)).

Dissosira Pascoe, 1865. 124, note [6].

Chaeromorpha (*Dissosira*): Aurivillius, 1922: 145 [7].

Agelasta (*Dissosira*): Breuning, 1939: 482 [2].

Anthriboscyla Thomson, 1868. 165 [8]; type species: *Anthriboscyla mima* Thomson, 1868.

Mesosa (*Anthriboscyla*): Breuning, 1939: 411 [2].

Pseudaemocia Breuning, 1935. 269 [9]; type species: *Pseudaemocia rufa* Breuning, 1935 syn. nov.

Mutatocoptops (*Pseudaemocia*): Breuning, 1939: 506 [2].

Redescription (Modified the Diagnosis of Yamasako and Ohbayashi [4]). Body ovoid in shape; eyes subdivided; lower lobes relatively large, slightly wider than long. Antennal tubercles hardly elevated. Antennae well long and thick; each segment without apical spine, fringed beneath by suberect short setae; scape well long, slightly thickened apically, with a well-developed cicatrix on the apex; third segment distinctly longer than scape and fourth, respectively. Pronotum wider than long, with some indistinct discal tubercles; each side near apical margin usually with a small dull projection. Prosternal process with extremity usually well swollen posteroventrally, almost truncate in lateral view, but sometimes hardly swollen and more or less roundly sloped in lateral view. Mesosternal process with a well-developed tubercle on the center near the apex and roundly projected anteroventrally, almost truncate in lateral view.

Elytra without basal high bosses and lacking long suberect hairs. Legs with mesotibiae without distal notch on anterior side.

Endophallus. Endophallus well long and slender, approximately three times as long as median lobe, divided into BPH, MPH, and APH, with three kind spicule like sclerites as MSp, LSp, and SSp. BPH nearly 0.2 times as long as endophallus. MPH nearly 0.7 times as long as endophallus, subdivided into two membrane subdivisions as almost fused MT+CT and PB by a distinct constriction. APH well swollen and oval bursiform, nearly 0.2 times as long as endophallus, with ED on dorsal side, usually with AA which is lingulate shape and laid near ED, without AS.

MSp usually distributed in nearly apical half of MT+CT. LSp usually distributed in nearly basal half of MT+CT; dorsal side ones thick and short, arranged into two irregular longitudinal lines, unidentate in apical area, thence becoming thicker, denser, and multidentate toward basal area; ventral side ones rudimentary unidentate, and disappeared in some cases. SSp unidentate, short, and small, covered almost entire area of PB. MSp area and LSp area adjacent. LSp area and SSp area are separated but close to each other.

Remarks. Up to the present time, the genus *Agelasta* includes over 70 species which are separated into nine subgenera. *Agelasta* (*Dissosira*) has mainly been distinguished from the other subgenera by the following features (Breuning [2]): (1) pronotum without five distinct tubercules on disk, (2) elytra with basal margin not forming transversal edge, without high bosses near base and long suberect setae throughout, (3) humeri weakly projected laterad, (4) prosternal process well truncated in lateral view.

As already indicated by Yamasako and Ohbayashi [4], 24 known species belonging to the subgenus *Dissosira* could be separated into some species groups based on the external and male genital features. The redescription above is based on the type species group of the subgenus (Yamasako and Ohbayashi [4]).

The Species Transferred from Mesosa (Mesosa) to Agelasta (Dissosira). The following 12 species which are classified into *Mesosa* (*Mesosa*) are distinctly different from the type species of the subgenus, *M.* (*M.*) *curculionoides* (Figures 1(a)–1(c), 2(a)-2(b)) in the external features and the endophallic structures. These characteristics well coincide with *Agelasta* (*Dissosira*) except for the rounded prosternal process.

3.1. *Agelasta (Dissosira) perplexa* (Pascoe, 1858), Comb. Nov. (Figures 3(c)-3(d), 6(b), 7(e)–7(h), and 9(b))

Mesosa perplexa Pascoe, 1858: 243 [6].

Pachyosa perplexa: Matsushita, 1933: 344 [10].

Mesosa (*Mesosa*) *perplexa*: Breuning, 1939: 401 [2].

Saimia alternans Schwarzer, 1925: 60 [11].

Mimocoptops? *formosana* Pic, 1925: 30 [12].

Diagnosis. Body black, covered with ocher pubescence. Occiput with four narrow longitudinal black bands. Antennae with each basal part of third to the last segments with white pubescent annulations though it is very narrow on fifth, seventh, and ninth segments. Pronotum with three longitudinal narrow black bands on disk. Elytra with two light yellowish

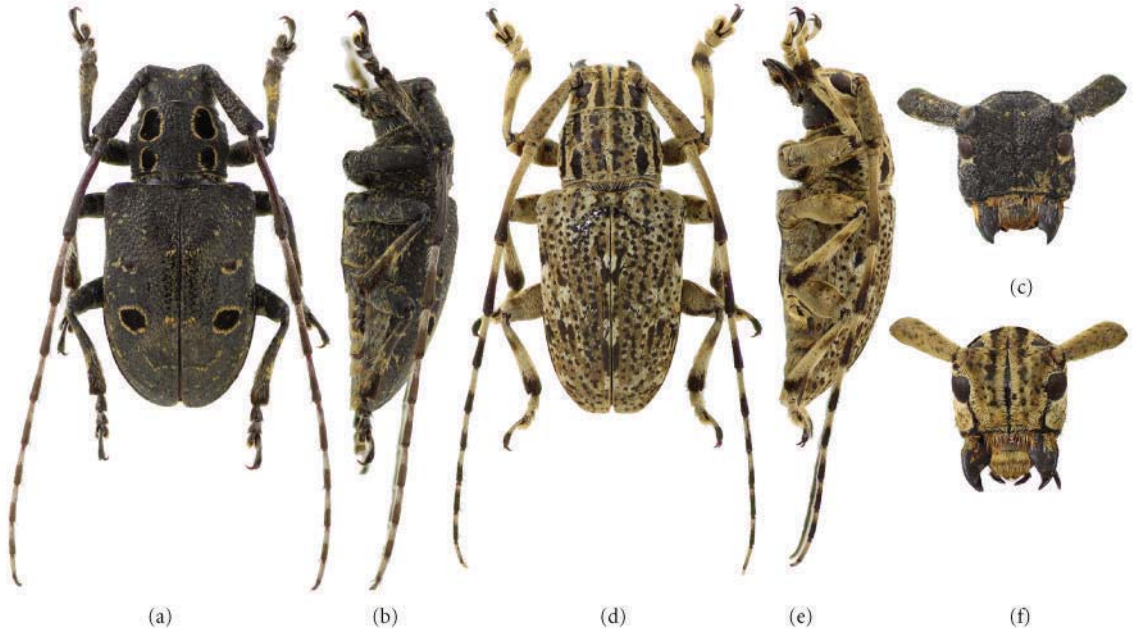
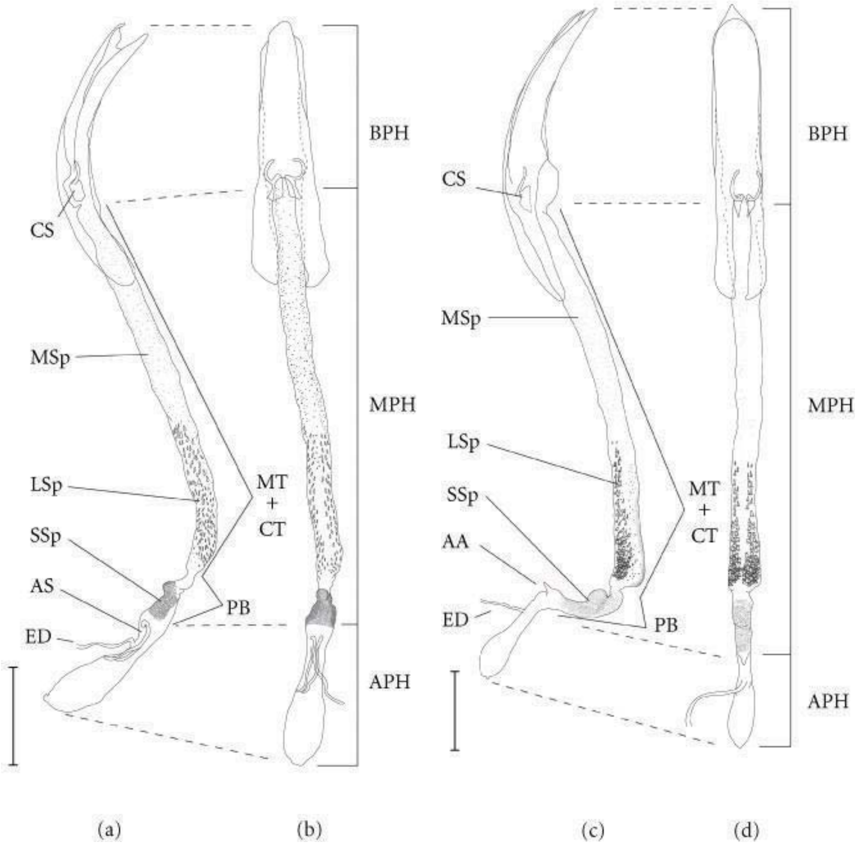


Figure 1: Comparison of the type species of *Mesosa* (*Mesosa*) and *Agelasta* (*Dissosira*). (a–c) *M.* (*M.*) *curculionoides*; (d–f) *A.* (*D.*) *catenata*; (a, d) male habitus in dorsal view; (b, e) ditto in lateral view; (c, f) ditto in frontal view.


BPH
CS
MSp
MPH
LSp
MT
+
CT
SSp
AS
PB
ED
APH
BPH
CS
MSp
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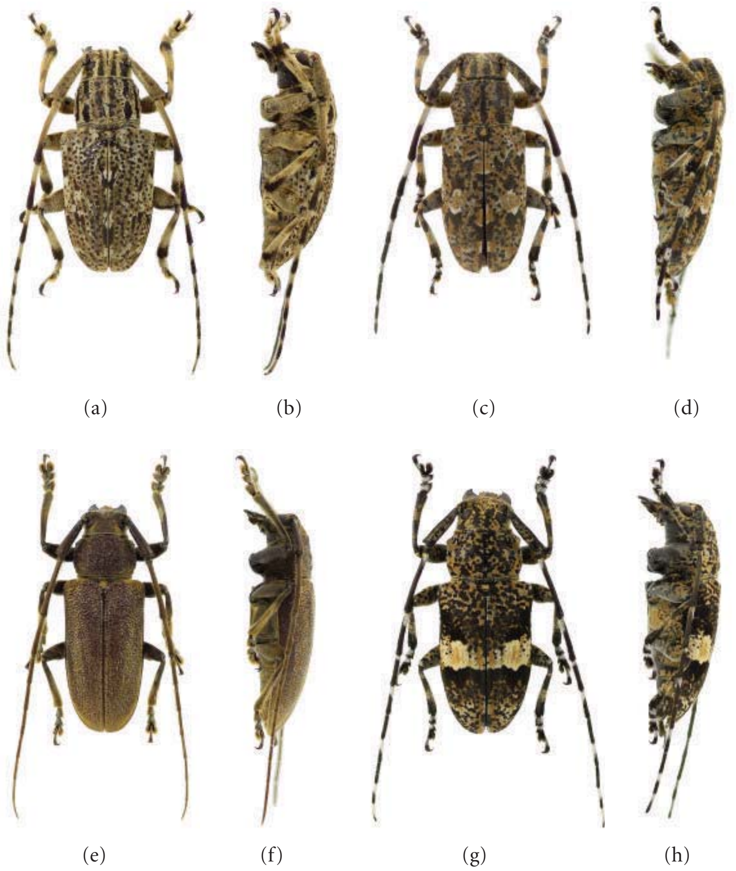
LSp
SSp
MT
+
CT
AA
ED
PB
APH
(a)
(b)
(c)
(d)

Figure 2: Comparison of the type species of *Mesosa* (*Mesosa*) and *Agelasta* (*Dissosira*). (a, b) *M.* (*M.*) *curculionoides*; (c, d) *A.* (*D.*) *catenata*; (a, c). median lobe with endophallus in lateral view; (b, d) ditto in dorsal view. Scale: 1.0 mm. For abbreviations see text.


(a) (b) (c) (d)

(e) (f) (g) (h)

FIGURE 3: Male habitus of *Agelasta* (*Dissosira*) spp. (a, b) *A.* (*D.*) *catenata*; (c, d) *A.* (*D.*) *perplexa*; (e, f) *A.* (*D.*) *rufa*; (g, h) *A.* (*D.*) *konoi*; (a, c, e, g) dorsal view; (b, d, f, h) lateral view.

or whitish brown bands which are marginated with black irregular spots. Prosternal process roundly sloped and not truncate in lateral view.

Male Genitalia (n = 2). Tegmen in lateral view slightly curved, slender rhombic, and widest near middle in ventral view. Ringed part slightly expanded laterad at near middle of tegmen, thence arcuately narrowed basad. Lateral lobes about 1/3 of total length of tegmen, with inner sides almost straight and outer sides nearly straight toward apical third, thence slightly narrowed to angularly rounded apex, provided with two kinds of setae of which one is long and thick, concentrated near apex, and another is rather short and thin, arising chiefly from outer sides of apical half.

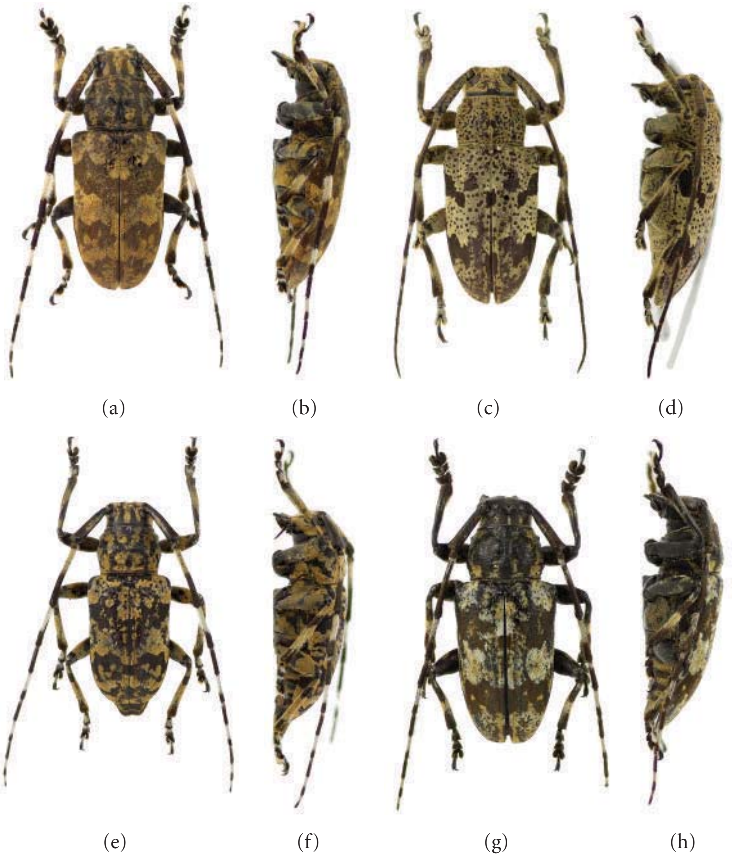
Median lobe in lateral view gentry curved; apex in ventral view weakly pointed; median strut dehiscent from basal 2/5.

Endophallus almost three times as long as median lobe in lateral view. The relative length of each broad membrane area of endophallus to median lobe are as follows: ML : TLE : BPH : MPH (MT+CT : PB) : APH = 3.7 : 10.0 : 2.2 : 5.9 (5.0 : 0.8) : 2.0. MPH with MT+CT well swollen and projected on ventral side near base. APH well swollen in oval shape, with ligulate shaped AA near apex.

MSp distributed in nearly apical 2/3 of MT+CT. LSp distributed in almost basal 1/3 of MT+CT on dorsal side, thick and short, arranged into two longitudinal irregular lines, unidentate in apical area, thence becoming thicker, denser, and multidentate toward basal area. SSp short, small and unidentate, covered almost of laterodorsal side and basal 1/3 of ventral side of PB. MSp area and LSp area adjacent. LSp area and SSp area close to each other.

Specimens Examined. [China] Syntype (BMNH): 1♀, "China" [printed on green oval label]; "Type" [printed on white label with red circle]; "Mesosa/perplexa/Pasc/N. China" [printed on white label]; "Pascoe/Coll./93–60." [printed on white label]; "Mesosa/perplexa/China Pasc." [printed on white label]; 1♂, She Shan, Shanghai, 9. VII, 2002, Hu and Tang leg. [Taiwan]: 1♂, Shouka forest road, Shizi township, Pingtung county, 4. VII, 2006, S-T. Hisamatsu leg. [Japan]: 3♂♂, 3♀♀, Yanaimachi, Matsuyama City, Ehime Pref., 20. VI, 1997, N. Ohbayashi leg.; 1♂, Shindate, Matsuyama City, Ehime Pref., 2. XI, 2004, J. Yamasako leg.; 1♂, same locality, 10. XI, 2004, J. Yamasako leg.

Distributions. China, Taiwan, Japan.



(a) (b) (c) (d)

(e) (f) (g) (h)

Figure 4: Male habitus of *Agelasta* (*Dissosira*) spp. (a, b) *A.* (*D.*) *yonaguni*; (c, d) *A.* (*D.*) *nigrostictica*; (e, f) *A.* (*D.*) *praelongipes*; (g, h) *A.* (*D.*) *kumei*; (a, c, e, g) dorsal view; (b, d, f, h). lateral view.

3.2. Agelasta (Dissosira) columba (Pascoe, 1859), Comb. Nov. (Figure 5(a))

Mesosa columba Pascoe, 1859: 40 [6].

Mesosa (*Mesosa*) *columba*: Breuning, 1939: 402 [2].

Diagnosis. This species is similar to *A. perplexa*, but distinguishable by the following features. Body black, covered with light brown pubescence. Pronotum with two pair of small rounded black maculae on disk. Elytra with several white spots which are sometimes forming transversal band near middle, scattered with some spots of dark brown pubescence which are sometimes forming indistinct transversal band before and after middle of elytra. Prosternal process roundly sloped and not truncate in lateral view.

Specimen Examined. Syntype (BMNH): 1♂, "Ceylon" [printed on light blue circle label]; "Mesosa/columba/Pascoe/type" [printed on white label]; "Type" [printed on white label with red circle].

Distribution. Sri Lanka.

Remarks. No specimen was available for dissection of male genitalia. However, this species distinctly differs from *Mesosa* (*Mesosa*) in the following structures: antennal scape elongate and slightly thickened apically; lower eye lobe relatively large. Besides, the external characteristics of this species are similar to *A. perplexa* and well coincided with *Agelasta* (*Dissosira*). Therefore, we treat this species as a member of this subgenus.

3.3. Agelasta (Dissosira) rufa (Breuning, 1935), Comb. Nov. (Figures 3(e)–3(f), 6(c), 7(i)–7(l), and 9(c))

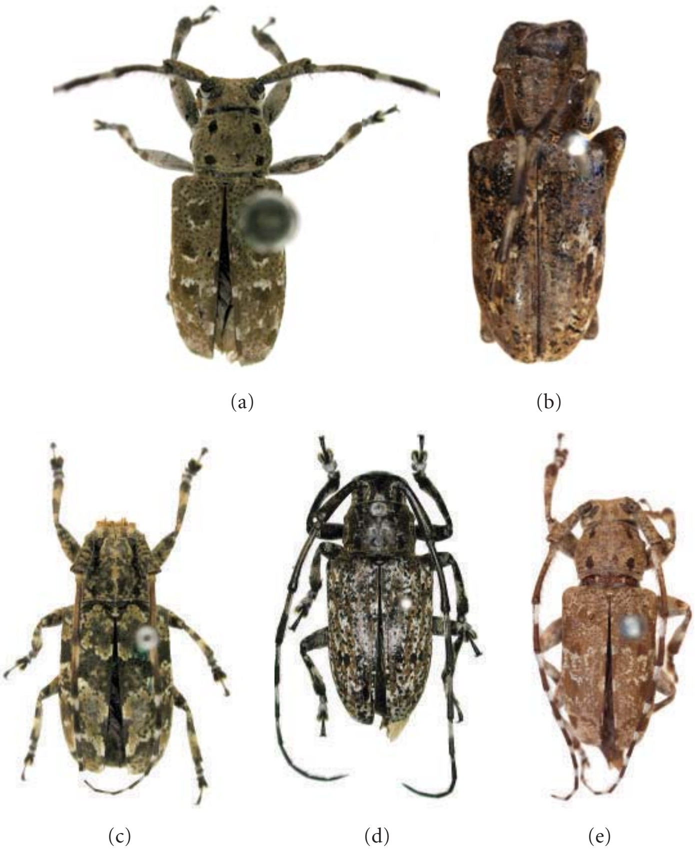
Pseudaemocia rufa Breuning, 1935: 269 [9].

Mutatocoptops (*Pseudaemocia*) *rufa*: Breuning, 1939: 506 [2].

Mesosa (*Mesosa*) *rufa*: N. Ohbayashi, 1992: 8 [13].

Diagnosis. Body reddish brown, sparsely covered with yellowish pubescence. Elytra sparsely with spots of yellowish pubescence which are sometimes forming some irregular longitudinal narrow maculae. Prosternal process rounded and not truncate at the apex.

Male Genitalia (n = 2). Tegmen in lateral view slightly curved, rhombic, and widest near middle in ventral view. Ringed part slightly expanded laterad at near middle of tegmen, thence arcuately narrowed basad. Lateral lobes about 1/4 of



(a) (b)

(c) (d) (e)

Figure 5: Habitus of *Agelasta* (*Dissosira*) spp. in dorsal view. (a) *A.* (*D.*) *columba* (syntype); (b) *A.* (*D.*) *catenatoides* (holotype); (c) *A.* (*D.*) *gardneri* (holotype); (d) *A.* (*D.*) *nigropunctata*; (e) *A.* (*D.*) *siamana* (holotype).

total length of tegmen, with inner sides almost straight and outer sides gently narrowed to rounded apex, provided with two kinds of setae of which one is long and thick, concentrated near apex, and another is rather short and thin, arising chiefly from outer sides of apical half.

Median lobe in lateral view gentry curved; apex in ventral view weakly pointed; median strut dehiscent from basal 1/3.

Endophallus almost 2.5 times as long as median lobe in lateral view. The relative length of each broad membrane area of endophallus to median lobe are as follows: ML : TLE : BPH : MPH (MT+CT : PB) : APH = 4.1 : 10.0 : 2.3 : 5.9 (4.9 : 1.0) : 1.8. MPH with MT+CT well swollen and projected on ventral side near base. APH well swollen in oval shape, with ligulate-shaped AA near apex.

MSp distributed in nearly apical 2/3 of MT+CT. LSp distributed in almost basal 1/3 of MT+CT on dorsal side, thick and short, arranged into two longitudinal irregular lines, unidentate in apical area, thence becoming thicker, denser, and multidentate toward basal area. SSp unidentate, short and small, covered almost of laterodorsal side and basal 1/3 of ventral side of PB. MSp area and LSp area adjacent. LSp area and SSp area slightly separated each other.

Specimens Examined. Holotype (BMNH): 1♀, “Parry’s Group/Bonin I./91–25” [printed on white label]; “Type” [printed on white label with red circle]; “Pseudaemocia/rufa mihi/Typ!/det. Breuning” [printed on white label]. Ogasawara Isls., Tokyo Pref., Japan, [Is. Chichijima]: 1♀, 25. X, 1974, M. Iga leg. [Is. Hahajima]: 1♂, 3♀♀, Chibusa-yama, 15. VI, 1992, N. Ohbayashi leg.; 1♂, Okimura, 16. VI, 1992, N. Ohbayashi leg.; 1♂, Mt. Funaki, 16. VI, 1991, T. Ito leg.; 1♀, 17. V, 1984, M. Hasegawa leg.; 1♂, 2♀♀, 15–17. VI, 1985, H. Makihara leg.; 1♂, 2♀♀, Funamidai, 24–27, VI. 1987, M. Nishimura leg.; 1♂, 1♀, Motochi, 25, VI. 1987, M. Nishimura leg. [Is. Mukojima]: 1♂, 19. X, 2002, H. Karube leg.

Distribution. Japan (Ogasawara Isls.).

Remarks. This species is endemic to the Ogasawara Islands, Japan. It was first described based on a female specimen as a unique species of the genus *Pseudaemocia* Breuning, 1935, which was downgraded to a subgenus of *Mutatocoptops* Pic, 1925 by Breuning [2]. Later, Ohbayashi [13] transferred it to *Mesosa* (*Mesosa*) by reason of the resemblance of its larval characters to *M.* (*M.*) *yonaguni*. In spite of its unique appearance of reddish body, very rough punctures on the body and indistinct maculae on the elytra, this species has male genital structures almost in common with *M.* (*M.*) *yonaguni*, and the external features basically coincide with the congeners of

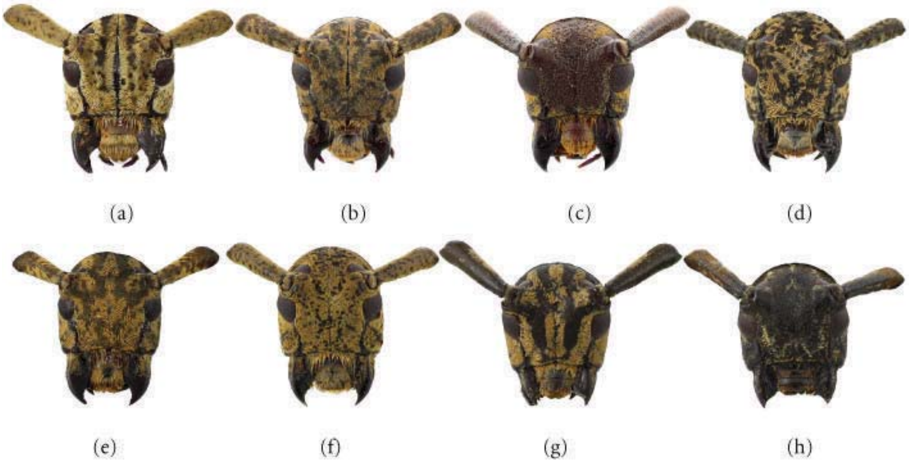


FIGURE 6: Male frontal view of *Agelasta* (*Dissosira*) spp. (a) *A.* (*D.*) *catenata*; (b) *A.* (*D.*) *perplexa*; (c) *A.* (*D.*) *rufa*; (d) *A.* (*D.*) *konoi*; (e) *A.* (*D.*) *yonaguni*; (f) *A.* (*D.*) *nigrostictica*; (g) *A.* (*D.*) *praelongipes*; (h) *A.* (*D.*) *kumei*.

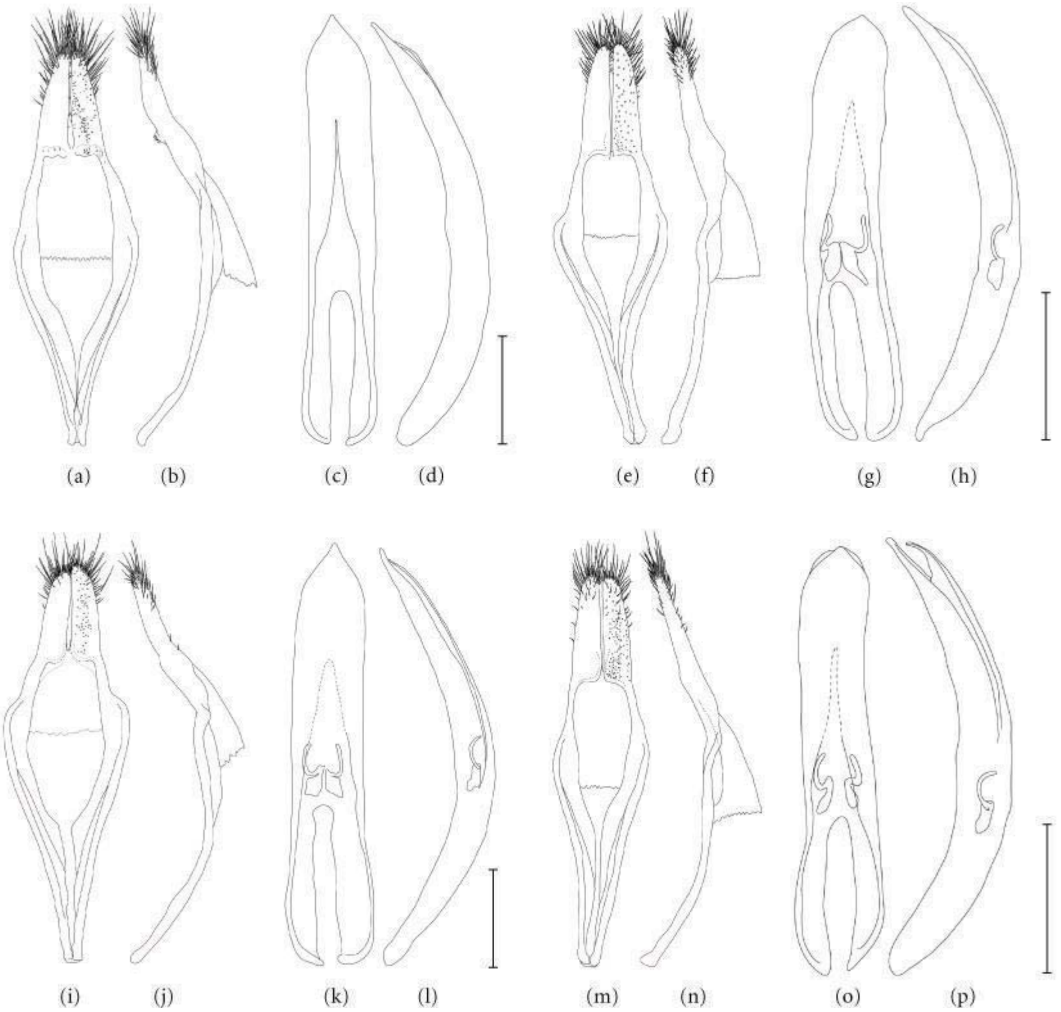


FIGURE 7: The male genital organ of *Agelasta* (*Dissosira*) spp. (a–d) *A.* (*D.*) *catenata*; (e–h) *A.* (*D.*) *perplexa*; (i–l) *A.* (*D.*) *rufa*; (m–p) *A.* (*D.*) *konoi*; (a, e, i, m) tegmen in ventral view; (b, f, j, n) ditto in lateral view; (c, g, k, o) median lobe in ventral view; (d, h, l, p). ditto in lateral view. Scale: 1.0 mm.

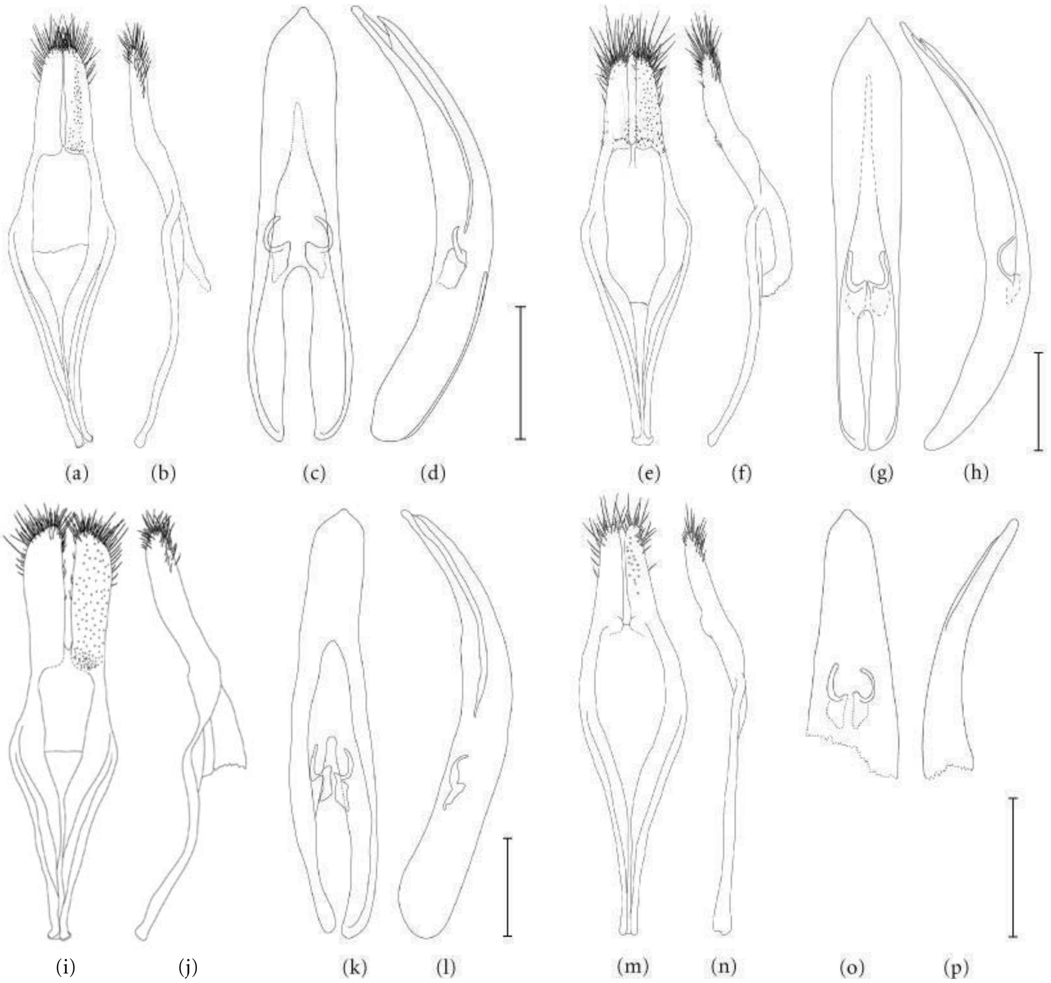


Figure 8: The male genital organ of *Agelasta* (*Dissosira*) spp. (a–d) *A.* (*D.*) *yonaguni*; (e–h) *A.* (*D.*) *nigrostictica*; (i–l) *A.* (*D.*) *praelongipes*; (m–p) *A.* (*D.*) *kumei*; (a, e, i, m) tegmen in ventral view; (b, f, j, n) ditto in lateral view; (c, g, k, o) median lobe in ventral view; (d, h, l, p) ditto in lateral view. Scale: 1.0 mm.

Agelasta (*Dissosira*). Therefore, we treat this species as a member of this subgenus.

3.4. *Agelasta (Dissosira) catenatoides* Yamasako and N. Ohbayashi, Nom. Nov. (Nomen Preoccupied by *Agelasta laosensis* Pic, 1925) (Figure 5(b))

Mesosa laosensis Breuning, 1935: 274 [9].

Mesosa (*Mesosa*) *laosensis*: Breuning, 1939: 402 [2].

Diagnosis. This species is quite similar to *A. catenata*, but distinguishable by body covered with brown pubescence.

Specimen Examined. Photographs of the holotype (MNHN): ♂, "Mesosa/laosensis/mihi Typ/det. Breuning" [printed on white label], "Vieng Kiet/5. oct. 1915/Vitalis" [printed on white label], "type" [printed on yellow label], "laosensis Br." [printed on white label], "TYPE" [printed on red label].

Distribution. Laos.

Remarks. No specimen was available for dissection of the male genitalia. The diagnosis described above is based on some photographs of the holotype. Judging from the photographs, this species is without doubt close to *A.* (*D.*) *catenata* which is the type species of *Agelasta* (*Dissosira*). Therefore, we treat this species as a member of *Agelasta* (*Dissosira*). On the other hand, the species name, *Agelasta laosensis*, is already occupied by Pic [12], and *A. laosensis* (Breuning, 1935 nec Pic, 1925) should be replaced by the secondary homonym. Therefore, we propose a new replacement name, *A.* (*D.*) *catenatoides* nom. nov., for the latter. The specific epithet refers to the resemblance to *A.* (*D.*) *catenata*.

3.5. *Agelasta (Dissosira) gardneri* (Breuning, 1938), Comb. Nov. (Figure 5(c))

Mesosa gardneri Breuning, 1938: 204 [14].

Mesosa (*Mesosa*) *gardneri*: Breuning, 1939: 402 [2].

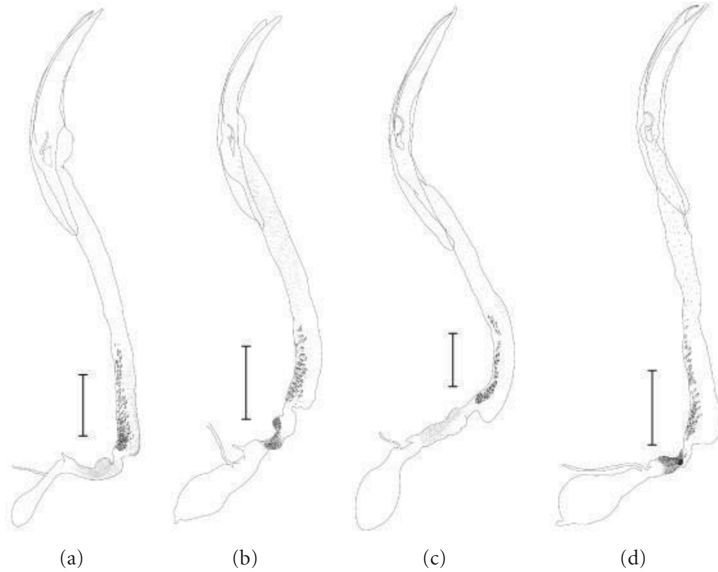

(a) (b) (c) (d)

Figure 9: The median lobe with endophallus of *Agelasta* (*Dissosira*) spp. in lateral view. (a) *A.* (*D.*) *catenata*; (b) *A.* (*D.*) *perplexa*; (c) *A.* (*D.*) *rufa*; (d) *A.* (*D.*) *konoi*. Scale: 1.0 mm.

Diagnosis. This species is similar to *A. perplexa*, but distinguishable by the following features. Body black, mingled with light and dark brown pubescence. Antennae with each basal part of third to the last segments with white pubescent annulations, which are getting narrower toward the last segment. Elytra with indistinct transversal bands of light brown pubescence near base and after middle, indistinct dark brown transversal bands before middle and near apices, scattered with white spots.

Specimen Examined. Holotype (BMNH): "Lachiwala/Dehra Dun, U. P./F. Ent./17. VII. 1929" [printed on white label], "R. R. D. 892/B. C. R. 284/Cage 712" [printed on white label], "Ex Bauhinia/retusa." [printed on white label], "37" [printed on white label], "Mesosa/gardneri/mihi Type/det. Breuning" [printed on white label], "Type" [printed on white circle label with red margined].

Distribution. North India.

Remarks. No specimen was available for dissection of the male genitalia. However, this species basically shares external characteristics with *A.* (*D.*) *perplexa*. Therefore, we treat this species as a member of *Agelasta* (*Dissosira*).

3.6. *Agelasta (Dissosira) nigropunctata* (Breuning, 1938), Comb. Nov. (Figure 5(d))

Mesosa nigropunctata Breuning, 1938: 203 [14].

Mesosa (*Mesosa*) *nigropunctata*: Breuning, 1939: 402 [2].

Diagnosis. This species is very similar to *A. catenata*, but distinguishable by the following features. Body black, mingled with black, brown, and white pubescence. Occiput with four narrow longitudinal black bands. Antennae with each basal part of third to 7th segments with white pubescent annulations, which are getting narrower toward 7th segment; the reminders covered with black pubescence. Pronotum with two longitudinal narrow black bands on disk. Elytra irregularly scattered with longitudinal spots of black, brown, and white pubescence. Prosternal process roundly sloped and not truncate in lateral view.

Specimen Examined. 1♂, Mulayit Taung, SE-Burma, 11, 1989 (ZSM, determined by Hüdepohl in 1995).

Distribution. Myanmar.

Remarks. No specimen was available for dissection of the male genitalia. However, this species basically shares external characteristics with *A.* (*D.*) *catenata* which is the type species of *Agelasta* (*Dissosira*). Therefore, we treat this species as a member of *Agelasta* (*Dissosira*).

3.7. *Agelasta (Dissosira) konoi* (Hayashi, 1956), Comb. Nov.

Remarks. This species is mainly distributed in the northeastern part of the Ryukyu Islands, Japan, and is divided into five subspecies as described below. Here we limited the description to the nominotypical subspecies only, and that of the other subspecies are omitted.

3.7.1. *Agelasta (Dissosira) konoi konoi* (Hayashi, 1956), Comb. Nov. (Figures 3(g)-3(h), 6(d), 7(m)–7(p), and 9(d))

Mesosa (*Mesosa*) *konoi* Hayashi, 1956: 13, pl. 4, Figure 1 [15].

Mesosa (*Mesosa*) *konoi konoi*: Hayashi, 1962: 33 [16].

Diagnosis. This species is similar to *A. perplexa*, but distinguishable by the following features. Body black, covered with ocher pubescence. Elytra with a yellowish white maculae marginated with black pubescence near middle.

Male Genitalia ($n = 2$). Tegmen in lateral view slightly curved, slender rhombic, and widest near middle in ventral view. Ringed part slightly expanded laterad near middle of tegmen, thence arcuately narrowed basad. Lateral lobes about 1/4 of total length of tegmen, with inner sides almost straight, and outer sides nearly straight toward apical third, thence slightly narrowed to rounded apex, provided with two kinds of setae of which one is long and thick, concentrated near apex, and another is rather short and thin, arising mainly from laterodorsal sides of apical half.

Median lobe in lateral view gentry curved; apex in ventral view weakly pointed; median strut dehiscent from basal 2/5.

Endophallus almost three times as long as median lobe in lateral view. The relative length of each broad membrane area of endophallus to median lobe is as follows: ML : TLE : BPH : MPH (MT+CT : PB) : APH = 3.5 : 10.0 : 2.1 : 6.1 (5.5 : 0.6) : 1.9. MPH with MT+CT well swollen and projected on ventral side near base. APH well swollen in oval shape, with ligulate-shaped AA near apex.

MSp distributed in nearly apical half of MT+CT. LSp distributed in nearly basal half of MT+CT on dorsal side, thick and short, arranged into two longitudinal irregular lines, unidentate in apical area, thence becoming thicker, denser, and multidentate toward basal area. SSp unidentate, short and small, covered almost of laterodorsal side and basal 1/3 of ventral side of PB. MSp area and LSp area adjacent. LSp area and SSp area close to each other.

Specimens Examined. 1♂, 1♀, Is. Nakanoshima, Tokara Isls., 7. VII, 1960, M. Satô leg.; 3♂♂, 1♀, same locality, 21–22. VII, 1969, M. Sakai leg.; 10♂♂, 10♀♀, same locality, 23. VI, 2003, J. Yamasako leg.

Distribution. Japan (Tokara Isls., Kagoshima Pref.; Is. Izu-Ôshima, Tokyo Pref.).

3.7.2. *Agelasta (Dissosira) konoi amamiana* (Hayashi, 1962) Comb. Nov.

Mesosa (*Mesosa*) *konoi amamiana* Hayashi, 1962: 13, pl. 3, Figure 11 [17].

Specimens Examined. 1♂, 1♀, Hatsuno, Is. Amami-Ôshima, 10. VII, 1962, N. Ohbayashi leg.; 1♀, same locality, 29. VII, 1962, N. Ohbayashi leg.; 1♂, 1♀, same locality, 13. VI, 1962, M. Satô leg.; 1♂, Shinokawa, Is. Amami-Ôshima, 18. VI, 1997, S. Yoshimichi leg.

Distribution. Japan (Is. Amami-Ôshima, Kagoshima Pref.).

3.7.3. *Agelasta (Dissosira) konoi okinoerabuensis* (Ohbayashi, 1959), Comb. Nov.

Mesosa (*Mesosa*) *konoi okinoerabuensis* Ohbayashi, 1959: 3 [18].

Specimens Examined. 2♂♂, 1♀, Is. Okinoerabu, 6. VI, 1957, M. Umebayashi leg.; 1♀, same locality, 13. VII, 1963, N. Ohbayashi leg.; 2♂♂, same locality, 27. VI, 1964, M. Nishikawa coll.

Distribution. Japan (Is. Okinoerabu, Kagoshima Pref.).

3.7.4. *Agelasta (Dissosira) konoi okinawana* (Hayashi, 1960), Comb. Nov.

Mesosa (*Mesosa*) *perplexa okinawana* Hayashi, 1960: 27 [19].

Mesosa (*Mesosa*) *konoi okinawana*: Hayashi, 1962: 13 [17].

Mesosa (*Saimia*) *cervinopicta*: Gressitt, 1951: 220 (part.: Is. Okinawa, Japan) (nec Fairmaire, 1897) [20].

Specimens Examined. 1♂, Takari, Is. Okinawa, 29. VI, 1993, N. Ohbayashi leg.; 1♀, Mt. Yonahadake, Is. Okinawa, Okinawa Pref., Japan, 2. VII, 1993, N. Ohbayashi leg.

Distribution. Japan (Is. Okinawa, Okinawa Pref.).

3.7.5. *Agelasta (Dissosira) konoi kumejimana* (Kusama and Takakuwa, 1984) Comb. Nov.

Mesosa (*Mesosa*) *konoi kumejimana* Kusama and Takakuwa, 1984: 356 [21].

Specimen Examined. 1♂, Nakadomari, Is. Kumejima, Okinawa Pref., Japan, 25. III–30. VII, 1990, T. Ito leg.

Distribution. Japan (Is. Kumejima, Okinawa Pref.).

3.8. *Agelasta (Dissosira) yonaguni* (Hayashi, 1962), Comb. Nov.

Remarks. This species is distributed in the southwestern part of the Ryukyu Islands and is divided into four subspecies as described below. Here we limited the description to the nominotypical subspecies only, and that of the other subspecies are omitted.

3.8.1. *Agelasta (Dissosira) yonaguni yonaguni* (Hayashi, 1962) Comb. Nov. (Figures 4(a)-4(b), 6(e), 8(a)–8(d), and 10(a))

Mesosa (*Mesosa*) *cervinopicta yonaguni* Hayashi, 1962: 5, pl. 1, Figure 5 [22].

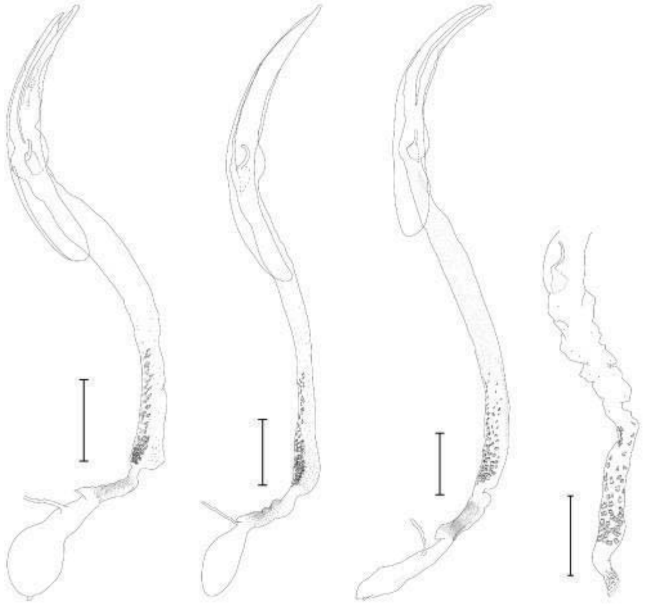

(a) (b) (c) (d)

FIGURE 10: The median lobe with endophallus of *Agelasta* (*Dissosira*) spp. in lateral view. (a) *A.* (*D.*) *yonaguni*; (b) *A.* (*D.*) *nigrostictica*; (c) *A.* (*D.*) *praelongipes*; (d) *A.* (*D.*) *kumei*. Scale: 1.0 mm.

Mesosa (*Pachyosa*) *cervinopicta yonaguni*: Samuelson, 1965: 100 [23].

Mesosa (*Mesosa*) *yonaguni*: Kusama and Irie, 1976: 20 [24].

Pachyosa cervinopicta: Miwa, 1935: 37 (Is. Yonaguni, Japan) (nec Fairmaire, 1897) [25].

Mesosa (*Saimia*) *cervinopicta*: Gressitt, 1951: 220 (nec Fairmaire, 1897) [20].

Diagnosis. This species is similar to *A. perplexa*, but distinguishable by the following features. Body black, mingled with yellowish ocher pubescence and black pubescence. Pronotum with disk with some irregular longitudinal black bands. Elytra mingled with yellowish ocher and black patches, of which yellowish ones are forming transversal irregular bands on post humeri, near middle, and near apical fourth. Prosternal process roundly truncated in lateral view.

Male Genitalia (n = 1). Tegmen in lateral view slightly curved, slender rhombic, and widest near middle in ventral view. Ringed part slightly expanded laterad near middle of tegmen, thence arcuately narrowed basad. Lateral lobes about 1/4 of total length of tegmen, with inner sides almost straight, and outer sides gently narrowed to angularly rounded apex, provided with two kinds of setae of which one is long and thick, concentrated near apex, and another is rather short and thin, arising chiefly from outer sides of apical half.

Median lobe in lateral view gentry curved; apex in ventral view weakly pointed; median strut dehiscent from basal 1/3.

Endophallus almost three times as long as median lobe in lateral view. The relative length of each broad membrane area of endophallus to median lobe are as follows: ML : TLE : BPH : MPH (MT+CT : PB) : APH = 3.8 : 10.0 : 2.2 : 6.0 (5.1 : 0.9) : 1.8. MPH with MT+CT well swollen and projected on ventral side near base. APH well swollen in oval shape, with ligulate-shaped AA near apex.

MSp distributed in nearly apical 2/3 of MT+CT. LSp distributed in nearly basal 1/3 of MT+CT on dorsal side, thick and short, arranged into two longitudinal irregular lines, unidentate in apical area, thence becoming thicker, denser and multidentate toward basal area. SSp unidentate, short and small, covered laterodorsal side of PB. MSp area and LSp area adjacent. LSp area and SSp area close to each other.

Specimens Examined. 1♂, Sonai, Is. Yonaguni, 10, V, 1963, Y. Arita leg.; 2♀♀, Tendabaru, Is. Yonaguni, 13, V, 1963, Y. Arita leg.; 2♀♀, Tabaru-gawa, Is. Yonaguni, 14, V, 1963, Y. Arita leg.; 5♀♀, Mt. Urabedake, Is. Yonaguni, 6–7, VII, 1969, Y. Hori leg.; 2♀♀, same locality, 11. VII, 1964, N. Ohbayashi leg.; 1♂, Hikawa, Is. Yonaguni, Okinawa Pref, Japan, 16. V, 1963, Y. Arita leg.; 1♂, Mt. Urabedake, Is. Yonaguni, Okinawa Pref, Japan, 20. V, 1989, N. Ohbayashi leg.

Distribution. Japan (Is. Yonaguni, Okinawa Pref.).

3.8.2. *Agelasta (Dissosira) yonaguni subkonoi* (Hayashi, 1962) Comb. Nov.

Mesosa (*Mesosa*) *subkonoi* Breuning, 1964: 91 [26].

Mesosa (*Mesosa*) *cervinopicta cervinopicta* f. *subkonoi*: Hayashi, 1964: 70, Figure 1 [27].

Mesosa (*Mesosa*) *yonaguni subkonoi*: Kusama and Irie, 1976: 20, Figures 3(a) and 3(b) [24].

Mesosa (*Mesosa*) *perplexa*: Hayashi, 1962: 32 (nec Pascoe, 1858) [16].

Pachyosa cervinopicta: Miwa, 1933: 12 (nec Fairmaire, 1897) [28].

Mesosa (*Saimia*) *cervinopicta*: Gressitt, 1951: 220 (nec Fairmaire, 1897) [20].

Mesosa (*Mesosa*) *cervinopicta cervinopicta*: Hayashi, 1962: 35, pl. 4, Figure 3 (nec Fairmaire, 1897) [15].

Specimens Examined. [Is. Ishigaki, Okinawa Pref., Japan]: 3♂♂, 1♀, Kawarayama, 29. VI, 1964, N. Ohbayashi leg.; Arakawa, 16. VI, 1965, K. Hatta leg.; 2♀♀, Nosoko-dake, 21. VII, 1998, A. Komada leg. [Is. Iriomote, Okinawa Pref., Japan]: 2♂♂, 1♀, Ushikuno-mori, 7. VIII, 1962, Y. Arita and M. Satô leg.; 1♀, same locality, 26. VI, 1965, Y. Hori leg.; 1♂, Sonai, 24. VI, 1965, Y. Hori leg.

Distribution. Japan (Is. Ishigaki, Is. Iriomote and Is. Taramajima, Okinawa Pref.).

3.8.3. *Agelasta (Dissosira) yonaguni kashiwaii* (Kusama and Takakuwa, 1984), Comb. Nov.

Mesosa (*Mesosa*) *yonaguni kashiwaii* Kusama and Takakuwa, 1984: 11, 358 [21].

Mesosa (*Mesosa*) *cervinopicta* f. *subkonoi*: Hayashi and Nomura, 1964: 67 (Is. Hateruma, Japan) (nec Breuning, 1964) [29].

Mesosa (*Mesosa*) *yonaguni subkonoi*: Kusama and Irie, 1976: 20 (part.: Is. Hateruma) (nec Breuning, 1964) [24].

Specimens Examined. 3♂♂, 1♀, Is. Hateruma, 26. IV, 1975, K. Shimizu leg.

Distribution. Japan (Is. Taketomi, Is. Kohama, Is. Kuroshima and Is. Hateruma, Okinawa Pref.).

3.8.4. *Agelasta (Dissosira) yonaguni similaris* (Kusama and Takakuwa, 1984), Comb. Nov.

Mesosa (*Mesosa*) *yonaguni similaris* Kusama and Takakuwa, 1984: 11 [21].

Pachyosa cervinopicta: Matsushita, 1933: 344 (nec Fairmaire, 1897) [10].

Mesosa (*Saimia*) *cervinopicta*: Hayashi, 1960: 27 (nec Fairmaire, 1897) [19].

Mesosa (*Pachyosa*) *cervinopicta cervinopicta*: Samuelson, 1965: 99 (nec Fairmaire, 1897) [23].

Mesosa (*Mesosa*) *yonaguni subkonoi*: Kusama and Irie, 1976: 20 (nec Breuning, 1964) [24].

Mesosa (*Mesosa*) *yonaguni semipraelongipes* Kusama and Takakuwa, 1984: 358, errata [21].

Specimens Examined. 1♂, Hirara-shi, Is. Miyako, 14. VII, 2001, N. Ohshige leg.; 1♂, Is. Tarama, 1. VI, 1993, H. Kanazawa leg.

Distribution. Japan (Is. Miyako, Is. Irabu, Is. Ikema, Is. Ôgami and Is. Tarama of Miyako Isls., Okinawa Pref.).

3.9. *Agelasta (Dissosira) nigrostictica* (Breuning, 1967), Comb. Nov. (Figures 4(c)-4(d), 6(f), 8(e)–8(h), and 10(b))

Mesosa (*Mesosa*) *nigrostictica* Breuning, 1967: 185 [30].

Diagnosis. This species is similar to *A. catenata*, but distinguishable by the following features. Body black or reddish brown, evenly covered with light brown pubescence. Antennae with each basal part of third to sixth or seventh segments with white pubescent annulations, which are getting narrower toward apical segment; the reminders covered with black pubescence. Pronotum with two indistinct longitudinal narrow black bands on disk. Elytra with two pairs of black fragment maculae on lateral side before and after middle. Prosternal process roundly sloped and not truncate in lateral view.

Male Genitalia ($n = 1$). Tegmen in lateral view slightly curved, rhombic, and widest near middle in ventral view. Ringed part slightly expanded laterad near middle of tegmen, thence arcuately narrowed basad. Lateral lobes about 1/4 of total length of tegmen, with inner sides almost straight, and outer sides nearly straight toward apical third, thence gentry narrowed to rounded apex, provided with two kinds of setae of which one is long and thick, concentrated near apex, and another is rather short and thin, sparsely distributed in ventral side, apical third of laterodorsal sides; each ventral side near base with a transversal obtuse ridge which is haired in mass on the edge of ridge.

Median lobe in lateral view gentry curved; apex in ventral view weakly pointed; median strut dehiscent from basal 2/5.

Endophallus about 2.5 times as long as median lobe in lateral view. The relative length of each broad membrane area of endophallus to median lobe is as follows: ML : TLE : BPH : MPH (MT+CT : PB) : APH = 4.3 : 10.0 : 2.5 : 6.2 (5.3 : 0.9) : 1.2. MPH with MT+CT well swollen and weakly projected on ventral side near base. APH well swollen in oval shape, with ligulate-shaped AA near apex, which is relatively indistinct.

MSp sparsely distributed in nearly apical half of MT+CT. LSp on dorsal side distributed in nearly basal half of MT+CT, thick and short, arranged into two longitudinal irregular lines, unidentate in apical area, thence becoming thicker, denser, and multidentate toward basal area; LSp on ventral side irregularly distributed in nearly basal 1/3 of MT+CT, short, indistinct and rudimentary unidentate. SSp unidentate, short and small, covered almost of laterodorsal side and basal 1/3 of ventral side of PB. MSp area and LSp area adjacent. LSp area and SSp area close to each other.

Specimens Examined. [Java, Indonesia]: Photographs of the holotype (MNHN): 1♂, "Mesosa/nigrostictica/mihi typ/Breuning dét." [printed on white label], "TYPE" [printed on red label], "MUSÉUM PARIS/1952/COLL R OBERTHUR" [printed on white label margined with black line], "Java/Malang" [printed on white label margined with black line]. [Bali, Indonesia]: 1♂, Bali, Indonesia, 30. V, 1998, Native coll.; 1♂, West Bali, Indonesia, 19–21. XII, 2002, Y. Yokoi leg.; 1♂, Bali, Indonesia, X, 2005, Native leg.

Distribution. Indonesia (Java, Bali).

Remarks. This is first record of this species from Is. Bali. The male genital features above are described based on the specimen from Is. Bali.

3.10. *Agelasta (Dissosira) siamana* (Breuning, 1974), Comb. Nov. (Figure 5(e))

Mesosa (*Mesosa*) *siamana* Breuning, 1974: 73 [31].

Diagnosis. This species is very similar to *A. columba*, but distinguishable by the following features. Body black, covered with light brown pubescence. Antennae with each basal part of third to the last segments with white pubescent annulations, which are getting narrower toward the last segment; the reminders covered with black pubescence. Pronotum with two pair of small longitudinal black maculae on disk. Elytra with indistinct transversal white band near middle, scattered with several brown spots.

Specimen Examined. Photographs of a syntype (MNHN): 1♂, "Mesosa/siamana/mihi typ/Breuning dét." [printed on white label], "TYPE" [printed on red label], "Siam" [printed on white label], "MUSEUM PARIS/COLL. H. W. BATES/1952" [printed on white label margined with black line].

Distribution. Thailand.

3.11. *Agelasta (Dissosira) praelongipes* (Kusama and Irie, 1976), Comb. Nov. (Figures 4(e)-4(f), 6(g), 8(i)–8(l), and 10(c))

Mesosa (*Mesosa*) *praelongipes* Kusama and Irie, 1976: 20, Figures 2(a) and 2(b) [24].

Diagnosis. This species is very similar to *A.* (*D.*) *yonaguni*, but distinguishable by the following features. Prosternal process nearly truncated in lateral view. Fore legs of male distinctly longer than female. Seventh abdominal sternite twice as long as the sixth; pygidium distinctly exposed from the apices of elytra in male. The pubescence on abdominal sternites sparsely arranged or almost disappeared.

Male Genitalia (n = 2). Tegmen in lateral view slightly curved, rhombic, and widest near middle in ventral view. Ringed part slightly expanded laterad near middle of tegmen, thence arcuately narrowed basad. Lateral lobes about 1/3 of total length of tegmen, with inner side almost straight, and outer side expanded toward apical 4/5, thence obliquely narrowed to angularly rounded apex, provided with two kinds of setae of which one is long and thick, concentrated near apex, and another is rather short and thin, arising chiefly from outer sides of apical half.

Median lobe in lateral view gentry curved; apex in ventral view weakly pointed; median strut dehiscent from basal half.

Endophallus almost three times as long as median lobe in lateral view. The relative length of each broad membrane area of endophallus to median lobe is as follows: ML : TLE : BPH : MPH (MT+CT : PB) : APH = 3.5 : 10.0 : 2.5 : 6.0 (5.2 : 0.8) : 1.5. MPH with MT+CT well swollen and projected on ventral side near base. APH swollen in elongate oval shape, with ligulate-shaped AA near apex.

MSp distributed in nearly apical 2/3 of MT+CT. LSp on dorsal side distributed in nearly basal 1/3 of MT+CT, thick and short, arranged into two longitudinal irregular lines, unidentate in apical area, thence becoming thicker, denser and multidentate toward basal area; LSp on ventral side irregularly distributed in nearly basal 1/3 of MT+CT, short and rudimentary unidentate. SSp unidentate, short and small, covered basal 3/4 of PB. MSp area and LSp area adjacent. LSp area and SSp area close to each other.

Specimens Examined. 1♂, 1♀, Uinpia, Is. Miyako, 30–31. V, 1975, H. Makihara leg.; 5♂♂, 3♀♀, Aragusuku-kaigan, Is. Miyako, 7. VIII, 1999, T. Mizoguchi leg.; 1♂, 1♀, Is. Kurima, collected the logs at III, 1996 and emerged on VI, 1996, K. Shimizu leg.; 1♂, same locality, IV, 2003, K. Shimizu leg.

Distribution. Japan (South area of Is. Miyako and Is. Kurima of Miyako Isls., Okinawa Pref.).

3.12. *Agelasta (Dissosira) kumei* (Takakuwa, 1991), Comb. Nov. (Figures 4(g)-4(h), 6(h), 8(m)–8(p), and 10(d))

Mesosa (*Mesosa*) *kumei* Takakuwa, 1991: 51, illustration [32].

Diagnosis. This species is similar in the appearance to *A. konoi* or *A. perplexa*, but distinguishable by its large body size. Body black, covered with yellowish light ocher pubescence. Occiput with four longitudinal narrow black bands. Pronotum with three longitudinal black bands on disk. Elytra with three transversal yellowish white bands on behind humeri, near middle, and near apices. Pronotum with three rudimentary tubercles on disk. Prosternal process rounded in lateral view, and not truncate at the apex.

Male Genitalia (n = 1*, Partly Broken).* Tegmen in lateral view slightly curved, slender rhombic, and widest near middle in ventral view. Ringed part weakly expanded laterad near middle of tegmen, thence arcuately narrowed to basad. Lateral lobes slightly narrowed toward rounded apex, provided with two kinds of setae of which one is long and thick, concentrated on the apex, and another is rather short, thin, mainly arising from apical half.

Median lobe with apex roundly pointed in ventral view.

Basal half of endophallus is lost. LSp thick and short, arranged into two longitudinal irregular lines on dorsal side of MT+CT, unidentate in apical area, thence becoming thicker, denser, and multidentate toward basal area.

Specimens Examined. 1♀, Is. Lu-dao, Taitung county, Taiwan, 16–20. VI, 1989, Native leg.; 2♀♀, same locality, 5–10. VII, 1989, Native leg.; 1♂, Gangguan, alt. 0–10 m, same island, 5. IV, 2004, T. Kurihara leg.

Distribution. Taiwan (Is. Lu-Dao).

Remarks. The male genitalia of the examined specimen was partly broken, and we could not observe the basal half of median lobe and the basal part of endophallus. However, this species basically shares the external features, the shape of the tegmen of the male genitalia, and the characteristics of LSp of the endophallus with *A. konoi* or *A. yonaguni* and *A. perplexa*. It suggests that this species is closely related to these species. Therefore, we treat this species as a member of *Agelasta* (*Dissosira*).

4. Discussion

Among the species which have been classified into the nominotypical subgenus of *Mesosa*, 12 species distributed in the Oriental region are different from *Mesosa* (*Mesosa*) and have close relationship with *Agelasta* (*Dissosira*).

The genera *Mesosa* and *Agelasta* have mainly been distinguished from each other by the prosternal process, rounded or truncated in lateral view (e.g., Breuning [2]). The shape of the prosternal process is essentially stable in the Mesosini group, and it is worth defining the genera or subgenera by the external features. However, this structure is exceptionally variable and unstable in some groups of the genus *Agelasta*. Therefore, several species included in *Mesosa* have been confused with *Agelasta*, especially *Agelasta* (*Dissosira*), because of this variable structure (Yamasako and Ohbayashi [4]). Yamasako and Ohbayashi [4, 5] had already pointed out that the basic structure of the endophallus is very useful for defining the groups of Mesosini such as the genera or subgenera. Also, it is considered to be useful for analysis of the phylogenetic relationship. Therefore, the generic definition of Mesosini should be decided by the combination of the external and the genital features.

According to this point of view, the genus *Agelasta* is essentially distinguishable from *Mesosa* by the following characteristics: (1) antennal scape elongate, slightly thickened apicad, (2) lower lobes of eyes relatively large, (3) endophallus with LSp on the dorsal side arranged into two irregular longitudinal lines, unidentate in apical area, thence becoming thicker, denser and multidentate toward the basal area, rudimentary unidentate or almost disappeared on the ventral side, almost without AS. These differences suggest that the genera *Agelasta* and *Mesosa* are different phyletic groups.

On the basis of these characteristics, we transferred 12 species to *Agelasta* (*Dissosira*) that were previously classified into *Mesosa* (*Mesosa*) in spite of their rounded prosternal process in lateral view. They should be included in *Agelasta* (*Dissosira*) (sens. str. by Yamasako and Ohbayashi [4]) because they share external features and endophallic structures with the type species of the subgenus, *A.* (*D.*) *catenata*.

Agelasta (*Dissosira*) is widely distributed in the Oriental geographic region except for the Philippines, and its distribution extends northwardly to the Tokara Islands of Japan which is the northern end of the Oriental region.

Appendix

The examined specimen data for the type species of *Mesosa* (*Mesosa*) and *Agelasta* (*Dissosira*) in this study.

Mesosa (Mesosa) curculionoides

Specimens Examined. 1♂, Vestec, Czech, 30. V, 1946, Lenesch leg.; 1♂, 1♀, Leitha-Geb., b. Eisenstadt, 6. VI, 1976, BGLD; 3♂♂, Olympie-Péloponèse, Greece, 2. VII, 1981, A. Le Restif leg.; 1♂, Murauen, b. Mureck, 14. V, 1983, S-STMK; 1♂, Góry, Plock Dist., Poland, 18. V, 2002, M. Szewczyk leg.; 1♀, same locality, 20. V, 2003, M. Szewczyk leg.

Agelasta (Dissosira) catenata

Specimens Examined. 1♂, 1♀, Sayaboury, Laos, 14. VII. 1965, J. A. Rondon leg.; Same locality and collector, 9. VIII. 1965; 1♂, Ban Van Heua, Vientiane, Laos, 15. VIII. 1965 J. A. Rondon leg.; 1♀, Ile de Khong, Laos, 15. IV. 1965 J. A. Rondon leg.

Abbreviations

AA:	Appendix of apical bulb
APH:	Apical phallomer
AS:	Sclerite of apical phallomer
BPH:	Basal phallomer
CS:	Crescent shaped sclerites
CT:	Central trunk
LSp:	Large spicules
ML:	Median lobe
MPH:	Median phallomer
MSp:	Micro spicules
MT:	Medial tube
PB:	Preapical bulb
SSp:	Small spicules
TLE:	Total length of endophallus.

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Research Article

Improved Visualization of *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae)—Part II: Alimentary Canal Components and Measurements

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Alphitobius diaperinus (Panzer) (Coleoptera: Tenebrionidae) is a pest of stored food products and problematic to every type of poultry production facility. Larvae and adults can ingest and harbor foodborne and poultry pathogens. Determining the efficiency of this insect's capacity to transmit disease is critical to improving management of *A. diaperinus* on poultry facilities and providing a safe food supply for human consumption. However, a deficiency exists in the literature reporting measurements of the gut and its defined segments. Previous reports include line drawing depictions, which aid little in the determination of the pathogen reservoir potential of these insects. Advances in technology allowed more accurate visualization and precise measurement of gross anatomical features of the alimentary canal. A photographic depiction to aid the researcher in the visualization of anatomical features and accurate measurements of the alimentary canal for these insects is presented here.

1. Introduction

The of high-density poultry feeding operations to increase production output in order to meet market demand has changed the environment in which poultry is raised. The close quarters and high bird density favors the survival of arthropod pests. *Alphitobius diaperinus* (Panzer) (1797) (Coleoptera: Tenebrionidae), a pest of stored food products, is a common and persistent pest in every type of poultry production facility: breeders, grow-out, caged-layers and pullets. These insects have adapted well to the artificially controlled environment within poultry houses and what were once only minor pests in low density flocks have become large infestations in high-density rearing facilities. Their presence generates economic and management concerns. For example, *A. diaperinus* survive on the floor of a broiler production house in the accumulated mix of bedding material, excreta, feathers, spilt feed, carcasses, and other debris, referred to as litter. The high density of birds in production results in increased litter moisture, both from the excreta and automatic drinkers. Combined with the controlled temperature in the houses these conditions are highly conducive to beetle survival and population expansion.

Chickens and turkeys readily feed on *A. diaperinus*, and young birds preferentially ingest larvae, even in the presence of starter feed [1, 2]. Chicks fed solely *A. diaperinus* larvae for 9 days gained 37% less body weight than chicks on starter feed and, in addition, showed signs of stress [2]. This weight was not recovered when returned to starter feed through 14 days of age [2]. The omnivorous diet of *A. diaperinus* also means that they can compete with the birds for their feed. Furthermore, *A. diaperinus* can ingest and harbor foodborne and poultry pathogens (reviewed in: [3]). Consequently, *A. diaperinus* represents a health issue to the birds and to the humans which consume the birds [4].

During their life cycle *A. diaperinus* larvae migrate into the insulation of the building walls and the soil beneath the litter for pupation and eventual eclosion. Their tunneling behavior disrupts the compacted earth floors and dense wall insulation [5]. Their activity in the walls reduces the building insulating capacity, causing enough damage to raise energy costs 67% and require replacing of insulation every two to three years [6–9]. Their activity in the compacted earthen floors, on which bedding is spread, results in an irregular and hollowed floor surface [6]. This hollowing can retain bedding and reduce the effectiveness of litter clean outs by tractor loaders. Therefore, *A. diaperinus* also represent a structural pest for the producer. Economic effects of *A. diaperinus* infestations on poultry production are difficult to quantify. Of financial concern for the producer is the issues that these insects cause facilities structural damage, affect bird growth and health, and vector poultry diseases.

A primary concern for the consumer is that these insects vector foodborne pathogens. Determination of the efficiency of this insect's capacity to harbor pathogens is critical to improving management of *A. diaperinus* in poultry facilities and providing a safe food supply for human consumption. Understanding the anatomy and physiology of the insect to model pathogen movement and transport within the insect is vital [10–12]. Line drawings of the alimentary canal for larvae and adult *A. diaperinus* have been provided in previous studies, but these aid little in determining reservoir potential of the alimentary canal [13–15]. No single reference exists with a photographic depiction to aid the researcher in the visualization of anatomical features and measurements of the alimentary canal for these insects. Improved reference images are needed to more accurately describe the larval and adult *A. diaperinus* alimentary canal, and these are presented here.

2. Experimental

2.1. Beetles. The Southern Plains Agricultural Research Center (SPARC) starter colony of *A. diaperinus* was from a colony originally isolated from a poultry farm located in Wake County, NC. The SPARC colony has remained in production since 2004. The adult colony was reared in 1000 mL wheat bran (Morrison Milling Co., Denton, TX) in plastic containers (15 × 15 × 30 cm) with screened bottoms. Insects were provided a 6 cm^2 sponge, placed atop a piece of aluminum foil, and moistened with deionized water as needed, and a 0.5 cm thick slice of a medium-sized apple was replenished twice per week. Fishmeal (30 mL; Omega Protein, Inc., Hammond, LA) was added to the wheat bran once per week, and new wheat bran was added as it was depleted by dropping through the screened bottom of the cage. Eggs were collected as needed on layered black construction paper (6 × 6 cm) and transferred to a separate container; emergent larvae were maintained as described above in a solid bottom container, until pupation. Pupae were transferred to a screened bottom container and emerging adults reared as described above. The entire colony was maintained at 30°C in an 8 : 16 hr (light : dark) photoperiod.

2.2. Morphometrics of Insects and Alimentary Canal

2.2.1. Insect Measurements. Immediately before dissection for removal of alimentary canals, as described below, male and female adults and late instars were measured using imagery software described by Esquivel [16]. Head capsule widths were also recorded for larvae to determine stadia, and, based on previous head capsule width measurements [17], the larvae used in this study were 7th instar or older and the adults were more than 4 weeks after eclosion.

2.2.2. Alimentary Canal Measurements. To determine size and capacity of the sections comprising the alimentary canals, intact alimentary canals were removed from male ($n = 5$) and female ($n = 5$) adults and late instars ($n = 10$). Equipment and dissection methodologies described by Esquivel [16] were slightly modified for excision of alimentary canals. Briefly, individual specimens were examined under an Olympus SZ60 dissecting stereomicroscope (Olympus, Kalamazoo, MI, USA). Lumenera INFINITY software and INFINITY 1–3 C camera (Lumenera, Ottawa, ON, Canada) were interfaced with a computer to record images and measurements of each specimen. Because the adults and larvae *A. diaperinus* were smaller than those insects examined previously, dissection technique, pins, and forceps varied, as described below.

2.2.3. Adults. Beetles were taken from rearing cages and placed in a vial at −20°C for ca. 15 min. Individual adults were removed from the vial and pinned (no. 00, BioQuip, Rancho Dominguez, CA, USA) dorsolaterally through the right elytron and through the body. Positioning of the pinning site was closer to the right margin of the abdomen to prevent piercing of the alimentary canal. Pinning at this location also provided an "anchor" during the dissection process. The beetle was then pinned into one of the "dissection wells" [16]. Distilled (RO) water was added to the well to facilitate dissection and excision of the alimentary canal.

The technique to remove the wings and abdominal dorsal cuticle was similar to Esquivel [16], with the exception that the right pair of wings was not removed and the cuticle was cut only at the left lateral margin of the abdomen. Two pair of forceps (no. 55 Rubis, BioQuip, Rancho Dominguez, CA, USA) were used to grasp the thorax dorsally at the midline and break each half open, allowing access to the ventral connective tissue between the head and the thorax. This connective tissue was severed to allow removal of the head intact and the alimentary canal was excised by teasing away the tracheae and connective tissue along the length of the body. The tissue between the ultimate and penultimate ventral abdominal plates was severed, allowing removal of the intact alimentary canal. The intact canal was then placed into a separate well and the head was grasped dorsally at midline and gently pried open. Pieces of the exoskeleton and tissue were teased away leaving only the mandibles and alimentary canal. Similarly, abdominal plates still attached around the rectum were teased away. The mandibles were pinned using minuten pins (BioQuip, Rancho Dominguez, CA, USA) and the rectum was grasped and pulled taut to lay the alimentary canal in a straight line, exercising care to not distend the canal past its normal length. A minuten pin held the rectum in place.

Following distension of the alimentary canal, measurements were recorded for the foregut (from the mouth—including buccal cavity, pharynx, and esophagus—to distal end of proventricular valve), the midgut (distal end of proventricular valve to distal end of pyloric valve), the small intestine (distal end of pyloric valve to enlargement of the intestine), the large intestine (enlarged intestine), and the rectum. Section assignments closely follow designations of McAllister et al. [13] and Snodgrass [18] except the rectum, which was not delineated or measured separately in those studies.

Total exterior body lengths ($n = 10$ per group) were measured along the anteroposterior axis for comparison to total alimentary canal lengths. For the adult, the head was measured from the anterior end of head to the first anterior thoracic segment. The thorax was measured from the first anterior thoracic segment to the anterior elytra attachment. The abdomen was measured from the anterior elytra attachment to the distal end of abdomen. For the late instar a measurement from the anterior end of head to the distal end of abdomen was performed.

2.2.4. Larvae. Late instars were taken from rearing cages and placed in a modified plastic centrifuge vial (JFE, unpublished data) and killed by exposure to ethyl acetate for 10–15 min. Dead larvae were placed in a dissection well and held down using a modified no. 00 pin (JFE, unpubl. data) allowing anchoring of the larva so that the alimentary canal was not pierced by the conventional pinning technique. Distilled water was added to the well to facilitate dissection and excision of the alimentary canal.

The dorsal cuticle of the larvae was cut along the left margin from the penultimate abdominal segment to the first thoracic segment. The cuticle was pulled to the right while removing tracheae and other tissue. Similar to the adults, the head was removed intact from the larva and the last abdominal segment (i.e., pygidium) was also removed intact. Removal of the alimentary canal from the body, subsequent clearing of the attached material (head and abdominal segments), distension of the alimentary canal, and measurement of alimentary canal sections were as described for adults.

Total exterior body lengths ($n = 10$) were measured along the anteroposterior axis for comparison to total alimentary canal lengths. For the late instar, a measurement from the anterior end of head to the distal end of abdomen was performed.

2.3. Data Analysis. Data were analyzed using commercially available statistical software (Prism ver. 5.01, GraphPad Software Inc., La Jolla, CA). Descriptive statistics were generated and are presented in table formats. Within each anatomical segment, life stage, and sex of insect, a means comparison of length was performed using a two-way ANOVA followed by Bonferroni posttests ($P < 0.05$).

3. Results and Discussion

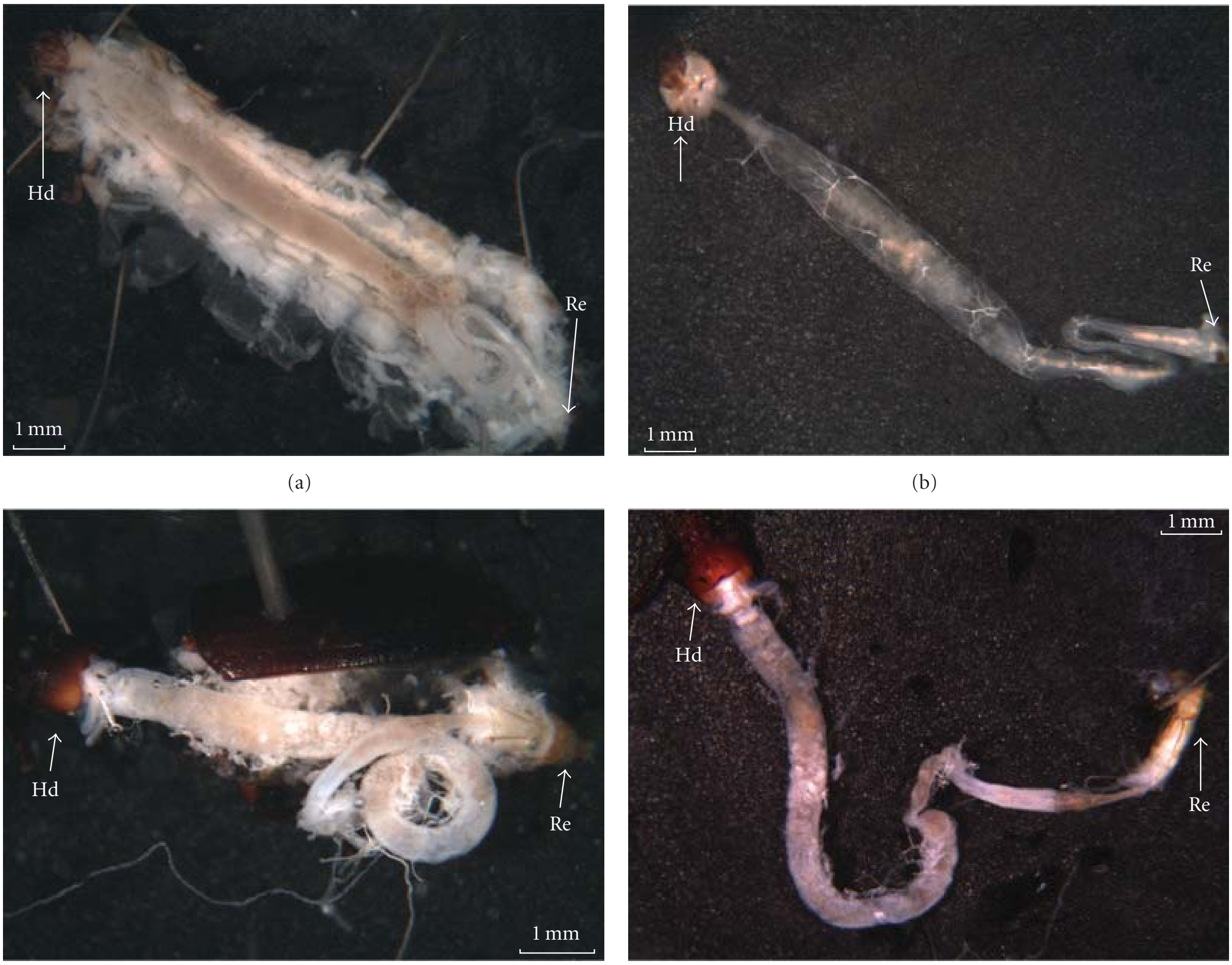
The digestive tract is arranged into fore-, mid-, hindgut and rectum sections, which can be easily demarcated by visual examination. Figures 1(a) and 1(b) of *in situ* and extracted digestive tracts reveal the simplistic gut structure with a sigmoid bend distinctive to larval *A. diaperinus.* Conversely, adult *A. diaperinus* possess a more convoluted alimentary canal containing a complete loop before reaching the pyloric valve (Figures 1(c) and 1(d)). These images correspond with existing line drawn schematics presented by McAllister et al. [13] and Rahman et al. [14, 15] but provide more details regarding morphology and reservoir potential of the alimentary canal. Figure 2 displays the distended adult alimentary canal used during measurement of gut segments and insets of the proventricular valve and expanded views of the female and male rectum. Figure 3 displays the extracted larval alimentary canal demonstrating features used to delineating gut segments, with insets of the demarcating proventricular and pyloric valves, and the distended position of hindgut showing features used during measurement of the large and small intestinal segments. These delineating features are similar on both the larva and adult.

The alimentary canal of *A. diaperinus* is a tubular structure with similarities to beetles consuming stored grain products, as well as characteristics distinctive to its more omnivorous feeding habits when in a poultry production environment, as previously described by McAllister et al. [13]. Larvae in particular are known to be cannibalistic and have chitinase activity in their digestive secretions [19]. However, a study comparing the alimentary canal of *A. diaperinus* of individuals fed herbivorous or carnivorous diets determined no anatomical differences between the larvae on the two disparate diets [15]. Therefore our laboratory-raised insects were used as representative specimens for this study.

Adults of 2 to 4 months of age and late instar larvae were measured for this study. The mean head capsule width of the late instars ($n = 10$) used in this study was 1.084 (± 0.013) mm and ranged from 0.960 to 1.320 mm; representing 7th to 9th instar. Barké and Davis [17] reported head capsule widths of 0.95, 1.08, and 1.28 mm, and Francisco and Prado [20] reported widths of 1.061, 1.208, and 1.339 mm for 7th, 8th, and 9th instars, respectively. Overall measurements of the alimentary canal and its sections resemble those reported by McAllister et al. [13] for fully tanned adults and 8th- to 11th-instar larvae. However, some variation was expected due to the likely differences in the range of ages of the insects used in the two studies.

The ten adult foreguts in this study, measured from the epipharynx to the posterior end of the proventricular valve, averaged 1.73 mm in length and the ten late instars foreguts averaged 2.36 mm (Table 1). No significant differences in the length of the foregut between male and female, nor between adult and late instar, were found. In comparison, foregut measurements from the mouth to the proventricular valve previously yielded mean lengths of 1.25 mm in adults and 1.5 mm in larvae (McAllister et al. [13]). However, measurements by McAllister et al. [13] were made using a dissecting scope and a calibrated ocular micrometer which may have affected precision.

The midgut was demarcated from the distal end of proventricular valve to the distal end of pyloric valve and measured 9.98 mm in larvae and 9.00 mm adults after distension.



(a) (b)

(c) (d)

Figure 1: Alimentary canals for larval and adult *Alphitobius diaperinus*: ((a) and (b)), larval canal *in situ* and extracted, respectively; ((c) and (d)) adult canal *in situ* and extracted, respectively. Hd: head; Re: rectum.

Table 1: Mean length (mm) and standard deviation (SD) of alimentary canal of the *Alphitobius diaperinus* female and male beetles (>4 weeks post eclosion) and the late instars (7–9th). Measured by microscopy.

	Foregut		Midgut		Small intestine		Large intestine		Rectum		Total alimentary canal	
	Length	SD	Length	SD	Length	SD	Length	SD	Length	SD	Length	SD
Adult female	1.70	$\pm0.30^{a}$	9.22	$\pm0.71^{a,b}$	3.24	$\pm0.35^{a}$	2.30	$\pm0.18^{a}$	2.29	$\pm0.32^{a}$	18.74	$\pm1.11^{a}$
Adult male	1.75	$\pm0.12^{a}$	8.77	$\pm1.89^{a}$	3.14	$\pm0.40^{a}$	2.43	$\pm0.32^{a}$	1.26	$\pm0.19^{a,b}$	17.35	$\pm1.83^{a}$
Late instar	2.36	$\pm0.38^{a}$	9.98	$\pm1.94^{b}$	3.50	$\pm0.46^{a}$	2.14	$\pm0.50^{a}$	0.64	$\pm0.10^{b}$	18.61	$\pm2.30^{a}$
Adult*	1.73	±0.22	9.00	±1.37	3.19	±0.36	2.36	±0.25	1.78	±597.5	18.04	±1.61

*Mean compilation of adult male and adult female measurements.

[a-b] Sample groups (late instar, female, and male) with the same letter are not significantly different ($P < 0.05$) as compared within the anatomical segment of the gut (nonparametric 2-way ANOVA with Bonferroni posttests).

A significant difference was found in the length of the midgut between the male, which was shorter than that of the late instar. In comparison, McAllister et al. [13] defined the midgut as extending from the proventricular valve and terminating at the pyloric valve, measuring 7.5 mm in larvae and 4.1 mm adults.

The hindgut is divided into a small and large intestine. The small intestine was demarcated from the distal end of pyloric valve extending to the enlargement of the canal, signifying the start of large intestine, and measured 3.50 mm in larvae and 3.19 mm adults. The large intestine was demarcated from the enlargement of intestine to the origination of

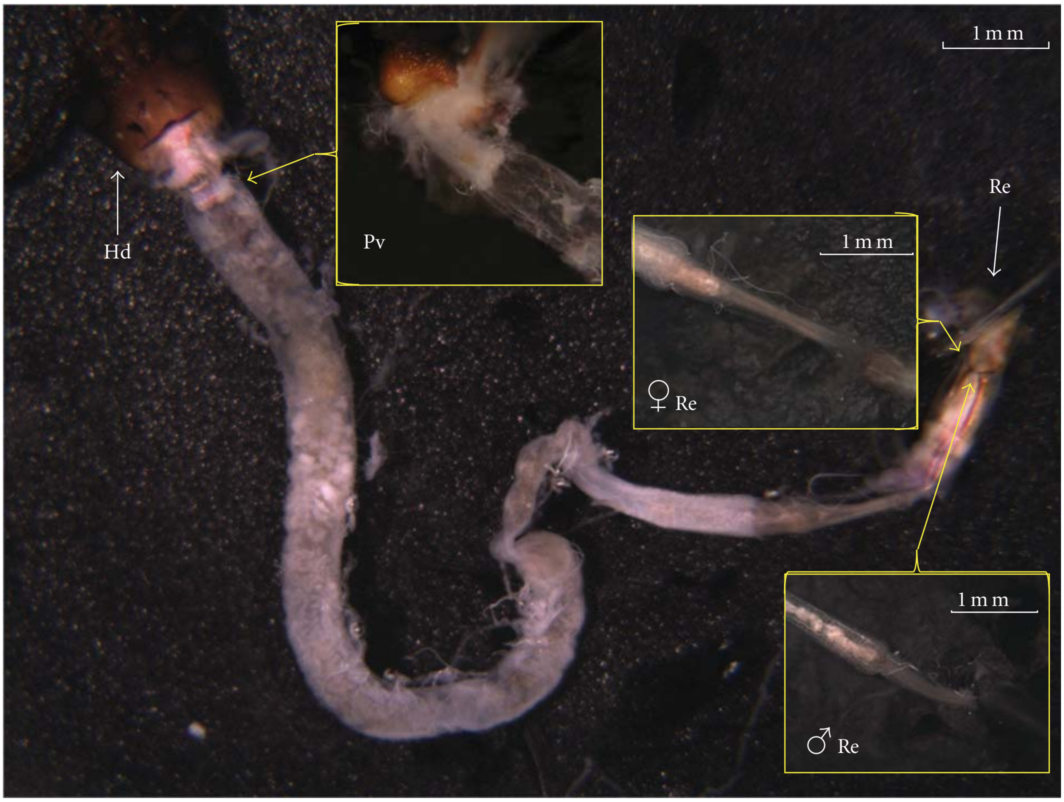


FIGURE 2: Distended alimentary canal of adult *Alphitobius diaperinus* canal; yellow lines indicate area of expanded detail for Pv: proventricular valve, male and female rectal areas. Hd: head; Re: rectum.

the rectum and measured 2.14 mm in larvae and 2.36 mm adults. No significant differences were found in the length of the hindgut between male and female, nor between the adult and late instar. In comparison, McAllister et al. [13] defined the anatomy in the larvae of the small intestine as a straight tube which begins near the pyloric valve and extends anteriorly to the posterior margin of the fifth abdominal segment for a total length of 2.0 mm. At that point, it reverses direction and extends posteriorly as the large intestine for a length of 2.9 mm. In the adult, the small intestine was described as a single loop beginning from the pyloric valve and extending to the posterior margin of the third abdominal segment for a length of 3.5 mm. It then extends posteriorly as the large intestine for a length of 2.4 mm.

The rectum was also measured as demarcated from the posterior end of large intestine to the anus and measured 0.64 mm in larvae and 1.78 mm adults. The female rectum was significantly longer than that of the late instar. Neither the ovipositor of the female nor the aedeagus of the male was included in these measurements; their anatomy is discussed in a counterpart paper in this journal issue [21].

The largest observed discrepancy was in the length of the midgut. McAllister et al. [13] reported lengths of 7.5 and 4.1 mm for larvae and adults, respectively, while current results indicated lengths of 9.98 and 9.00 mm for larvae and adults, respectively. Methodology differences between studies may account for these differences. In the current study, the alimentary canal was distended to normal length, to ensure a straight line measurement. However, methodologies in McAllister et al. [13] did not clearly indicate measurement technique and suggests measurements of the alimentary canal as it lay *in situ*. Discrepancies in measurements may also be attributed to definitions of sections comprising the alimentary canal. In the current study, measurements involving the proventricular and pyloric valves reached to the distal side of the valve. In contrast, language in McAllister et al. [13] suggests that measurements were taken from the proximal side of the respective valves. Inclusion of the valves in the measurements would likely bring their estimates closer to those reported in the current study.

According to Dunford and Kaufman [22] the average length of an adult *A. diaperinus* is approximately 5.8 to 6.3 mm, therefore the fore-, mid-, and hindguts are more than 2.5 times the length of the insect. Barké and Davis [17] noted that average adult female ranged from 6.75 to 8.00 mm and male from 5.50 to 7.00 mm; however, the method of measurement collection was not presented. Rahman et al. [14] used a micrometer to measure characteristics of the adult and determined the foregut was "about" 2 mm in length and the hindgut (including the rectum) was 0.9 cm. The entire canal was reported to be 3 times the body length, 21 mm in the female (body length of 7 mm) and 19 mm in the male (body length of 5 mm). The average length of an adult beetle, in this study, was 7.01 mm, and the average

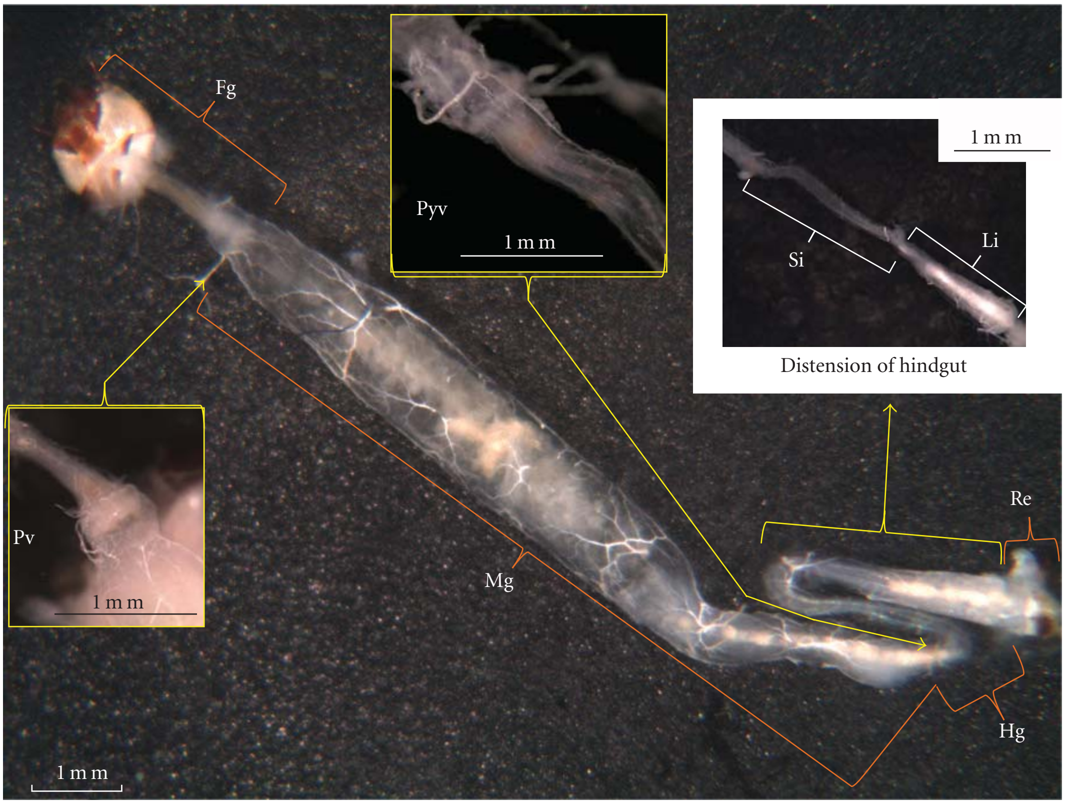


Figure 3: Components of larval *Alphitobius diaperinus* alimentary canal; yellow lines indicate area of expanded detail. Fg: foregut; Hg: hindgut; Li: large intestine; Mg: midgut; Pv: proventricular valve; Pyv: pyloric valve; Re: rectum; Si: small intestine.

Table 2: Mean length (mm) and standard deviation (SD) of segment and total body length of *Alphitobius diaperinus* beetles and total body length of late instars (7–9th). Measured by microscopy on the anteroposterior axis.

Segment	Female ($n = 10$)		Male ($n = 10$)		Late instars ($n = 10$)	
	Length	±SD	Length	±SD	Length	±SD
Head	1.094	±0.008^{a}	1.165	±0.013^{a}	—	
Thorax	1.355	±0.010^{a}	1.382	±0.008^{a}	—	
Abdomen	4.682	±0.030^{b}	4.333	±0.020^{b}	—	
Total	7.131	±0.041^{c}	6.883	±0.031^{c}	12.80	±1.169^{d}

$^{a–d}$Sample groups (late instar, female, and male) with the same letter are not significantly different ($P < 0.05$) (nonparametric 2-way ANOVA with Bonferroni posttests).

adult female ranged from 6.49 to 7.77 mm and male from 6.50 to 7.42 mm (Table 2). The mean alimentary canal length (foregut through rectum) was 2.6 times the length of the adult insect (Table 3).

The mean length of the late instars was 12.80 mm, ranging from 9.81 to 14.78 mm (Table 2), and the total alimentary length was 1.5 times the body length of the insect (Table 3). According to Dunford and Kaufman [22], the average length of a late instar was approximately 7 to 11 mm in length and the fore-, mid-, and hindguts were 1.6 to 2.5 times the length of the insect. Rahman et al. [15] determined larval alimentary canal length 1.5 times (21 mm) that of an 8th-instar body length (14 mm). They also stated that fore-, mid-, and hindgut measurements were 2, 12, and 7 mm, respectively; however the method of measurement collection was not presented. In addition, rectal lengths were included in the hindgut measurement.

4. Conclusions

A handful of studies have reported measurements of various parts of the alimentary canal of *A. diaperinus*. However, the exact method used for measurement and the anatomical structures used to define segment features were not always provided. No single study encompassed the scope of measurements on the same group of insects presented in this study. Advances in current technology also allowed more accurate visualization and precise measurement of gross anatomical features of the alimentary canal. These images and measurements provide additional perspective on the

Table 3: The length of the segments of the alimentary canal as percent of total alimentary canal length (ACL) and body length (BL) of the *Alphitobius diaperinus* female and male beetles (>4 weeks after eclosion) and the late instars (7–9th). Measured by microscopy.

	Foregut		Midgut		Small intestine		Large intestine		Rectum		Total alimentary canal
	ACL	BL	ACL	BL	ACL	BL	ACL	BL	ACL	BL	BL
Adult female	9.0	23.8	49.2	129.1	17.3	17.3	12.2	32.1	12.2	32.1	262.5
Adult male	10.1	25.5	50.6	127.4	18.1	18.1	14.0	35.2	7.3	18.3	252.0
Late instar	12.7	18.4	53.6	77.9	18.8	27.4	11.5	16.7	3.4	5.0	145.4
Adult*	9.6	24.6	49.9	128.3	17.7	45.5	13.1	33.6	9.9	25.4	257.1

* Compilation of adult male and adult female measurements.

pathogen reservoir potential of *A. diaperinus* and the magnitude of potential disease agents which could be harbored.

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Research Article

Dung Beetles (Coleoptera: Scarabaeinae) Attracted to *Lagothrix lagotricha* (Humboldt) and *Alouatta seniculus* (Linnaeus) (Primates: Atelidae) Dung in a Colombian Amazon Forest

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Dung beetles are among the most important insects in the Neotropics. Some species use a wide range of food sources, whereas other species are highly specialized. This study compares the use of two-primate excrement by an assemblage of dung beetles in a tropical forest in Colombia. Dung of *Lagothrix lagotricha* and *Alouatta seniculus* was used to attract beetles. A total of 32 species (47.7% of the species recorded for the area) were found on the two types of excrement studied, demonstrating that primate excrement is an important resource. The niche overlap between both feces is 27.03%, which indicates a high degree of resource specialization. Although these two primate species are found in the same areas, their diets vary greatly to permit a high degree of differentiation in beetle species. A study that includes dung of others primates would create a more complete panorama of resource overlap in the assemblage.

1. Introduction

Dung beetles are among the most important insect assemblages in the Neotropics, due to their important role in nutrient recycling, seed dispersal, and helminthes control, as they use omnivorous and herbivorous mammals dung as their food resource [1, 2].

Some species of dung beetles can use a wide range of food sources, from carrion to dung or more specific resources such as mushrooms, fruits, diplopods, decomposing vegetation, detritus, and eggs [1, 3–10]. Other species are highly specialized, using certain mammal dung [4, 11–15] or a more specific resource like primate dung of *Alouatta* spp. [3, 16–24] as well as *Lagothrix* spp. [25, 26].

Although, diverse aspects of the natural history and ecology of dung beetles have been widely studied [2, 27–30], little is known about their specialized use of some resources, especially primate dung. For this reason, this paper presents a study comparing the use of two primate excrement types by an assemblage of dung beetles in a tropical forest in Colombian Amazon region. Up to the present, no study has compared the preferences between two different primates excrement in the same locality.

2. Materials and Methods

2.1. Study Area. This study was carried out at the Center for Ecological Research of the Macarena (CIEM), located at 2° 40′ N and 74° 10′ W at an altitude of 350 m. The area is in a lowland tropical wet forest, located on the right bank of the Duda River, at the eastern border of Tinigua National Natural Park (Meta Department, Colombia). The predominant vegetation is mature forest [31] with a single rain cycle (dry period between December and March). The annual precipitation average is 2400 mm, with the least rainfall recorded in January (0 mm) and the greatest amounts in May–July (530 mm) [32].

The study was conducted in the primary habitat of the area, mature land forest, characterized by a continual tree canopy with a height of 25 to 30 m and emergent trees that reach up to 35 m [33]. Seven species of primates coexist in the area and of these, *Lagothrix lagotricha* (Humboldt, 1812) (Woolly Monkeys) and *Alouatta seniculus* (Linnaeus, 1766) (Red Howler Monkeys) are the most abundant [34].

2.2. Field Sampling

2.2.1. Lagothrix lagotricha Dung (see [25]). From january to July 1997 (including part of dry and rainy season), a group of woolly monkeys was followed for 60 h each month, and dung from a single focal individual was collected during the day. Dung beetles attracted to the dung were collected in plastic bags with the monkeys dung sample ($n = 520$). Dung was collected between 5 minutes after defecation. Additionally, five pitfall traps on soil surface were used during 72 hours in mature forest (replaced the dung daily), in order to complement the sample for nocturnal and delayed visitors.

2.2.2. Alouatta seniculus Dung. During January 1998 (dry season), a group of howler monkeys was followed and in the morning, when the monkeys defecated all the species attracted to the dung for 10 minutes were collected. Afterwards, the largest possible quantity of dung was collected in a hermetic container. The collected excrement was placed the same day in 25 mL cups along a 300 m transect in 10 pitfall traps. The traps were placed 30 m apart for a period of 24 hours. This methodology was carried out on three occasions with two days between each sampling effort.

The specimens collected were preserved in a 70% alcohol and taken to the Museum of Natural History at the University of Los Andes (ANDES-E), where they were deposited. They were identified to species level using keys, in comparison with the other specimens and the assistance of specialists.

2.3. Data Analysis. Percentage overlap between the dung types was determined using the Jaccard similarity analysis by PAST software. In addition, to compare the composition of species recorded on the two types of dung, an analysis was carried out using the Levin's breadth of niche index with the standardization proposed by Hurlbert [35]. The MacArthur and Levin's index of niche overlap was calculated with the modification proposed by Pianka [36], using the species as resources and the types of dung as the species [37].

3. Results

A total of 32 species were found on the two types of excrement studied, seven of those found on *L. lagotricha* were only identifiable to the genus level and thus appear as morph species in Table 1. It is probable that these morph species are contained in the species already identified for *A. seniculus*, but the material obtained for the two excrement types could not be compared. The analysis comparing the two excrements was thus conducted for two possible extreme scenarios: (a) where the seven morph species not identified by *L. lagotricha* are contained within the species identified for *A. seniculus* and (b) where these species are not contained and are different species.

The species found on the primate dung sample account for 47.7% of the 67 total species recorded for the area [15], demonstrating that the excrement of both primate species is without doubt an important resource in the area. 18 species were found on the excrement of *L. lagotricha*, of which seven were exclusive to this type of excrement (or 13 given scenario (a)). Likewise, 19 species were found on the excrement of *A. seniculus* of which 14 are exclusive (or 8 in scenario (a)). Five species were recorded on both types of excrement (11 in scenario (a)).

The similarity between both dung types is higher in scenario (a) (Jaccard's mean value = 42.5%, SD = 9.04%) than in scenario (b) (Jaccard's mean value = 18.5%, SD = 6.85%). In addition, the structure of the assemblage in each scenario fluctuates in the species in common related with the dung type they use (Figure 1). The Levin's niche breadth index does not present significant differences between both species (*L. lagotricha* = 0.016, *A. seniculus* = 0.017, mean value $IL = 1.958$). The niche overlap index presented a mean value of 0.2703 (72.97%), with no significant differences between both species (*L. lagotricha* over *A. seniculus* = 0.277, *A. seniculus* over *L. lagotricha* = 0.2631), while the Renkonen overlap percentage was 15%.

In addition, it is interesting to note that on both dung types, it is possible to found rolling species (telecoprids) and tunnelers (paracoprids), while the dwellers (endocoprids) were only collected on the dung of *A. seniculus* (Figure 2).

4. Discussion

Estrada et al. [14] registered an overlap greater than 80% for the dung beetles assemblage on the excrement of *A. seniculus* and *Nasua narica* (L., 1776), presenting a high number of common species with some variations in abundance, as a few were recorded exclusively on a single bait. In this study, the relation found between the overlap percentages in the two excrements was the opposite, a range between 15.6% (scenario (b), $n = 5$ spp.) and 34.3% (scenario (a), $n = 11$ spp.), which indicates that, for this locality, few species are generally associated with both types of dung.

These results indicate that there is a high degree of food resource specialization, despite both excrements coming from primates, there are sufficient differences in diet, microhabitat, and behavior of each species to permit a degree of differentiation between the species that make use of each dung [38–42].

Although these two species are found in the same areas, their diets vary greatly, as *L. lagotricha* consumes insects, fruits, and leaves, while the diet of *A. seniculus* is primarily foliage [25], and these differences affect the consistency, nutritional composition and smell of their respective excrements. Additionally, these species use different forest strata, produce excrement at different places, and times and the mobility and number of individuals per group are different [41]. It is probable that the use of different forest strata [41] affects the spatial disposition of the dung and distribution of the species that use these food resources. In addition, it is possible that seasonal difference in diet proportion affects the number of shared species between primates dung because

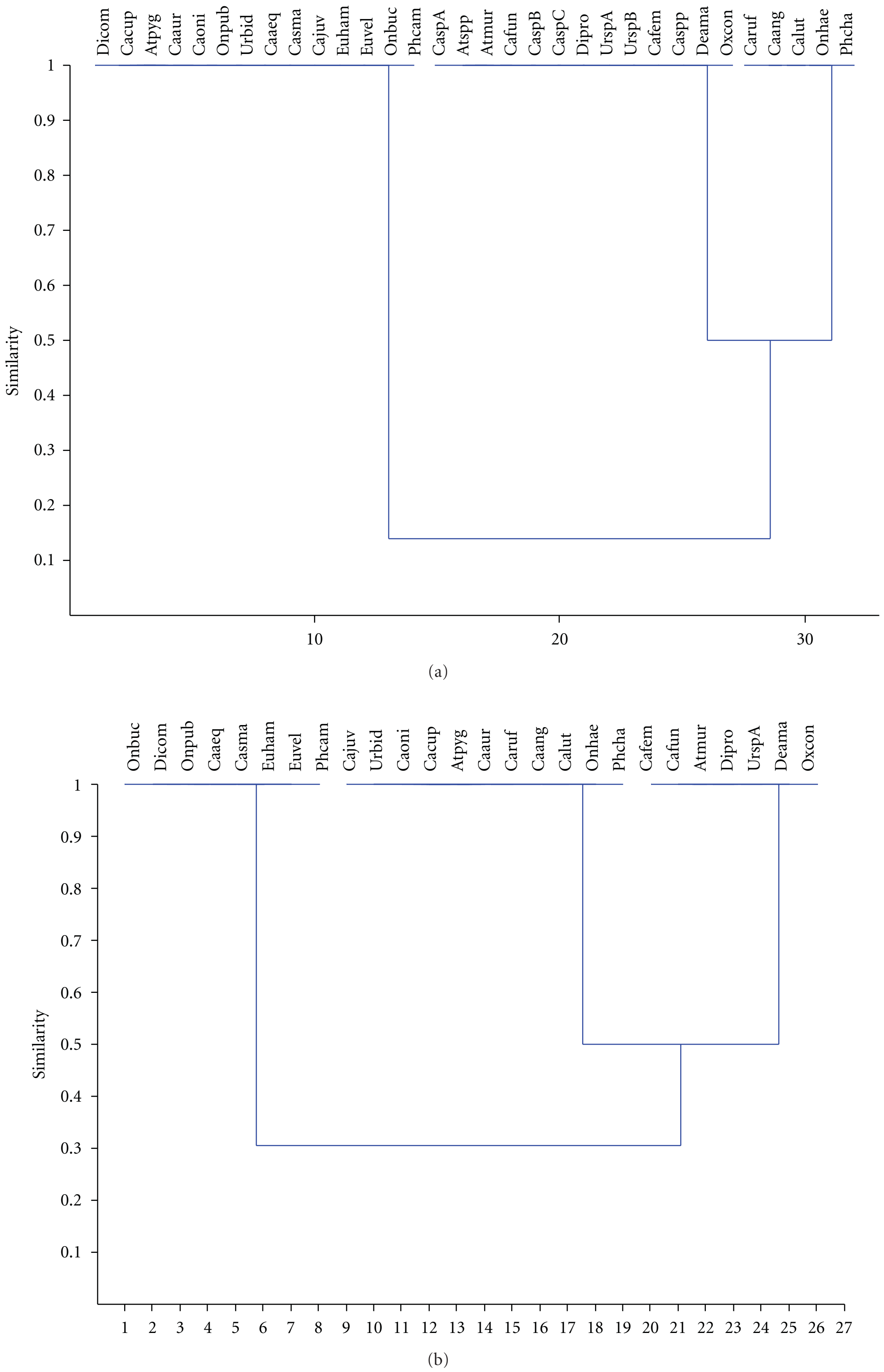

Figure 1: Dendrogram cluster analysis of similarity (Jaccard's index) among species and the dung type they use in scenarios (a) and (b), CIEM-Meta, Colombia.

Table 1: List of the species associated with each of the primate's dung, with their relocation habits (T: tunneler, R: roller, and D: dweller), in the CIEM station, Meta, Colombia.

Tribe	Species	rh	*L. lagotricha*	*A. seniculus*
Ateuchini	*Ateuchus murrayi* (Harold, 1868)	T	X	—
	Ateuchus pygidialis (Balthasar, 1939)	T	—	X
	Ateuchus sp.	T	X	—
Coprini	*Canthidium aurifex* (Bates, 1887)	T	—	X
	Canthidium cupreum (Blanchard, 1843)	T	—	X
	Canthidium funebre (Balthasar, 1939)	T	X	—
	Canthidium onitoides (Perty, 1830)	T	—	X
	Canthidium ruficolle (Germar, 1824)	T	X	X
	Canthidium sp. A	T	X	—
	Canthidium sp. B	T	X	—
	Canthidium sp. C	T	X	—
	Dichotomius compressicollis (Luederwaldt, 1929)	T	—	X
	Dichotomius problematicus (Luederwaldt, 1922)	T	X	—
	Ontherus pubens (Genier, 1996)	T	—	X
Coptodactylini	*Uroxys bidentis* (Howden and Young, 1981)	T	—	X
	Uroxys sp. A	T	X	—
	Uroxys sp. B	T	X	—
Deltochilini	*Canthon aequinoctialis* (Harold, 1868)	R	—	X
	Canthon angustatus (Harold, 1867)	R	X	X
	Canthon femoralis (Chevrolat, 1834)	R	X	—
	Canthon fulgidus (Redtenbacher, 1867)	R	—	X
	Canthon juvencus (Harold, 1868)	R	—	X
	Canthon luteicollis (Erichson, 1847)	R	X	X
	Canthon sp.	R	X	—
	Deltochilum amazonicum (Bates, 1887)	R	X	—
Oniticellini	*Eurysternus hamaticollis* (Balthasar, 1939)	D	—	X
	Eurysternus velutinus (Bates, 1887)	D	—	X
Onthophagini	*Onthophagus buculus* (Mannerheim, 1829)	T	—	X
	Onthophagus haematopus (Harold, 1875)	T	X	X
Phanaeini	*Oxysternon conspicillatum* (Weber, 1801)	T	X	—
	Phanaeus cambeforti (Arnaud, 1982)	T	—	X
	Phanaeus chalcomelas (Perty, 1830)	T	X	X

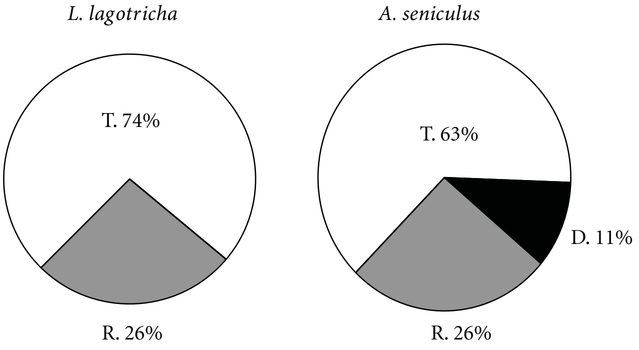

Figure 2: Richness of the three relocating guilds (R: rollers, T: tunnelers, D: dwellers) in the two dung types (*L. lagotricha* and *A. seniculus*) in the area, CIEM-Meta, Colombia.

the diet of these species is more similar in the rain season, but at dry time it is very different (P. Stevenson pers. comm.).

The rolling species can use the dung that remains on leaves, while tunnelers and dwellers do not, but even so the dominant group on both excrement types is the tunnelers. It is interesting to note that many of the species recorded on the two excrements were frequently found perching in the study area (Noriega, unpublished data). The amount of dung that remains on leaves is very small compared to what reaches the ground, but some of the species that utilize the dung that remains on leaves did not fall into the traps were placed at ground level [43].

A study that includes other possible resources, such as the dung of others primates species in the area and that of other mammals, would create a more complete panorama of resource overlap in the beetle assemblage, clarifying which species have generalist habits and which really are specialists, allowing to approach the quantification of the interspecific competition for this locality.

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Research Article

Cytogenetics of *Oryctes nasicornis* L. (Coleoptera: Scarabaeidae: Dynastinae) with Emphasis on Its Neochromosomes and Asynapsis Inducing Premature Bivalent and Chromosome Splits at Meiosis

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The chromosomes of specimens of *Oryctes nasicornis* from three locations in France and two locations in Greece were studied. All karyotypes have an X-Y-autosome translocation: 18, neoXY. Two male specimens from France (subspecies *nasicornis*) displayed an unusual behaviour of their meiotic chromosomes in 30–50% of spermatocytes, with asynapsis at pachynema, premature bivalent and chromosome split at metaphases I and II. The karyotypes remained balanced at metaphase I, but not at metaphase II. These particularities mimic the meiotic behaviour of B chromosomes and question about their existence, reported earlier in Spanish specimens. Due to the variable character of B chromosomes, complementary analyses are needed. To our knowledge, such meiotic particularities have not been described, beside cases of infertility. In specimens from Corsica (subspecies *laevigatus*) and Greece (subspecies *kuntzeni*), all spermatocytes I and II had a normal appearance. The meiotic particularity may thus be limited to male specimens from subspecies *nasicornis*.

1. Introduction

Beside pathological conditions such as malignancies or chromosome-instability syndromes, intraindividual variations of chromosomes are rare. Because of its usual stability, the karyotype of a limited number of cells is thought to represent that of a whole individual. This stability prevails for germ cells, so that parental and descendant karyotypes are similar. Consequently, the chromosome analysis of a limited number of cells from a limited number of individuals most frequently gives valuable information about the karyotype of their species. Exceptions exist, however, among which the presence of B chromosomes represents a major cause of numerical variation and polymorphism. B chromosomes have been described in plants and animals. They are characterized by a number of criteria among which is their particular meiotic behaviour: they do not pair like autosomes and tend to undergo premature centromere cleavage and non-disjunction at anaphase. This leads to variations of their number from cell to cell and descendant to descendant [1].

Insect cytogenetics has essentially been developed through spontaneously dividing germ cells at diakinesis/metaphase I and metaphase II. At these stages, chromosome morphology is not optimal for analysis. Among several thousand of chromosome formulas reported in coleopterans, the presence of B chromosomes was noticed in about 40 instances [1–5]. *Oryctes nasicornis* L. 1758 (Coleoptera: Scarabaeidae: Dynastinae) is one of the very first insects in which dispensable supernumerary chromosomes were described [6] and later on considered as B chromosomes. This observation was quoted in reviews on both insect cytogenetics [5, 6] and B chromosomes [1].

Having analysed the mitotic chromosomes of a male specimen of *O. nasicornis* L. 1758, we were surprised to observe a karyotype different from its earlier descriptions. It had neither a Xyp (p for parachute, [6]) sex formula nor supernumerary B chromosomes, but neoXY as a consequence of an X-Y-autosome translocation. B chromosomes being dispensable, we studied specimens from other localities and performed meiotic analyses to understand the causes of these discrepancies. We did not find B chromosomes, but, in two out of seven specimens, there were quite unexpected meiotic particularities. From pachytene to spermatocyte II stages, recurrent asynapsis, nonpairing, and premature centromeric cleavages mimic the behaviour of B chromosomes. Checkpoints controlling meiotic chromosome behaviour have been identified, from yeast to mammals [7, 8]. They monitor elimination of spermatocytes with abnormal chromosome synapsis [9]. In some *Oryctes nasicornis* specimens, the anomalies at metaphase I and II, as consequences of pachytene asynapsis, suggest the low stringency of these checkpoints.

2. Material and Methods

Two male specimens (number 1 and 2) of *O. nasicornis* were obtained from the breeding developed at the Museum of Besançon (France). They were captured as larvae in the Besançon area and are assumed to correspond to the *nasicornis* subspecies. They metamorphosed in June 2006. Two adult male specimens were captured in April 2007 (specimen number 3) and September 2010 (specimen number 4), at Bois-le-Roi, at the Fontainebleau forest border (48°27′ N, 2°42′ E). They are assumed to belong also to the *nasicornis* subspecies. Another male (specimen number 5) was captured near Porto Vecchio, Corsica (41°36′ N, 9°11′ E), in June 2007. It is assumed to belong to the *laevigatus* Heer 1841 subspecies. Finally, two males were captured in Greece, one (specimen number 6) near Oros Kallidromo (38° 44′ N, 22°39′ E) in may 2010 and one (specimen number 7) near Kalambaka (39°47′ N, 21°55′ E) in June 2011. They are assumed to belong to the *kuntzeni* Minck subspecies. Pachytene bivalent chromosome preparations were obtained following a long hypotonic shock and meiotic and mitotic metaphases after treatment with O.88 M KCL for 15 min. and another 15 min. in diluted calf serum (1 vol.) in distilled water (2 vol.) [10, 11]. Chromosomes at various mitotic and meiotic stages were studied after Giemsa and silver stainings and Q- and C-banding. Image capture was performed on a Zeiss Phomi 3 equipped with a high-resolution camera JAI M4+ and IKAROS (Metasystems) device or a Leica Aristoplan equipped with a JAI M300 camera and ISIS (Metasystems) device.

3. Results

Mitotic Karyotype (Figure 1). It is composed of 18 chromosomes, including three sub-metacentric (number 1, 2 and 8) and five acrocentric (number 3–7) autosomal pairs. All of them carry large and variable heterochromatic segments around the centromeric region. The X chromosome is submetacentric and the Y acrocentric. Their size is much larger than that of gonosomes of most other Scarabaeid beetles. All heterochromatin is positively stained after C-banding and heterogeneously stained after Q-banding (not shown) which indicates its heterogeneous composition. Beside the variations of the amounts of heterochromatin, all specimens had the same chromosome complement, as reported [4, 12].

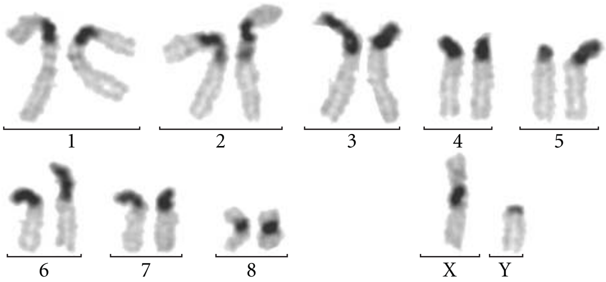

Figure 1: Mitotic karyotype of *Oryctes nasicornis* male (specimen number 1 from Besançon) after C-banding.

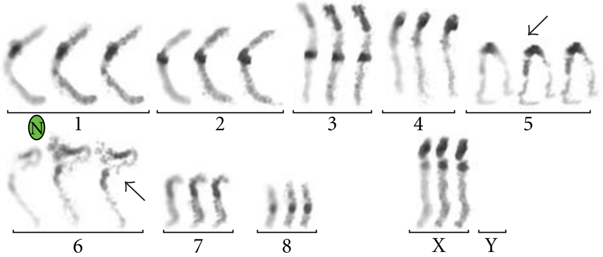

Figure 2: Karyotype from a spermatocyte at pachynema after the Giemsa staining (left), NOR staining displaying nucleoli (N) (centre), and C-banding treatment (right). Acrocentric bivalents 5 and 6 are not synapsed, but associated by their heterochromatic short arms (arrows). Heterochromatin is more compact than on mitotic chromosomes. Specimen number 3 from Bois-le-Roi, as is the case in the next figures.

Pachytene Chromosomes (Figures 2 and 3). As expected from the mitotic karyotype, nine bivalents were generally observed. They could be identified by the amount and position of their heterochromatin, although heterochromatin was globally more compact than in mitotic cells. The sex bivalent was quite characteristic. It had a large synapsed segment, similarly to autosomes, followed by juxtacentromeric heterochromatin, and a compact segment. This was interpreted as the result of an X-Y-autosome translocation, the autosomal portion forming the long arm and the sex chromosomes forming the short arm. This translocation explains the low number of chromosomes (18 instead of 20 in most Scarabaeidae) and the large size of the sex chromosomes (the short arm relative length matches that of the X of other Dynastinae with a free X). Thus, the mitotic karyotype formula is 18, neoXY. Silver staining displayed a strong staining of all heterochromatin, as in most coleopterans. In addition, round nucleolar-like structures were recurrently associated with the short arm of a small acrocentric bivalent that we defined as number 6. Thus, according to previous studies [13], the Nucleolar Organizer Region (NOR) is located on chromosome 6 short arm (Figure 2). The above description refers to observed spermatocytes. However, one or several bivalents displayed

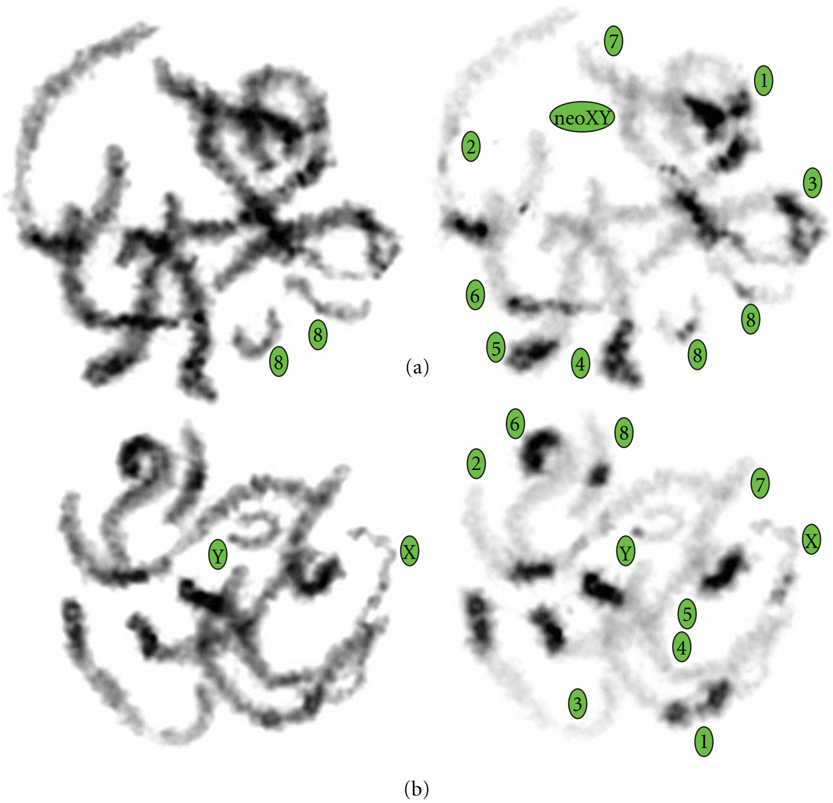

Figure 3: Spermatocytes at pachynema after the Giemsa staining (left) and C-banding (right) displaying asynapsis of chromosomes 8 (a) and sex chromosomes (b).

either asynapsis or incomplete synapsis in 29% and 41% of the spermatocytes from specimen number 2 and 3 from Besançon and Bois-le-Roi, respectively (Table 1). Smaller acrocentrics (numbers 6 and 7) were the most frequently involved, but all bivalents, including the sex bivalent (Figures 2, 3(a) and 3(b)), could be occasionally affected. In all instances, the non-synapsed autosomes were lying close to each other, suggesting either their premature desynapsis or deficient synapsis. The two homologues remained frequently at contact by their heterochromatic regions (Figure 2). Conversly the neoX and neoY chromosomes could be completely separated (Figure 3(b)). Specimen number 1 was immature and spermatocytes at pachynema of specimen number 5 could not accurately be studied and could not be considered as control. In specimen number 4 from Bois-le-Roi and specimens number 6 and 7 from Greece, the synapsis was strictly normal. We applied the same cytological techniques to specimens from more than other 100 species and observed such pachytene asynapsis only once and at a low frequency.

Diakinesis/Metaphase I (Figure 4). This stage was the most frequent in all the specimens studied: a total of 696 cells could be examined. Most of them displayed nine bivalents (biv), among which the sex bivalent could be identified by its asymmetrical constitution, as in other species with translocation-derived neoXY. No particularities were noticed in specimens number 4 to 7, whereas 43% and 34% of cells from the specimens number 2 and 3 (Table 1) displayed univalents (univ), respectively. Their number was inversely proportional to that of bivalents: 9 biv + 0 univ; 8 biv + 2 univ; 7 biv + 4 univ; 6 biv + 6 univ, demonstrating that two univalents replaced one bivalent. The univalent occurrence, observed at both early diakinesis and late metaphase I, did not seem to depend on the progression towards anaphase. It preferentially involved smaller and sex chromosomes.

Table 1: Numbers and percentages of mitotic and meiotic cells analysed in specimen number 2 from Besançon and number 3 from Bois le Roi. Cells were scored as abnormal (abnl) when they displayed asynapsis (pachynema), univalents (diakinesis/metaphase I) or monochromatidic chromosomes (metaphase II), and normal (nl), when all chromosomes were in correct phase.

Cell stage	Besançon-image no. 2			Bois-le-Roi-image no. 3		
	nl	abnl	% abnl	nl	abnl	% abnl
Mitotic Metaphase	32	0	0	5	0	0
Pachynema	34	10	29	36	25	41
Diakinesis/ Metaphase I	41	31	43	195	99	34
Diakinesis/ Metaphase II	25	11	31	40	58	59

Metaphase II (Figures 5 and 6). No particularities were noticed among the 56, 48, 50 and 27 metaphases II analyzed

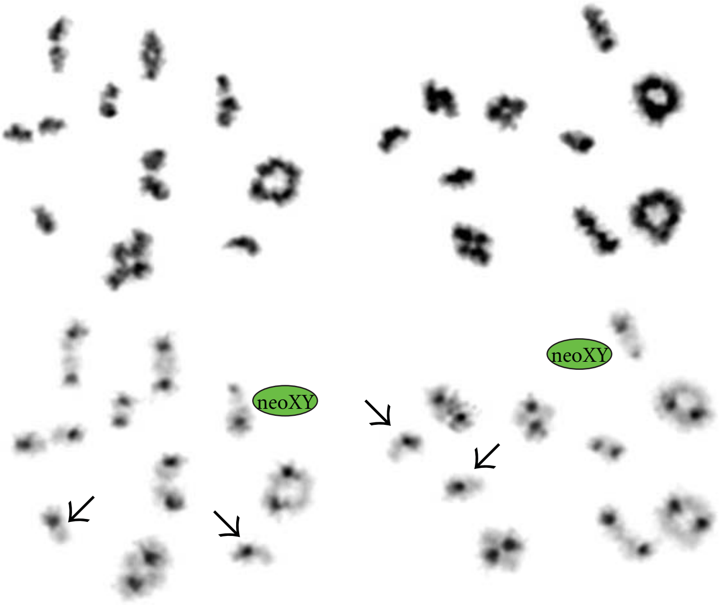

Figure 4: Spermatocytes at metaphase I after the Giemsa staining (top) and C-banding (bottom) with eight bivalents and two univalents (arrows).

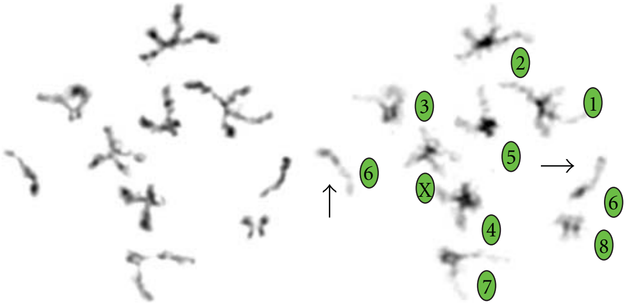

Figure 5: Spermatocyte at metaphase II displaying eight bi-chromatidic and two single-chromatid chromosomes, presumably number 6 (arrows).

from specimens 4, 5, 6, and 7, respectively. All were composed of 9 double-chromatid chromosomes. In specimens 2 and 3, 31% and 59% of metaphases II, respectively, comprised more than 9 chromosomes. C-banding allowed us to differentiate mono-chromatidic (monoc) and bi-chromatidic (bic) chromosomes. The number of monoc was roughly inversely proportional to that of bic: 9 bic + 0 monoc, 8 bic +2 monoc (Figure 5), 7 bic + 4 monoc, and 6 bic + 6 monoc. In a proportion of metaphases II, however, the ratio bic/monoc was different, indicating that aneuploidies occurred, as consequence of segregation errors at anaphase I (Figure 6). The premature centromeric split preferentially involved the small acrocentrics, the metacentric 8, and the sex chromosomes.

4. Discussion

The karyotypes of the specimens of *O. nasicornis* studied here obviously do not contain B chromosomes. *O. nasicornis* is a widespread species in Western Europe, with eleven subspecies identified. The first mention of its karyotypic particularities was reported on Spanish specimens, which belong to the *grypus* Illiger 1803 subspecies [4]. The specimens from Besançon and Bois-le-Roi belong to the subspecies *nasicornis*. These two locations cover only a small part of the whole distribution area of the subspecies, but they are sufficiently distant (about 300 km) to assume that they do not constitute an isolate with abnormal gametogenesis. The specimens from Corsica and Greece, in which we failed to detect any meiotic particularity, belong to the subspecies *laevigatus* and *kuntzeni*, respectively, and there are no available data on the chromosomes of other specimens from this subspecies. Thus, the question of both the presence of B chromosomes and/or atypical meiosis, in relation with subspecies, remains open and needs further investigations.

The high recurrence of asynapsis and premature centromeric cleavage may be an artifact induced by hypotonic shock and spreading. However, the techniques used for pachynema and other meiotic stages were different, and we found fairly similar rates of aberrations at all stages. Furthermore, technical artifacts can hardly explain aneuploidies at metaphase II. We applied these techniques on meiotic chromosomes from many species of coleopterans without B chromosomes and observed such particularities only once at a low rate. Conversely, when B chromosomes were duly identified, they had a particular pairing leading to non-disjunctions at anaphase I, hence duplications and losses in spermatocytes II and variable numbers in descendants. It has no effect upon the phenotype, which indicates they carry no genes with major effect on the phenotype [1]. Here, all chromosomes can be involved in abnormal meiotic pairing. At metaphase II, 30–50% of spermatocytes displayed premature chromosome cleavage, which should induce a high rate of

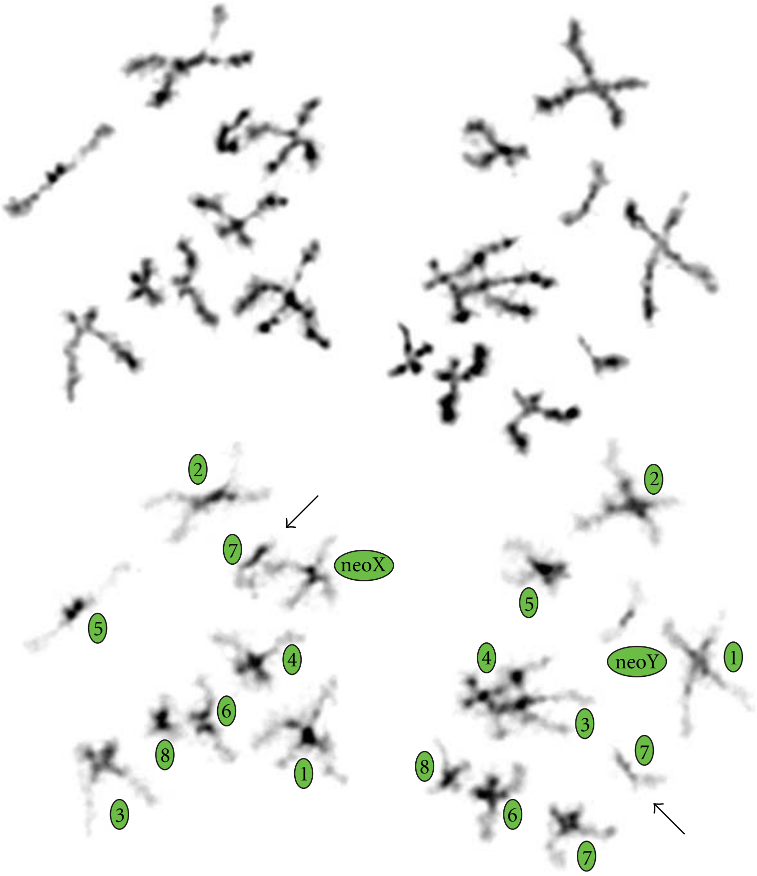

Figure 6: Unbalanced sister metaphases II after the Giemsa staining (top) and C-banding (bottom). One acrocentric (presumably number 7) is single-chromatid on the left, while the complementary mono-chromatidic chromosome is in excess on the right (arrows).

unbalanced gametes. Indeed, aneuploid spermatocytes II were observed and a reduction of reproductive fitness should be expected, but we have no indication that it is the case. Furthermore, it is noteworthy that the rates of asynapsis at pachynema, premature bivalent cleavage at metaphase I, and premature centromere split at metaphase II are roughly similar at both intra- and interindividual levels. This suggests that metaphase I and II anomalies are direct consequences of pachytene asynapsis, and that there is both synapsis and checkpoint flaws at pachynema [8, 9]. It will be interesting to establish karyotypes of a series of eggs laid by parents with these meiotic particularities to know whether or not they induce a high rate of aneuploidies at early stages of development.

Another point of interest, in the karyotype of *O.nasicornis*, is the presence of neo-sex chromosomes. As described in the Scarabaeid beetles *Dynastes hercules* and *Jumnos ruckeri*, their meiotic behaviour, with an autosome-like synapsis of a long portion, indicates they originated from an X-Y-autosome translocation [13, 14]. As in these species too, the autosomal portion is separated from the original X component by the centromere, that is, constitutive heterochromatin. The insulating role of heterochromatin has been discussed for long in mammals, where it prevents inactivation spreading from the late replicating X to the attached autosome in female somatic cells [15]. In meiotic prophase of the male, heterochromatin also isolates euchromatin from the inactivated sex chromosomes [16]. In *Drosophila*, the gene dosage compensation between males and females somatic cells is achieved by the overexpression of genes from the single X of the males [17]. This may also be the case of the beetle *Dynastes hercules*, but this was shown only for NOR expression [13]. In *Gryllotalpa fossor* (Orthoptera), the dosage compensation is of the mammalian type [18]. Finally, in *Musca domestica* (Diptera), no dosage compensation seems to exist [19]. These different situations demonstrate the existence of several regulatory mechanisms for X-linked gene expression in insect somatic cells. Whatever this mechanism, that is, over- or underexpression, there is an important character which is the existence of an epigenetic control spreading over large chromosome segments, if not whole chromosomes. We proposed that, in insects with overexpression of the X-linked genes in the male, as *Drosophila*, heterochromatin might play this insulating role [13]. This fits with the observation that in the few instances where an X-autosome translocation carrier *Drosophila* is fertile, the break point originating the translocation occurred within heterochromatin of the X ([20] and references herein). The presence of heterochromatin between gonosomal and autosomal components in the neo-sex chromosomes of *O. nasicornis* provides another example suggesting the role of heterochromatin to avoid spreading of cis-acting epigenetic control elements.

In conclusion, this study shows that two chromosomal particularities exist in *O. nasicornis*. One is an X-Y-autosome translocation, frequently deleterious for reproduction, unless specific conditions prevent position effect, due to the different regulation of sex chromosomes and autosomes. Such translocations are not exceptional in Coleoptera, compared to other animals such as mammals. The other particularity is much more exceptional: two male specimens of *O. nasicornis nasicornis* display meiotic alterations usually considered as deleterious for fertility. These specimens were caught at two distant localities, which suggests these alterations are spread in the population and do not drastically prevent reproduction. Progress in the molecular biology of meiosis has shown the multiplicity of genes involved in synaptonemal complex formation and recombination [21, 22]. One of them may be altered in some specimens of *O. n. nasicornis* and maintained if associated with some hypothetical advantage. A third particularity, that is, the presence of B chromosomes, reported in specimens from Spain, may be an incorrect interpretation of the meiotic particularity described here and warrants further studies.

Acknowledgments

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Research Article

Australian Alleculinae: New Genera, New Combinations, and a Lectotype Designation (Coleoptera: Tenebrionidae)

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Litopous baehri gen. et sp. nov. and *Scaphinion clavatus* gen. et sp. nov. are described. New combinations (previous genus in parentheses) are *Aethyssius minor* (Carter) (*Tanychilus* Newman), *Homotrysis subopaca* (Carter) (*Metistete* Pascoe), *Metistete armata* Carter (*Homotrysis* Pascoe), *M. opaca* (Carter) (*Tanychilus*), *M. punctata* (Carter) (*Melaps* Carter), and *Nocar subfasciatus* Carter (*Taxes* Champion). A lectotype and two paralectotypes are designated for *Dimorphochilus diversicollis* Borchmann which is removed from synonymy, and the genus is briefly discussed.

1. Introduction

Australian Alleculinae are poorly known and there are numerous unnamed species. Many species that are now included in genera such as *Homotrysis* Pascoe, *Metistete* Pascoe, and *Aethyssius* Pascoe apparently belong to other genera, but no consistently reliable morphological characters have been proposed to distinguish them. In the present paper two such taxa from Western Australia are included in two new genera based on the discovery of diagnostic characters. Another taxon from the same state was considered as possibly belonging to a third distinct genus with simple tarsal claws, a character very unusual in the Alleculinae. However, examination of the claws under high magnification revealed that the inner edges are minutely crenulated, and also in other respects, such as the apically unidentate mandibles, cultriform terminal maxillary palpomere and front of head not produced, this species (unnamed) falls within the current concept of *Aethyssius*.

The terms frontal index and stomatiform punctures follow Matthews and Bouchard [1], and the checklist of names included in that work gives the dates and citations of all descriptions.

2. Material

Material from the following collections was examined:

AM: Australian Museum, Sydney;

ANIC: Australian National Insect Collection, Canberra;

ANIC(MM): Macleay Museum material on permanent loan to ANIC;

BMNH: Natural History Museum, London;

NMV: Museum of Victoria, Melbourne;

QM: Queensland Museum, South Brisbane;

SAMA: South Australian Museum, Adelaide;

WAMA: Western Australian Museum, Perth;

ZSM: Zoologische Staatssammlung, Munich.

3. New Genera

3.1. Litopous gen. nov.

Type Species. *Litopous baehri* sp. nov.

Description. Oblong, densely setose with long erect bristles on all dorsal surfaces and legs. Head not prolonged. Antennomeres elongate, bearing dense long bristles, only a few compound sensoria on apical 3 segments. Eyes deeply emarginate anteriorly. Mandibles bluntly bidentate. Apical maxillary palpomere roundedly securiform, symmetrical.

Tarsi bearing long bristles only, without lobes or cupuliform segments. Claws not pectinate, with only small teeth on basal half of inner edges (Figure 2(d)) . Intercoxal process of first ventrite acutely angular. Length 8.3 mm.

Distribution (Figure 3). Known only from the type locality near Wurarga inland from Geraldton, WA.

Etymology. Greek litos = plain, pous = foot.

Discussion. This is the most unusual Australian alleculine examined, having no trace of lobes or cupuliform tarsomeres. The penultimate segment of the protarsus bears two stout diverging acute projections, probably coalesced bristles. The teeth on the tarsal claws are very short (Figure 2(d)), not typically pectinate as in all other known Australian Alleculinae except the unrelated *Hemicistela* Blackburn and the simple-clawed species mentioned in the introduction. The antennae are also unusual in bearing numerous long stiff setae and very few compound sensoria. According to current classifications of the subfamily (e.g., Matthews et al. [2]) *Litopous* does not fit into the subtribe Alleculina as other Australian alleculines because of the absence of tarsal lobes, and it does not conform to the diagnostic characters of any of the other higher taxa of the subfamily as discussed by Campbell [3]. For the present, this genus will be treated as *incertae sedis* within the tribe Alleculini.

3.2. Litopous baehri sp. nov. (Figures 1(a), 2(d), 3)

Description of Female. Elongate-oblong, uniformly dark brunneous, with dense erect setae, total length 8.3 mm, width at humeri 2.7 mm.

Head. Frons and clypeus not prolonged, frontal index 0.42, edges indented at frontoclypeal suture, which is impressed. Basal membrane of labrum short, labrum very transverse bearing long stiff bristles. Eyes moderate, separate dorsally by distance equal to a little more than width of one eye. Antennomere 3 longer than 4 but shorter than 4+5, segments gradually shortening distad, terminal segment fusiform and apically acute. Antennomeres 1–7 bearing long bristles, 8–11 short stout setae, only a few compound sensoria on apical 3 segments. Mandibles short, bluntly bidentate.

Prothorax. Subquadrate, 1.2 times as wide as long, sides strongly convex, maximum width a little before middle. Pronotal surface densely and coarsely punctuate, punctures bearing long erect bristles. Prosternal process about as wide as half of one procoxa.

Pterothorax. Much wider than prothorax, sides of elytra straight, subparallel for anterior 2/3, roundedly tapering posteriorly. Striae deeply impressed, strial punctures round, simple, separated by distance about equal to one-puncture diameter. Interstriae with numerous punctures bearing long erect bristles. Wings fully developed.

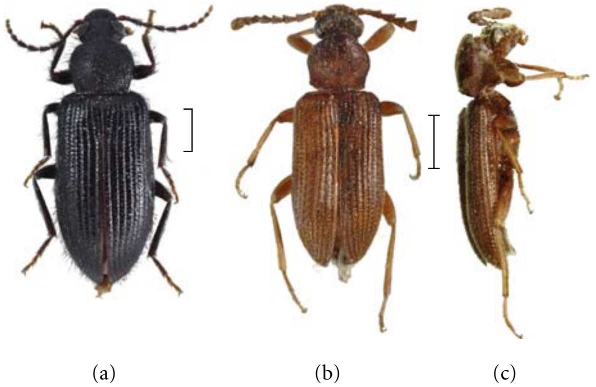

Figure 1: Habitus. (a) *Litopous baehri* sp. nov. female; (b) *Scaphinion clavatus* sp. nov. male, dorsal; (c) ditto, lateral. Scale lines 1 mm.

Legs. Slender, unmodified, femora projecting beyond body outline for about half their length. Tarsi a little longer than half tibial length, basal metatarsomere a little longer than next 3 combined.

Abdomen. Intercoxal process of first ventrite acutely triangular.

Male. Unknown.

Type. Holotype female: Australia, WA 06/168, 2 km SE Wurarga, 28.37913 S, 116.33105 E, 371 m, 3-4.3.2006, M. Baehr. WAMA Reg. no. 82708.

Etymology. The author takes pleasure in naming this species after its collector, Martin Baehr of ZSM.

3.3. Scaphinion gen. nov.

Type Species. Scaphinion clavatus sp. nov.

Description. Elongate-oblong, bearing long recumbent setae. Head not prolonged. Eyes large, deeply emarginate. Antennomeres sometimes greatly enlarged distally. Mandibles small, acutely bidentate. Apical maxillary palpomere broadly securiform, somewhat asymmetrical. Occiput deeply transversely excavate and receiving prolongation of anterior part of pronotal disc. Elytral punctures uniformly large, deep, with sides thickened (stomatiform). Tarsi with small square lobes on penultimate segments. Claws pectinate. Total length ±5 mm (based on 3 available specimens of the genus).

Distribution (Figure 3). The Kimberley District of north-western Australia where all three known species were collected.

Etymology. Greek scaphe = hollowed out, inion = back of head.

Discussion. Scaphinion is related to *Metistete* Pascoe because the elytral punctures are entirely stomatiform. All three

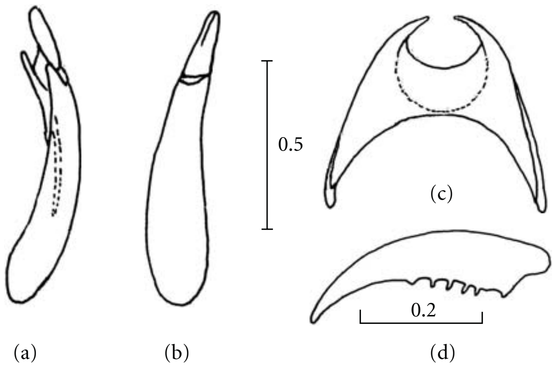

Figure 2: Outlines. (a) *Scaphinion clavatus* aedeagus, lateral; (b) ditto, dorsal; (c) *S. clavatus* sternite 8; (d) *Litopous baehri*, tarsal claw.

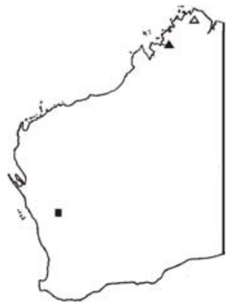

Figure 3: Known distributions in Western Australia. (■) *Litopous behri*; (▲) *Scaphinion clavatus* and *S.* sp. 1; (△); *Scaphinion* sp. 2.

known species differ from the latter genus in having the pronotum anteriorly prolonged and received in a concavity of the occiput. The enlargement of the antennae of two of the known specimens (Figures 4(a), 4(b)) is also unknown in *Metistete*. The third specimen has simple antennae (Figure 4(c)). The three male specimens known of this genus each belong to a different species as discussed below. The author believes that in general it is not desirable to describe new species of known genera on the basis of single specimens, but where new genera are clearly involved, as is the case in the present paper, they should be described even though their type species are initially represented by uniques. The two species of *Scaphinion* which are not types of the genus are briefly characterized but not named now in order to illustrate some of the specific diversity which is to be expected in the genus. A forthcoming faunal survey of the Kimberley District by the Western Australian Department of Environment and Conservation should discover more specimens of the genus and thus permit detailed descriptions of both sexes.

3.4. Scaphinion clavatus sp. nov. (Figures 1(b), 1(c), 2(a), 2(b), 2(c), 3, and 4(a))

Description of Male. Elongate oblong, uniformly fuscous, with dense recumbent setae, total length 5.3 mm, width at humeri 1.8 mm.

Head. Clypeus broadly truncate, sides diverging and forming a straight line with edges of canthi. Head moderately prolonged, frontal index 1.0. Basal membrane of labrum very short, labrum very transverse, bearing moderately long setae. Eyes deeply emarginate anteriorly, large, separated by a distance subequal to about 1.5 eye width as seen from above. Antennomere 3 about as long as 4, 3–9 subtriangular, gradually widening and becoming asymmetrical, 10 very transverse, wider than 9, 11 transverse, smaller than 10 and contained within a cavity of latter (Figure 4(a)). All segments bearing short setae, 3–11 with numerous compound sensoria. Mandibles small, acutely bidentate.

Prothorax. Anterior edge of pronotum strongly produced to broadly rounded apex which overhangs base of head, pronotum widest just behind middle, sides slightly convergent to base, basal edge straight. Ratio of maximum width to length 1 : 1. Pronotal surface densely and coarsely punctate with long recumbent setae. Prosternal process as wide between coxae as 1/3 width of 1 coxa.

Pterothorax. Much wider than prothorax, sides of elytra subparallel for anterior 2/3, broadly tapering to apices. Striae not impressed, consisting of very large and deep, close-set rounded stomatiform punctures. Interstriae with numerous small punctures bearing long recumbent setae. Wings fully developed.

Legs. Moderately slender and setose, femora with about 1/2 of their length projecting beyond elytral edges. Tibiae subparallel, slightly bowed, not distorted. Tarsi about 1/3 length of tibiae. Lobes of preapical tarsomeres small.

Abdomen. Intercoxal process of first ventrite acutely triangular. Aedeagus (Figures 2(a) and 2(b)) simple.

Female. Unknown.

Type. Holotype male. 16.22S 125.12E W.A. Charnley Riv. 2 km SW Rolly Hill CALM site 25/2 16–20 June 1988, I.D. Naumann, at light, open forest near closed forest margin, ANIC.

Discussion. The three known male specimens belong to three different species, *S. clavatus* and two others which remain unnamed for now for the reasons discussed above. The latter two have the following locality data: Species 1, 16.38S 125.15E CALM site 28/3 4 km W of King Cascade, W.A., 12–16 June 1988 T.A. Weir, at light closed forest margin, ANIC; species 2, 14.39S 126.57E Drysdale River, W.A. 18–21 Aug. 1975, I.F.B. Common and M.S. Upton, ANIC. The most obvious difference between the three is in the form of the antennae, sp. 1 having only moderately enlarged antennae (Figure 4(b)) and sp. 2 unmodified filiform ones (Figure 4(c)). In addition, in both unnamed species the eyes are larger than in *clavatus*, the interocular distance being equal to about half an eye width and the shape of abdominal sternite 8 is different in all three species, this being the most

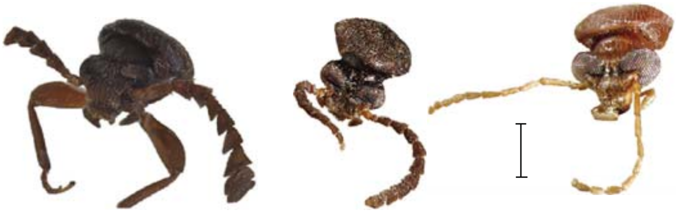
(a) (b) (c)

Figure 4: Head and prothorax of *Scaphinion*. (a) *S. clavatus*; (b) *S.* sp. 1; (c) *S.* sp. 2. Scale line 1 mm for all figures.

reliable indication of specific distinctness. The aedeagi are identical.

4. New Combinations

A recent examination of Carter types of Alleculinae in NMV revealed that a number of species were incorrectly assigned to genus in [1]. The following names are transferred to different genera from those listed in the latter work. The correct generic name is first and the name in [1] is in parentheses.

Aethyssius minor (Carter) (*Tanychilus* Newman).

Homotrysis subopaca (Carter) (*Metistete* Pascoe).

Metistete armata Carter (*Homotrysis* Pascoe). Carter placed this species in the correct genus but it was wrongly assigned by [1].

Metistete opaca (Carter) (*Tanychilus*).

Metistete punctata (Carter) (*Melaps* Carter).

Nocar subfasciatus Carter (*Taxes* Champion). Also placed correctly by Carter but misplaced by [1].

5. Previous Lectotype Designations

Carter and other authors of that time did not specifically designate holotypes in their descriptions, but they always labelled one specimen of the type series as the "Type" and the others as cotypes, clearly intending that they should be considered holotype and paratypes, respectively. In all unpublished museum catalogs and lists, the specimens which bear the label "Type" are listed as holotypes and often given numbers. However, according to Article 74 of the ICZN [4] this is not sufficient for the specimens in question to be considered primary types unless they were clearly identified (or indicated to be unique) in the original descriptions. On the other hand, many Carter and Macleay numbered specimens are listed in print as holotypes in AM by McKeown [5] and in this case this action is tantamount to lectotype designation according to Article 74.6 of [4]. The names involved, listed in their present genera, are *Aethyssius cyaneus* (Macleay), *A. cylindricollis* (Carter), *A. major* (Carter), *A. mastersi* (Macleay), *A. metallica* (Carter), *A. oculatus* (Carter), *A. piceus* (Macleay), *A. puncticeps* (Blackburn), *A. ruficollis* (Macleay), *A. rugosulus* (Macleay), *A. suturalis* (Carter), *A. tenuicornis* (Carter), *A. vitticollis* (Macleay), *Dimorphochilus pascoei* (Macleay), *Euomma palpalis* (Macleay), *E. mastersi* (Macleay), *Homotrysis albolineata* Carter, *H. interstitialis* Carter, *H. mastersi* (Macleay), *H. montium* Carter, *H. ruficornis* Macleay, *H. rufobrunnea* Carter, *H. sexualis* Carter, *H. silvestris* Carter, *H. subgeminata* Macleay, *Metistete clermontia* (Carter), *M. elongata* (Macleay), *M. illidgei* (Carter), *M. pascoei* (Macleay), *M. planicollis* (Macleay), *M. punctipennis* (Macleay), *M. rufipilis* (Carter), *M. subsulcata* (Macleay), *M. yeppoonensis* (Carter), *Nocar convexus* (Macleay), *N. depressiusculus* (Macleay), *N. suttoni* Carter, *Ommatophorus mastersi* Macleay, and *Scaletomerus politus* (Macleay). For all of these, "ST" in the checklist of [1] should be changed to LT.

The remaining approximately 80 specimens listed as holotypes or the equivalent in the catalogs of ANIC(MM), BMNH, NMV, QM, SAMA, and ZSM must be considered only syntypes unless the original descriptions were based on single specimens. In the checklist of [1] they are appropriately listed as ST and HT, respectively. In most cases the specimens labelled "Type" by their original describers will eventually be selected as lectotypes, but this action should be deferred until the taxa in question are revised.

5.1. Dimorphochilus Borchmann, 1908. Borchmann [6] described this as a new genus with three new species, *D. apicalis, D. diversicollis*, and *D. sobrinus*, collected during the Hamburg Museum Southwest Australia expedition of 1905. The concept of this genus was uncertain because *D. sobrinus* should be assigned to *Tanychilus*, which has apically unidentate mandibles unlike *Dimorphochilus*. *Tanychilus gouldi* (Hope) was also misplaced in *Dimorphochilus* by Carter [7] partly for this reason. The fixation of the type species of *Dimorphochilus* as *D. apicalis* Borchmann by [1] restricts the diagnostic characters of the genus as follows: apices of mandibles bidentate, or bilobate with a longitudinal groove between lobes; dorsal surfaces entirely glabrous, the body castaneous with legs paler, often flavous; elytra with simple punctures only and a sutural gap. The latter term refers to a widening of the suture at the apices of the elytra, the space thus opened being covered with overlapping expansions of the marginal membranes of the suture. This character is illustrated for *D. apicalis* by [1] in Figure 80 K, which shows the extremely widened gap of a female on the right and the more normal one of a male on the left. Carter [8] erroneously synonymised *D. diversicollis* with *D. apicalis.* The former species is here resurrected following examination of the types listed below.

The genus *Dimorphochilus* as now understood includes the following five named species known from the states indicated in parentheses: *apicalis* (WA), *diversicollis* (WA), *caudatus* (Carter) (Qld), *luctuosus* (Champion) (Tas), and *pascoei* (Macleay) (Qld, NSW). In addition, there are two unnamed species in SA, probably others elsewhere. There is some doubt about *D. luctuosus* as it is entirely piceous and the elytral gap is minimal.

The material collected during the Hamburg Southwest Australia expedition of 1905 was largely taken to Hamburg and subsequently destroyed during World War II. It was considered that Borchmann material of Alleculinae was likewise destroyed until three female specimens of *D. diversicollis* agreeing with the original description and bearing the expedition labels were discovered among miscellaneous accessions in WAMA. Borchmann had five males and numerous females of the species but did not designate a holotype. The specimens discovered are here designated lectotypes of *Dimorphocilus diversicollis* Borchmann, 1908 as follows, with the sequence of labels indicated by numbers in parentheses.

Lectotype Female. (1) (printed) Hamb. S. W. Austr. Exped. 1905. Stat. 76 Day Dawn 9.-10. VII; (2) (handwritten) *Dimorphochilus diversicollis* Borch. Cotype! 4238; (3) (yellow, printed) Western Australian Museum Entomology Reg no 57620; (4) (red, handwritten by author) LECTOTYPE Female *Dimorphochilus diversicollis* Borch. Sel. by E. Matthews.

Paralectotype Females (Two). (I) (1) as above Stat. 71 Northampton 4238a 15. VII; (2) as in 3 above Reg no. 57621; (3) (blue, handwritten by author) PARALECTOTYPE Female *Dimorphochilus diversicollis* Borch. (II) (1) as above Stat. 77 Yalgoo 4238b 11. VII. *Dimorphochilus diversicollis* originally handwritten on underside; (2) as in 3 above Reg no. 57622; (3) as in 3 for previous paralectotype.

Acknowledgments

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Research Article

Improved Visualization of *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae)—Part I: Morphological Features for Sex Determination of Multiple Stadia

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The lesser mealworm, *Alphitobius diaperinus* (Panzer), is a perennial pest of poultry facilities and known to transmit pathogens of poultry and humans. Ongoing research examining reservoir potential of *A. diaperinus* revealed the need for a comprehensive, user-friendly guide for determining sex of *A. diaperinus* at different stadia. This paper is unique in providing a comprehensive illustrated guide of characters used for differentiation of sexes in *A. diaperinus.*

1. Introduction

The lesser mealworm, *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae), is a perennial pest of poultry facilities. The species is of tropical origin from sub-Saharan Africa and has adapted to the moist, temperature controlled environments of poultry production facilities [1]. As a nocturnal species, the low-light conditions within broiler grow-out houses are conducive for populations to thrive [2]. Peak population densities occur during the warmer summer season but the pest is present throughout the year [3]. Omnivorous feeding habits allow *A. diaperinus* to feed on manure, feed, chicken carcasses, detritus, and other larvae and beetles [4–7].

Generally, 5 to 8 wks are required for *A. diaperinus* to complete a life cycle within a broiler grow-out house; ambient temperature and humidity will affect the life span [8]. Female beetles oviposit in sheltered areas of the house, depositing 1000 to 1800 fertile eggs over their life span [2, 9–11]. Eggs hatch in 4–7 days and larvae live in the moist litter and along the building walls [12]. The larvae progress through 6 to 11 stadia over a span of 30 to 100 days before reaching adulthood [9, 13, 14]. Late instars nearing pupation seek a drier environment by tunneling into compacted earthen floors beneath the litter or into the insulation and crevices of the building walls [15]. Adults emerge after 7 to 8 days. Under laboratory conditions, life span of an adult can exceed a year; however, in field setting, longevity of adults and larvae is influenced by predation from poultry and abiotic conditions.

In addition to causing structural damage to poultry houses, *A. diaperinus* has been associated with the transmission of pathogens (e.g., *Salmonella*) which can be potentially fatal to poultry and, more importantly, humans [16]. Consequently, studies are ongoing to further determine the reservoir capacity of lesser mealworm. Factors that may affect reservoir capacity and transmission potential are sex of beetles and larvae and size of the respective alimentary canals. Size of the *A. diaperinus* alimentary canal and its respective sections are presented in a companion paper in this issue [17]. Rapid determination of sex in the field settings of the poultry facilities would enable more timely and proactive approaches to beetle management. However, during the course of examining, the reservoir potential and alimentary canal capacity of *A. diaperinus,* it was determined that a comprehensive assessment of characters for determining sex of *A. diaperinus* was lacking. Schematics of genitalia for determining sex of pupae and beetles are available [18–20], but these schematics fail to clearly reflect the orientation and morphology of said features. Further, secondary sexual characteristics, such as orientation of tibial spurs within *A. diaperinus* adults differ and reports vary in the description and use of either mesothoracic or metathoracic tibial spurs to determine sex [21, 22]. The objective of this paper is to improve upon extant schematics and establish a definitive illustrated guide of morphological characters for determining sex of *A. diaperinus*.

2. Materials and Methods

2.1. Insect Rearing. The Southern Plains Agricultural Research Center (SPARC) starter colony of *A. diaperinus* was obtained from a colony originally isolated from a poultry farm in Wake County, NC, maintained by Dr. D. W. Watson (North Carolina State University, Raleigh, NC). The SPARC colony has remained in production since 2004. The colony was reared in plastic containers (15 × 15 × 30 cm) with screened tops; containers held 1000 mL wheat bran (Morrison Milling Co., Denton, TX) on the floor surface. A 6 cm^2 sponge moistened with deionized water and a slice (0.5 cm thick) of apple provided a water and food source, respectively. Both were replenished twice per week. The moistened sponge was placed on a piece of aluminum foil to prevent contact with the bran. Also, 30 mL of fishmeal (Omega Protein, Inc., Hammond, LA) were added to the wheat bran once per week; new wheat bran was added as it was depleted by dropping through the screened bottom of the cage. Layered black construction paper (6 × 6 cm) was provided as an oviposition substrate to allow collection of eggs to sustain the colony. Eggs were transferred to a separate container and resulting larvae, pupae, and adults were maintained as described above. The colony was maintained at 30°C in an 8 : 16 hr (light : dark) photoperiod.

2.2. Validity of Characters for Determining Sex. Late instars reportedly retain pygopods upon pupation and resulting pygopods on pupae reflect female pupae [18]. Three cohorts of 25, 85, and 96 larvae were sorted based on presence of pygopods and development was monitored from larvae to adulthood. Resulting pupae were sorted based on previously reported characters [18, 23] and eclosing adults were sexed to confirm relationship with pygopods on pupae. Paired pygopods in the larval stage, present ventrally on the 10th abdominal segment, aid in locomotion [24].

2.3. Preparation of Specimens. Late-instar larvae, pupae, and adults were collected from rearing containers and placed in respective 25 dram vials. Vials containing larvae and pupae were placed in a freezer to kill the insects; adults were killed by adding 80% ethyl alcohol (EtOH) to vials. Adults in EtOH-filled vials were briefly agitated by hand to remove adhering diet and frass. Larvae and pupae were cleaned by brushing with a fine-tipped paintbrush dipped in EtOH, since full immersion increased transparency of their more weakly sclerotized, lighter pigmented cuticle.

Specimens were allowed to air dry on a clean Kimwipe towel. Larvae and pupae were pinned through the thorax to prepare them for imaging. For adults, slight pressure was applied to the abdomens using fine-tip forceps to evert genitalia before pinning. Additional adults were processed to document differences and orientation of tibial spurs between sexes. Tibiae of the mesothoracic legs were excised and individually point mounted by their proximal ends for imaging apical spurs.

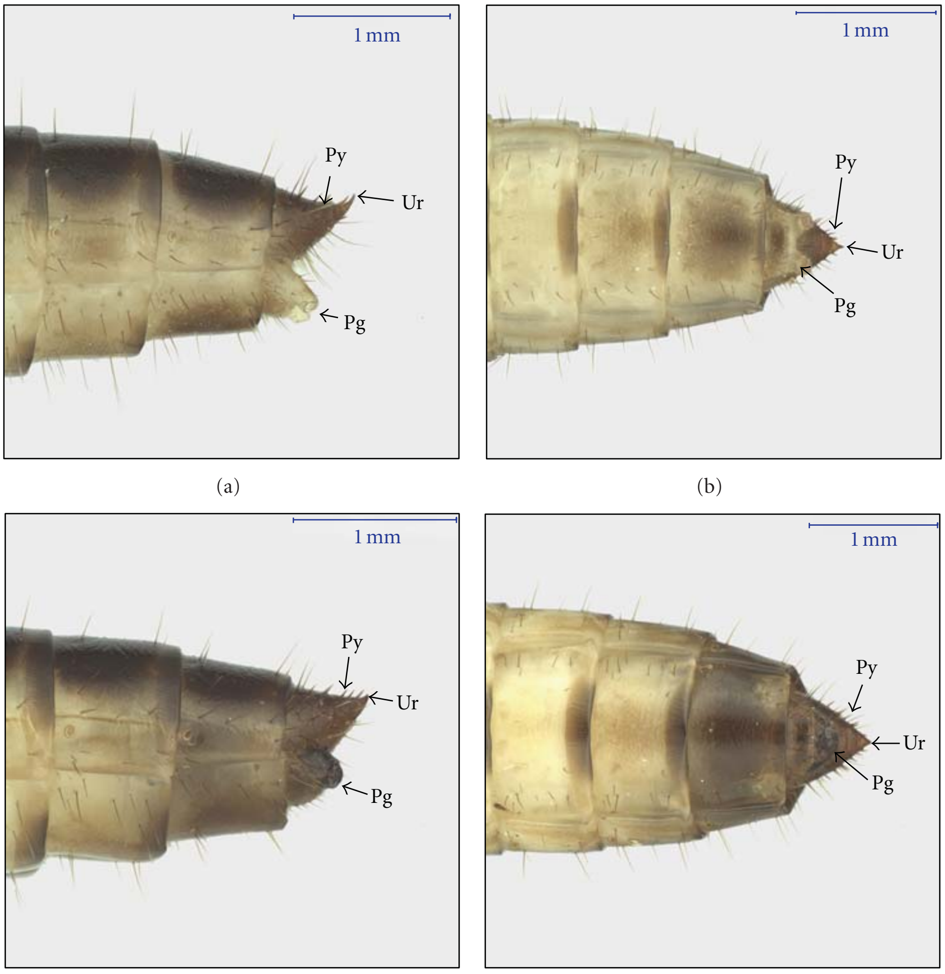
2.4. Imaging. A specially constructed viewing arena consisted of an 80 LED ring light (Model KD-200; Gain Express Holdings Ltd, Hong Kong) with a modified pinning stage located within the center of the ring. A small (7.62 × 2.54 × 1.27 cm) Styrofoam bar attached to the internal face of the ring light provided an additional, vertical pinning surface to facilitate orientation of specimens. A removable hemisphere-shaped dome with a 2.7 mm viewing aperture fit over the outer edge of the ring to uniformly distribute light within the arena.

Point mounted or direct-pinned specimens of all stadia and excised tibiae were placed in the viewing arena and manipulated for best orientation under a Leica MZ16 microscope equipped with APO lens (Leica Microsystems, Wetzlar, Germany). A ProgRes 3008 digital camera mounted on the microscope interfaced with a Windows-driven operating system. Sequentially focused images of each specimen were captured using PictureFrame 2.3 software (Optronics, Goleta, CA). Each image series was subsequently processed using Auto-Montage Pro software 5.01.0005 (Syncroscopy USA, Frederick, MD) to construct one composite image with enhanced depth of field. Adobe Photoshop CS5 (Adobe Systems, Inc., San Jose, CA) was used to improve clarity of composite images.

3. Results

3.1. Larvae. All late-instars in two cohorts ($n = 110$ total) possessed a pair of prominent fleshy pygopods ventral to the pygidium (Figures 1(a) and 1(b)). In the third cohort, a small percentage of larvae (12.5%; $n = 96$) did not exhibit prominent pygopods; instead the pygopods were unapparent, or much reduced (Figures 1(c) and 1(d)). A previous report suggested that the pygopods [24] were retained to the female pupal stage (discussed below). However, sex ratios of resulting pupae from sorted larval cohorts indicate that presence of pygopods does not exclusively reflect females. Of the larvae with prominent pygopods surviving to adulthood, 56.9% ($n = 86$) of adults were males. Similarly, adult males comprised 58.3% ($n = 12$) of the larvae with unapparent pygopods. Both sexes possess a urogomphus (Figure 1).

3.2. Pupae. The pupae of both sexes of *A. diaperinus* possess a pair of urogomphus dorsally (Figure 2). The larval urogomphus are retained to the pupal stage [18], and,

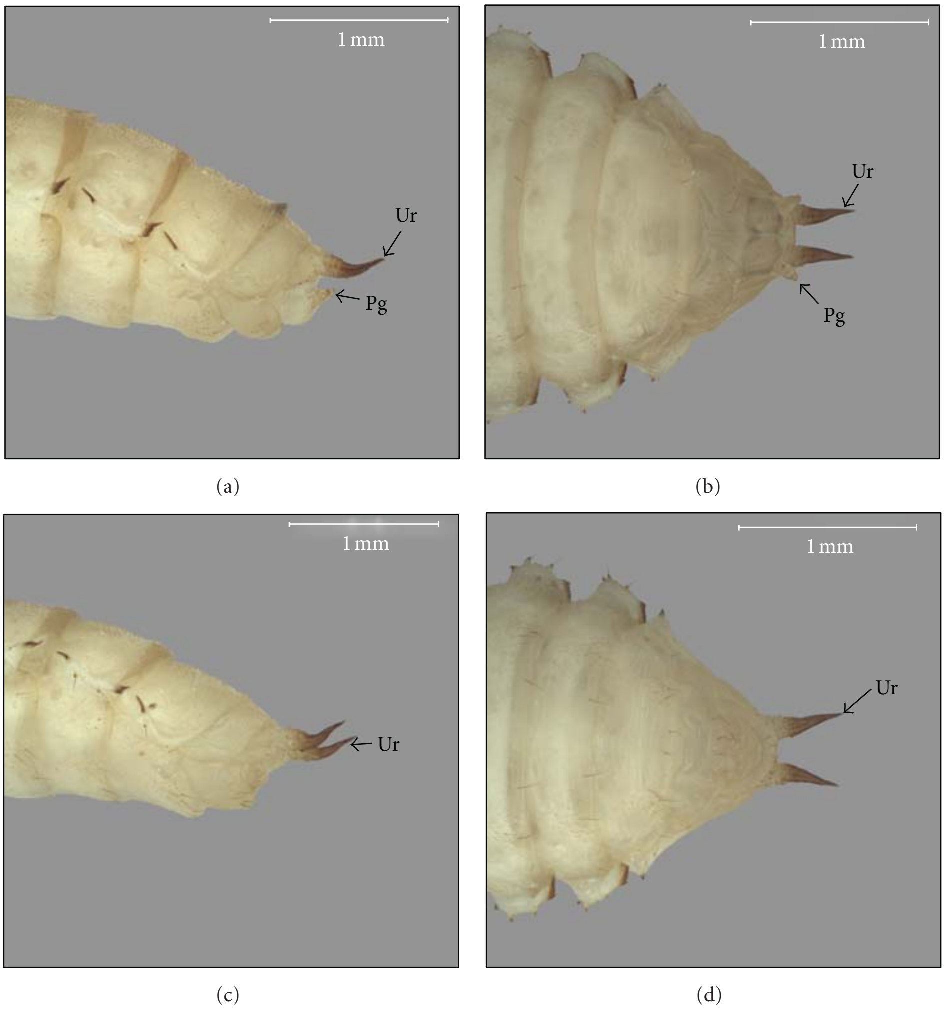


(a) (b) (c) (d)

Figure 1: External posterior characteristics of late-instar *A. diaperinus*: with prominent pygopods ((a) lateral view and (b) ventral view) and unapparent, or much reduced, pygopods ((c) lateral view and (d) ventral view). Pg, pygopod; Py, pygidium; Ur, urogomphus.

reportedly, the additional pair of prominent processes seen ventrally in female pupae (Figures 2(a) and 2(b)) are the paired pygopods retained from the larval stage (Figure 1(a)) [19]. However, as previously demonstrated, not all larvae with prominent pygopods are females. Conversely, all pupae with prominent pygopods ($n = 37$) yielded female beetles; males resulted from all pupae without pygopods ($n = 49$) (Figures 2(c) and 2(d)). Thus, in the pupal stage, pygopod presence is a reliable indicator of sex. Newly formed pupae are typically a pearly white color [18] but the urogomphus and the pygopods, if present, darken as the pupae age [25].

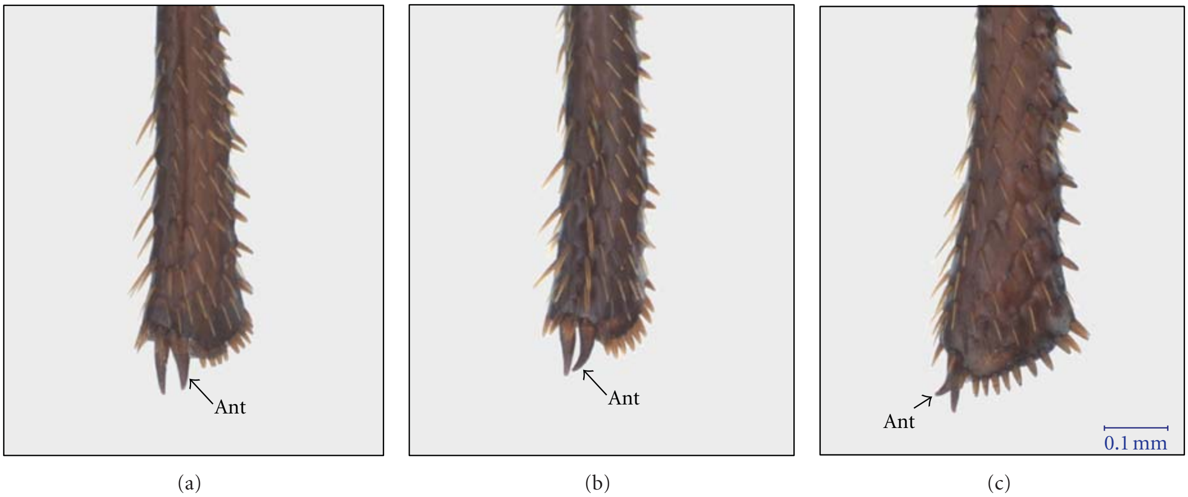
3.3. Tibial Spurs of Adults. Paired tibial spurs are present apically on the tibiae of all legs, but orientation of the spurs on the mesothoracic and metathoracic legs can aid in differentiation of sexes. The spurs arise from the anterior and posterior apical corners of the tibiae (Figure 3). In females, both spurs of the mesothoracic tibiae are parallel to each other and align along the longitudinal axis of the tibia (Figure 3(a)). Conversely, the mesothoracic tibial spurs on the male are not parallel to each other. In males, the anterior spur curves away from the longitudinal axis of the tibia without much deviation in the horizontal axis (i.e., not curved in the direction of either the anterior or posterior face of the tibia; Figures 3(b) and 3(c)). In fact, the small size and lack of color contrast often obscure the spur's curvature when the tibia is examined directly from a dorsal or ventral perspective. In these cases, the anterior spur may superficially appear shortened relative to the posterior spur until the segment is rotated (or higher magnification/better lighting/etc. is utilized). The posterior spur remains straight and generally in line with the longitudinal axis of the male tibiae. This same orientation of the tibial spurs is present on the metathoracic tibiae of males. However, the curvature of the anterior spur on the metathoracic tibiae of the males is less extreme than the curvature of the spur on the mesothoracic tibiae [21]. Only images of mesothoracic spurs on males are provided here because their sharper curvature relative to the metathoracic spurs enables more rapid determination of sex.



(a) (b)

(c) (d)

Figure 2: External posterior characteristics for determining sex of *A. diaperinus* pupae: female with urogomphus and prominent pygopods ((a) lateral view and (b) ventral view) and male with urogomphus, lacking prominent pygopods ((c) lateral view and (d) ventral view). Pg, pygopods; Ur, urogomphus.



(a) (b) (c)

Figure 3: External characteristics of mesothoracic tibiae for determining sex of *A. diaperinus* adults: (a) anteroventral view of parallel apical spurs on females; (b) and (c) anteroventral and anterior views, respectively, of apical spurs on males, note curvature of anterior spur. Ant, anterior spur; scale bar applicable to all frames; tibiae from left legs shown.

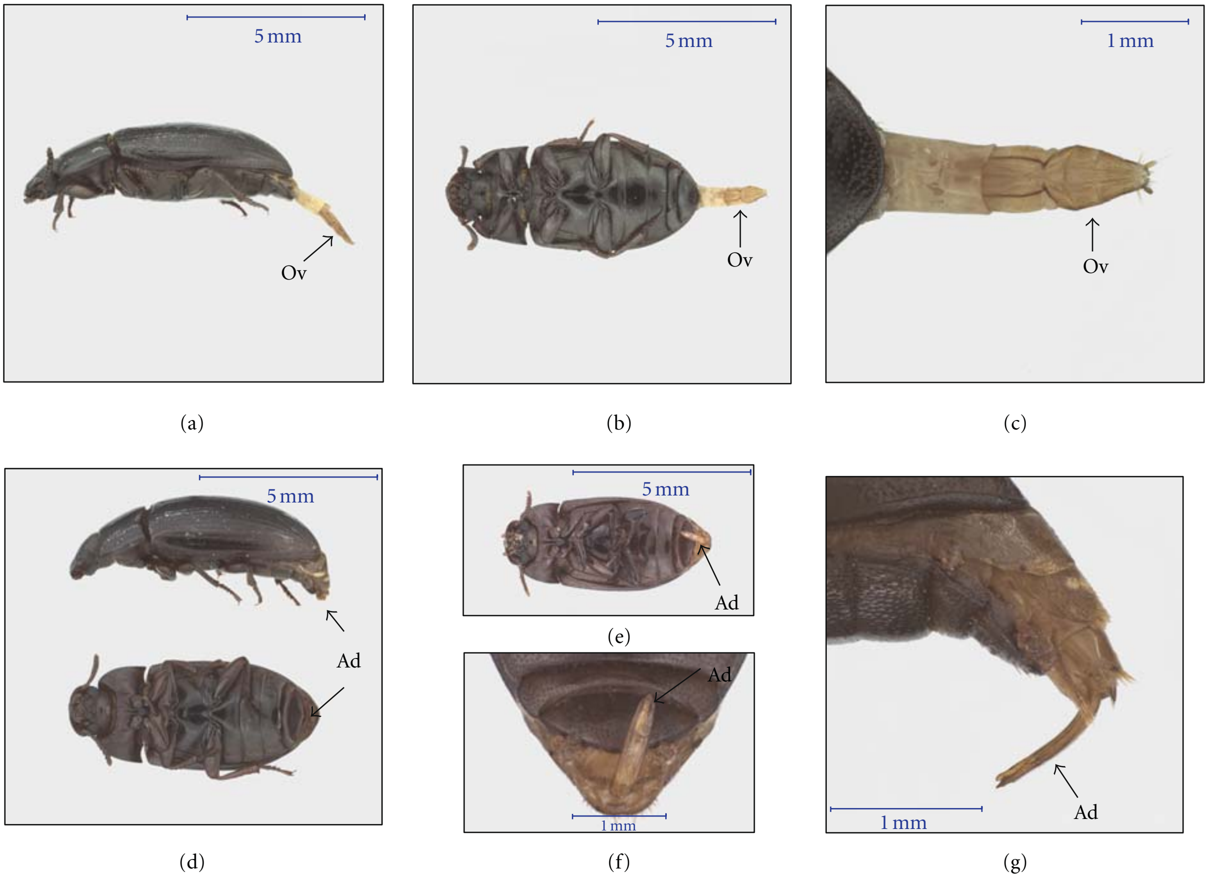


Figure 4: Genitalia of *A. diaperinus* adults: female ovipositor:protracted, lateral (a), and ventral views (b, c); male aedeagus:retracted, lateral, and ventral views (d); protracted, ventral (e, f), and lateral views (g). Ad, aedeagus; Ov, ovipositor.

3.4. Adult Genitalia. Images of *A. diaperinus* genitalia (Figure 4) improve upon previous drawings [19, 20]. Coloration of the distal end and along the length of the ovipositor indicates sclerotization within the organ membrane (Figures 4(a)–4(c)). Two dark longitudinal lines dorsally and ventrally within the ovipositor suggest sclerotization along the length which may aid in movement of the ovipositor (i.e., protraction and retraction, directionality). In fact, the dorsal lines were observed to aid in the opening and closing of the anus. The paired cerci protruding from the end of the ovipositor each possesses a solitary seta, likely to aid in site selection for oviposition. The male aedeagus is a sclerotized organ and, if not readily visible (Figure 4(d)), can be protracted by squeezing of the abdomen (Figures 4(e)–4(g)), although more pressure is required than that used on females. When protracted, the aedeagus may have a curvature to either side of the longitudinal midline of insect body (Figures 4(e) and 4(f)) and is projected away from the body (Figure 4(g)).

4. Discussion

This paper is unique in providing a comprehensive photographic guide for differentiation of sexes in *A. diaperinus.* Further, this paper clarifies that the prominent fleshy processes observed ventrally on female larvae are pygopods. Although general dorsal, lateral, and ventral views of *A. diaperinus* larvae have been provided [9, 22], sex determination of late-instars has been previously alluded to because the pygopods were reportedly retained from late instars to the female pupal stages [18]. Visual aides were not provided to unequivocally establish the external morphology of late instars; however, our observations indicate determination of sex in the larval stages based on the presence of pygopods is unreliable. The urogomphus has been used to differentiate between species of *Alphitobius* [26], but this is the first record to visually demonstrate the variation of pygopods in *A. diaperinus* larvae.

This paper clarifies the definition of the ventral paired processes in female pupae as remnants of the paired pygopods observed in larvae. The pygopods were previously noted but incorrectly identified as “genital appendages” and “second valvifers” [18, 23]. Line drawings of external characters on pupae for differentiating between sexes [18] fail to show potential coloration of urogomphus and pygopods. Figure 2 allows more clear identification of these external characters, including representation of coloration for these characters. Newly formed pupae are completely white [18]. Coloration of external characters suggests these pupae

(Figure 2) are not newly formed; thus, coloration could potentially be used as an indicator of pupal age. However, the latter was outside the scope of this paper.

Differences in the orientation of tibial spurs of *Alphitobius laevigatus* (F.) have been previously shown [21, 22]. The curvatures of tibial spurs on *A. laevigatus* reportedly [21] resemble those of *A. diaperinus* but comparative illustrations of *A. diaperinus* tibial spurs were not provided. Viewing angle of the specimen is critical when assessing spur orientation to determine sex; more so, if using the metathoracic tibial spurs [22] because the curvature of male metathoracic spurs is "so slight as to be barely noticeable" [21]. Thus, it is recommended that the mesothoracic spurs be used for determination of sexes for adults, in addition to examination of genitalia. Descriptions presented here regarding location and orientation of the spurs are more thorough and images more clearly delineate differences between mesothoracic spurs of respective sexes.

Differing intensity of coloration was observed between ovipositors of different females. Whether coloration intensity of ovipositors reflects age remains to be determined. In sexing dead adults, either the cerci were protruding slightly from the last abdominal segment or the ovipositor was protruding altogether. If neither were evident, application of slight pressure to the abdomen caused the cerci to protrude. Generally, if the ovipositor and cerci are not immediately visible after applying pressure to the abdomen, in all likelihood the insect is a male. However, to confirm male gender, applying more pressure to the abdomen caused protrusion of the aedeagus for confirmation (Figures 4(e)–4(g)). Detailed descriptions and morphometrics of the ovipositor and the aedeagus were previously reported [19, 20].

This comprehensive guide is user-friendly towards novice entomologists and nonentomologists (e.g., microbiologists, pathologists) and will be an invaluable tool for those entering the study area of *A. diaperinus* and pathogen interactions affecting poultry and humans.

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Research Article

Insects of the Subfamily Scolytinae (Insecta: Coleoptera, Curculionidae) Collected with Pitfall and Ethanol Traps in Primary Forests of Central Amazonia

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An experiment was conducted in a primary forest area of the Tropical Forest Experimental Station, 45 km from Manaus-Boa Vista Highway, in order to compare the insect fauna of the subfamily Scolytinae, in flight activity and on the ground. Five impact traps of the type Escolitideo/Curitiba, with ethanol baits, were installed at the height of 3 m above the ground, and five pitfall traps were buried in the same area of the above ground traps. The data collections were evaluated through abundance, richness, and Simpson diversity index, and, to compare these data with the pitfalls and the months collection, the ANOVA was used. The Pearson correlation test was also carried out to evaluate the meteorological factors (temperature and rainfall). From the total of 2,910 Scolytinae, 2,341 were captured in pitfall traps representing 80.45% and 569 with Escolitideo/Curitiba traps representing 19.55%. The most abundant species in the collections were *Xyleborus volvulus* Fabricius and *Xyleborus affinis* Eichhoff, and this was classified as constant in both habitats. The result of the analysis indicates that the Simpson's index was high and that the abundance of insects was affected by the types of trap and by the month of collection. The analysis of correlation with meteorological factors showed that only *Xyleborus spinulosus* species presented significant correlation with temperature.

1. Introduction

The Scolytinae are mostly secondary predators by developing under natural conditions in trees injured, hit by lightning, fire, plants nutritionally deficient, drooping, and so forth, but can attack healthy plants also [1]. This subfamily presents species phyllophagous (bark beetles), which feed on the phloem tissues, that is, the inner part of the bark of the tree, and xylomycetophagous (beetles of ambrosia) which have as their main food symbiotic fungi, which introduce and cultivate in the host plant [2–4]. The beetles xylomycetophagous attack preferably sapwood which is richer in nutrients, but in some species of wood, the attack also happens in the heartwood [5].

The Scolytinae attack usually begins within twenty-four hours after the tree is cut and each forest species has greater or lesser resistance, but none is totally free of infestation by these insects. The resistance presented by each species is probably related to the attractive substances and the wood hardness which undoubtedly influences the speed of penetration and the general importance of the attack.

These insects have been captured in various regions of the world. In the Amazon region they were also found attacking several hosts, such as forest and fruit trees, as shown in some studies carried out by Mendes [6], Abreu and Dietrich [7], Abreu [8], Abreu and Bandeira [9], Barbosa [10], Dall'Oglio and Peres-Filho [11], and Matias and Abreu [12].

Studies involving biology and ecology of this subfamily have also been conducted in the Amazon, using mainly flight intercept traps, in which an attractive substance is used [13–15]. These insects have also been found in other substrates, as shown by surveys conducted by Schubart and Beck [16], Penny and Arias [13], Morais [17, 18], and Rodrigues [19], in samples collected from trees, leaf litter and on the ground, where various types of traps were used, including the pitfall trap. Therefore, the capture of these insects on the ground and in flight activity is important for the complete knowledge of their life cycle, and at the same time, to know if the species caught in flight are the same found on the ground.

2. Material and Methods

This work was carried out with beetles of the subfamily Scolytinae collected in the Tropical Forestry Station, 45 km from Manaus, on the BR-174 highway, with an area of 21,000 ha and geographical coordinates 02° 35′ 55.5′′ South and 60° 02′ 14.8′′ West of Greenwich. In this area the average minimum annual temperature is 20°C and the maximum 26°C, with an average relative air humidity of 77% [20]. The soil of the region is clayey, and it can be classified as Oxisol and Ultisol [21].

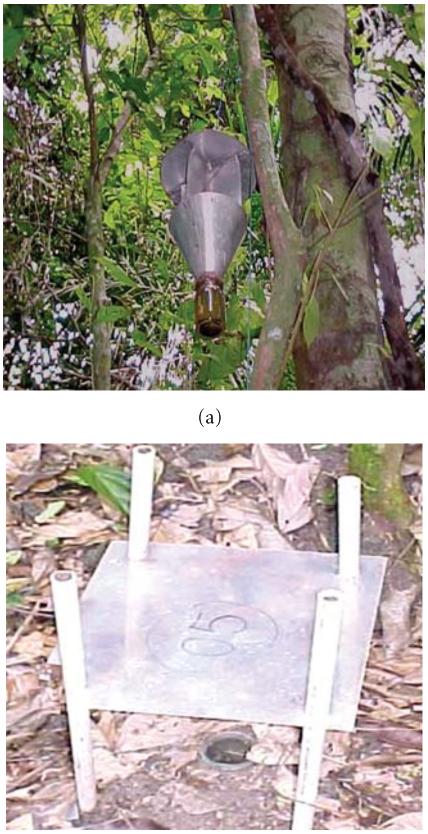
Five modified impact traps of the type Escolitídeo/Curitiba (EC) were used for this survey, using 100% commercial alcohol as attraction [22], for the capture of the insects in flight, and five pitfall traps for capture of those with activity on the ground. The collection period happened from July 2005 to July 2006.

The impact trap consists of a conical cover, a panel of impact, a funnel, and a bottle for collection, containing alcohol 30% with detergent. The bait is placed inside a glass bottle attached to the panel with a pierced cap to allow volatilization. The traps were installed at a height of 3 m above the ground, and with, the aid of a nylon rope, they were tied to two trees at a distance of 30 m from each other (Figure 1(a)).

The pitfall trap consists of a 500 mL glass bottle, an acrylic sheet, 25 cm × 25 cm, and four PVC tubes with 40 cm in length. During the assembly, the bottles were buried, leaving the openings at the ground level to allow the insects to fall inside them. Picric acid at 0.003% concentration, which is considered neutral, that is, does not attract or repel the insects, was used to preserve the insects. The acrylic sheets were used as coverage, which were supported by four PVC tubes, buried to half of their lengths (Figure 1(b)).

The samples were collected weekly, when the renewal of the bait and liquid preservatives were made. The collected beetles were identified by means of direct comparison with specimens of the Invertebrate Collection of the National Institute for Amazon Research. Taxonomic identification keys were also used [1, 23–28].

The faunistic analysis was obtained through absolute and relative abundance, constancy, species richness, and the calculation of the Simpson diversity index referring to each month of collection. Absolute abundance was done by direct count of the individuals and the relative abundance, by the calculations of the percentages of individuals of each species in relation to the total number of captured individuals [29]. The constancy was determined by the percentage of occurrence of the species in the collections. According to the obtained percentages, the species were separated into the following categories: (a) constant species (W) present in more than 50% of the collections, (b) accessory species (Y) present in 25 to 50% of the collections; (c) accidental species (Z) in less than 25% of the collections [30]. The Simpson diversity index was calculated according to the formula: $D = 1 - \sum(n_i(n_i - 1)/N(N - 1))$, where n is the number of sampled individuals for each i species and N is the total number of sampled individuals.


(a)

(b)

Figure 1: Traps used to capture insects. (a) Escolitídeo/Curitiba trap-EC and (b) Pitfall trap.

The analysis of abundance, the Simpson's index, and the richness of the species as a function of the type of trap and the months of collection was done by ANOVA. For these analyses data were transformed into log $(x + 1)$, and the significance level was $P < 0.01$. The monthly fluctuation analysis of the four most abundant species was also carried out, relating the number of collected insects in each type of trap with the data of temperature and rainfall by Pearson Correlation [31].

3. Results and Discussion

According to the data shown in Tables 1 and 2, 2,910 Scolytinae specimens were captured, from which 2,341 with the pitfall trap, representing 80.45% and 569 with the EC trap, representing, 19.55%. The data show the existence of nine genera and 26 species. The *Xyleborus* genus stood out from the others because it represented 97% of the collection in the pitfall trap and 57.14% in the EC trap. The predominance of this genus has already been observed in the work carried out also in primary forest by Abreu [14].

From all the species captured with the pitfall trap, the ones that stood out are the species *Xyleborus volvulus*

Fabricius, representing 50.11% (1,173 individuals.); *Xyleborus affinis* Eichhoff, with 38.02% (890 individuals); *Xyleborus ferrugineus* Fabricius with 4.36% (102 individuals); *Xyleborus spinulosus* Blandford, with 4.06% (95 individuals). The others represented 3.46% (81 individuals). In the EC trap, *X. affinis* represented 34.62% (197 individuals); *X. volvulus,* with 18.28% (104 individuals); *Premnobius cavipennis* Eichhoff, with 11.6% (66 individuals); *Hypothenemus eruditus* Westwood, with 9.67% (55 individuals); *H. obscurus* (Fabricius) with 5.98% (34 individuals). The remaining accounted for 19.85% (113 individuals) (Tables 1 and 2).

Quantitatively there was some numerical superiority for the pitfall trap because it was responsible for almost 90% of the collected specimens, but, in richness, there was some advantage for the EC trap. While this trap was responsible for the capture of 25 species, the pitfall trap captured only 13. From the studied species, *X. affinis*, *X. volvulus*, *X. ferrugineus, X. spinulosus*, *Theoborus solitariceps*, *X. flavus*, *P. cavipennis, H. obscurus*, *H. eruditus*, *Monarthrum* sp. 2, *Amphicranus* sp. 1 and *Coccotrypes palmarum* were captured with the two traps (Tables 1 and 2).

In relation to the analysis of richness, abundance, and the Simpson's index, the results indicate that the of Simpson's index ($R^2 = 0.098$; $F_{11,96} = 0.953$; $P = 0.4939$) was not affected neither by the type of trap, nor by the month. On the other hand, the abundance ($R^2 = 0.4361$; $F_{11,96} = 6.75$; $P < 0.001$) is related to the month of collection, and the type of trap and richness ($R^2 = 0.337$; $F_{11,96} = 4,436$; $P < 0.001$) is only affected by the month of collection. The result of these indexes for each sampled point and month of collection is represented in Tables 3 and 4.

The greatest abundance of species in pitfall traps was recorded in the months of August and November 2005 and May 2006, while, in the EC trap, it was in the months of January and July 2006. In the analysis of richness with the EC trap, the largest and smallest numbers of species were registered in the months of October and December 2005, respectively. For the pitfall trap, that happened in the months of August and July 2005.

In general, the Simpson diversity index was high, reflecting less diversity and the dominance of two species, *X. volvulus* and *X. affinis*. For the EC trap, this index varied from 0.12 (trap 4, January 2006) to 1 (traps 2, 3, and 5, December 2005). For the pitfall trap, it varied from 0.170 (trap 3, March 2006) to 1 (trap 1, July 2005; trap 5, March 2006; trap 5, July 2006; trap 1, 3, 5, July 2006).

Regarding the constancy, in the pitfall trap, three species have been considered constant, two accessory and 6 accidental. In the EC trap, three species have been considered constant, five accessory and 16 accidental. It was also observed that the *X. affinis* species was constant for the two traps, becoming evident the importance of this species in the studied environment.

The analysis of correlation of the number of insects with rainfall and temperature indicates that only the *X. spinulosus* species was affected by the temperature ($r = 0.443$; $P = 0.039$), since the number of individuals increased as a function of the temperature. Although the temperature is considered one of the most important climatic factors in the development and survival of the beetles of the Scolytinae subfamily, it tends to be higher in the microclimate of the host, but less prone to sudden fluctuations in the external environment [1, 32]. This was observed in the course of this work and probably that is why this factor did not influence the activity of these beetles on external environment, that is, in flight activity and on the ground.

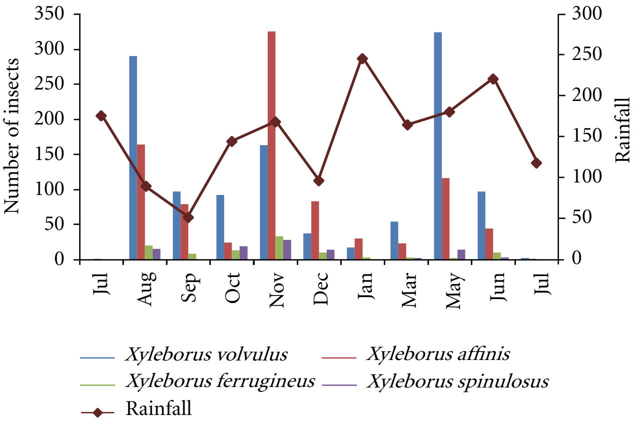


Figure 2: Monthly rainfall and total number of four of the most abundant species of Scolytinae subfamily collected with pitfall traps from July 2005 to July 2006, in primary forest of Central Amazon.

Data from insects collected with pitfall traps and Escolitideo/Curitiba traps relating rainfall and temperature with the number of insects of the four most abundant species are represented in Figures 2, 3, 4, and 5. In these figures it can be observed that for the pitfall trap, the species *X. affinis,* and *X. volvulus* presented a population peak in August and November 2005 and May 2006. It can also be noticed that in the EC trap, *X. affinis* presented peaks in January, May and June 2006, while *X. volvulus* presented a peak only in July 2006. The months of August 2005 and July 2006 showed low values of rainfall, while, in November 2005 and May 2006 these values were high.

When analyzing the rainfall data some studies suggest that this factor influences on the population and the behavior of the Scolytinae, observing that high values of precipitation affect the abundance of these insects [32, 33]. Other authors presented the opposite, as shown in a study carried out by Flechtmann et al. [34], where the capture peak of Scolytinae coincides with high intensity of rainfall.

In this context and based on the outcome of this work, it can be affirmed that temperature and precipitation did not affect the activity of most of these insects, both in flight and on the ground. These results agree with Hulcr et al. [35] claiming that circumtropical species, like *X. affinis* and *X. volvulus*, are usually not affected by climatic factors, since they are found in rainy and dry weather.

In this work it has been observed that, despite the 25 species captured with the EC trap using alcohol as attraction, the number of specimens is considered small if compared to the pitfall trap. In forest plantations in Brazil with a single forest species, the capture of these insects using this kind of attraction has been more efficient [22, 36–38]. In a primary or secondary forest, the substrate is very rich, due to

Table 1: Absolute and relative abundance of insects of the subfamily Scolytinae collected with pitfall traps during the period of July 2005 to July 2006, in primary forest of Central Amazon.

Species	Months														
	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Total	%
Xyleborus volvulus	0	290	97	92	163	37	17	0	54	0	324	97	2	1173	50.11
Xyleborus affinis	1	164	79	24	325	83	30	0	23	0	116	44	1	890	38.02
Xyleborus ferrugineus	0	20	8	13	33	10	3	0	3	0	2	10	0	102	4.36
Xyleborus spinulosus	0	15	0	19	28	14	0	0	2	0	14	3	0	95	4.06
Theoborus solitariceps	0	4	0	0	18	0	0	0	0	0	0	0	0	22	0.94
Xylosandrus compactus	0	2	0	3	4	3	4	0	2	0	0	0	1	19	0.81
Xyleborus flavus	0	0	0	0	0	12	0	0	0	0	0	0	1	13	0.56
Coccotrypes palmarum	2	0	1	0	0	0	4	0	0	0	3	0	0	10	0.43
Amphicranus sp. 1	2	1	4	2	0	0	0	0	0	0	0	0	0	9	0.38
Premnobius cavipennis	0	2	0	0	0	0	0	0	0	0	0	1	1	4	0.17
Hypothenemus obscurus	0	1	1	0	0	0	0	0	0	0	0	0	0	2	0.09
Hypothenemus eruditus	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0.04
Monarthrum sp. 2	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0.04
TOTAL	6	499	190	154	571	159	58	0	84	0	459	155	6	2341	100

Table 2: Absolute and relative abundance of insects of the subfamily Scolytinae collected with Escolitídeo/Curitiba traps during the period of July 2005 to July 2006, in primary forest of Central Amazon.

Species	Months														
	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Total	%
Xyleborus affinis	10	17	7	8	9	1	55	0	15	0	35	28	12	197	34.62
Xyleborus volvulus	0	0	1	2	0	0	3	0	0	0	0	5	93	104	18.28
Premnobius cavipennis	2	5	13	7	4	1	4	0	6	0	16	6	2	66	11.60
Hypothenemus eruditus	1	1	3	2	3	0	1	0	2	0	7	9	26	55	9.67
Hypothenemus obscurus	1	3	8	4	3	0	0	0	0	0	10	2	3	34	5.98
Monarthrum durum	0	0	1	1	4	2	6	0	1	0	6	8	0	29	5.10
Sampsonius dampfi	0	0	5	3	2	0	0	0	0	0	6	0	0	16	2.81
Sampsonius prolongatus	0	0	0	2	1	0	6	0	2	0	2	0	0	13	2.28
Xyleborus spinulosus	0	1	0	1	3	0	3	0	0	0	0	0	3	11	1.93
Xyleborus ferrugineus	0	0	0	0	6	0	0	0	1	0	1	1	1	10	1.76
Sampsonius detractus	0	1	1	0	2	0	2	0	0	0	2	0	0	8	1.41
Coccotrypes palmarum	0	0	0	0	0	1	0	0	0	0	0	4	0	5	0.88
Amphicranus sp. 1	0	0	0	1	0	1	0	0	1	0	1	0	0	4	0.70
Theoborus solitariceps	0	2	0	1	1	0	0	0	0	0	0	0	0	4	0.70
Monarthrum semipaleans	0	2	0	0	0	0	0	0	0	0	0	0	0	2	0.35
Sampsonius pedrosai	0	0	1	0	0	0	1	0	0	0	0	0	0	2	0.35
Xyleborus flavus	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0.18
Xyleborus solitarinus	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0.18
Hypothenemus sp. 1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0.18
Sampsonuis giganteus	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0.18
Cryptocarenus diadematus	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0.18
Cryptocarenus seriatus	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0.18
Cryptocarenus heveae	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0.18
Monarthrum sp. 1	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0.18
Monarthrum sp. 2	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0.18
TOTAL	15	36	42	34	38	6	81	0	28	0	86	63	140	569	100

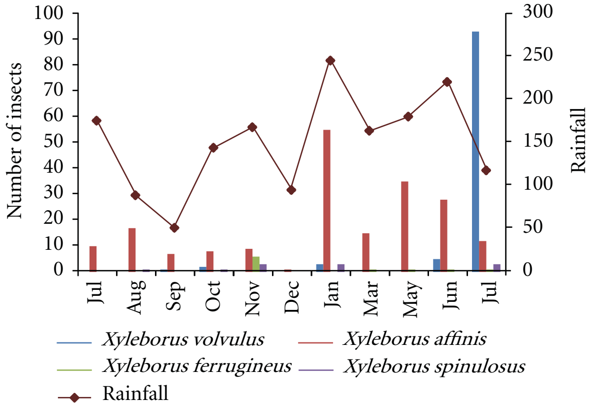


Figure 3: Monthly rainfall and total number of four of the most abundant species of Scolytinae subfamily collected with Escolitídeo/Curitiba traps from July 2005 to July 2006, in primary forest of Central Amazon.

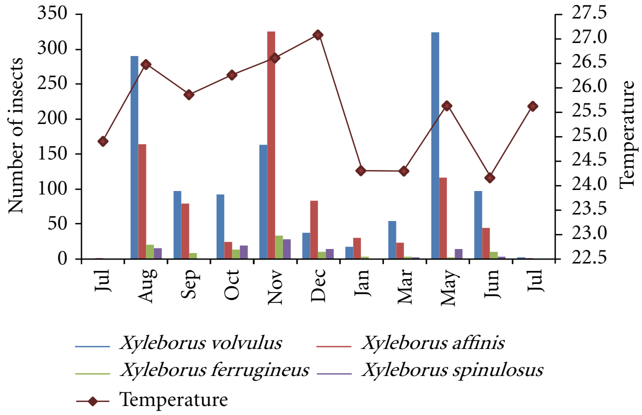


Figure 4: Monthly temperature and total number of four of the most abundant species of Scolytinae subfamily collected with pitfall traps from July 2005 to July 2006, in primary forest of Central Amazon.

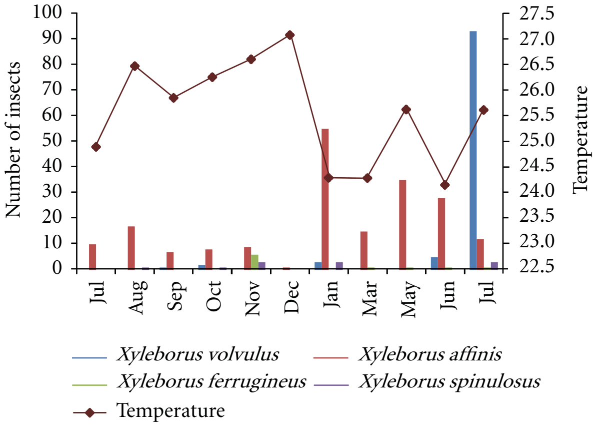


Figure 5: Monthly temperature and total number of four of the most abundant species of Scolytinae subfamily collected with Escolitídeo/Curitiba traps from July 2005 to July 2006, in primary forest of Central Amazon.

Table 3: Abundance, richness, and Simpson index of the species collected with five pitfall traps from July 2005 to July 2006.

(a)

	2005		
Month	Abundance	Richness	Simpson
Jul	0	0	1
	2	1	0
	10	3	0,46
	0	0	1
	0	0	1
Aug	118	6	0,589
	102	4	0,470
	84	4	0,596
	95	6	0,618
	104	6	0,433
Sep	3	3	0,667
	8	3	0,594
	45	4	0,593
	59	3	0,561
	75	2	0,461
Oct	36	5	0,690
	32	5	0,375
	18	3	0,494
	36	4	0,437
	32	4	0,689
Nov	218	6	0,526
	102	4	0,552
	72	5	0,588
	108	4	0,573
	71	4	0,492
Dec	65	5	0,455
	33	5	0,571
	22	4	0,479
	27	5	0,694
	12	4	0,681

(b)

	2006		
Month	Abundance	Richness	Simpson
Jan	7	1	0
	15	4	0,516
	11	3	0,562
	2	2	0,5
	23	4	0,567
Mar	14	3	0,541
	5	3	0,64
	32	2	0,170
	33	5	0,630
	0	0	1

(b) Continued.

	2006		
Month	Abundance	Richness	Simpson
May	44	4	0,539
	188	4	0,201
	118	4	0,373
	41	3	0,604
	75	4	0,543
Jun	21	5	0,522
	82	3	0,361
	15	1	0
	20	4	0,415
	0	0	1
Jul	0	0	1
	4	3	0,625
	0	0	1
	2	2	0,5
	0	0	1

many different species of trees, producing different attractive substances when they fall, die, or decay. This may have contributed to the reduced number of specimens collected with the EC trap although the number of species was greater.

The majority of the captured species belong to the Xyleborini tribes, with 13 species, distributed in the genera *Xyleborus, Sampsonius*, *Premnobius,* and *Theoborus*, followed by Corthylini, with the genera *Monarthrum* and *Amphicranus*. These species are prevalent in tropical regions, with xylomycetophagous habits, that is, feed on fungi that grow inside the plant. Another tribe found was Cryphalini, with the genera *Cryptocarenus* and *Hypothenemus,* also common in tropical regions, with varied eating habits, being considered myelophagous because they feed on pith and buds, phloephagous, feed on the tissues of the phloem, and xylophagous, feed on the xylem [1, 39]. This was also observed in the work of Abreu [14].

The *Xyleborus* genus was represented by six species, but *Xyleborus volvulus* and *X. affinis* were dominant and found both in flight and on ground. These species are quite common and abundant in primary forest of Amazonas State, according to the work of Abreu [14] and Matias and Abreu [12], and, from what can be seen, they also have outstanding preference for the region of the collections, since there is a large difference between them and the other species, including when compared with those that have the same eating habits.

The species *X. ferrugineus,* despite being regarded as one of the most important and abundant in tropical regions, including being the vector of *Ceratocystis fimbriata* (Ellis & Halsted) fungus that causes the death of several plants [1, 40, 41] presented low abundance, confirming the work carried out by Abreu [8], Abreu and Bandeira [9], Matias and Abreu [12], and Abreu [14]. In contrast, many works carried out in the South, Southeast and Midwest of Brazil show that this species is abundant in those regions [22, 33, 34, 36, 37], being considered fairly common in Brazil.

Table 4: Abundance, richness, and Simpson index of the species collected with five Escolitideo/Curitiba traps from July 2005 to July 2006.

(a)

	2005		
Month	Abundance	Richness	Simpson
Jul	6	4	0,722
	2	1	0
	3	2	0,444
	2	1	0
	2	1	0
Aug	11	5	0,744
	6	4	0,667
	12	7	0,708
	4	2	0,375
	3	3	0,667
Sep	6	3	0,667
	10	5	0,76
	10	7	0,78
	7	3	0,571
	9	4	0,617
Oct	5	5	0,8
	10	8	0,86
	9	6	0,741
	9	6	0,790
	1	1	0
Nov	14	8	0,837
	11	5	0,711
	4	3	0,625
	4	3	0,625
	5	5	0,8
Dec	5	4	0,72
	0	0	1
	0	0	1
	1	1	0
	0	0	1

(b)

	2006		
Month	Abundance	Richness	Simpson
Jan	20	6	0,64
	18	4	0,377
	10	5	0,68
	13	2	0,142
	20	5	0,545
Mar	4	2	0,375
	2	1	0
	5	4	0,72
	9	4	0,667
	8	4	0,656

(b) Continued.

	2006		
Month	Abundance	Richness	Simpson
May	20	4	0,575
	19	8	0,731
	13	5	0,734
	19	7	0,765
	15	6	0,658
Jun	12	5	0,764
	12	5	0,764
	12	3	0,486
	13	4	0,485
	13	4	0,675
Jul	27	3	0,565
	46	3	0,163
	11	4	0,645
	38	7	0,636
	19	5	0,504

Another very representative genus was the *Sampsonius*, with five species, including *S. dampfi*. The species in this genus also feed on fungi that grow in the host. As females are unable to construct an entrance tunnel in the plant, they look for recently constructed galleries of *Xyleborus*, appropriate for their bodies. After entering, they wait until the tunnels are extended, cleaned, and after that they expel the lodgers [1].

Many Scolytinae are attracted by resin-oils, terpene hydrocarbons or alcohols and other substances emanating from the vascular tissues of newly felled trees, decayed and still with high levels of humidity [3, 4]. In accordance with Hulcr et al. [35], ambrosia beetles are strongly attracted by hosts that liberate high levels of alcohol.

This work reinforces the theory that ambrosia beetles are common in tropical forests, because these environments present favorable climatic conditions for the development of these insects, as well as their fungal symbionts. Studies carried out by Hulcr et al. [35, 42] in forests of Thailand and Papua New Guinea confirmed this theory.

4. Conclusions

The capture of Scolytinae in primary forest of Central Amazon shows a low diversity of these insects and the existence of two predominant species in the region. It also shows that many species, in addition to flying, also have activities on forest ground. In this area there are many lignocellulosic materials as trunks and branches of trees, where they can cultivate the fungi they feed on.

The majority of the insects collected in the studied area have no correlation with the temperature and rainfall.

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Research Article

Effect of Plant Characteristics and Within-Plant Distribution of Prey on Colonization Efficiency of *Cryptolaemus montrouzieri* (Coleoptera: Coccinellidae) Adults

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Cryptolaemus montrouzieri (Coleoptera: Coccinellidae) has been widely used in classical and inundative biological control of mealybugs, including the long-tailed mealybug, *Pseudococcus longispinus* (Hemiptera: Pseudococcidae). This study was conducted to investigate colonization and establishment efficiency of *C. montrouzieri* to manage *P. longispinus* on three different ornamental plant species (*Ficus elastica, Lilium longiflorum*, and *Dieffenbachia seguine*). Within-plant distribution pattern of *P. longispinus* and the colonization ecology of adult *C. montrouzieri* were investigated. Significantly more *P. longispinus* were found on the upper parts of the plants regardless of plant species, and *C. montrouzieri* adults discovered *P. longispinus* significantly faster when they were released on the top of the plants than on the bottom. Choice tests revealed that *C. montrouzieri* adults preferred smaller *P. longispinus* nymphs. The implications for utilization of *C. montrouzieri* for biological control of mealybugs on various ornamental plants are discussed.

1. Introduction

The long-tailed mealybug, *Pseudococcus longispinus* (Targioni-Tozzetti) (Hemiptera: Pseudococcidae), is a key pest of fruit trees and ornamental plants. *P. longispinus* feeds on various plant parts including roots, trunks, cordons, canes, leaves, and fruits, causing aesthetic damage on ornamental plants or yield loss of crops [1]. Fungal pathogens that grow on the honeydew excreted by *P. longispinus* can cause further damage. For example, high *P. longispinus* densities often cause leaf drop and reductions of crop quality and yield; Uygun [2] reported that yield loss of citrus due to *P. longispinus* could be up to 80–90%. Also, *P. longispinus* can transmit viral diseases in grapevines [3]. Chemical management of *P. longispinus* is difficult because it produces thick layers of protective wax and can hide in bark crevices, spurs, or canes. In general, chemical control is only effective when *P. longispinosus* is in the crawler stage and when host plants do not afford physical refuges from chemical sprays [4]. Therefore, biological control using natural enemies has been a major alternate method to manage *P. longispinus* [5].

Natural enemies utilized to manage *P. longispinus* include lady beetles, parasitic wasps, and lacewings [6]. Among the natural enemies, the mealybug destroyer, *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae), is one of the key natural enemies of *P. longispinus*. *C. montrouzieri* is native to Australia and has been introduced to manage many mealybug species throughout the world [7, 8]. In the United States, *C. montrouzieri* was first imported in the late 1800s to manage mealybugs in California [9]. Since then, well-defined and efficient rearing techniques were developed [10], and thus *C. montrouzieri* has been commercially available to growers throughout the United States.

Cryptolaemus montrouzieri has been used for different biological applications: classical biological control [11] and augmentative biological control [12, 13]. In an established population, immature stages of *C. montrouzieri* dominate the stable age distribution, and most prey is consumed by the larvae [14]. However, adult stage of *C. montrouzieri* is released when biological control of *P. longispinus* is initiated because of their ability to disperse and colonize. Also, it is assumed that adults will lay eggs in suitable locations and give rise to another generation that provides the majority of pest suppression when they are in the larval stage.

Effectiveness of natural enemies is dependent upon the ability of the organism to establish populations in a given environment and find prey rapidly [15]. Previous studies showed that natural enemies' ability to establish and search for prey was affected by plant structure and size [16–20]. Garcia and O'Neil [15] showed that plant size and variegation affected the searching efficiency of *C. montrouzieri,* and Merlin et al. [21] found that oviposition of *C. montrouzieri* was stimulated by wax filaments produced by its prey. Also, these studies indicated that successful biological control of *P. longispinus* would be affected by how efficiently newly released *C. montrouzieri* adults search for *P. longispinus*. Specifically, there is a high chance for *C. montrouzieri* adults to successfully establish when they can start to search and find the suitable prey as soon as they are released. In addition, prey-size choice could affect successful colonization of predators [22]. Therefore, key factors influencing prey search efficiency of *C. montrouzieri* may include release location of *C. montrouzieri* based on within-plant distribution of *P. longispinus*, plant characteristics, and stages of *P. longispinus* that *C. montrouzieri* adults prefer.

This study was conducted to investigate colonization efficiency of *C. montrouzieri* to manage *P. longispinus* on three different types of ornamental plants. The objectives of this study were (1) to investigate within-plant distribution of *P. longispinus*, (2) to quantify the searching time of *C. montrouzieri* related to release location, and (3) to determine preference of *C. montrouzieri* adults for the size of *P. longispinus*.

2. Materials and Methods

All experiments were conducted in the greenhouse and the entomology laboratory of West Virginia University, Morgantown, WV, U.S.A.

2.1. Within-Plant Distribution of P. longispinus . We obtained three common species of ornamental plants from the greenhouse at West Virginia University (Monongalia County, WV, USA). The ornamental plants in this study include *Ficus elastica* (Urticales: Moraceae) (86–94 cm in height), *Lilium longiflorum* (Liliales: Liliaceae) (42–61 cm in height), and *Dieffenbachia seguine* (Alismatales: Araceae) (23–27 cm in height). These plants were selected because they are very common ornamental plants produced in the greenhouse. These plants had been infested with *P. longispinus* for at least one year before experiments to obtain moderate-to-high density of *P. longispinus*. Five plants of each plant species with similar infestation levels were selected, and the total numbers of *P. longispinus* nymphs and adults were counted on the upper and lower halves of each plant. Densities of *P. longispinus* on upper and lower parts of each plant species were compared using two-way ANOVA at 5% error rate [23].

2.2. Prey Searching Time of C. montrouzieri Adults on Three Different Plant Species. *C. montrouzieri* adults were maintained in ventilated cages with *P. longispinus* and a honey-water solution under laboratory conditions of 25°C and 16 : 8 (L : D) h photoperiod. *C. montrouzieri* were reared on *P. longispinus*. All *C. montrouzieri* adults were denied prey but had access to water for the 12 h period preceding all experiments. One randomly chosen *C. montrouzieri* adult was introduced onto either top (i.e., top shoot) or bottom (i.e., bottom part of stem within 2 cm above the soil line) of a plant. Once one *C. montrouzieri* adult was introduced to a plant, searching time of *C. montrouzieri* was measured. Searching time was measured as the duration between introduction and finding the first prey. This experiment was replicated five times for each plant species and each release location. Each *C. montrouzieri* adult was used for the test only once. The searching time of *C. montrouzieri* was recorded and analyzed with two-way (i.e., releasing locations and plant species) ANOVA at 5% error rate (SAS Institute, 2008).

Because we used the same plants for repeated release of different *C. montrouzieri* adults in the experiment, any leftover chemical cues by previously used *C. montrouzieri* adults could affect the next adults introduced to the plant. Therefore, we examined the effect of leftover chemical cues by previously used *C. montrouzieri* adults on the next adults introduced to the plant by dividing the data into two groups: first ten and last ten introductions of *C. montrouzieri*. The searching time of the two groups was compared ANOVA at 5% error rate [23].

2.3. C. montrouzieri Preference to Prey Body Size. A total of 20 *C. montrouzieri* adults were denied prey but had access to plain water for the 12 h period preceding the experiment. Preference of *C. montrouzieri* for three different sizes of *P. longispinus* (0.3 ± 0.07, 1.3 ± 0.10, and 3.0 ± 0.14 mm) was investigated using an empty 9-cm-diameter petri dish (LAB-TEK Division Miles Laboratories, Inc., Naperville, IL, USA) containing an excised leaf of *F. elastic* on the bottom of the Petri dish. Three *P. longispinus* with different body sizes were randomly placed on the leaf for each replication. One *C. montrouzieri* adult was placed in the center of the Petri dish and allowed to search for *P. longispinus*. *C. montrouzieri* adults' choice among the three different sizes of *P. longispinus* and handling and cleaning time was recorded. Handling time was measured from the start of first contact of *P. longispinus* by *C. montrouzieri* to cessation and included feeding. Cleaning time was measured as duration of waxy residue removal from the body. This choice test was run until the first nymph was consumed and replicated 20 times. Searching, handling, and cleaning times were compared using ANOVA at 5% error rate [23], and the first choices by *C. montrouzieri* adults to feed on the three different body sizes of *P. longispinus* were compared using Chi-square test [24].

Table 1: Mean (±SD) number of *P. longispinus* on the upper and lower parts of three different plants. Note that there were no significant differences within columns.

Plant species	Upper part of plant	Lower part of plant
Ficus elastica	415 ± 264.5a*	110 ± 93.2b
Lilium longiflorum	564 ± 172.4a	51 ± 26.8b
Dieffenbachia seguine	441 ± 154.1a	51 ± 40.4b

*Means within rows followed by the same letter are not significantly different (F test, $P < 0.05$).

3. Results

3.1. Within-Plant Distribution of P. longispinus. There were no significant differences in the total number of *P. longispinus* among the plants used in this study ($F = 1.21$; df = 2,57; $P > 0.05$). However, significantly ($F = 57.4$; df = 1,29; $P < 0.0001$) more *P. longispinus* were found on the upper parts of the plants regardless of the plant species (Table 1). There were no significant differences in *P. longispinus* densities among the three plant species when *P. longispinus* densities on the upper and lower parts were compared: upper parts of plants ($F = 0.46$; df = 2,14; $P = 0.650$) and the lower part of plants ($F = 0.94$; df = 2,14; $P = 0.443$).

3.2. Prey Searching Time of C. montrouzieri Adults on Three Different Plant Species. The time *C. montrouzieri* spent to find the first *P. longispinus* was significantly different among the three different plant species ($F = 7.9$; df = 2,24; $P = 0.002$) and the two different release points (top and bottom) ($F = 29.73$; df = 1,24; $P < 0.001$). Because interactions between plant species and release points ($F = 7.37$; df = 2,24; $P < 0.001$) were significant, we separately compared the time *C. montrouzieri* spent to find the first *P. longispinus* between two release locations for each plant height category. We found that *C. montrouzieri* spent significantly more time searching for *P. longispinus* when they were released from the bottom of the plants regardless of plant species (Figure 1). This indicates that prey-searching time for *C. montrouzieri* adults to find the first *P. longispinus* can be reduced by releasing them from the top of the plant.

We found that there were no differences in the searching ($F = 1.93$; df = 1,18; $P > 0.05$), handling, and cleaning time ($F = 1.21$; df = 1,58; $P > 0.05$) between the first ten *C. montrouzieri* introduced to the plants and the next ten introduced. This indicates that there were no significant effects of chemical cues left, if any, on the searching behavior of *C. montrouzieri* adults in this study.

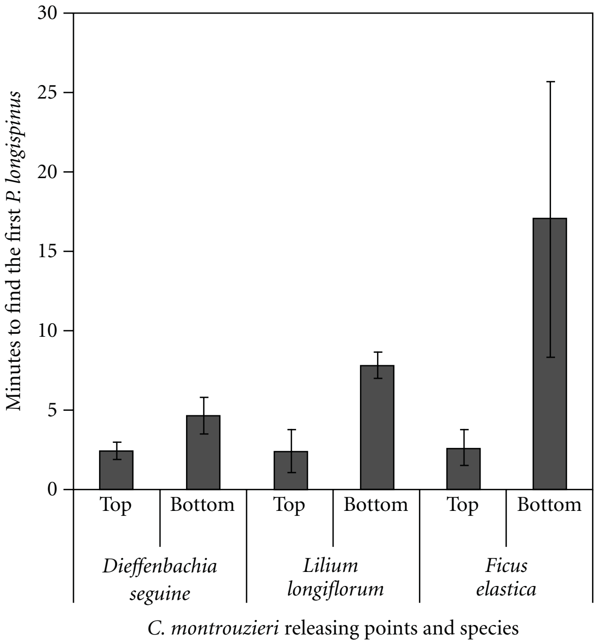

Figure 1: Effects of *C. montrouzieri* release locations and plant species on the time *C. montrouzieri* spent to find the first *P. longispinus*. Note that there were significant differences (F test; $P < 0.05$) between two release locations regardless of plant species.

Table 2: Handling and cleaning time (minutes ± SD) of *C. montrouzieri* adults feeding on different sizes of mealybug.

Mealybug size (mean ± SD)	Handling time	Cleaning Time	Total
Small (0.3 ± 0.07 mm)	6.9 ± 2.18a*	3.5 ± 2.03b	10.4 ± 4.21b
Medium (1.3 ± 0.10 mm)	10.3 ± 4.35a	4.5 ± 2.08b	14.8 ± 6.43ab
Large (3.0 ± 0.14 mm)	12.0 ± 7.00a	8.7 ± 1.53a	20.7 ± 8.53a

*Means within columns followed by the same letter are not significantly different (F test, $P < 0.05$).

3.3. C. montrouzieri Preference to the Body Size of P. longispinus. The results of the preference test of *C. montrouzieri* adults for three different sizes of *P. longispinus* showed that there was significant ($\chi^2 = 9.109$; df = 2; $P < 0.05$) preference of *C. montrouzieri* adults for smaller *P. longispinus* compared to medium ($\chi^2 = 7.619$; df = 1; $P < 0.01$) and larger ($\chi^2 = 8.314$; df = 1; $P < 0.01$) sizes. Although there were no significant differences in handling time of *C. montrouzieri* feeding on different sizes of *P. longispinus* ($F = 3.25$; df = 2,17; $P = 0.064$), there were significant differences in cleaning time among *C. montrouzieri* feeding on different sizes of *P. longispinus* ($F = 8.14$; df = 2,17; $P = 0.003$) (Table 2). This result indicates that *C. montrouzieri* adults choose smaller *P. longispinus* more frequently and spend significantly more time to clean after feeding on larger *P. longispinus*.

4. Discussion

Within-plant distribution of pests is key information for determining where to release natural enemies. To maximize efficiency of biological control, prey-searching time could be reduced depending on where natural enemies are released [18, 25]. In this study, we observed that significantly more *P. longispinus* inhabited the upper part of plants regardless of plant species. This observation is in agreement with the finding by Flaherty et al. [1] who showed higher population and movement of mealybugs toward the top of grapevines. Because *C. montrouzieri* is known to be more effective when *P. longispinus* populations are high [8, 26], releasing *C. montrouzieri* adults from the top of the plants could reduce prey-searching time because *P. longispinus* is abundant on the upper part of plants. In addition, plant species could affect prey-searching efficiency by natural enemies [15]. The results of our study indicated that effectiveness and establishment of *C. montrouzieri* increased when they were released on *Dieffenbachia seguine,* the smallest plant tested in this study. Therefore, the effectiveness of *C. montrouzieri* managing *P. longispinus* could be maximized when *C. montrouzieri* are released from the top and on smaller plants.

This study demonstrated that *C. montrouzieri* chose to feed on smaller *P. longispinus.* When *C. montrouzieri* fed on larger nymphs, they spent longer time handling and cleaning after feeding and before searching for another prey. This result is congruent with Merlin et al. [21] who found that *C. montrouzieri* consumed smaller *P. longispinus* nymphs first and then fed on larger nymphs or adults. Because establishment of natural enemies after release determines the success of augmentational biological control (i.e., inoculative release), finding the first prey by the natural enemies could increase the chance of establishment. Although a difference of 5–15 minutes in time to initial prey encounter may not make much of a difference to the final biological control outcome in a greenhouse, it could influence the chance of establishment because we frequently observed that *C. montrouzieri* adults could fly away to escape from the greenhouse when they cannot find the first prey in a reasonable period. Therefore, *C. montrouzieri* adults have higher chance to establish when the period for finding the first prey is shorter.

The results of this study suggest a major consideration for the use of *C. montrouzieri* to manage *P. longispinus.* The efficiency of *P. longispinus* management by *C. montrouzieri* depends on the location of *C. montrouzieri* release and the plant species. Our study showed that effectiveness and establishment of *C. montrouzieri* managing *P. longispinus* could be maximized when *C. montrouzieri* were released from the top of the plants and on the smaller plants, *C. montrouzieri* adults can reduce prey search time when they are released where *P. longispinus* is abundant. Future study needs to investigate the effect of plant age and stage on vertical distribution of *P. longispinus* on the plant.

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Research Article

Effect of Larval Density on Development of the Coconut Hispine Beetle, *Brontispa longissima* (Gestro) (Coleoptera: Chrysomelidae)

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The coconut hispine beetle, *Brontispa longissima* shows, aggregation in the field. To elucidate the effect of aggregation on larval developmental aspects, we examined the effects of larval density on various aspects of larval development and on survival rates. Recently we found that *B. longissima* was divided into two monophyletic clades by genetic analysis. Therefore, we also compared the results between two populations, from Ishigaki, Japan (ISH) and Papua New Guinea (PNG), which were representative of the two monophyletic clades of *B. longissima*. In both ISH and PNG, the larval developmental period was shorter and the survival rate higher with rearing under high-density conditions than under isolated conditions. Similarly, fewer instars were required before pupation under high-density conditions than under isolated conditions. *Brontispa longissima* therefore developed better under high-density conditions, and the trends in the density effect were similar between two monophyletic clades.

1. Introduction

The coconut hispine beetle, *Brontispa longissima* (Gestro) (Coleoptera: Chrysomelidae) is one of the most serious insect pests of *Cocos nucifera* (L.) and other palms [1, 2]. This beetle is considered to be native to Papua New Guinea and Indonesia [3], but since the 1930s it has gradually invaded Australia and the Pacific Islands including Vanuatu and Samoa [3, 4]. Since heavy infestations of *B. longissima* were first found in the Mekong Delta of Vietnam in 2003, it has been spreading rapidly and widely throughout Southeast Asian countries such as the Maldives, Thailand, Cambodia, and the Philippines [1, 5, 6]. In these countries, the coconut palms are very important for food, fuel, and industrial materials, and as part of landscape at tourist destinations [7, 8]. It is therefore important to establish a method of protecting coconut palms in Southeast Asian countries from the damage caused by *B. longissima*. However, there is insufficient information on the fundamental ecology of this species.

For these reasons, we have been studying the beetle's ecological properties. Phylogenetic analysis based on the mtDNA sequences has revealed two monophyletic clades in *B. longissima* [9]. One group is referred to as the Pacific clade and is distributed in Australia, Papua New Guinea, Samoa, and Sumba Island in Indonesia. The other is the Asian clade and is distributed over a wide area of Asia and the Pacific region. Morphological traits do not differ between the two clades [9], but little has known whether there are any differences in their ecological properties.

In the field, *B. longissima* aggregates within the folded leaflets of the coconut palm [6, 10]. We therefore assumed that development at high density is promoted within the populations of this insect. Here, to clarify developmental differences in *B. longissima* during the immature developmental stages under different density conditions, we investigated the effects of changes in larval density on the length of the insect's developmental period and on the number of instars required before pupation, the survival rate, and body size.

Furthermore, we examined whether the effects of density differed between the two monophyletic clades.

2. Materials and Methods

2.1. Insects. *Brontispa longissima* populations were collected from Ishigaki Island, Okinawa Prefecture, Japan, and from East New Britain Province, Papua New Guinea. The populations from Ishigaki (ISH) and Papua New Guinea (PNG) have been, respectively, categorized according to genetic analysis into the Asian group and the Pacific group [9]. The two populations under the present experiment were same as the populations used in Takano et al. [9]. Larvae and adults were provided with fresh leaves of *C. nucifera*. The pieces of leaves (15 cm length) were bundled with elastic bands, because this beetle prefers to hide between the leaves. Until pupation, larvae were maintained with a bundle of leaves in a plastic container (15.5 cm long, 11.5 cm wide, 5.0 cm high) covered with a screened ventilated lid. Pupae were placed in a Petri dish and the adults were reared in a plastic container in the same way as the larvae. Rearing was conducted at 25 ± 1°C under a 12 : 12-h (L/D) photoperiod and 65% ± 5% relative humidity.

2.2. Experiments. ISH hatchlings less than 24 h after emergence were transferred into a Petri dish (5.5 cm diameter, 1.5 cm high) containing a bundle of fresh-cut leaves of *C. nucifera*. Some Petri dishes contained 1 individual (isolated conditions) and some contained 10 (crowded conditions). We considered a density of 10 individuals per dish to be crowded, but not overcrowded, because a preliminary experiment had shown that at this density there was still enough food for larvae of final instar which is the lifecycle stage at which the greatest amounts of food are ingested. The treatment for isolated condition was replicated 30 times (i.e., 30 individuals) and for crowded condition 10 times (i.e., 100 individuals). We transferred the larvae to a new Petri dish containing fresh leaves every day, and we checked the lengths of developmental periods of the larvae and the pupae. Survival rates and the occurrence of molting were recorded every day until adult emergence. Molting was checked by counting exuviae and measuring the head width of larvae under a stereomicroscope. Emerged adults were sexed and the length from the head to the tip of abdomen was measured under the stereomicroscope. The same procedures were followed with *B. longissima* PNG as with ISH. Experiments were conducted at 28 ± 1°C under a 12 : 12-h (L/D) photoperiod and 65% ± 5% relative humidity.

2.3. Statistical Analyses. Differences in survival rate were statistically compared by using Fisher's exact probability test. The chi-squared test was used to examine differences between the two rearing densities in terms of the proportions of instars each number immediately before pupation. These analyses were conducted with version 2.11.0 of R software [11]. A *t*-test was used to compare the differences in length of the developmental period and body size. These analyses were conducted with version 5.1 of JMP software (SAS Institute Inc. Cary, NC, USA). Developmental period length and body size were compared between isolated and crowded conditions within the same monophyletic group and between ISH and PNG under each density condition.

3. Results

In both populations the survival rate tended to decrease with developmental stage, and from the third instar larval stage onward it was higher under crowded conditions than under isolated conditions (Figure 1). The adult emergence rate under crowded conditions was 86% in both populations. In contrast, under isolated conditions the adult emergence rate was 70.0% in ISH and 53.3% in PNG (Figure 1).

We examined the lengths of the developmental periods in ISH and PNG populations at the two different densities (Table 1). The larval development period was significantly shorter under crowded conditions than under isolated conditions in both ISH and PNG. The length of the pupal development period did not differ significantly between the two density conditions in either population.

We then investigated the variations in the larval instar number at which pupation occurred (Figure 2). ISH larvae pupated at the fourth or fifth instar. In contrast, PNG larvae pupated at the fourth to sixth instars. The proportion of each of the instar numbers at which pupation occurred differed significantly between the two density conditions in each clade (chi-squared test, ISH: $\chi^2 = 16.961$, $P < 0.001$; PNG: $\chi^2 = 9.122$, $P = 0.010$). At pupation, the proportion of fourth instars under crowded conditions was greater than that under isolated conditions in both ISH and PNG.

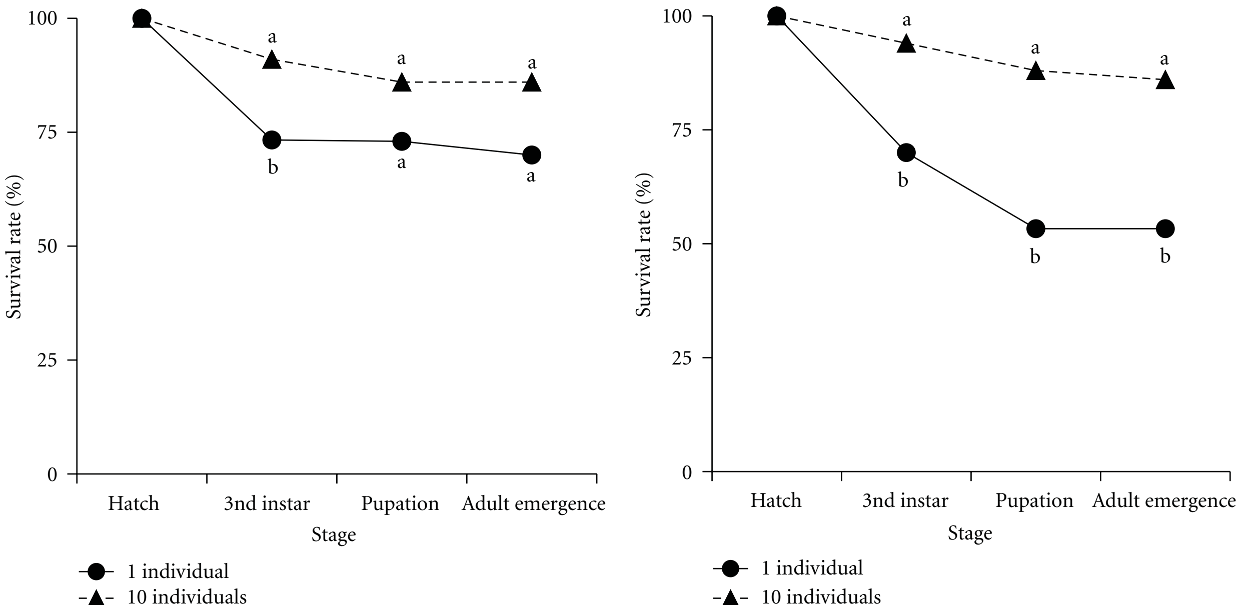
The heads of last-instar larvae were significantly wider under isolated conditions than under crowded conditions (Table 2). In both ISH and PNG, body length in adult males was significantly greater under isolated conditions than under crowded conditions, whereas female body length did not differ significantly.

Within each density condition, the larval development period was significantly shorter in ISH than in PNG (Table 1). Body length in adult males under isolated conditions was significantly greater in PNG than in ISH (Table 2). The other data did not differ significantly between the two clades (Tables 1 and 2).

4. Discussion

Our results revealed that larval density influenced various ecological aspects in *B. longissima* and that the overall trends in the density effect were similar between the two populations, ISH and PNG.

Survival rates were higher under crowded conditions than isolated conditions. This trend has been observed in many other species of insects, including the leaf beetle, *Chrysolina aurichalcea* Mannerheim (Coleoptera: Chrysomelidae), and the pleasing fungus beetle *Dacne picta* Crotch (Coleoptera: Erotylidae) [12, 13]. Our results support the findings of these studies. Utida [14] described that low density causes stress in individuals, and in our study, the survival rates when only 1 individual was present were low.



(a) (b)

Figure 1: Survival rates from hatching to adult emergence in *B. longissima* reared under isolated conditions (1 individual) or crowded conditions (10 individuals). (a) ISH (individuals obtained from a stock culture initiated from insects collected on Ishigaki Island). (b) PNG (obtained from a stock culture of insects collected in Papua New Guinea). Values with the same letters do not differ significantly (Fisher's exact probably test, $P > 0.05$).

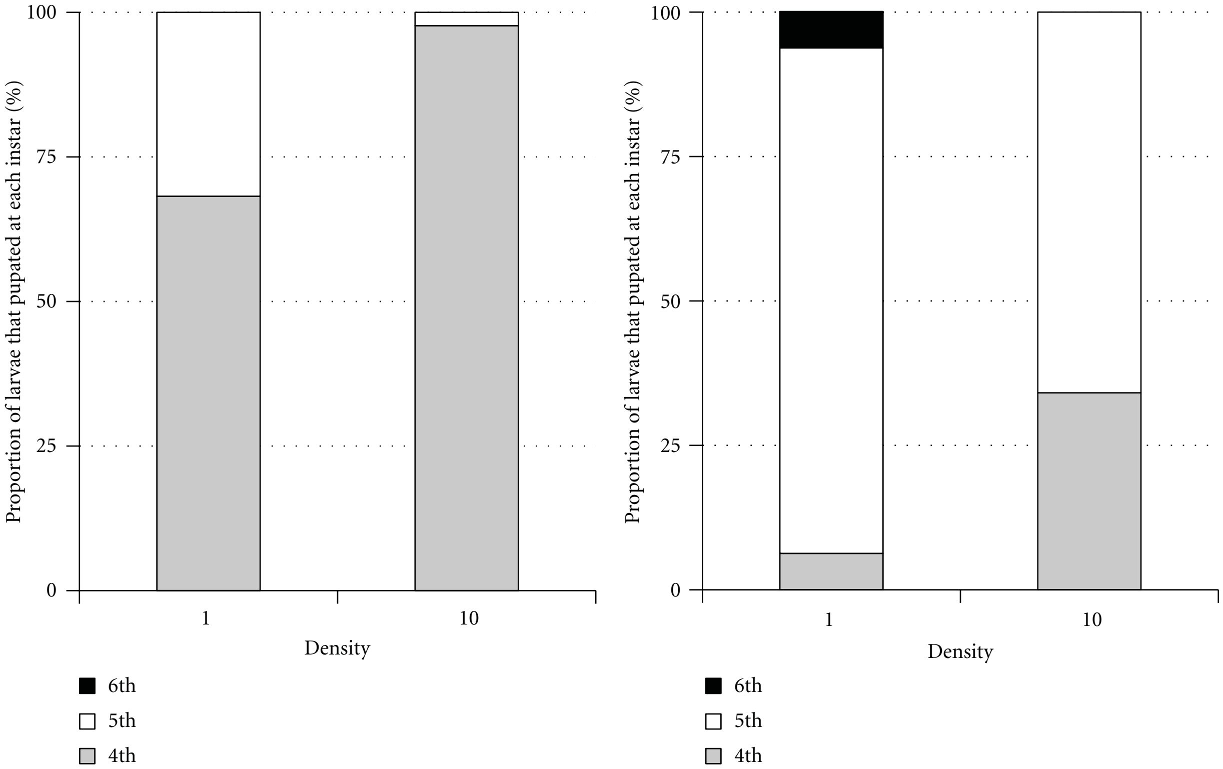
Table 1: Developmental periods (mean ± SE, days) of *B. longissima* reared under the density of one individual or 10 individuals.

Developmental stage	Density	Population				*t*-value	*P*
		ISH		PNG			
Larva	1 individual	22	24.8 ± 0.7	16	28.1 ± 0.8	−2.85	0.0073
	10 individuals	86	20.5 ± 0.3	88	25.8 ± 0.8	−13.88	<0.0001
	t-value		−6.83		−3.07		
	P		<0.0001		0.0027		
Pupa	1 individual	21	5.1 ± 0.1	16	4.9 ± 0.1	1.42	0.1647
	10 individuals	86	4.9 ± 0.1	86	4.8 ± 0.1	1.20	0.2300
	t-value		−1.73		−0.75		
	P		0.0873		0.4563		

One reason is might therefore be that extremely low density is a stressor for *B. longissima* larvae.

The length of the larval development period was clearly influenced by the density conditions, but larval density had no effect on the length of pupation in *B. longissima*. This effect of crowding on larval development is found in some other species of insects such as *Diploptera punctata* (Eschscholtz) (Blattodea: Blaberidae), and *Mythimna separata* (Walker) (Lepidoptera: Noctuidae) [15, 16]. Applebaum and Heifetz [17] described that food consumption, metabolic rate, and general activity are enhanced by crowded conditions in the insects that show density-dependent responses. We did not examine the quantities of leaves ingested in each dish. Possibly, larvae under crowded conditions might grow more quickly than isolated larvae because of competitive need to feed more actively. Moreover, during the experiments, we found that several larvae overlapped with, or touched, each other, even though there was enough space for them to eat on the leaves while apart. In *B. longissima*, this body-touching behavior and/or the presence of feces from other individuals might shorten the larval development period under crowded conditions. However, we cannot make this inference from our current data.

In some species of insects, body size diminishes with increasing population density [18]. Besides, Goulson and Cory [19] explained that this phenomenon occurs unrelated to a direct shortage of food. Also, here, we provided enough leaves for larvae every day, but we observed an inverse relationship between larval density and body length of adult males. By comparison, female body length was not influenced by density. These findings suggest that males of *B. longissima*, but not females, are susceptible to density effects.



(a)

(b)

Figure 2: Variations in the proportions of pre-pupation larval instars (fourth, fifth, and sixth) in *B. longissima* reared under isolated conditions (density, 1 individual per Petri dish) or crowded conditions (10 individuals) (a) ISH, (b) PNG.

Table 2: Head width of last-instar larvae and body length of adults (mean ± SE, mm) of *B. longissima* reared under the density of one individual or 10 individuals.

Site	Density	Population				*t*-value	*P*
			ISH		PNG		
Head width of last-instar larvae	1 individual	22	1.24 ± 0.02	16	1.25 ± 0.01	−0.51	0.6098
	10 individuals	86	1.19 ± 0.01	88	1.20 ± 0.01	−0.96	0.3375
	t-value		−3.08		−2.67		
	P		0.0027		0.0089		
Male adult body length	1 individual	11	8.6 ± 0.1	8	9.1 ± 0.2	−2.47	0.0246
	10 individuals	44	8.3 ± 0.0	45	8.3 ± 0.0	0.33	0.7423
	t-value		−2.02		−6.44		
	P		0.0487		<0.0001		
Female adult length	1 individual	10	9.2 ± 0.1	8	9.3 ± 0.1	−0.70	0.4940
	10 individuals	42	9.3 ± 0.0	41	9.3 ± 0.0	−0.10	0.9205
	t-value		1.50		0.30		
	P		0.1391		0.7628		

Regarding head width, it seems that the wider heads under isolated conditions were due to the size of male larvae.

The last-instar larvae of *B. longissima* included more 5th or 6th instars under isolated conditions than under crowded conditions. Generally, insects initiate to pupation when the larvae have reached a critical body size [20, 21]. Some species of insects increase the number of prepupation instars as a form of compensatory growth when the larvae fail to reach their threshold size for metamorphosis under adverse conditions [22]. In our experiments, the head width of the penultimate instar under isolated conditions was smaller than that of the final instar under crowded conditions (data not shown). We therefore consider that *B. longissima* larvae under isolated conditions needed to undergo more larval molts to reach the threshold size for pupation. The number of pre-pupation instars in *B. longissima* has been reported as five to six by Waterhouse and Norris [3] and four to six by Yamauchi [23]. In many species of insects, the number of pre-pupation instars is affected by rearing conditions, including food quality, density, temperature, and humidity [22]. Therefore, any differences between our results and these past reports are likely associated with differences in environmental factors, including temperature and food. Furthermore, we observed the differences in proportions of last instars (fourth, fifth, and sixth) between ISH and PNG. We therefore think that the number of pre-pupation instars varies among not only density conditions but also monophyletic clades in *B. longissima*.

Our findings suggest that *B. longissima* reared under high-density conditions has a survival advantage, and therefore high-density conditions allowed *B. longissima* to increase the number of its generations in the field. In other words, once *B. longissima* invades into the coconut palm field, it might be able to increase acceleratingly as the population increases and spread in the field. The density-dependent phenomena have the potential to influence fecundity or dispersion during the adult stage. In future, we need to investigate the effect of population density on ecological aspects in *B. longissima* adults. Our findings will help to elucidate the factors involved in the rapid spread of *B. longissima* and its damage, in the coconut palm field in Southeast Asian countries.

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Research Article

Feeding Preferences of the Endangered Diving Beetle *Cybister tripunctatus orientalis* Gschwendtner (Coleoptera: Dytiscidae)

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The numbers of *Cybister tripunctatus orientalis* Gschwendtner diving beetles are declining in most regions of Japan, and it is included in the Red Data List of species in 34 of 47 prefectures of Japan. However, basic ecological information about *C. tripunctatus orientalis*, such as its feeding habits, remains unknown. In order to elucidate the feeding habits of *C. tripunctatus orientalis* larvae, feeding preference experiments were carried out in 2nd and 3rd instar larvae. The number of Odonata nymphs consumed was significantly higher than the number of tadpoles consumed, indicating that *C. tripunctatus orientalis* larvae prefer Odonata nymphs to tadpoles. In addition, all the first instar larvae of *C. tripunctatus orientalis* developed into second instars when they were supplied with motionless Odonata nymphs, but their survival rate was lower when they were supplied with motionless tadpoles. These results suggest that *C. tripunctatus orientalis* larvae prefer insects to vertebrates.

1. Introduction

Cybister tripunctatus orientalis Gschwendtner (adult body length: 24–29 mm) is found in China, the Korean Peninsula, Taiwan, and Japan excluding Hokkaido [1]. The numbers of *C. tripunctatus orientalis* are declining in most regions of Japan, and it is included in the Red Data List of species in 34 of 47 prefectures of Japan [2, 3]. *Cybister tripunctatus orientalis* has become extinct in Tokyo, Kanagawa, Aichi, Kyoto, Osaka, Wakayama, and Hyogo (it was rediscovered in Hyogo in 2010: [4]). Contributing factors, such as a decreasing number of suitable aquatic habitats due to the abandonment of rice paddies, water pollution, pesticide application, and invasion by alien species, are of great concern [2, 5]. In addition, the sizes of predatory invertebrate populations are limited by their food resources, as is true for any predatory insect [6–8]. Thus, understanding their trophic ecology is necessary to support an insect conservation program. However, basic ecological information about *C. tripunctatus orientalis*, such as their feeding habits, remains unknown.

A number of descriptive reports [9–12] have suggested that *Cybister* larvae feed on tadpoles, fish, and aquatic insects. In studies of *C. tripunctatus orientalis*, Kunimoto [13] saw 3rd instar larvae capturing tadpoles and Odonata larvae (*Pantala flavescens*) in rice fields in Tottori, western Japan. Ohba [14, 15] revealed the larval feeding habits of two congeneric *Cybister* species based on a field census and rearing experiment: *C. brevis* Aubé and *C. chinensis* Motschulsky (formerly *Cybister japonicus* Sharp, see Nilsson and Petrov [16]) larvae preyed mainly on aquatic insects and did not eat vertebrate animals such as tadpoles, except for the 3rd instar of *C. chinensis*. Moreover, these studies showed that the results of feeding preference experiments performed under laboratory conditions were in accordance with field observations of the *Cybister* species and their growth performance [14, 15]. Therefore, feeding preference experiments performed under laboratory conditions can contribute to deducing the natural feeding habits of *Cybister* species. The objective of this study was to reveal whether the larvae of *C. tripunctatus orientalis* prefer invertebrate prey (Odonata nymph) over vertebrate prey (tadpole). For that purpose, two laboratory experiments were carried out in order to determine the feeding preferences of *C. tripunctatus orientalis* larvae.

2. Materials and Methods

2.1. Study Animals. Three male and two female *C. tripunctatus orientalis* adults were collected as breeding stock from an irrigation pond in southern Kochi, Shikoku, Japan, in September 2010 and kept in an aquarium (45 cm × 34 cm in dimension, 20 cm in height) maintained under natural water temperature and day length conditions for overwintering. From April 2011 onwards, they were maintained at a water temperature of 25°C under a 16L : 8D light cycle to stimulate reproduction. River gravel was laid onto the bottom of the aquarium in a 2 cm thick layer, and dechlorinated tap water was added to a depth of 15 cm over the gravel surface. Three water hyacinths (*Eichhornia crassipes*; ca. 5 cm in stock diameter) were planted in the aquarium as oviposition sites. Hatched larvae were reared individually in small plastic containers (10 cm diameter × 10 cm in height) filled to a depth of 2 cm with dechlorinated tap water and kept under natural temperature and day length conditions from June to July 2011. Larvae of *Culex* spp., chironomids, and notonectid nymphs were supplied to the *C. tripunctatus orientalis* larvae as food for the first experiment. The prey animals used in this study were collected from rice fields and irrigation ponds.

2.2. First Experiment. To investigate the feeding preferences of *C. tripunctatus orientalis*, a feeding preference experiment was conducted (see Ohba [14, 15]) using 2nd and 3rd instar larvae. As the first instar larvae did not capture live tadpoles in the preliminary experiment, we did not use first instar larvae in the feeding preference experiment. Small damselfly nymphs (Platycnemididae: *Copera* spp. or Lestidae: *Lestes* spp., 15–20 mm) and large dragonfly nymphs (*Sympetrum* spp. 20–30 mm) were provided as food to the 2nd and 3rd instar larvae, respectively. In addition, small (snout to vent length: 10–20 mm) and large (20–30 mm) tadpoles of the tree frog *Hyla japonica* were provided as food to these larval instars, respectively. The prey density in each plastic container was kept constant (3 tadpoles and 3 Odonata nymphs). Each beetle larva was fasted for a day before the experiment. Before the start of the experiment, the Odonata nymphs were fed larval *Aedes* spp. on a daily *ad libitum* basis in order to prevent intra- and interspecific predation (attacking the tadpoles) during the experiment. The number of carcasses (consumed by the *C. tripunctatus orientalis* larva) of each prey animal was counted at 24 hours after the beginning of the experiment. The experiments using the 2nd and 3rd instar nymphs were replicated 10 and 14 times, respectively. To determine the diet choices of *C. tripunctatus orientalis* larvae, the Wilcoxon signed-rank test was used to compare the number of prey consumed between the tadpoles and Odonata nymphs for each larval instar. In this case, paired nonparametric test should be applied to this data because of discrete-valued data.

2.3. Second Experiment. In the preliminary experiment, *C. tripunctatus orientalis* larvae hardly ever consumed tadpoles but they did routinely eat Odonata nymphs. This might have been caused by different escape capabilities of tadpoles and Odonata nymphs. To examine only the influence each prey item on the development of *C. tripunctatus orientalis* larvae when the prey were prevented from escaping, motionless tadpoles and motionless Odonata nymphs were used. The bases of the tadpole tails (*H. japonica*, ca. 10 mm) or the thoraces of the Odonata nymphs (*Lestes* spp. ca. 15 mm) were squeezed using forceps for 5 seconds to immobilize them. A first instar *C. tripunctatus orientalis* larva and one motionless preys specimen were put into a small plastic container. The individual prey were exchanged for new prey every day, and this process was continued for 15 days or until the *C. tripunctatus orientalis* larva died. The motionless tadpole and motionless Odonata nymph treatments were replicated 6 times each. Survival analysis was used to test for survival curve differences between the motionless tadpoles and motionless Odonata nymphs. The Kaplan-Meier method for estimating survival and the nonparametric Mantel-Cox log-rank test were used. Survival analysis has been regularity employed in medical science to analyze "incomplete" data recorded before the termination of the event of interest. Because many insects died before the present experiments were terminated, the incomplete data thus resulting is not suitable for traditional nonparametric techniques (e.g., Moore and Townsend [17]). A statistically different significance was assumed to be at $P < 0.05$. All statistical tests were conducted using the Statcel [18].

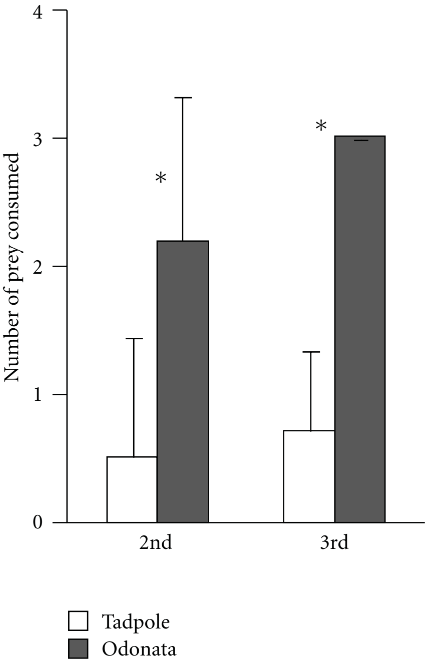

Figure 1: The number of tadpoles and Odonata nymphs consumed by *Cybister tripunctatus orientalis* larvae. $^*P < 0.05$, the Wilcoxon signed-rank test. Data are shown as the mean + S.D.

3. Results and Discussion

In the first experiment, the number of Odonata nymphs consumed was significantly higher than the number of tadpoles consumed in 2nd and 3rd instars (the Wilcoxon signed-rank test, 2nd: $Z = 2.55$; 3rd: $Z = 2.98$, $P < 0.02$ for both; Figure 1).

Although we did not carry out any field observations, it is assumed that *C. tripunctatus orientalis* larvae consume mostly aquatic invertebrates in their natural habitats. However, Kunimoto [13] recorded 3rd instar larvae of *C. tripunctatus orientalis* capturing tadpoles in rice fields. This discrepancy would most likely have been caused by differences in prey density; that is, their study was carried out in June, when there is a high density of tadpoles in rice fields [13]. According to Ohba [15], the emergence of first and second instar larvae of closely related species, *Cybister chinensis* Motschulsky, coincided with the appearance period of Odonata nymphs and tadpoles but these larvae fed on Odonata nymphs and did not eat tadpoles. Therefore, larvae of *C. tripunctatus orientalis* may not eat tadpoles well in their fields. In fact, *C. tripunctatus orientalis* larvae consumed more Odonata nymphs than tadpoles when supplied with the same number of Odonata nymphs and tadpoles (Figure 1). Thus, the 2nd and 3rd instar larvae of *C. tripunctatus orientalis* preferred Odonata nymphs over tadpoles, as is also observed for closely related species, *Cybister brevis* Aubé, larvae [14].

In the second experiment, the survival rates of *C. tripunctatus orientalis* larvae differed significantly between those feeding on motionless tadpole and Odonata nymph treatments (survival analysis, Mantel-Cox $\chi^2 = 5.87$, $P = 0.015$). In larvae fed motionless tadpoles, four died after 12.3 ± 4.27 (mean ± S.D.) days and two developed into 2nd instar larvae after 14.5 ± 0.71 days. On the contrary, all six larvae fed motionless Odonata nymphs developed into 2nd instar larvae after 6.2 ± 0.41 days. The mean larval duration of the first instar was longer in the motionless tadpole treatment than in the motionless Odonata nymph treatment irrespective of survival. The results show that *C. tripunctatus orientalis* larvae can consume tadpoles but obtain insufficient levels of nutrients from them for optimal development. Interestingly, *C. brevis* larvae died from starvation after being supplied with only motionless tadpoles, indicating that *C. brevis* larvae do not consume tadpoles; not because they cannot capture tadpoles but because they dislike and/or do not recognize tadpoles as prey animals [14]. These different feeding habits might be attributable to differences in digestive enzymes between the two species. This should be examined in future studies. These results strongly suggest that environments with abundant aquatic invertebrates are favorable for maintaining *C. tripunctatus orientalis* populations.

Rice fields are an important breeding habitat for aquatic insects [19] including *C. tripunctatus orientalis* in Japan. However, the species diversity in rice fields has been declining due to recent land consolidation, the modification of traditional earth ditches to *U*-shaped concrete ditches in Japan [5, 20]. Therefore, poorly drained paddies, which are wet in winter and kept flooded throughout summer, are suitable to conserve the aquatic invertebrates as well as *C. tripunctatus orientalis*.

Acknowledgments

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Research Article

Incorporating a Sorghum Habitat for Enhancing Lady Beetles (Coleoptera: Coccinellidae) in Cotton

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Lady beetles (Coleoptera: Coccinellidae) prey on insect pests in cotton. The objective of this 2 yr on-farm study was to document the impact of a grain sorghum trap crop on the density of Coccinellidae on nearby cotton. *Scymnus* spp., *Coccinella septempunctata* (L.), *Hippodamia convergens* Guérin-Méneville, *Harmonia axyridis* (Pallas), *Coleomegilla maculata* (De Geer), *Cycloneda munda* (Say), and *Olla v-nigrum* (Mulsant) were found in sorghum over both years. Lady beetle compositions in sorghum and cotton and in yellow pyramidal traps were similar. For both years, density of lady beetles generally was higher on cotton with sorghum than on control cotton. Our results indicate that sorghum was a source of lady beetles in cotton, and thus incorporation of a sorghum habitat in farmscapes with cotton has great potential to enhance biocontrol of insect pests in cotton.

1. Introduction

Lady beetles (Coleoptera: Coccinellidae) have a significant impact on aphids (Hemiptera: Aphididae) [1–4], including the cotton aphid (*Aphis gossypii* Glover) attacking cotton (*Gossypium hirsutum* L.) [5] and the corn leaf aphid (*Rhopalosiphum maidis* (Fitch) and greenbug (*Schizaphis graminum* (Rondani) attacking grain sorghum (*Sorghum bicolor* (L.) Moench spp. *bicolor*) [6–8]. In the southeastern USA, cotton fields commonly are closely associated with other agronomic crops, especially corn (*Zea mays* L.) and peanut (*Arachis hypogea* L.), and in these farmscapes polysphagous pest species are known to move from corn and peanut into cotton to find newly available, suitable food, or oviposition sites [9]. As part of a larger pest management strategy, strips of grain sorghum planted between a source crop and cotton have proved useful as a trap crop to reduce pest movement, especially stink bugs (Hemiptera: Pentatomidae), into cotton [10, 11]. Additionally, a grain sorghum trap crop is beneficial to natural enemies by hosting the corn leaf aphid and greenbug [12]. Thus, grain sorghum, when planted adjacent to cotton, can perform as a trap crop for stink bugs and possibly as a source of natural enemies moving into cotton. In fact, many species of Coccinellidae are commonly found inhabiting grain sorghum: *Harmonia axyridis* (Pallas), *Hippodamia convergens* Guérin-Méneville, *H. sinuata* Mulsant, *H. parenthesis* (Say), *Coccinella septempunctata* L., *Coleomegilla maculata* (De Geer), *Cycloneda munda* (Say), *Scymnus* spp., *Olla v-nigrum* (Mulsant), *Exochomus* sp., and *Psyllobora vigintimaculata* (Say) [13–16]. These same species colonize cotton [4, 16, 17], and their presence within a grain sorghum trap crop may lead to these predators moving into cotton and facilitating insect pest management.

The objective of this study was to document the impact of a grain sorghum trap crop on the density of Coccinellidae on nearby cotton. Two treatments were used: (1) cotton fields without sorghum and (2) cotton fields bordered on one side by a strip of grain sorghum. Within the grain sorghum, Coccinellidae were not only sampled on plants but also from yellow pyramid traps that predominantly served in the larger pest management scheme to kill stink bugs in sorghum.

2. Materials and Methods

2.1. Study Sites. Six cotton fields, ranging from 5 to 18 ha in size, were sampled each year, 2006 and 2007, in Irwin County GA (Table 1). Recommended agricultural practices

Table 1: Planting date (PD) and variety for cotton (Ct) with sorghum trap crops, control cotton, and sorghum (So) in 2006 and 2007.

Year	Treatment	Rep	Crop	Variety[a]	PD
2006	Cotton w/trap crop	1	Ct	DP 555	4/28
	Cotton w/trap crop	2	Ct	DP 555	5/4
	Cotton w/trap crop	3	Ct	DP 555	5/10
	Sorghum trap crop	1–3	So	DK 54	4/14
	Control cotton	1	Ct	DP 555	5/1
	Control cotton	2	Ct	DP 555	5/4
	Control cotton	3	Ct	DP 555	5/26
2007	Cotton w/trap crop	1	Ct	DP 555	5/9
	Cotton w/trap crop	2	Ct	DP 555	6/7
	Cotton w/trap crop	3	Ct	DP 555	6/11
	Sorghum trap crop	1–3	So	DK 54	6/13
	Control cotton	1	Ct	DP 555	5/11
	Control cotton	2	Ct	DP 555	5/11
	Control cotton	3	Ct	DP 555	6/11

[a] Seed companies; DK: DeKalb; DP: Deltapine.

for production of sorghum [18] and cotton [19] were followed. Row width was 0.91 m for each crop, and rows for each crop were parallel to each other.

2.2. Yellow Pyramidal Traps. These traps consisted of a 2.84-liter clear plastic polyethylene terephthalate jar (United States Plastic Corp., Lima, OH, USA) on top of a 1.22 m-tall yellow pyramidal base [20, 21]. An insecticidal ear tag (Saber Extra, Coppers Animal Health Inc., Kansas City, KS, USA) was placed in the plastic jar at the beginning of a test to prevent escape of captured specimens. Active ingredients in the ear tag were lambda-cyhalothrin (10%) and piperonyl butoxide (13%). As part of the larger strategy to reduce pest movement into cotton, stink bug attraction to the traps was enhanced by placing *Euschistus* spp. stink bug lures (40 μL of the *Euschistus* spp. pheromone, methyl (*E, Z*)-2,4-decadienoate (CAS registry no. 4493-42-9) (Degussa AG Fine Chemicals, Marl, Germany, loaded onto rubber septa) in traps and replacing lures weekly. Insects from weekly collections were taken to the laboratory for identification.

2.3. Experimental Design. Two treatments were used each year: control cotton (without a sorghum trap crop) and cotton bordered by a sorghum trap crop and yellow pyramidal traps within the trap crop. At the beginning of the study, six commercial cotton fields were selected in Irwin County, Georgia, and each treatment was assigned randomly to three cotton fields similar to a completely randomized design. For the sorghum trap crop treatment, sorghum was planted in a strip (4 rows) along one edge of the cotton field; row 1 of sorghum was adjacent to a peanut field and row 4 was adjacent to the cotton field. Then 25–28 yellow pyramidal traps (depending on field width) were placed 12 m apart in row 1 of sorghum.

2.4. Insect Sampling. Each year of the study, crops and yellow pyramidal traps were examined weekly for the presence of lady beetles; from the week of 5 July to the week of 16 August in 2006 and from the week of 19 July to the week of 23 August in 2007. Due to time constraints of sampling these large fields, not all farmscapes were sampled on the same day of the week, but crops and/or yellow pyramidal traps within a field were sampled on the same day. For each sorghum sample, all plant parts within a 1.83 m length of row were visually checked for all lady beetles. For each cotton sample, all plants within a 1.83 m length of row were shaken over a drop cloth, and the aerial parts of all plants were visually checked thoroughly for all lady beetles. Voucher specimens are stored in the USDA-ARS, Crop Protection and Management Research Laboratory in Tifton, GA, USA.

For sampling purposes, the edge of a cotton field adjacent to a peanut field was labeled as side A, and in a clockwise direction the other 3 sides of a field were labeled as sides B, C, and D. Each year, samples were obtained from within the cotton field at 3 distances from the edge of side A (i.e., at rows 1, 2, and 5), and at 6 interior locations along the length of the field (i.e., rows 16, 33, 100, 167, 233, and 300 from the edge of the field on side A). In both years, the 300-row samples were not close to the edge of side C; 24–31 m from side C in 2006 and 61 m from side C in 2007. For sides B–D, samples were taken from 2 edge locations, rows 1 and 5 from the edge of the field. The number of samples from each field on each date was as follows: 9 from each row on side A, 3 from each row on sides B–D, and 6 from each interior location. For both years, the 4-row strip of sorghum was sampled by taking 9 samples from each of the 4 rows.

2.5. Statistical Analysis. Lady beetle species compositions in sorghum strips, cotton fields, and yellow pyramidal traps were similar for both years, and then data for the two years were combined. Means were obtained for number of lady beetle adults per sample for sorghum and yellow pyramidal traps using PROC MEANS [22]. The number of lady beetle adults per sample in cotton with sorghum trap crops and control cotton was compared using *t*-tests. In 2007, one cotton field with a sorghum trap crop was excluded from data analysis on week 6 due to an insecticide application after sampling on week 5. One control cotton field was excluded from data analysis on weeks 5 and 6 due to an insecticide application after sampling on week 4.

3. Results and Discussion

Scymnus spp., *C. septempunctata*, *H. convergens*, *H. axyridis*, *C. maculata*, *C. munda*, and *O. v-nigrum* were found in crops and yellow pyramidal traps over both years in Georgia. The corn leaf aphid was observed feeding on sorghum mainly during the vegetative stage, and the greenbug was observed mainly feeding in sorghum grain heads. Cotton aphids were present on cotton for much of the growing season, but they were mainly observed on this crop early in the season (late June-early July).

Scymnus spp. and *C. septempunctata* were the predominant species in sorghum; however, *C. septempunctata* and

TABLE 2: Percentage composition (within columns) of lady beetle species in sorghum trap crops, yellow pyramidal traps, cotton with sorghum trap crops, and control cotton.

Species	Percentage in sorghum trap crops ($n = 1789$)	Percentage in yellow pyramidal traps ($n = 20,313$)	Percentage in cotton w/sorghum trap crops ($n = 4804$)	Percentage in control cotton ($n = 2879$)
Scymnus spp.	51.9	16.5	28.6	43.0
C. septempunctata	33.9	38.1	13.7	14.8
H. convergens	6.3	6.3	31.7	22.6
H. axyridis	5.4	32.1	24.6	18.3
C. maculata	2.0	5.7	1.1	0.9
O. v-nigrum	0.4	0.1	0.2	0.2
C. munda	0.1	1.2	0.1	0.2

H. axyridis were the most abundant coccinellids captured in the yellow pyramidal traps (Table 2). Species composition was similar for cotton with or without (i.e., control) a trap crop with the predominant species being *Scymnus* spp., *C. septempunctata*, *H. convergens*, and *H. axyridis*. The lady beetle species in sorghum and cotton have been previously reported to colonize these crops [4, 13–17].

It was not surprising that yellow pyramidal traps (baited with an aggregation pheromone for *Euschistus* spp. stink bugs) captured adult lady beetles. Captures of lady beetles in this yellow trap, with or without the stink bug pheromone, are common (T.E.C., personal observation), and yellow sticky cards have been used in previous studies to sample adult Coccinellidae [23–27]. The similarity in lady beetle captures in traps and those sampled on sorghum may indicate that the yellow trap itself does not attract lady beetles from significant distances; lady beetle capture in the trap was likely facilitated by the attractiveness of the surrounding sorghum. Nevertheless, modifying the yellow pyramidal traps (intended to attract and kill stink bugs) to reduce lady beetle capture could conserve these predators in sorghum.

In 2006, lady beetle density remained relatively low in flowering and milking sorghum and then peaked during the soft dough stage of seed development (Figure 1). Generally, corn leaf aphids are first observed on sorghum when plants have three to five leaves, and then their numbers increase on vegetative sorghum until declining around the boot or early bloom stages [7]. Greenbugs also are present on sorghum during the three to five leaf stage, but their numbers do not increase until after plants have about 10 leaves, with peak abundance at the half bloom or soft dough stage and then declining as sorghum matures [7]. After week 4, density of adult lady beetles began an overall decline in sorghum, a likely result of prey depletion on sorghum. Apparently, as prey were depleted on sorghum, beetles moved from sorghum to yellow pyramidal traps during week 5 but their capture in these traps dropped precipitously thereafter (Figure 1). Density of lady beetles increased slightly on sorghum during the hard dough stage (i.e., when 75% of the grain dry weight has accumulated) and then declined as sorghum heads matured. In cotton, lady beetles first appeared in relatively low numbers in early July and peaked on cotton in late July-early August. Lady beetle density was significantly higher on cotton with sorghum trap crops than on control cotton during weeks 2 through 6 (Table 3). Altogether, these results indicate that sorghum was a source of adult lady beetles moving into cotton fields. Because cotton aphids were observed on cotton early in the season, lady beetles were likely responding to populations of cotton aphids in cotton. Aphidophagous lady beetles, though, can be generalist predators; therefore, when they moved into fruiting cotton, they were likely also preying on other pest insects that feed on cotton fruit such as lepidopteran pests and stink bug eggs [28, 29].

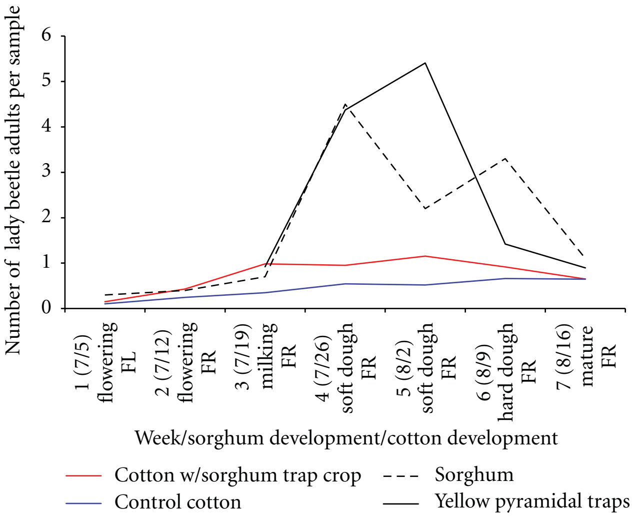

FIGURE 1: Mean number of lady beetle adults per sample in cotton with a sorghum trap crop, control cotton, sorghum, and yellow pyramidal traps in 2006. FL: flowers; FR: fruit. Number of lady beetles in yellow pyramidal traps divided by 10. Date refers to middle of sampling week.

In 2007, lady beetle abundance on sorghum followed a similar pattern as seen during 2006. Beetles first moved to flowering sorghum, and density peaked when sorghum heads reached the soft dough stage (Figure 2). Lady beetle density was significantly higher on cotton with sorghum trap crops than on control cotton during weeks 1 through 5 (Table 3). As above, these results suggest that sorghum can serve as a source of lady beetles dispersing to cotton.

Table 3: Number (mean ± SE) of lady beetle adults per 1.83 m of row in cotton with sorghum trap crops and control cotton in 2006 and 2007.

Year	Week	Cotton w/sorghum trap crop	Control cotton	$\lvert t \rvert$	df	P
2006	1	0.15 ± 0.02	0.106 ± 0.022	1.45	937	0.1465
	2	0.431 ± 0.039	0.246 ± 0.04	3.2	937	0.0014
	3	0.982 ± 0.076	0.349 ± 0.038	7.47	1132	0.0001
	4	0.952 ± 0.075	0.545 ± 0.052	4.51	1132	0.0001
	5	1.153 ± 0.094	0.52 ± 0.057	5.79	1132	0.0001
	6	0.918 ± 0.072	0.66 ± 0.053	2.91	1132	0.0037
	7	0.65 ± 0.059	0.648 ± 0.065	0.02	1327	0.9819
2007	1	0.611 ± 0.051	0.302 ± 0.039	4.6	503	0.0001
	2	0.447 ± 0.045	0.309 ± 0.036	2.4	536	0.0168
	3	0.732 ± 0.058	0.411 ± 0.045	4.39	563	0.0001
	4	0.637 ± 0.059	0.487 ± 0.049	1.97	413	0.049
	5	0.696 ± 0.075	0.479 ± 0.066	2.03	476	0.0432
	6	0.412 ± 0.047	0.338 ± 0.05	1.07	341	0.2845

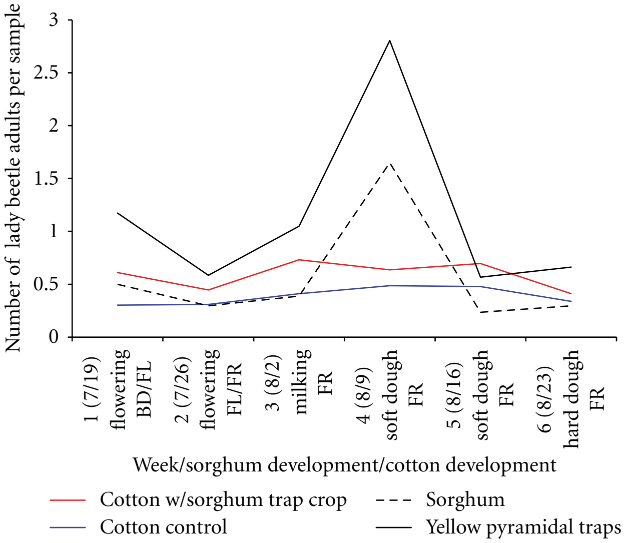

Figure 2: Mean number of lady beetle adults per sample in cotton with a sorghum trap crop, control cotton, sorghum, and yellow pyramidal traps in 2007. BD: buds; FL: flowers; FR: fruit. Number of lady beetles in yellow pyramidal traps divided by 10. Date refers to middle of sampling week.

In the current study, results suggest that adult lady beetles dispersed from sorghum into cotton. Previous studies also have demonstrated sorghum as a source of lady beetles moving into cotton. For example, populations of insect predators, including *H. convergens*, increased when feeding on greenbug in grain sorghum fields adjacent to cotton in Arizona [30, 31]. These predators dispersed into cotton as sorghum matured and the greenbug population declined. Cage studies also indicate that adult lady beetles disperse from sorghum into cotton in response to crop phenology and prey abundance [32]. In the current study, lady beetle density similarly declined as sorghum matured. In a study in Texas, as greenbug and corn leaf aphid numbers increased in sorghum, predators, including the predominant *Hippodamia* spp. predators, also increased [33]. They reported that predator levels in cotton began to increase at about the same time that predator density began to decrease in sorghum indicating that predators dispersed from sorghum into cotton. In fact, fluorescent dust marking demonstrated predator dispersal from sorghum into cotton. In another study using rubidium to mark predators in sorghum and cotton, *H. convergens* and *Scymnus loewii* Mulsant were documented to move from sorghum into cotton [32].

In these previous studies, adult lady beetles dispersed from sorghum into cotton in response to sorghum senescence and prey decline, but in our study, lady beetles continuously moved from sorghum to cotton throughout development of fruit in cotton. Although the reason for lady beetles continuously moving from sorghum into cotton was not determined, it was likely due to resource availability (e.g., prey, pollen, and extrafloral nectaries) in cotton. Perhaps, planting sorghum earlier would result in relaying lady beetles from senescing sorghum into cotton as documented in a 3 yr relay intercropping study in Texas [34]. There, the intercrops acted as a reservoir for predators, including lady beetles, during the noncotton season. These intercrops “relayed” the aphid predators from canola and wheat in the winter to sorghum in the spring and finally to cotton in the summer. Of the intercrop species tested, predator numbers were highest in sorghum. Average aphid abundance was lower in relay intercropped cotton than in isolated cotton, and average predator numbers were higher in relay intercropped cotton than in isolated cotton. Predators appeared in higher numbers earlier in the summer in relay intercropped cotton than in isolated cotton suggesting that this management strategy aids early colonization of predators in cotton, thereby inhibiting increase of the cotton aphid. In a 2 yr study in Texas, a sorghum relay strip-crop system enhanced numbers of predators, including lady beetles, and suppressed cotton aphid abundance in cotton [35]. It can be concluded from these two studies and the current study that incorporating a source crop for lady beetles in a cotton field can be a successful management tactic for control of cotton aphids. Also, a multifunctional habitat of sorghum to detract stink bugs from feeding and ovipositing on cash crops, and using pheromone traps to capture and kill stink bugs has great potential for suppressing stink bugs in cotton while preserving lady beetles.

Lady beetles in this study were present in cotton fields with or without sorghum indicating that these natural enemies disperse into cotton from other plants. Peanut fields were adjacent to all the cotton fields, but early-season host plants such as corn and rye were also prevalent in these agricultural landscapes. Because each of these crops harbors lady beetles [29, 36, 37], they likely contributed lady beetles to sorghum and cotton. Nevertheless, placement of a strip of sorghum along the cotton field edge near peanut enhanced abundance of lady beetles in cotton. Possible explanations for this enhancement include providing newly abundant prey during senescence of corn and rye, providing new or preferred prey for adults developing in peanut, concentrating abundant prey, and thus lady beetles, next to cotton fields. Regardless of the mechanisms involved, a habitat of sorghum can be utilized in conserving biocontrol of these natural enemies in cotton.

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Research Article

Classification, Natural History, and Evolution of Tarsosteninae (Coleoptera: Cleridae)—Part I: Generic Composition of the Subfamily and Key and Phylogeny of Genera

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Four new genera and one new species of the subfamily Tarsosteninae (Coleoptera: Cleridae) are described. The new genera are: *Agapetilus* Opitz, gen. nov., *Fallopylus* Opitz, gen., nov, *Globoclava* Opitz, gen. nov., and *Pseudopylus* Opitz, gen. nov. The new species involves *Agapetilus vietus* Opitz. sp. nov. *Liostylus* Fairmaire is synonymized with *Rhophaloclerus* Fairmaire. New combinations, *Fallopylus pallipes* (MacLeay, 1872), comb. nov., *Globoclava quadrimaculata* (Chevrolat, 1876), comb. nov., *Parapylus sedlaceki* (Kolibáč, 2003), comb. nov., *Pseudopylus okei* (Elston, 1929), comb. nov., and *Rhophaloclerus pictus* (Fairmaire, 1902), comb. nov., are established. A key and phylogeny of the genera of Tarsosteninae is provided.

1. Introduction

According to Opitz [1] there are six subfamilies in the Cleridae whose specimens have the fourth tarsomere reduced. The elucidation of the generic composition of these subfamilies, and their intrasubfamilial relationships, is the focus of the research program of the author. This contribution involves Tarsosteninae. It is the fourth of a series of works that makes known the generic composition of the six subfamilies referenced above. The first three contributions involve Epiphloeinae Kuwert [2], Neorthopleurinae Barr [3], and Korynetinae Laporte [4]. The revisions of the remaining two subfamilies, the Peloniinae Opitz and Enopliinae Gistel, are in various stages of preparation.

2. Taxonomic History

The majority of the generic taxa herein classified in Tarsosteninae were originally grouped under other subfamilies: *Rhophaloclerus* Fairmaire in Tillinae [5]; *Abeliella* Peracchi, *Curacavi* Solervicens, *Apteropilo* Lea, and *Neopylus* Solervicens in Enopliinae [5]; *Apopylus* Kolibáč, *Blackburniella* Chapin, *Parapylus* Blackburn, *Pylus* Newman, *Tarsostenodes* Blackburn, *Thriocera* Gorham; *Riotenerus* Pic in Peloniinae [1]; *Tarsostenosis* Heller and *Thriocerodes* Wolcott & Dybas in Korynetinae [6]. It is likely that the monotypic *Pallenothriocera* Pic, presently classified in Korynetinae, also belongs in Tarsosteninae. However, the specimen representing this nominal genus has not been found.

3. Material and Methods

For the most part the entire inventory of species of each genus was examined and several nonconspecific specimens of genera were disarticulated to examine the more cryptic structures of the integument. Methods and concepts involving dissection, measurements, terminology, specific and generic delimitations, and preparation of illustrations were similar to those implemented in [1].

4. Systematics

4.1. Phylogenetics of Genera. The concepts of Hennig's phylogenetics were implemented in this treatise [7]. This involved the preparation of a suite of character states and a character matrix (Table 1), which was analyzed via NONA [8] in combination with Winclada version 1.00.08 [9]. The analysis generated 58 trees, with 46 steps, index of consistency of 58, and an index of retention of 74. The 58 trees were examined and the one selected (Figure 24) most closely approximates a tree prepared manually. Heuristic analysis (maximum trees (hold) = 100, number of replications 9 (mult) = 100, and multiple TBR (mult max)) was used.

4.2. Character States. Twenty-seven character states were used to analyze the phylogenetic relationships among the genera of Tarsosteninae. Outgroups included taxa of Korynetinae [4]. Character states valued "0" are considered plesiotypic, whereas those assigned a value of "1" are interpreted as apotypic (Table 1). The methods by which the phylogenetic state of a characteristic is determined are well documented [10, 11].

Character 0

Unguis denticle: (0) absent; (1) present

Character 1

Pronotal tubercle: (0) absent; (1) present

Character 2

Pronotal tubercle: (0) slightly developed; (1) highly developed

Character 3

Asetiferous punctations: (0) present; (0) absent

Character 4

Ninth row of elytral asetiferous punctations: (0) not reduced; (1) reduced

Character 5

Elytral 2°: (0) present; (1) absent

Character 6

Terminal maxillary palpomere: (0) digitiform; (1) somewhat securiform

Character 7

Terminal maxillary palpomere: (0) subsecuriform; (1) securiform

Character 8

Tibial spur formula: (0) 2-2-2; (1) 0-1-1

Character 9

Tibial spur formula: (0) 2-2-2; (1) 1-2-1

Character 10

Tibial spur formula: (0) 2-2-2; (1) 0-0-0

Character 11

Tarsal pulvillar formula: (0) 3-3-3; (1) third pulvillus reduced

Character 12

Ommatidia: (0) large; (1) small

Character 13

Capitulum: (0) compact; (1) lax

Character 14

Pronotal sides: (0) smooth; (1) crenulated

Character 15

Eye: (0) large; (1) small

Character 16

Ocular plate: (0) small; (1) large

Character 17

Elytral asetiferous punctations nodes: (0) absent; (1) present

Character 18

Pronotal indentations: (0) absent; (1) present

Character 19

Pronotal collar: (0) not extended; (1) extended

Character 20

Elytral asetiferous punctations: (0) to elytral apex; (1) to elytral half

Character 21

Pronotal disc: (0) without glabrous elevations; (1) with glabrous elevations

Character 22

Pronotal disc: (0) without glabrous spots; (1) with glabrous spots

Character 23

Pronotal disc: (0) without narrow glabrous streaks; (1) with narrow glabrous streaks

Character 24

Gular process: (0) not confluent; (1) confluent

Character 25

Pronotal commissure: (0) absent; (1) present

Character 26

Capitulum: (0) not much shorter than rest of antennal length; (1) much shorter than rest of antenna.

4.3. Key to Genera of Tarsosteninae.

1 Unguis with denticle (Figure 15(j))	2
1′. Unguis without denticle (Figure 15(i))	4
2(1). Last antennomere globose, about three times larger than penultimate antennomere (South Africa)	***Globoclava* gen. nov**
2′. Last antennomere only slightly larger than penultimate antennomere	3
3(2′). Body form oblong narrow (Figure 22(b)), pronotum oblong (Tanzania)	***Agapetilus* gen.nov**
3′. Body form oblong broad (Figure 23(h)), pronotum transverse (Democratic Republic of the Congo, Kenya, Mozambique, South Africa, Tanzania)	***Thriocera* Gorham**
4(1′). Pronotal sides without vestige of tubercle (Figure 14(b))	5
4′. Pronotal sides with shallow (Figure 1(d)) or with well defined projecting tubercle (Figure 4(c))	7
5(4). Pronotum subquadrate (Madagascar)	***Rhophaloclerus* Fairmaire**
5′. Pronotum distinctly oblong	6
6(5′). Elytral disc with 8 rows of punctations; pronotal disc uniformly scabrous (Bolivia, Brazil, Uruguay)	***Abiliella* Peracchi**
6. Elytral disc with 10 rows of punctations; pronotal disc with glabrous streaks (Cosmopolitan)	***Tarsostenus* Spinola**
7(4′). Pronotal sides with shallow tubercle (Figure 1(d))	8
7′. Pronotal sides with well defined projecting tubercle (Figure 4(c))	14
8(7). Last maxillary palpomere distinctly securiform (Figure 12(a))	9
8′. Last maxillary palpomere subsecuriform (Figure 6(c))	13
9(8). Pronotal side margins serrulated (Chile)	***Curacavi* Solervicens**
9′. Pronotal side margins not serrulated	10
10(9′). Metabasitarsal pulvillus well developed	11
10′. Metabasitarsal pulvillus not well developed or absent	12
11(10). Pronotum distinctly oblong (Australia)	***Tarsostenodes* Blackburn**
11′. Pronotum quadrate (Australia)	***Blackburniella* Chapin**
12(10′). Elytral asetiferous punctations nodulated (Australia)	***Apopylus* Kolibáč**
12′. Elytral asetiferous punctations not nodulated (New Caledonia).	***Tarsostenosis* Heller**
13(8′). Pronotum distinctly oblong (Argentina)	***Riotenerus* Pic**
13′. Pronotum subquadrate (Australia)	***Thriocerodes* Wolcott & Dybas**
14(7′). Apical maxillary palpomere distinctly securiform	15
14′. Apical maxillary palpomere subsecuriform	17
15(14). Rows of elytral asetiferous punctations not defined (Chile)	***Neopylus* Solervicens**
15. Rows of elytral asetiferous punctations clearly defined	16
16(15). Pronotum with glabrous tumescences (Australia)	***Apteropilo* Lea**
16. Pronotum without glabrous tumescences (Australia)	***Pseudopylus* gen.nov.**
17(14′). Elytral base with tumescence (Australia)	***Parapylus* Blackburn**
17′. Elytral base without tumescence	18
18(17′). Tibial spur formula 2-2-1 (Australia)	***Pylus* Newman**
18′. Tibial spur formula 1-2-2 (Australia)	***Fallopylus* gen. nov.**

4.4. Description of Tarsosteninae

Type Genus. Tarsostenus Spinola [12].

Diagnosis. These beetles have a reduced 4th tarsomere, do not have a pair of pronotal trichobothria, and have long capitate antennae in which the funicular antennomeres are filiform and the length of the capitulum is not as long as the combined length of the remainder of the antennomeres. The incomplete dorsolateral pronotal carina is confluent with the pronotal hem at the posterior angles of the pronotum.

Description. Shape: ranges from narrow rectangulate to short rectangulate. *Size*: length 2.2–14.0 mm; width 0.6–6.0 mm. *Integumental color*: Varies from uniformly reddish-brown to multicolored conditions where the integument is mostly dark brown and the elytral disc shows a paler fascia, in very few cases the integument may be shiny blue or shiny multicolored with red, yellow and brown. *Head*: transverse, strongly deflexed, usually narrower than pronotum, surface usually finely punctated; epistomal suture faintly indicated; internal epistomal ridge poorly developed; clypeus bipartite, comprised of pigmented upper region and nonpigmented

Table 1: Character matrix for 27 morphological characters of Tarsosteninae genera.

	Characters																										
Taxa											1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2
	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6
Outgroup	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0
Abeliella	0	0	0	0	0	0	1	1	1	0	0	1	0	1	0	0	0	1	0	0	0	0	0	0	0	0	1
Agapetilus	1	0	0	1	0	0	1	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1
Apopylus	0	1	0	0	1	1	1	0	0	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	1
Apteropilo	*0*	1	1	0	0	0	1	1	0	0	0	0	0	0	1	1	1	1	1	0	0	1	1	0	0	0	1
Blackburniella	0	1	0	0	0	0	1	1	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1
Curacavy	0	1	0	0	0	1	1	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	1
Fallopylus	0	1	1	0	1	1	1	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	1
Globoclava	1	0	0	0	0	0	1	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Neopylus	0	1	1	0	0	0	1	1	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	1
Parapylus	0	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	1
Pseudopylus	0	1	1	0	1	1	1	0	0	0	1	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	1
Pylus	0	1	1	0	1	1	1	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	1
Rhophaloclerus	0	0	0	0	0	0	1	1	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1
Riotenerus	0	1	0	0	0	0	1	0	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1
Tarsostenodes	0	1	0	0	0	0	1	1	0	1	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	1
Tarsostenosis	0	1	0	0	0	0	1	1	0	1	0	1	0	1	0	0	0	0	0	0	1	1	0	1	0	0	1
Tarsostenus	0	0	0	0	0	0	1	1	0	1	0	0	0	1	0	0	0	0	0	0	0	1	0	1	0	0	1
Thriocera	1	0	0	0	0	0	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1
Thriocerodes	0	0	0	0	0	0	1	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1

lower region; antenna comprised of 11 antennomeres, capitate, capitulum shorter than length of combined other antennomeres, noncapitular antennomeres filiform; frontal preantennal angle not acute; eyes coarsely to finely faceted, slightly notched anteriorly; labrum shallowly incised, transverse tormal processes fused contiguous; epipharynx not complex; last palpomere of maxillary and labial palpus boldly or slightly securiform; mandible with well-developed dens, basal notch not large; gula large, gular processes widely separated, gular sutures strongly converging. *Thorax*: pronotum usually transverse-quadrate, or elongate, lateral tubercle absent or strongly developed, anterior transverse depression present or not, dorsolateral carina incomplete or complete, always posteriorly confluent with pronotal hem, pronotal commissure absent; pronotal projections vary in lengths, prointercoxal process linear or expanded distally; pronototergosternal suture complete; procoxal cavity open, procryptosternum incomplete; metendosternite with furcal lamina; elytral form usually elongate rectangulate or short rectangulate, anterior margin with carina, disc with asetiferous punctations, 1° and 2° setae usually present, epipleural fold laterally positioned, gradually narrowing to elytral apical four-fifths, elytral punctations, plain, or bimodal or tetranodal; metathoracic wings present or not; legs, tarsal formula 5-5-5, cursorial, tibial spur formula 2-2-2, 2-2-1, 1-2-2, 1-2-1, 0-2-2, or 0-0-0, tarsal pulvillar formula 3-3-3 or 3-3-2; unguis with (Figure 15(j)) or without denticle (Figures 2(f) and 15(i)); wedge cell of metathoracic wing present or not, when present closed or open. *Abdomen*: comprised of 6 visible sternites, 6th visible sternite usually beneath 5th, robust and compact; pygidium quadrate or scutiform; aedeagus sometimes inverted, well sclerotized, tegmen tubular very sclerotized or lightly sclerotized, bilobed distally, tegminal lobes usually fimbriate, phallobasic rod variously developed, phallobasic apodeme well developed, phallic plates variously developed; spicular fork well developed, intraspicular plate linear, spicular apodeme variously fused; ovipositor not longer than abdomen, with multilobed dorsal and ventral lamina; oblique and ventral bacculi well developed. *Alimentary canal* (Figure 18(m)): stomodaeum short, proventricular valve comprised of 4 primary lobes (Figure 18(j)); ventriculus well developed, ventricular crypts poorly developed; 4 cryptonephridial Malpighian tubules; proctodaeum short in males and long in females. *Mesodermal male reproductive organs*: typically with two pairs of accessory glands, rarely with one pair of glands; testes comprised of multiple follicles. *Mesodermal female reproductive organs*: spermathecal capsule from faintly to highly sclerotized, spermathecal gland attached to apex or subapex of spermathecal capsule; saccular bursal copulatrix well developed bursal sclerite present or not; ovaries comprised of multiple follicles.

4.5. Descriptions of Genera of Tarsosteninae

4.5.1. Abiliella Peracchi (Figures 1, 2, and 22(a)). *Abiliella* Peracchi [13]. *Type species: Abiliella fasciata* Peracchi [13]. By monotypy.

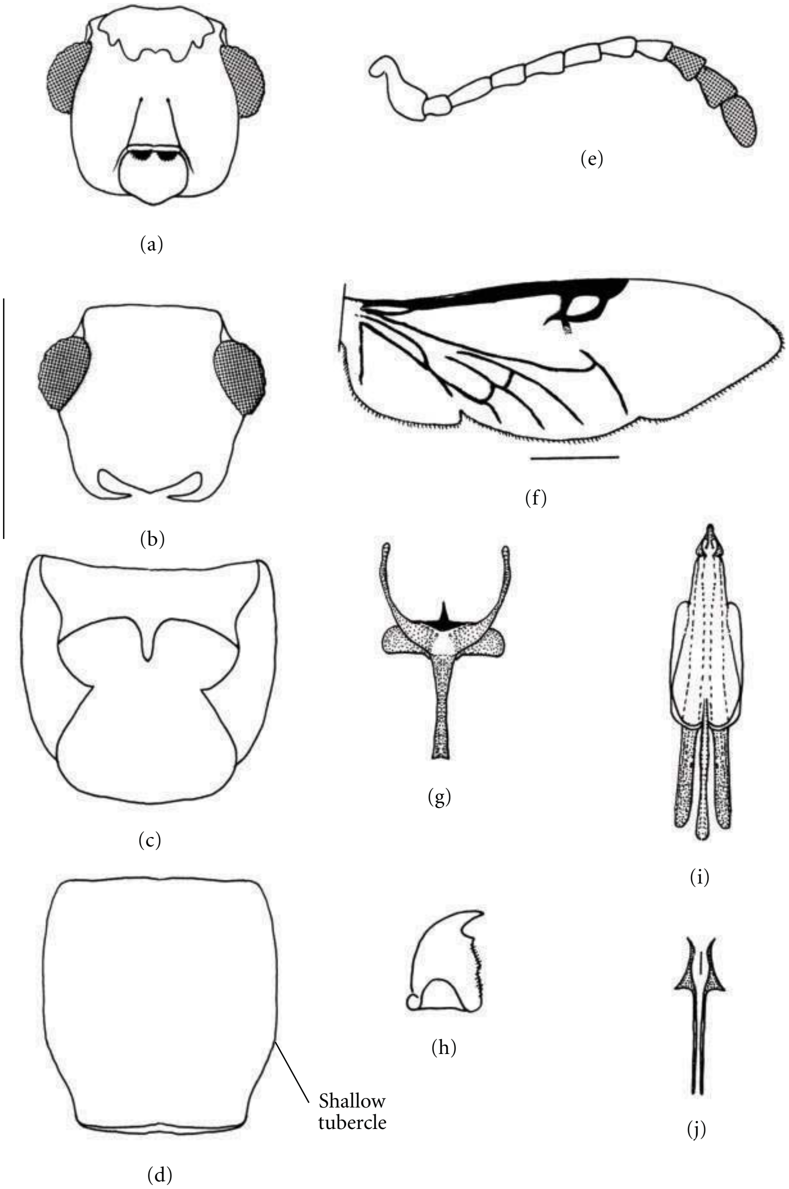


Figure 1: Various organs of *Abiliella fasciata*. (a, b) Head ((a) ventral, (b) dorsal). (c, d) Pronotum ((c) ventral, (d) dorsal). (e) Antenna. (f) Metathoracic wing. (g) Metendosternite. (h) Mandible. (i) Aedeagus. (j) Spiculum.

Synapotypic Characteristics. Pronotum elongate, elytral punctations binodal, epipleural margin serrulated, unguis without denticle, phallic plates very broad, and phallobasic lobes not fimbriate.

Diagnosis. Specimens of *Abiliella* are distinguishable from the superficially similar specimens of *Tarsostenus* by having only eight rows of elytral punctations; the elytral disc of *Tarsostenus* specimens have 10.

Description. Size: length 4.0–9.0 mm; width 1.2–2.8 mm. *Form* (Figure 22(a)): oblong rectangulate, about 3 times longer than broad. *Vestiture*: disc of cranium and pronotum vested with white setae, elytral disc vested with 1° setae and shorter profusely distributed 2° setae. *Head* (Figures 1(a), 1(b), and 2(a)): cranium quadrate, frons wider than or narrower than width of eye, indented with large setiferous punctations that give cranium rugose appearance; gula (Figure 1(a)), large, trapezoidal, sutures oblique, gular processes widely separated, processes in form of two setiferous tubercles; labrum short, medial incision shallow, transverse tormal processes curvate, not confluent, epipharyngeal plate very small; mandible (Figure 1(h)), body short, anterior, medial dens well developed, posterior dens not well developed, penicillus well developed; maxilla (Figure 2(b)), laterolacinia present, terminal palpomere securiform; labium (Figure 2(b)), ligula not deeply incised, terminal palpomere

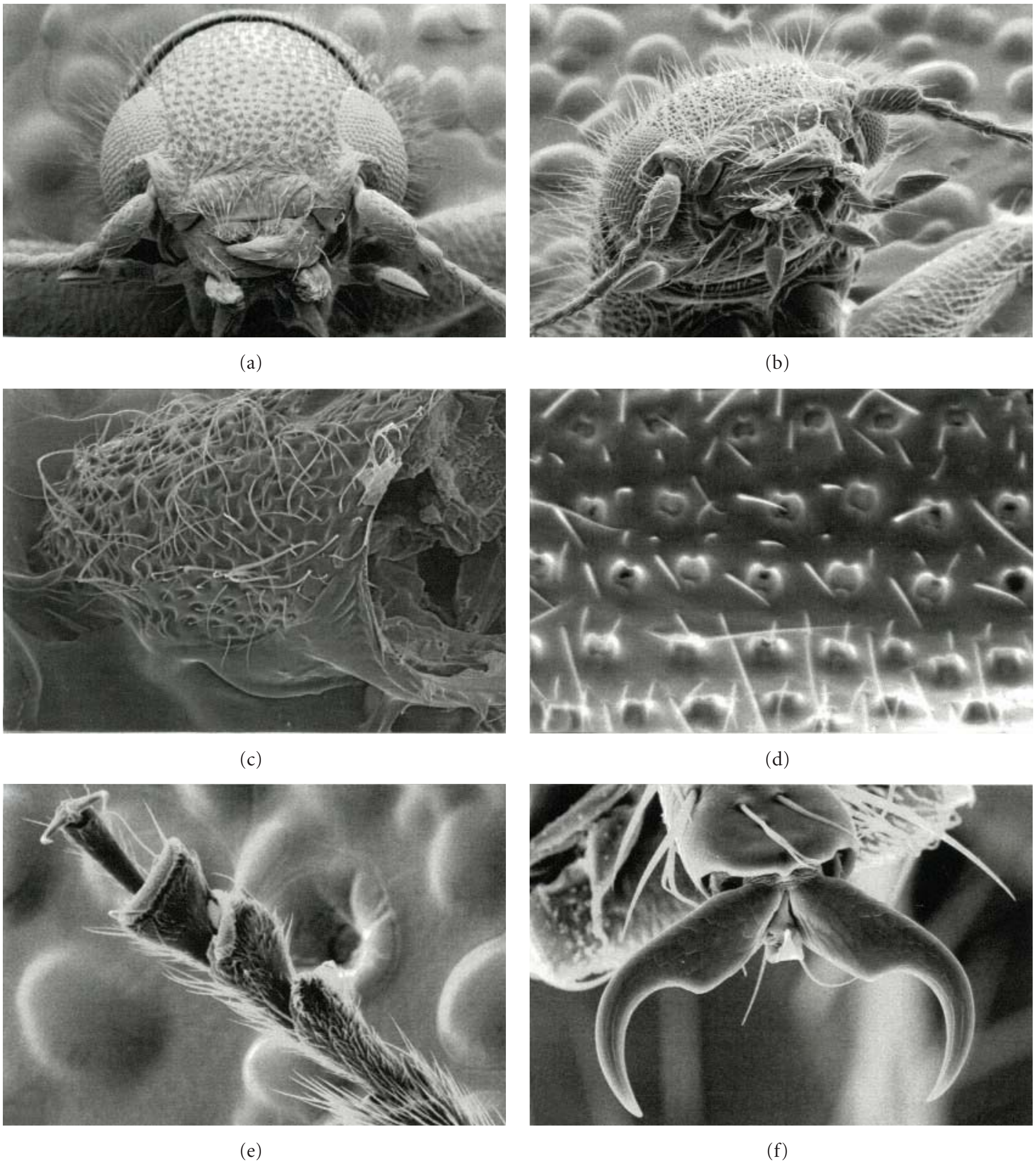


Figure 2: Various organs of *Abiliella fasciata*. (a) Head. (b) Mouthparts. (c) Pronotum (posterolateral angle). (d) Elytral surface (shows binodal asetiferous punctations). (e) Metatarsus. (f) Metatarsal unguis (shows absence of denticle).

securiform; eyes small or large, coarsely faceted, ocular notch large; antenna (Figure 1(e)), capitate, capitulum lax and narrow, scape about as long as combined length of pedicel and antennomere 3, funicular antennomeres filiform, capitular antennomeres narrow, antennomeres 9 and 10 subrectangular, antennomere 11 ovoid. *Thorax*: pronotum (Figures 1(c), 1(d), and 2(c)), elongate, convex, side margins slightly sinuous, sculptured with large round setiferous punctations, dorsolateral ridge extends from posterior angle to anterior angle (Figure 2(b)), surface smooth and not fractured by coarse punctations, prebasal fissure well developed, prointercoxal process not expanded distally; pronotal projections short; elytron sculptured with large spheroid asetiferous punctations, latter seriate and binodal (Figure 2(d)), 1° setae always adjacent to asetiferous punctations, 2° setae present, arranged serially, epipleural fold laterally positioned, extended to elytral apex, margin minutely serrulated, anterior margin carinate; metathoracic wing (Figure 1(f)), wedge cell open; metendosternite (Figure 1(g)), with furcal lamina, furcal anterior plate diminutive, acuminate; legs, tibial spur formula 0-1-1, tarsal pulvillar formula 3-3-3 (Figure 2(e)), unguis without denticle. *Abdomen*: aedeagus (Figure 1(i)), shorter than length of abdomen, phallobase lobate distally, lobes not fimbriate; phallic lateral plates very broad, spicular plates triangular, acuminate, rarely, spicular apodemes not fused (Figure 1(j)), intraspicular plate rod shaped; ovipositor, ventral and dorsal laminae unilobed, laminal rod present; distal margin of pygidium not incised, distal margin of male 6th sternite slightly incised. *Alimentary canal*: no information available. *Male mesodermal internal reproductive organs*: not studied. *Female mesodermal internal reproductive organs*: not studied.

Distribution. The members of this genus have been found only in Brazil and Bolivia.

Species Examined. Abiliella fasciata Peracchi and one new undescribed species.

4.5.2. Agapetilus gen. nov. (Figure 22(b)). Type species: Agapetilus vietus Opitz, sp. nov. Herein designated.

Synapotypic Characteristics. Pronotal wrinkles.

Diagnosis. The pronotal disc is profusely sculptured with wrinkles.

Description. Size: length 5.2 mm; width 1.2 mm. *Form* (Figure 22(b)): oblong, narrow rectangulate, about 5 times longer than broad. *Vestiture*: disc of cranium and pronotum densely vested with pale setae, elytral disc vested with 1° setae, 2° setae absent. *Head*: cranium quadrate, frons much wider than width of eye, indented with large setiferous punctations; gula large, trapezoidal, sutures oblique, gular processes widely separated, processes in form of two setiferous tubercles; labrum short, deeply incised, tormal processes not examined, epipharyngeal plate not examined; mandible, body short, anterior and medial dens well developed, posterior dens not well developed, penicillus not verified; maxilla well developed, laterolacinia not verified, terminal palpomere subsecuriform; labium, ligula deeply incised, terminal palpomere securiform; eyes small, coarsely faceted, ocular notch large; antenna, capitate, capitulum compact, scape about as long as combined length of pedicel and antennomere 3, funicular antennomeres filiform, capitular antennomeres slightly expanded, antennomeres 9 and 10 subtriangular, antennomere 11 ovoid. *Thorax*: pronotum oblong, disc sculptured with many wrinkles, prebasal fissure shallow, prointercoxal process not expanded distally, pronotal projections long; elytron sculptured with small setiferous punctations, basal tumescences present, asetiferous punctations absent, 1° setae present, 2° setae concentrated into medial fascia, interstitial spaces smooth, epipleural fold laterally positioned, very narrow to elytral apex, anterior margin not carinate; metathoracic wing not examined; metendosternite not examined; legs, tibial spur formula 0-2-2, tarsal pulvillar formula 3-3-3, unguis with denticle. *Abdomen*: aedeagus shorter than length of abdomen, phallobase not reduced, not lobate nor fimbriate; phallic lateral plates broad, phallobasic rod absent, phallic apex robust, spiculum not examined; ovipositor not examined. *Alimentary canal*: not studied. *Male mesodermal internal reproductive organs*: not studied. *Female mesodermal internal reproductive organs*: not studied.

Distribution. This monotypic genus is known only from Tanzania.

Species Examined. Agapetilus vietus Opitz, sp. nov.

Etymology. The generic name Agapetilus stems from the Latin petilus (=slender) and the intensive prefix *aga-* (=very). I refer to the slender body form of the type species.

4.5.3. Agapetilus vietus Opitz, sp. nov.

Type Material [Holotype ♂]. Tanzania, Tanga, Lushoto Dist., Mazumbai For. Res. 4°49′S 38°29′E, 1650–1730 m, 27. XI.1995, Fog 29 II, Zmuc Denmark (Institute Royal des Sciences Naturelles de Belgique).

Description. Form: oblong slender. *Size*: length 5.2 mm; width 1.2 mm. *Integumental color*: Antenna, legs, and posterior half of elytral disc, and abdomen yellow-brown, forebody, pterothorax, and anterior half of elytral disc brown, with white fascia across middle of elytral disc. *Male genitalia*: Aedeagus very short, tegmen without lobes and posterior limit not fimbriate; phallic plates broad and apex pronounced.

Distribution. Known only from Tanzania.

Etymology. The specific epithet *vietus* (=wrinkled) is a Latin adjective. I refer to the extensive wrinkling on the pronotal disc.

4.5.4. Apopylus Kolibáč (Figures 4(h) and 22(c)). Apopylus Kolibáč [6]. *Type species: Apopylus unumgarensis* Kolibáč [6]. By monotypy.

Synapotypic Characteristics. The restriction of the tetranodal punctations to the posterior four-fifths of the elytral disc is a uniquely derived characteristic of this genus.

Diagnosis. The restriction of the tetranodal punctations to the posterior four-fifths of the elytral disc will conveniently distinguish the members of this genus within Tarsosteninae.

Description. Apopylus Kolibáč and its type species were adequately described by Kolibáč [6]. The metendosternite of *Apopylus unumgarensis* specimens have well-developed laminae.

Species Examined. Apopylus unumgarensis Kolibáč and one undescribed species.

4.5.5. Apteropilo Lea (Figures 5(a)–5(h), 6(e), 6(f), and 22(d)). Apteropilo Lea [14]. Type species*: Apteropilo pictipes* Lea [14]. By monotypy. Corporaal [5]. Kolibáč [6] (*Pylusopsis* Elston), Bartlett [15].

Synapotypic Characteristics. Pronotal disc with two glabrous tumescences, pronotal sides with two extraordinarily large setiferous punctations, metendosternite without furcal anterior plate.

Diagnosis. The combination of glabrous tumescences on the pronotal disc present, elytral punctations binodal, and each side of the pronotum with two large setiferous punctations will conveniently distinguish the members of this genus within Tarsosteninae.

Description. Size: length 3.5–6.0 mm; width 1.2–2.0 mm. *Form*: Figure 22(d) oblong short rectangulate, rarely hind body suboval, about 2.5 times longer than broad. *Vestiture*: disc of cranium and pronotum vested with pale setae, elytral disc vested with 1° setae and shorter profusely distributed 2° setae. *Head* (Figures 5(a) and 5(b)): cranium quadrate, frons wider than width of eye, indented with large setiferous punctations that give cranium rugose appearance, or cranial indentations small and widely separated; gula (Figure 5(a)), large, trapezoidal, sutures oblique, gular processes widely separated, processes in form of two setiferous tubercles; labrum short, deeply incised, transverse tormal processes not confluent, epipharyngeal plate very small; mandible (Figure 5(d)), body short, anterior, medial dens well developed, posterior dens not well developed, penicillus well developed; maxilla, laterolacinia present, terminal palpomere securiform; labium, ligula not deeply incised, terminal palpomere securiform; eyes small, coarsely faceted, ocular notch small; antenna (Figure 5(h)), capitate, capitulum lax or not, scape about as long as combined length of pedicel and antennomere 3, funicular antennomeres filiform, antennomeres 9 and 10 triangular, antennomere 11 ovoid. *Thorax*: pronotum (Figures 5(c) and 5(g)), transverse, disc with two glabrous tumescences that sometimes are confluent with other elevations, side margins with distinct tubercles, usually two round punctations particularly large near sides, rest of disc with large round setiferous punctations, dorsolateral ridge extends from posterior angle to anterior angle, surface coarse and fractured by coarse punctations, sclerotized region above pronotal projection glabrous, prebasal fissure well developed, prointercoxal process not expanded distally; pronotal projections short; elytron sculptured with large spheroid asetiferous punctations, latter seriate and binodal, 1° setae always adjacent to asetiferous punctations, 2° setae densely distributed, not arranged serially, epipleural fold laterally positioned, extended to elytral apex, anterior margin carinate; metathoracic wing (Figure 5(f)), rarely absent, wedge cell open; metendosternite (Figure 5(e)), with furcal lamina, furcal anterior plate absent; legs, tibial spur formula 0-2-1, tarsal pulvillar formula 3-3-3, unguis without denticle (Figure 6(f)). *Abdomen*: Aedeagus shorter than length of abdomen, phallobase reduced, lobate distally, lobes fimbriate; phallic lateral plates very broad, spicular plates narrowly triangular, acuminate, spicular apodemes not fused, intraspicular plate rod-shaped; ovipositor, ventral and dorsal laminae unilobed, laminal rod present; distal margin of pygidium not incised, distal margin of male sixth sternite slightly incised. *Alimentary Canal*: Not studied. *Male mesodermal internal reproductive organs*: not studied. *Female mesodermal internal reproductive organs*: Spermathecal capsule well sclerotized, tubular, elongate, spermathecal gland attached to apex of spermathecal capsule.

Distribution. The members of this genus have been found in southeastern and Western Australia and on Kings Island, off the Bass Strait.

Species Examined. Apteropilo chrysocome (Elston), *A. pictipes* Lea, *A. raldae* Bartlett, and *A. volans* Bartlett.

Notes. Bartlett [15] provided habitus illustrations of each species and drawings of male genitalia.

4.5.6. Blackburniella Chapin (Figures 7(a)–7(h), 12(a), 12(b), and 22(e)).

Blackburniella Chapin [16]. *Type species: Thanasimomorpha intricta* Blackburn [17]. By original designation. Corporaal [5]. Matthews [18]. Kolibáč [6].

Erolestus Wolcott [19]. Synonymized by Wolcott, 1947.

Synapotypic Characteristics. Spermathecal duct very long, tuft of setae on elytral umbo, phallic apex extended.

Diagnosis. The tuft of black setae on the elytral umbo will distinguish the members of this genus from others in the subfamily.

Description. Size: length 4.2–8.0 mm; width 1.2–2.0 mm. *Form*: Figure 22(e), oblong long rectangulate, about 4 times longer than broad. *Vestiture*: disc of cranium and pronotum vested with pale setae, elytral disc vested with 1° setae and shorter profusely distributed 2° setae, elytral umbo covered with tuft of black setae, elytral setae very densely distributed in elytral apical half. *Head* (Figures 7(a), 7(b), and 12(a)): cranium quadrate, frons wider than width of eye, indented with large setiferous punctations that give cranium rugose appearance; gula (Figure 7(a)), large, trapezoidal, sutures oblique, gular processes widely separated, processes in form of two setiferous tubercles; labrum short, deeply incised, transverse tormal processes confluent, epipharyngeal plate very small; mandible, body short, anterior, medial dens well developed, posterior dens not well developed, penicillus well developed; maxilla (Figure 12(b)), laterolacinia present, terminal palpomere securiform; labium, ligula not deeply incised, terminal palpomere securiform; eyes small, coarsely faceted, ocular notch large; antenna (Figure 7(g)), capitate, capitulum lax, somewhat narrow, scape about as long as combined length of pedicel and antennomere 3, funicular antennomeres subfiliform, capitular antennomeres somewhat narrow, antennomeres 9 and 10 triangular, antennomere 11 ovoid. *Thorax*: pronotum (Figures 7(c) and 7(d)), from subquadrate to longer than broad, disc indented with large setiferous punctations, side margins sinuous, rounded near middle, dorsolateral ridge spans posterior third of pronotal sides, ridge surface coarse, sclerotized region above pronotal projection glabrous, prebasal fissure shallow, prointercoxal process not expanded distally; pronotal projections short; elytron sculptured with large spheroid asetiferous punctations in basal half, punctations minute in distal half, punctations seriate in basal half, not binodal, 1° setae always adjacent to asetiferous punctations, 2° setae densely distributed, not arranged serially, epipleural fold laterally positioned, extended to elytral apical three-fourths, anterior margin carinate; metathoracic wing (Figure 7(f)), wedge cell open; metendosternite (Figure 7(e)), with furcal lamina, furcal anterior plate prominent; legs, tibial spur formula 1-2-1, tarsal pulvillar formula 3-3-3, unguis without denticle. *Abdomen*: aedeagus shorter than length of abdomen, phallobase not reduced, lobate distally, lobes fimbriate; phallic lateral plates narrow, phallic apex extended, spicular plates very narrow, spicular apodemes fused at extremity, intraspicular plate rod-shaped; ovipositor, ventral and dorsal laminae unilobed, laminal rod present; distal margin of pygidium not incised, distal margin of male sixth sternite slightly incised. *Alimentary canal*: not studied. Male *mesodermal internal reproductive organs*: Not studied. *Female mesodermal internal reproductive organs* (Figure 7(h)): spermathecal capsule capitates, well sclerotized, spermathecal gland attached to base of spermathecal capsule, spermathecal duct very long, saccular bursa copulatrix present.

Distribution. The members of this genus have been found only in Australia.

Species Examined. Blackburniella intricata (Blackburn) and one new species.

Notes. Kolibáč [6] provided drawings of the male genitalia and female mesodermal reproductive organs.

4.5.7. *Curacavi Solervicens (Figures 3(a)–3(h), 6(a) and 6(b)).*

Curacavi Solervicens [20].*Type species: Curacavi dentatus* Solervicens [20]. By monotypy.

Synapotypic Characteristics. Pronotal punctations elongate, epipleuron in ventral position.

Diagnosis. The serrulated lateral margins of the pronotum will distinguish the members of this genus within Tarsosteninae.

Description. Size: length 4.0 mm; width 1.8 mm. *Form* (Figure 3(a)): oblong rectangulate, about 3 times longer than broad. *Vestiture*: disc of cranium and pronotum vested with dark setae, elytral disc vested with 1° setae and shorter sparsely distributed 2° setae. *Head* (Figure 6(a)): cranium quadrate, frons much wider than width of eye, indented with small setiferous punctations and lager asetiferous punctations; gula large, trapezoidal, sutures oblique, gular processes widely separated, processes in form of two setiferous tubercles; labrum short, medial incision shallow, mandible, body short, anterior and medial dens well developed, posterior dens not well developed, penicillus well developed; maxilla, laterolacinia present, terminal palpomere subsecuriform; labium, ligula not deeply incised, terminal palpomere securiform; eyes very small, coarsely faceted, ocular notch large; antenna (Figure 3(d)), capitate, capitulum lax and narrow, scape about as long as combined length of pedicel and antennomere 3, funicular antennomeres filiform, capitular antennomeres narrow, antennomeres 9 and 10 near capitate, antennomere 11 ovoid. *Thorax*: pronotum (Figures 3(b), 3(c), and 6(b)), transverse, convex, lateral margins serrulated, sculptured with large round setiferous punctations at anterior of disc, punctations elongate in remainder of disc, prebasal fissure well developed, prointercoxal process not expanded distally; pronotal projections short; elytron sculptured with large spheroid asetiferous punctations, latter seriate and binodal, 1° setae always adjacent to asetiferous punctations, 2° setae present, arranged serially, epipleural fold ventrally positioned, extended to elytral apex, margin minutely serrulated, anterior margin carinate; metathoracic wing with open wedge cell; metendosternite (Figure 3(e)), with furcal lamina, furcal anterior plate large, transverse; legs, tibial spur formula 2-2-2, tarsal pulvillar formula 3-3-3, unguis without denticle. *Abdomen*: aedeagus as long as length of abdomen, phallobase lobate distally (Figure 3(g)), lobes acuminate, not fimbriate; phallic lateral plate not very broad (Figure 3(h)), acuminate, with preapical uncus, spicular plates slender, with lateral uncus, spicular apodemes not fused (Figure 3(f)), intraspicular plate rod-shaped; ovipositor, ventral and dorsal laminae unilobed, laminal rod present; distal margin of pygidium not incised, distal margin of male sixth sternite slightly incised. *Alimentary canal*: stomodaeal valve comprised of four primary lobes, dorsal lobe very broad and half as long as other three lobes. *Male mesodermal internal reproductive organs*: Not studied. *Female mesodermal internal reproductive organs*: spermathecal capsule well sclerotized, barrel shaped, spermathecal gland attached to apex of spermathecal capsule.

Distribution. The members of this genus have been found only in Chile.

Species Examined. This is a monotypic genus.

Notes. For illustrations see Solervicens [21].

4.5.8. *Fallopylus gen. nov. (Figures 8(a)–8(j), 12(c), 12(d), 21(f), and 22(f)).*

Type species: Pylus pallipes MacLeay [22]. Kolibáč [6].

Synapotypic Characteristics. Bursal sclerites cyclic.

Diagnosis. Blackburniella Chapin, *Tarsostenodes* Blackburn, *Tarsostenus* Spinola, and *Fallopylus,* gen. nov., are all characterized by a 1-2-1 tarsal spur formula. However, in specimens of *Fallopylus* there is a distinct tubercle on the lateral margins of the pronotum, which is not the case in specimens of the other three aforementioned genera.

Description. Size: length 4.0–7.5 mm; width 1.4–3.2 mm. *Form* (Figure 22(f)): oblong short rectangulate, about 3 times longer than broad. *Vestiture*: disc of cranium and pronotum vested with pale setae, elytral disc vested with 1° setae and shorter 2° setae. *Head* (Figures 8(a) and 8(b)): cranium quadrate, frons slightly wider than width of eye, indented with very small setiferous punctations; gula (Figure 8(a)), large, trapezoidal, sutures oblique, gular processes widely separated, processes in form of two setiferous tubercles; labrum short, deeply incised, transverse tormal processes confluent, epipharyngeal plate very small; mandible (Figure 8(g)), body long, anterior and medial dens well developed, posterior dens not well developed, penicillus well developed; maxilla, laterolacinia present, terminal palpomere securiform or subsecuriform; labium, ligula deeply incised, terminal palpomere securiform; eyes large, coarsely faceted, ocular notch small; antenna (Figure 8(e) and 21(f)), capitate, capitulum lax, scape about as long as combined length of pedicel and antennomere 3, funicular antennomeres subfiliform, capitular antennomeres expanded, antennomeres 9 and 10 capitate, antennomere 11 ovoid, rarely acuminate. *Thorax*: pronotum (Figures 8(c) and 8(d)), transverse, disc indented with small setiferous punctations, lateral margins with tubercle sinuous, dorsolateral ridge extends from posterior angle to anterior angle, not fractured by coarse punctations, prebasal fissure prominent, prointercoxal process expanded distally; pronotal projections long; elytron sculptured with small or large bimodal asetiferous punctations, punctations seriate and extend to elytral apex, 1° setae always adjacent to asetiferous punctations, 2° setae sparsely distributed, arranged serially, epipleural fold laterally positioned, narrowed to elytral apex three, anterior margin carinate; metathoracic wing (Figure 8(h)), wedge cell closed; metendosternite (Figure 8(f)), with furcal lamina, furcal anterior plate prominent; legs, tibial spur formula 1-2-2, tarsal pulvillar formula 3-3-3, unguis without denticle. *Abdomen*: aedeagus (Figure 8(j)), shorter than length of abdomen, phallobase reduced, lobate and narrowed distally, lobes not fimbriate; phallobasic rod transverse, phallic lateral plates very spinous, phallic apex minute, spicular plates very narrow (Figure 8(i)), spicular apodemes not fused at extremity, intraspicular plate rod shaped; ovipositor, ventral and dorsal laminae unilobed, laminal rod present; distal margin of pygidium not incised, distal margin of male sixth sternite slightly incised. *Alimentary canal*: not studied. *Male mesodermal internal reproductive organs*: not studied. *Female mesodermal internal reproductive organs*: spermathecal capsule ovoid, well sclerotized, spermathecal gland attached to base of spermathecal capsule, spermathecal duct long, saccular bursa copulatrix present, with cyclic basal sclerites.

Distribution. The members of this genus have been found only in Australia.

Species Examined. Fallopylus pallipes (MacLeay) (new combination) and three new undescribed species.

Etymology. The generic name *Fallopylus* stems from the Latin *fallo* (=deceive) and the name of the genus *Pylus* Newman.

Notes. Kolibáč [6] provides drawings of the female mesodermal reproductive organs.

4.5.9. Globoclava gen. nov. (Figure 22(g)).

Type species: Pilus (=*Pylus*) *quadrimaculata* Chevrolat [23]. Shifting *Pilus quadrimaculata* Chevrolat from synonymy to a valid species of *Globoclava* represents a *new status* and *new combination*.

Synapotypic Characteristics. Last antennomere very large.

Diagnosis. The extensively globose development of the last antennomere will distinguish the members of this genus from other genera.

Description. Size: length 4.0–5.5 mm; width 1.2–1.6 mm. *Form* (Figure 22(g)): oblong short rectangular, about 3 times longer than broad. *Vestiture*: disc of cranium and pronotum vested profusely with erect setae, elytral disc vested with tall erect setae and shorter decumbent setae. *Head*: cranium quadrate, frons much wider than width of eye, indented with large setiferous punctations; gula large, trapezoidal, sutures oblique, gular processes widely separated, processes in form of two setiferous tubercles; labrum short, deeply incised, transverse tormal processes confluent, epipharyngeal plate very small; mandible, body short, anterior and medial dens well developed, posterior dens not well developed, penicillus well developed; maxilla, laterolacinia not studied, terminal palpomere subsecuriform; labium, ligula not incised, terminal palpomere subsecuriform; eyes large, finely faceted, ocular notch large; antenna, capitate, capitulum compact, scape about as long as combined length of pedicel and antennomere 3, funicular antennomeres filiform, capitular antennomeres expanded, antennomeres 9 and 10 expanded, antennomere 11 very large and ovoid. *Thorax*: pronotum quadrate, disc indented profusely with large setiferous punctations, lateral margins convex, lateral tubercle poorly developed, dorsolateral ridge extends from posterior angle to anterior angle, not fractured and with smooth surface, sclerotized region above pronotal projection glabrous, prebasal fissure shallow, prointercoxal process expanded distally; pronotal projections short; elytron sculptured with asetiferous punctations, punctations subseriate, epipleural fold laterally positioned, narrowed at elytral posterior two-thirds, anterior margin carinate; metathoracic wing not studied; metendosternite not studied; legs, tibial spur formula 0-1-1, tarsal pulvillar formula 3-3-3, pulvillus of metabasitarsomere very reduced, unguis with denticle. *Abdomen*: not examined. *Alimentary canal*: not studied. Male mesodermal internal reproductive *organs*: not studied. *Female mesodermal internal reproductive organs*: not studied.

Distribution. Known only from South Africa.

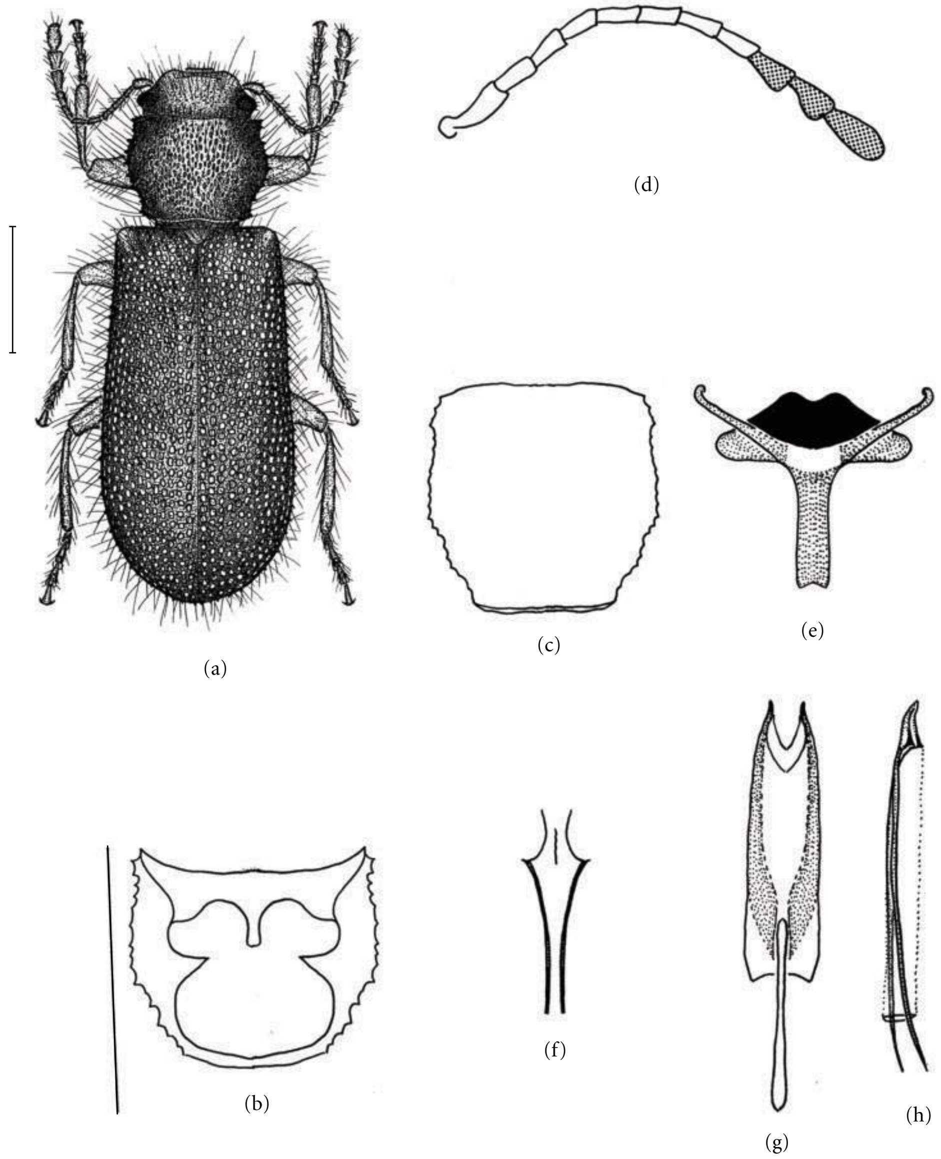


Figure 3: Various organs of *Curacavi dentatus*. (a) Habitus. (b, c) Thorax ((b) ventral, (c) dorsal). (d) Antenna. (e) Metendosternite. (f) Spiculum. (g) Tegmen. (h) Phallus.

Species Examined. Globoclava anthicides (Newman) (new combination).

Etymology. The generic name *Globoclava* stems from the Latin *globus* (=sphere) and the Latin *clavus* (= nail). I refer to the shape of the antenna in this beetle.

4.5.10. Neopylus Solervicens (Figures 9, 10, 12(e), and 22(h)). Neopylus Solervicens [24]. *Type species: Neopylus nahuelbutensis* Solervicens [24].

Synapotypic Characteristics. Pronotal and elytral disc vested with small white setal wisps.

Diagnosis. The small wisps of white setae on the dorsum of these beetles will distinguish them from superficially similar specimens of the Australian genus *Pylus* Newman.

Description. Size: length 9.0–10.0 mm; width 3.5–4.0 mm. *Form* (Figure 9): oblong long rectangulate, robust about 3 times longer than broad. *Vestiture*: disc of cranium vested profusely with white recumbent setae, pronotum and elytral disc vested with small wisps of white setae pale setae, elytral disc vested with 1° setae and shorter 2° setae. *Head* (Figures 10(a), 10(b), and 12(e)): cranium quadrate, frons wider than width of eye, indented with large setiferous punctations that give cranium rugose appearance; gula (Figure 10(a)), large, trapezoidal, sutures oblique,

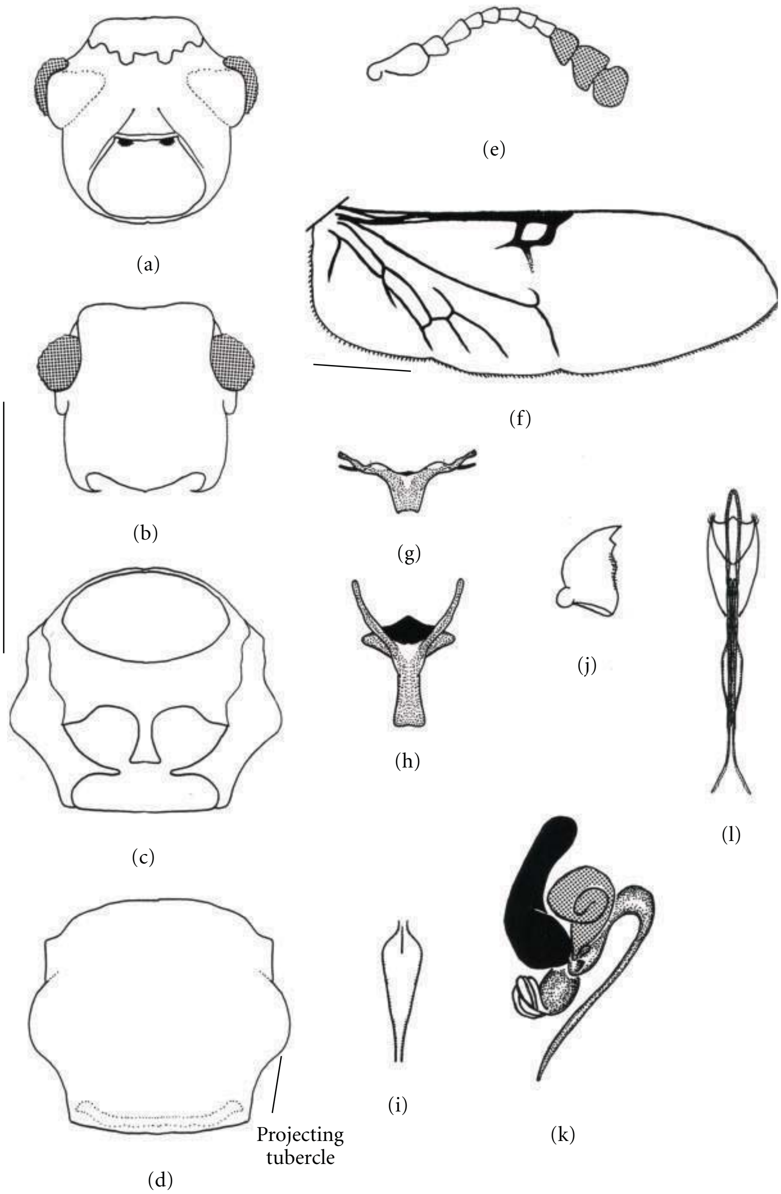


Figure 4: Various organs. (a)–(g) and (h)–(l) of *Pseudopylus okei*. (a, b) Head ((a) ventral, (b) dorsal). (c, d) Pronotum ((c) ventral, (d) dorsal). (e) Antenna. (f) Metathoracic wing. (g) Metendosternite. (h) Metendosternite of *Apopylus unumgarensis*. (i) Spiculum. (j) Mandible. (k) Male mesodermal internal reproductive organs. (l) Aedeagus.

gular processes widely separated, processes in form of two setiferous tubercles; labrum (Figure 10(j)), short, not deeply incised, transverse tormal processes confluent, epipharyngeal plate very small; mandible (Figure 10(k)), body short, anterior, medial dens well developed, posterior dens not well developed, penicillus well developed; maxilla (Figure 10(i)), laterolacinia present, terminal palpomere securiform; labium (Figure 10(g)), ligula not deeply incised, terminal palpomere securiform; eyes small, coarsely faceted, ocular notch large; antenna (Figure 10(f)), capitate, capitulum lax, somewhat narrow, scape about as long as combined length of pedicel and antennomere 3, funicular antennomeres subfiliform, capitular antennomeres somewhat narrow, antennomeres 9 and 10 triangular, antennomere 11 somewhat truncate. *Thorax*: pronotum (Figures 10(c) and 10(d)), transverse, disc indented with large setiferous punctations, central lineal fissure, and with small elevations, side margins with distinct tubercle, dorsolateral ridge extends from posterior angle to anterior angle, not fractured but made coarse by coarse punctations, sclerotized region above pronotal projection with setiferous punctations at base, glabrous in remainder, prebasal fissure shallow, prointercoxal process expanded distally; pronotal projections long; elytron sculptured with large spheroid asetiferous punctations, punctations smaller near elytral apex, punctations not seriate, 1° setae always adjacent to asetiferous punctations, 2° setae not

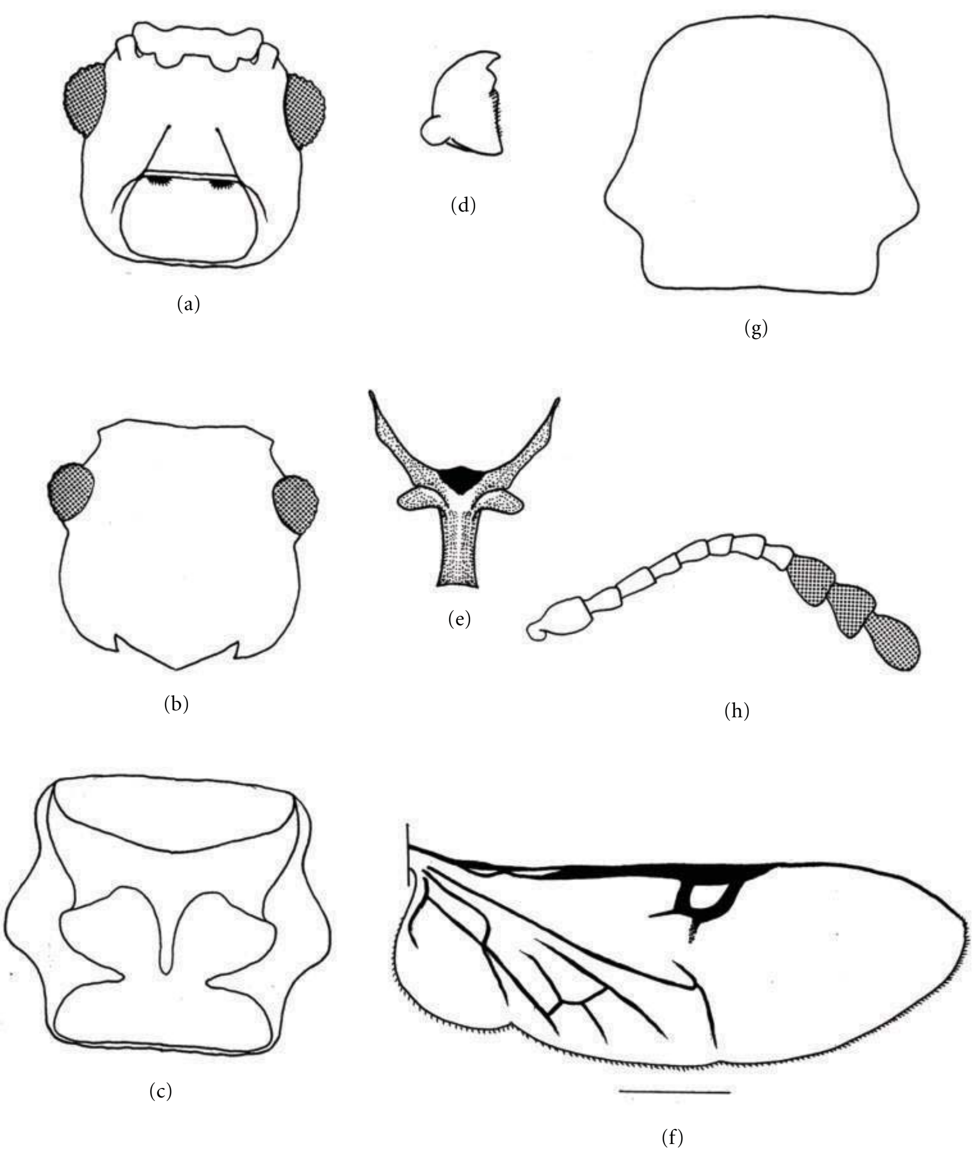


Figure 5: Various organs of *Apteropilo chrysocome*. (a, b) Head ((a) ventral, (b) dorsal). (c) Pronotum (ventral). (d) Mandible. (e) Metendosternite. (f) Metathoracic wing. (g) Pronotum (dorsal). (h) Antenna.

arranged serially, epipleural fold laterally positioned, narrow near elytral apex, anterior margin carinate; metathoracic wing (Figure 10(e)), wedge cell closed; metendosternite (Figure 10(h)), with furcal lamina, furcal anterior plate prominent; legs, tibial spur formula 1-2-2, tarsal pulvillar formula 3-3-3, unguis without denticle. *Abdomen*: aedeagus (Figures 10(m) and 10(n)), shorter than length of abdomen, phallobase not reduced, lobate distally, lobes not fimbriate; phallic lateral plates broad, phallic apex stout, spicular plates narrow (Figure 10(l)), slightly acuminate, spicular apodemes fused, intraspicular plate rod shaped; ovipositor, ventral and dorsal laminae unilobed, laminal rod present; distal margin of pygidium not incised, distal margin of male 6th sternite slightly incised. *Alimentary canal*: not studied. *Male mesodermal internal reproductive organs*: not studied. *Female mesodermal internal reproductive organs*: saccular bursa copulatrix present, bursa with two basal sclerites.

Distribution. The members of this genus have been found only in Chile.

Species Examined. This is a monotypic genus.

4.5.11. Parapylus Blackburn (Figures 11(a)–11(l), 12(f), and 22(i)). Parapylus Blackburn [17]. *Type species: Parapylus bicinctus* Newman [25]. By monotypy.

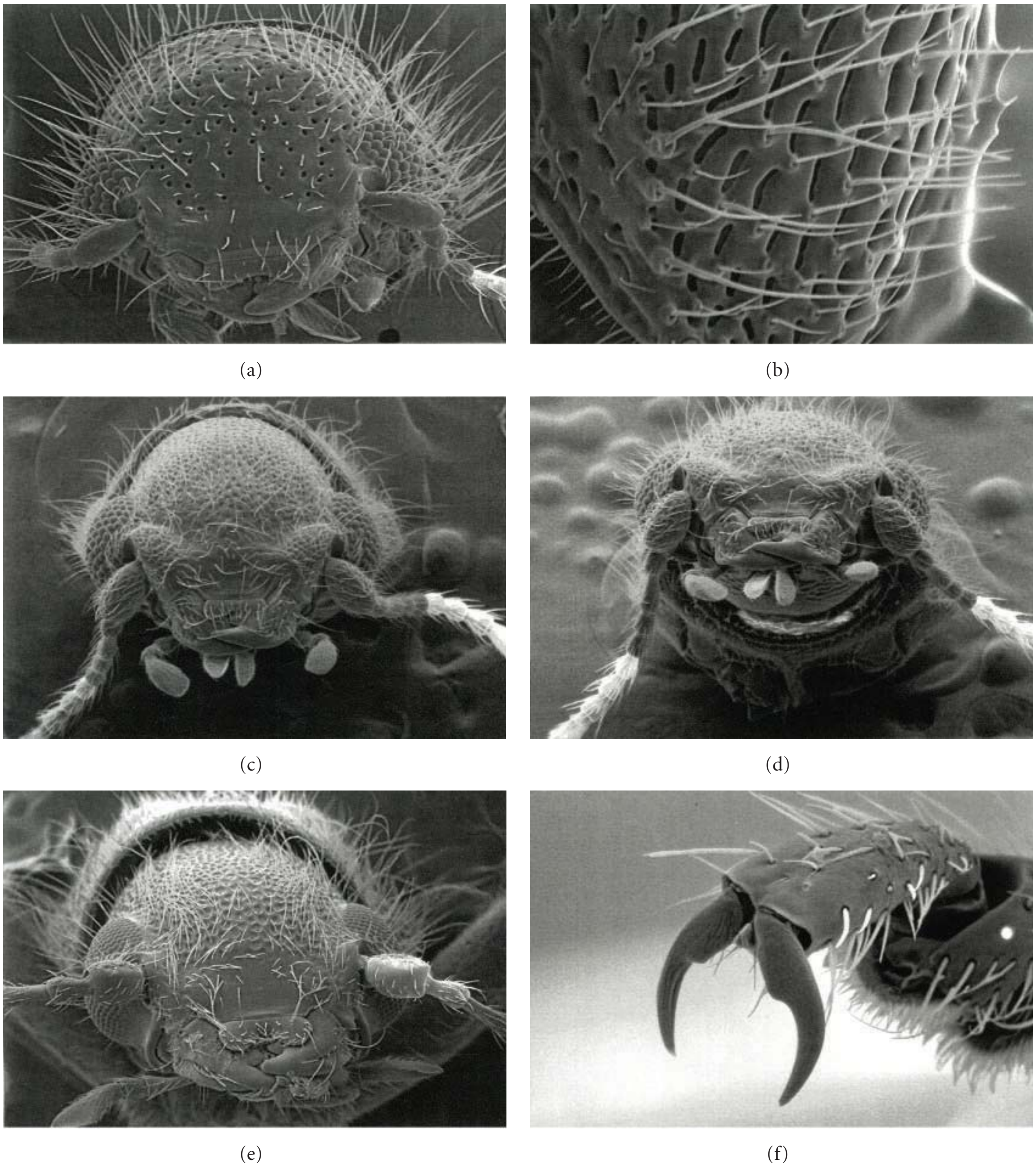

(a) (b) (c) (d) (e) (f)

Figure 6: Various organs. (a, b) *Curacavi dentatus* ((a) head, (b) pronotal disc). (c, d) *Pseudopilus okei* ((c) head, (d) mouthparts). (e, f) *Apteropilo chrysocome* ((e) head, (f) protarsus).

Synapotypic Characteristics. Tarsal spur formula 2-2-2, elytral disc vested with transverse setal fascia, only apex of spermathecal capsule visibly sclerotized.

Diagnosis. Transverse setal fascia on the elytral disc and a tarsal formula of 2-2-2.

Description. Size: length 4.0–7.0 mm; width 1.8–3.8 mm. *Form* (Figure 22(i)): oblong short rectangulate, robust, about 2 times longer than broad. *Vestiture*: disc of cranium vested profusely with dark setae, pronotum and elytral vested profusely vested with dark setae, elytral disc vested with 1° setae and shorter 2° setae, elytral disc narrow fascia of white setae. *Head* (Figures 11(a), 11(b), 12(f)): cranium quadrate, frons wider than width of eye, indented with large setiferous punctations that give cranium rugose appearance; gula (Figure 11(a)), large, trapezoidal, sutures oblique, gular processes widely separated, processes in form of two setiferous tubercles; labrum short, deeply incised, transverse tormal processes confluent, epipharyngeal plate not discernible; mandible, body long, anterior, medial dens well developed, posterior dens not well developed, penicillus well developed; maxilla (Figure 11(i)), laterolacinia present, terminal subdigitiform; labium (Figure 11(h)), ligula deeply incised, terminal palpomere securiform; eyes small, coarsely faceted, ocular notch large; antenna (Figure 11(e)), capitate, capitulum compact, wide, scape about as long as combined

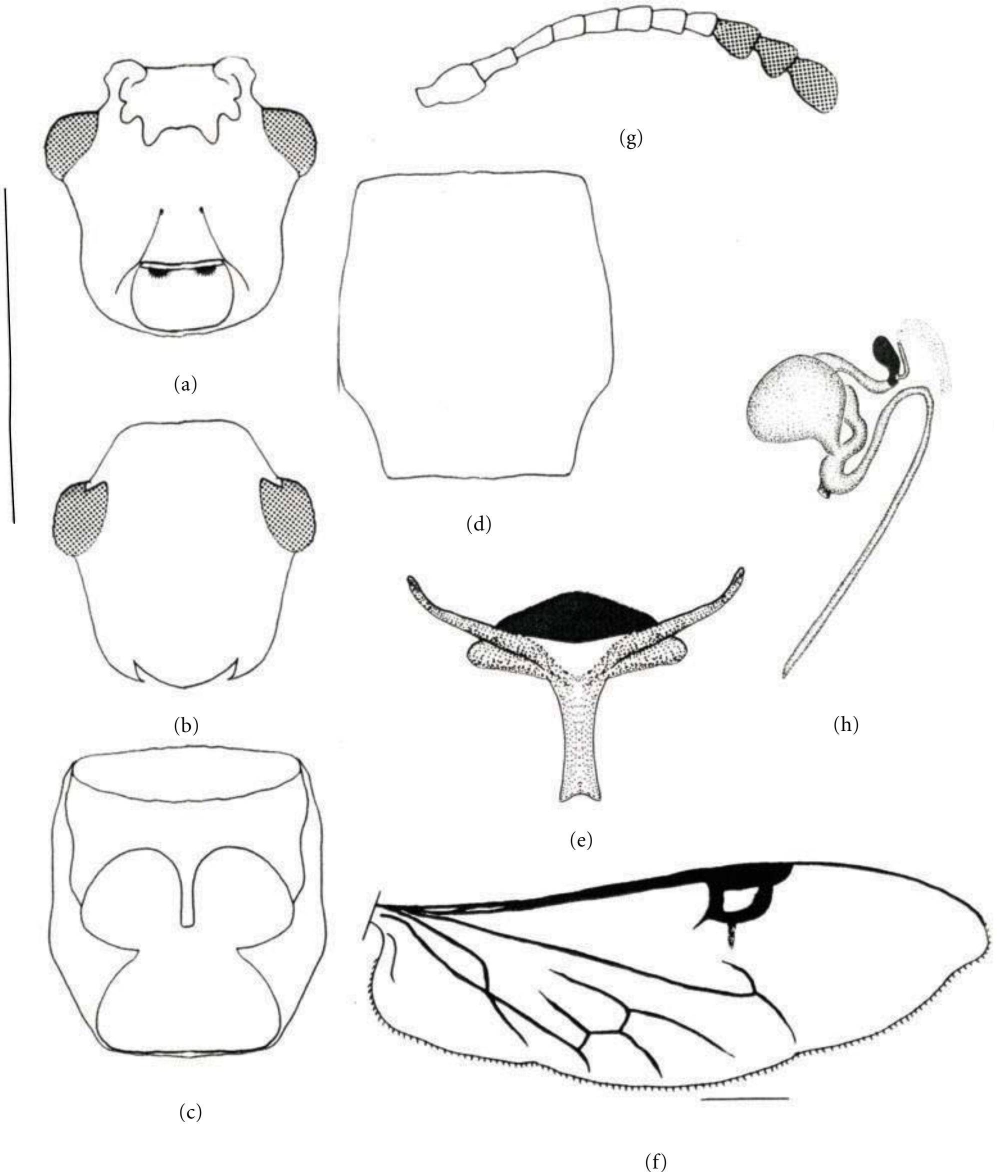


Figure 7: Various organs of *Blackburniella intricata*. (a, b) Head ((a) ventral, (b) dorsal). (c, d) Pronotum ((c) ventral, (d) dorsal). (e) Metendosternite. (f) Metathoracic wing. (g) Antenna. (h) Female mesodermal organs.

length of pedicel and antennomere 3, funicular antennomeres subfiliform, capitular antennomeres wide, antennomeres 9 and 10 expanded laterally, antennomere 11 transverse. *Thorax*: pronotum (Figures 11(c) and 11(d)), transverse, disc indented with large setiferous punctations, with central linear fissure, and small paralateral elevations, side margins with distinct tubercle, dorsolateral ridge extends from posterior angle to anterior angle, not fractured but made coarse by coarse punctations, sclerotized region above pronotal projection setose, not glabrous, prebasal fissure well developed, prointercoxal process expanded distally; pronotal projections long; elytron sculptured with large spheroid asetiferous punctations, punctations smaller in elytral apical half, punctations not seriate, 1° setae adjacent to asetiferous punctations, 2° setae not arranged serially, epipleural fold laterally positioned, narrows towards elytral apex, anterior margin carinate; metathoracic wing (Figure 11(f)), wedge cell closed; metendosternite (Figure 11(g)), with furcal lamina, furcal anterior plate prominent; legs, tibial spur formula 2-2-2, tarsal pulvillar formula 3-3-3, unguis without denticle. *Abdomen*: aedeagus shorter than length of abdomen, phallobase reduced, lobate distally, lobes fimbriate, phallobasic rod bifid distally; phallic lateral plates narrow, phallic apex minute, spicular plates triangular (Figure 11(j)), spicular apodemes not fused, intraspicular plate rod shaped; ovipositor, ventral and dorsal laminae unilobed, laminal rod present; distal margin of pygidium not incised, distal margin of male 6th sternite slightly incised. *Alimentary canal*: not studied. *Male mesodermal internal reproductive organs* (Figure 11(k)): two pairs of accessory glands, lateral pair divided. *Female*

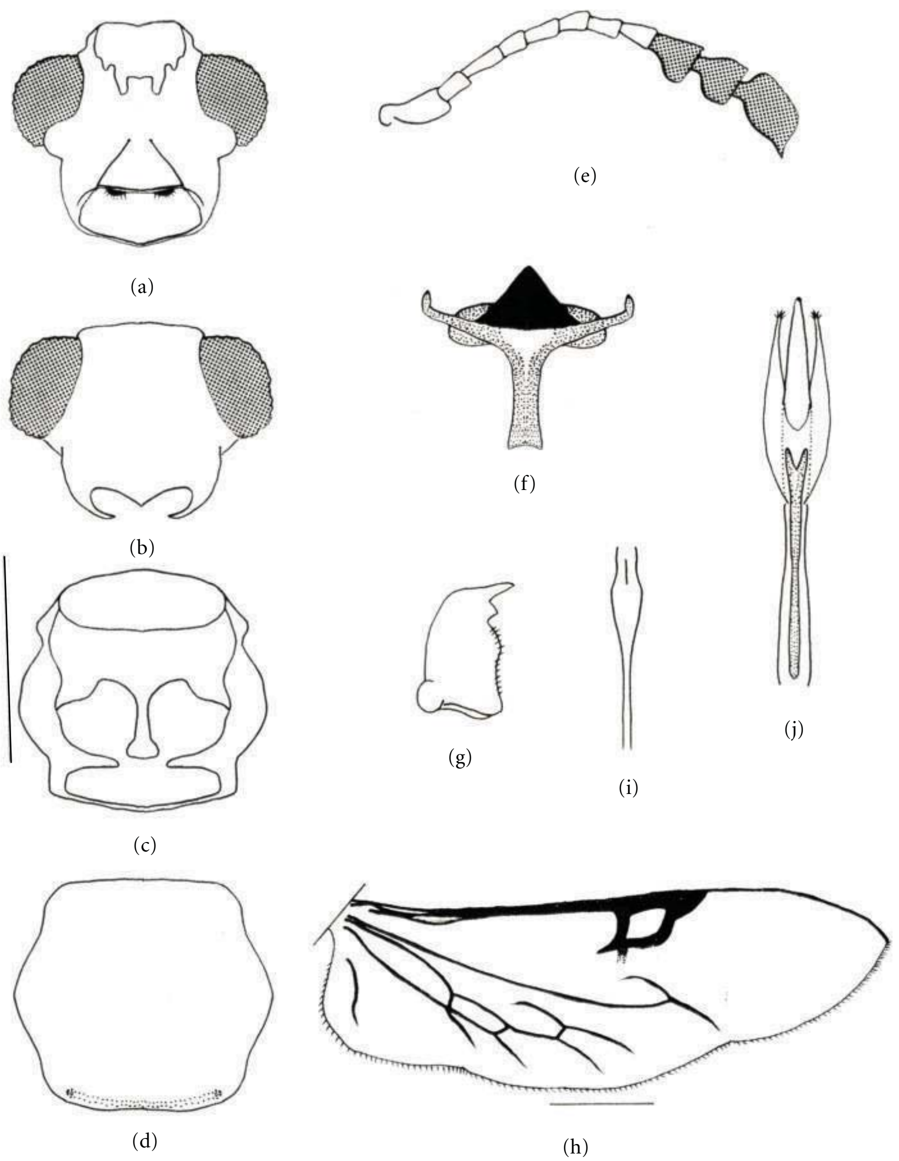


Figure 8: Various organs of *Fallopylus pallipes*. (a, b) Head ((a) ventral, (b) dorsal). (c, d) Pronotum ((c) ventral, (d) dorsal). (e) Antenna. (f) Metendosternite. (g) Mandible. (h) Metathoracic wing. (i) Spiculum. (j) Aedeagus.

mesodermal internal reproductive organs (Figure 11(l)): saccular bursa copulatrix present, bursa with two basal sclerites, only apex of spermathecal capsule sclerotized, spermathecal gland attached to apex of spermatheca.

Distribution. The members of this genus have been found only in Australia.

Species Examined. *Parapylus bicinctus* Newman and *P. sedlaceki* (Kolibáč) (new combination).

Notes. As *Parapylus* Blackburn Newman is characterized by having a tarsal spur formula of 2-2-2 its two species cannot be retained in *Pylus* Newman, which is characterized by a tarsal formula of 2-2-1. Other differences involve smaller ommatidia and the elytra of *Parapylus* have short longitudinal ridges on the posterior half of the elytral disc, the elytra are setose-fascia and show a basal umbo. Kolibáč [6] provides illustrations of various organs of *Parapylus bicinctus* Newman.

4.5.12. Pseudopylus, gen. nov. (Figures 4(a)–4(g), 4(i)–4(l), 6(c), 6(d), and 23(a)).

Type Species. *Pylus okei* Elston [26]. Kolibáč [6].

Synapotypic Characteristics. Tibial spur formula 0-0-0.

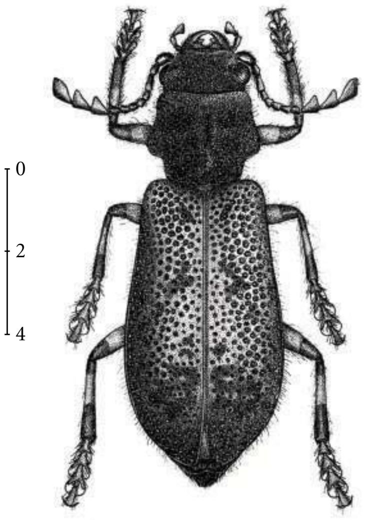


Figure 9: Habitus of *Neopylus nahuelbutensis*.

Diagnosis. The absence of glabrous tumescences on the pronotal disc of specimens of *Pseudopylus* will distinguish them from those of *Apteropilo*.

Size: length 3.8–7.0 mm; width 1.4–3.6 mm. *Form* (Figure 23(a)): oblong short rectangulate, about 3 times longer than broad. *Vestiture*: disc of cranium and pronotum vested with pale setae, elytral disc vested with 1° setae and shorter profusely distributed 2° setae. *Head* (Figures 4(a), 4(b), and 6(c)): cranium quadrate, frons wider than width of eye, indented with large setiferous punctations that give cranium rugose appearance; gula (Figure 4(a)) large, trapezoidal, sutures oblique, gular processes widely separated, processes in form of two setiferous tubercles; labrum short, deeply incised, transverse tormal processes not confluent, epipharyngeal plate very small; mandible (Figure 4(j)), body short, anterior, medial dens well developed, posterior dens not well developed, penicillus well developed; maxilla (Figure 6(d)), laterolacinia present, terminal palpomere sub securiform; labium, ligula not deeply incised, terminal palpomere subsecuriform; eyes small, coarsely faceted, ocular notch small; antenna (Figure 4(e)), capitate, capitulum compact, scape about as long as combined length of pedicel and antennomere 3, funicular antennomeres subfiliform, capitular antennomeres wide, antennomeres 9 and 10 triangular, antennomere 11 ovoid. *Thorax*: pronotum (Figures 4(c), and 4(d)), transverse, side margins with distinct tubercles, disc with large oblong or round setiferous punctations, dorsolateral ridge extends from posterior angle to anterior angle, surface coarse and fractured by coarse punctations, prebasal fissure well developed, prointercoxal process expanded distally; pronotal projections long; elytron sculptured with large spheroid asetiferous punctations, latter seriate, punctations bimodal, 1° setae always adjacent to asetiferous punctations, 2° setae arranged serially, epipleural fold laterally positioned, extended to elytral posterior four-fifths, anterior margin carinate; metathoracic wing (Figure 4(f)), rarely absent, when present wedge cell closed; metendosternite (Figure 4(g)), with furcal lamina, furcal lamina may be reduced in size, furcal anterior plate present or absent; legs, tibial spur formula 0-0-0, tarsal pulvillar formula 3-3-3, unguis without denticle. *Abdomen*: aedeagus (Figure 4(l)), as long as length of abdomen, phallobase reduced, lobate distally, lobes fimbriate; phallic lateral plates very narrow, particularly long, spicular plates very narrow, spicular apodemes not fused (Figure 4(i)), intraspicular plate rod shaped; ovipositor, ventral and dorsal laminae unilobed, laminal rod present; distal margin of pygidium not incised, distal margin of male sixth sternite slightly incised. *Alimentary canal*: six malpighian tubules. *Male mesodermal internal reproductive organs* (Figure 4(k)): two pairs of accessory glands, medial gland coiled, vesiculated at base, lateral glad digitiform, seminal vesicle bulbous, testis very small. *Female mesodermal internal reproductive organs*: spermathecal capsule well sclerotized, spermathecal gland attached near base of spermathecal capsule, saccular bursa copulatrix present, two denticulated basal bursal sclerites present.

Distribution. The members of this genus have been found only in Australia.

Species Examined. Pseudopylus okei (Elston) (new combination) and three undescribed species.

Etymology. The generic name *Pseudopylus* stems from the Greek *pseudo* (=fallacy) and the name of the genus *Pylus* Newman.

Notes. Kolibáč [6] provides excellent drawings of the aedeagus and female mesodermal internal reproductive organs of *Pseudopylus okei* (Elston). Note the poor development of the metendosternite of *P. okei* (Elston) (Figure 4(g)) a species in which the metathoracic wings are absent. The metendosternite is well developed in *A. unumgarensis* Kolibáč (Figure 4(h)) whose specimens show well-developed membranous wings.

4.5.13. Pylus Newman (Figures 13(a)–13(j), 16(a), 16(b), and 23(b)). Pylus Newman [27]. *Type species: Pylus fatuus* Newman [27]. Corporaal [5].

Ylotis Spinola [28]. Synonymized by Corporaal, 1950.

Ylotis Spinola [12]. Synonymized by Corporaal, 1950.

Synapotypic Characteristics. Spermathecal gland very long (Figure 13(i)).

Diagnosis. Within Tarsosteninae elytral disc punctations are serially rowed to the elytral apex in specimens of *Apopylus* Kolibáč, *Fallopylus* gen. nov., and in *Pylus* Newman. However, the tibial spur formula is 2-2-2 in *Pylus* Newman, 0-0-0 in *Apopylus* Kolibáč, and 1-2-1 in *Fallopylus* gen. nov.

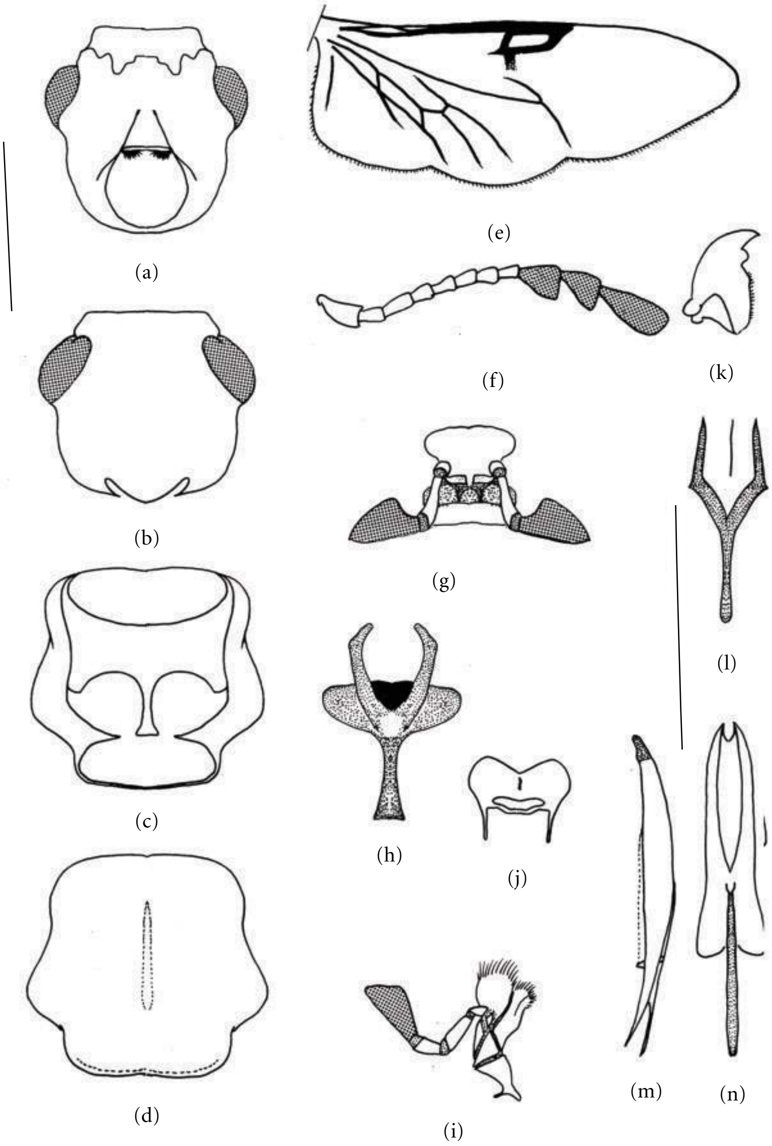


Figure 10: Various organs of *Neopylus nahuelbutensis*. (a, b) Head ((a) ventral, (b) dorsal). (c, d) Thorax ((c) ventral, (d) dorsal). (e) Metathoracic wing. (f) Antenna. (g) Labium. (h) Metendosternite. (i) Maxilla. (j) Labrum. (k) Mandible. (l) Spiculum. (m) Phallus. (n) Tegmen.

Description. Size: length 5.0–15.5 mm; width 2.0–5.2 mm. *Form* (Figure 23(b)): oblong long rectangulate, robust, about 3 times longer than broad. *Vestiture*: disc of cranium vested profusely with dark setae, pronotum and elytral vested profusely vested with dark setae, elytral disc vested with short 1° setae and shorter 2° setae. *Head* (Figures 13(a), 13(b), and 16(a)): cranium quadrate, frons wider than width of eye, indented with large setiferous punctations that give cranium rugose appearance; gula (Figure 13(a)), large, trapezoidal, sutures oblique, gular processes widely separated, processes in form of two setiferous tubercles; labrum short, not deeply incised, transverse tormal processes confluent, epipharyngeal plate small; mandible, body long, anterior, medial dens well developed, posterior dens not well developed, penicillus well developed; maxilla (Figure 16(b)), laterolacinia present, terminal subsecuriform; labium (Figure 16(b)), ligula deeply incised, terminal palpomere securiform; eyes large, coarsely faceted, ocular notch large; antenna (Figure 13(e)), capitate, capitulum compact, wide, scape about as long as combined length of pedicel and antennomere 3, funicular antennomeres subfiliform, capitular antennomeres wide, antennomeres 9 and 10 expanded laterally, antennomere 11 transverse. *Thorax*: pronotum (Figures 13(c) and 13(d)), transverse, disc indented with large setiferous punctations, with central fissure, and small paralateral elevations, side margins with distinct tubercle at middle and knob at

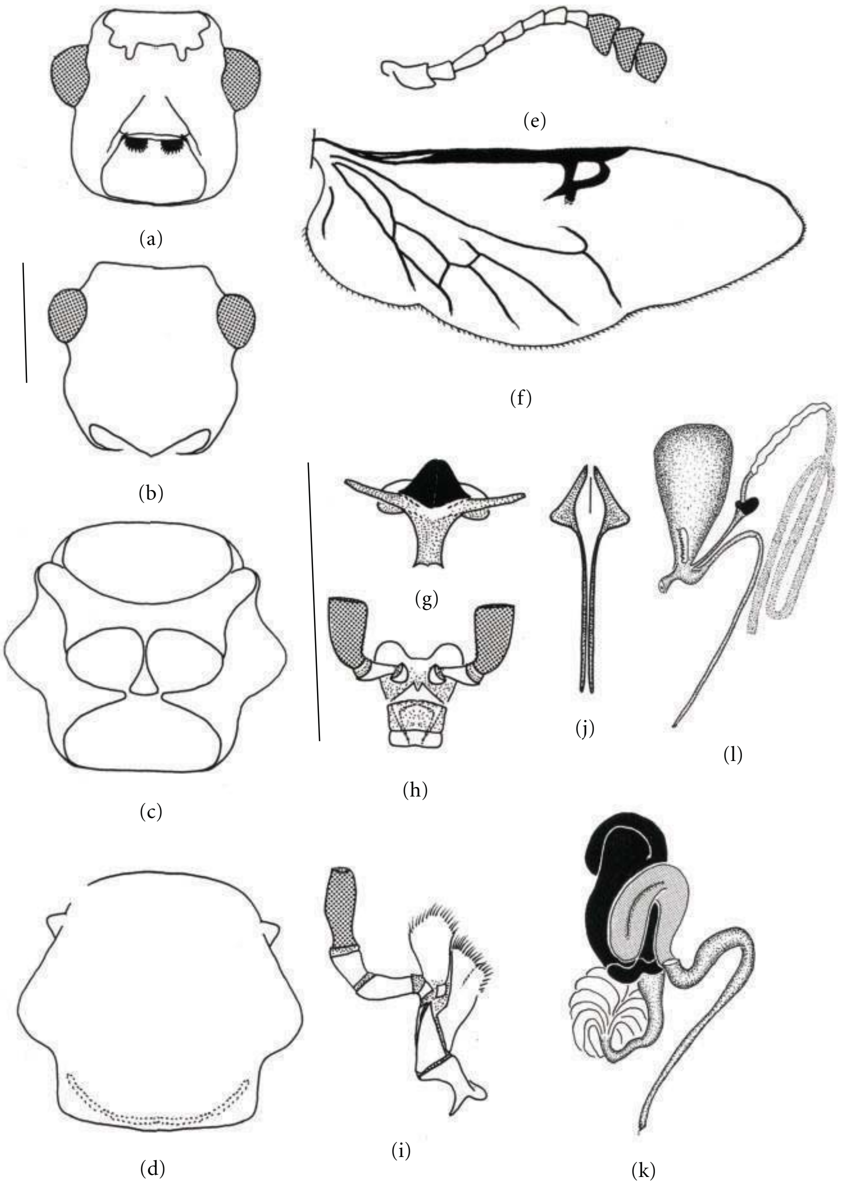


Figure 11: Various organs of *Parapylus bicinctus*. (a, b) Head ((a) ventral, (b) dorsal). (c, d) Pronotum ((c) ventral, (d) dorsal). (e) Antenna. (f) Metathoracic wing. (g) Metendosternite. (h) Labium. (i) Maxilla. (j) Spiculum. (k) Male mesodermal reproductive organs. (l) Female mesodermal reproductive organs.

anterior angles, dorsolateral ridge extends from posterior angle to anterior angle, not fractured but made coarse by coarse punctations, sclerotized region above pronotal projection not glabrous, setose, prebasal fissure well developed, prointercoxal process expanded distally; pronotal projections long; elytron sculptured with large binoded asetiferous punctations, punctations seriate, extend to elytral apex seriate, 1° setae adjacent to asetiferous punctations, 2° setae serially arranged, epipleural fold laterally positioned, narrows towards elytral apex, anterior margin carinate; metathoracic wing (Figure 13(f)), wedge cell closed; metendosternite with furcal lamina, furcal anterior plate prominent; legs, first tarsomere may be slender, acuminate and extended towards second tarsomere, tibial spur formula 2-2-1, tarsal pulvillar formula 3-3-3, unguis without denticle. *Abdomen*: aedeagus (Figures 13(h) and 13(j)), shorter than length of abdomen, phallobase reduced, lobate distally, lobes fimbriate, phallobasic rod not bifid distally; phallic lateral plates narrow, phallic apex minute, spicular plates narrow, with small rounded lateral extension, spicular apodemes not fused (Figure 13(g)), intraspicular plate rod shaped; ovipositor, ventral and dorsal laminae unilobed, laminal rod present; distal margin of pygidium not incised, distal margin of male 6th sternite slightly incised. *Alimentary canal*: not studied. *Male mesodermal internal reproductive organs*: not studied. *Female mesodermal internal reproductive*

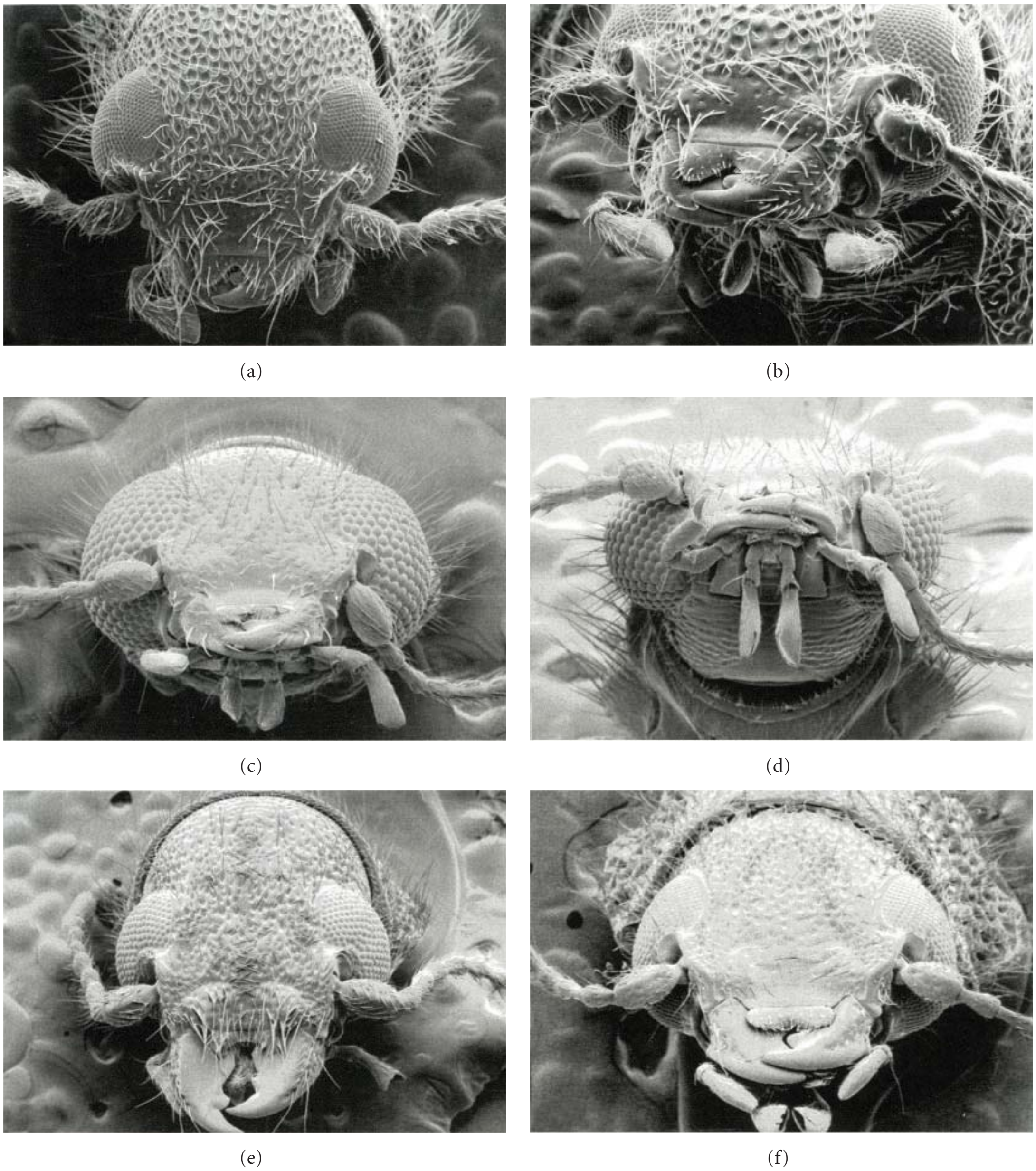


Figure 12: Various organs. (a, b) *Blackburniella intricata* ((a) head, (b) mouthparts). (c, d) *Fallopylus pallipes* ((c) head, (d) mouthparts). (e) *Neopylus nahuelbutensis* head. (f) *Parapylus bicinctus* head.

organs (Figure 13(i)): saccular bursa copulatrix present, bursa with two basal sclerites, spermathecal capsule well sclerotized, spermathecal gland very long, attached to base of spermatheca.

Distribution. The members of this genus have been found only in Australia.

Species Examined. Pylus fatuus Newman and one undescribed species.

Notes. Kolibáč [6] provides illustrations of various organs of *Pylus fatuus* Newman.

4.5.14. Rhophaloclerus Fairmaire (Figures 14(a)–14(g), 16(c), 16(d), and 23(c)). Rhophaloclerus Fairmaire [29, 30]. Type species: *Rhophaloclerus coquerelii* Fairmaire [29]. By monotypy. Kuwert [31]. Corporaal [5]. *Liostylus* Fairmaire [29]. *New Synonymy*.

Synapotypic Characteristics. Tibial spur formula 1-2-2.

Diagnosis. This is the only genus of Tarsosteninae with a tibial formula 1-2-2 and known to occur in Madagascar and the Comoros.

Description. Size: length 3.0–6.0 mm; width 0.7–3.0 mm. *Form* (Figure 23(c)): oblong rectangulate, most species

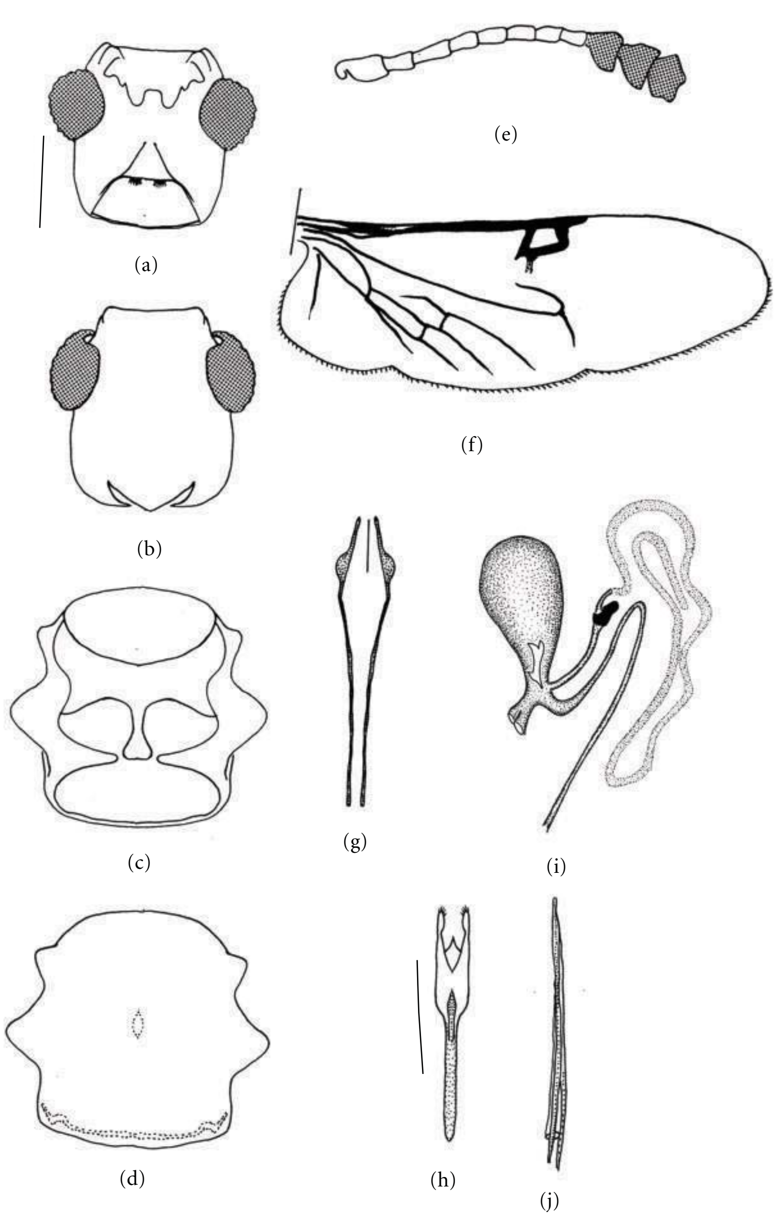


Figure 13: Various organs of *Pylus fatuus*. (a, b) Head ((a) ventral, (b) dorsal). (c, d) Pronotum ((c) ventral, (d) dorsal). (e) Antenna. (f) Metathoracic wing. (g) Spiculum. (h) Tegmen. (i) Female mesodermal reproductive organs. (j) Phallus.

narrow, about 3 times longer than broad. *Vestiture*: disc of cranium and pronotum vested with pale setae, elytral disc vested with 1° setae and shorter profusely distributed 2° setae. *Head* (Figures 14(a), 14(d), and 16(c)): cranium quadrate, frons much wider than width of eye, indented with very small setiferous punctations; gula (Figure 14(a)), large, trapezoidal, sutures oblique, gular processes widely separated, processes in form of two setiferous tubercles; labrum short, deeply incised, transverse tormal processes not confluent, epipharyngeal plate very small; mandible, body short, anterior and medial dens well developed, posterior dens not well developed, penicillus well developed; maxilla, laterolacinia present, terminal palpomere securiform; labium, ligula not incised, terminal palpomere securiform; eyes large, finely facetted, ocular notch large; antenna (Figure 16(d)), capitate, capitulum usually lax, rarely compact, scape about as long as combined length of pedicel and antennomere 3, funicular antennomeres subfiliform, capitular antennomeres expanded, antennomeres 9 and 10 expanded, antennomere 11 ovoid. *Thorax*: pronotum (Figures 14(b), and 14(c)), usually elongate, rarely transverse-ovoid, disc indented with small setiferous punctations, lateral margins variously convex, dorsolateral ridge extends from posterior angle to anterior angle, not fractured and with smooth surface, sclerotized region above pronotal projection glabrous, prebasal fissure shallow, prointercoxal process not expanded distally; pronotal projections short; elytron sculptured with shallow asetiferous punctations, punctations seriate and diminish in size to elytral apex, 1° setae always adjacent to asetiferous punctations, 2° setae densely distributed, epipleural

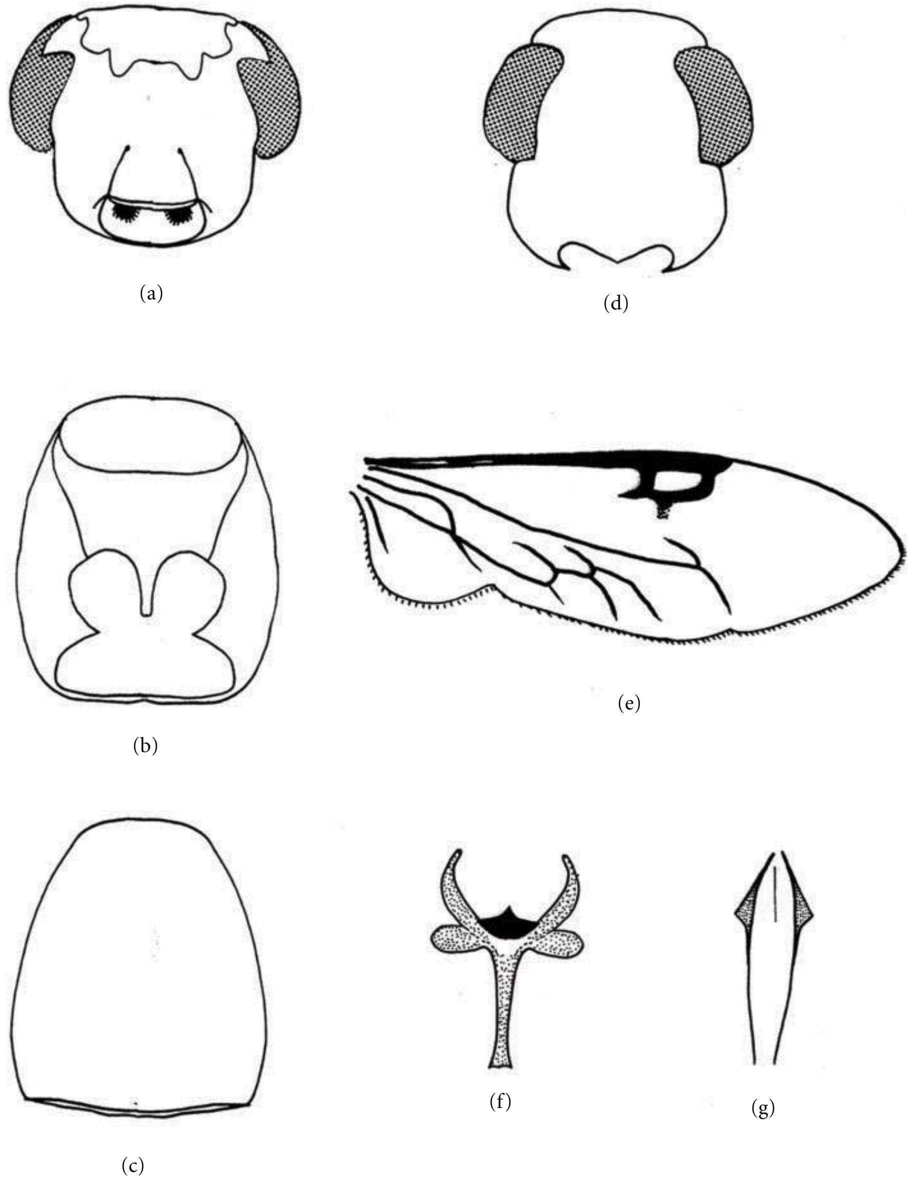


Figure 14: Various organs of *Rhophaloclerus coquerelii*. (a) Head (ventral). (b, c) Pronotum ((b) ventral, (c) dorsal). (d) Head (dorsal). (e) Metathoracic wing. (f) Metendosternite. (g) Spiculum.

fold laterally positioned, narrowed to elytral apex, anterior margin carinate; metathoracic wing (Figure 14(e)), wedge cell open; metendosternite (Figure 14(f)), with furcal lamina, furcal anterior plate prominent; legs, tibial spur formula 1-2-2, tarsal pulvillar formula 3-3-3, unguis without denticle. *Abdomen*: aedeagus shorter than length of abdomen, phallobase not reduced, lobate and narrowed distally, lobes fimbriate; phallic lateral plates narrow, phallobasic rod linear or transverse, furcated distally or not furcated, phallic apex minute, spicular plates very narrow (Figure 14(g)), spicular apodemes not fused at extremity, intraspicular plate rod shaped; ovipositor, ventral and dorsal laminae unilobed, laminal rod present; distal margin of pygidium not incised, distal margin of male 6th sternite slightly incised. *Alimentary canal*: not studied. *Male mesodermal internal reproductive organs*: not studied. *Female mesodermal internal reproductive organs*: spermathecal capsule subovoid, well sclerotized, spermathecal gland attached to middle of spermathecal capsule.

Distribution. Known only from Madagascar and Comoros.

Species Examined. Rhophaloclerus coquerelii Fairmaire, *R. pictus* (Fairmaire) (new combination), *R. vadoni* Pic, and five species that may or may not be described.

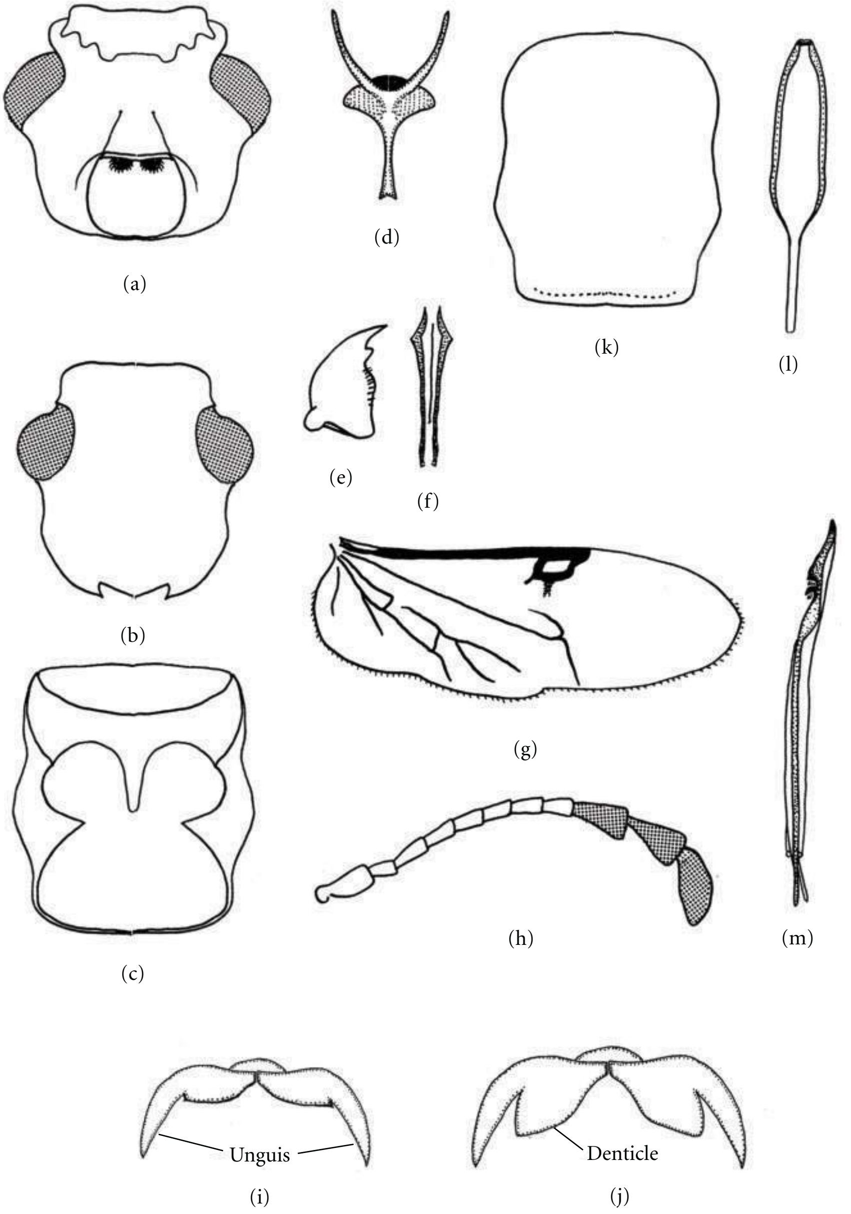


Figure 15: Various organs. (a)–(h), and (k)–(m) *Riotenerus fossipenne*. (a, b) Head ((a) ventral, (b) dorsal). (c) Pronotum (ventral), (d) Metendosternite. (e) Mandible. (f) Spiculum. (g) Metathoracic wing. (h) Antenna. (i) and (j) Generalized unguis ((i) without denticle, (j) with denticle). (k) Pronotum (dorsal). (l) Tegmen. (m) Phallus.

Notes. By placing the description of *Rhophaloclerus* Fairmaire amidst descriptions of other Tillinae genera Fairmaire [29, 32] gave the impression to subsequent authors [5, 33] that the genus in question belongs in Tillinae. The reduced 4th tarsomere and the characteristics of the antenna clearly indicate that *Rhophaloclerus* Fairmaire is appropriately classified in Tarsosteninae.

4.5.15. Riotenerus Pic (Figures 15(a)–15(h), 15(k)-15(m), 16(e), 16(f), 21(e), and 23(d)). Riotenerus Pic [34]. Type species: *Pelonium fossipenne* Schenkling [21]. By monotypy. Solevicens [35]. Opitz [1].

Synapotypic Characteristics. Elytral disc devoid of 2° setae; aedeagus with uncinate projections; interspicular plate very long (Figure 15(f)).

Diagnosis. Distinguishable from the superficially similar specimens of *Abiliella* Peracchi by the lack of elytral 2° setae.

Description. Size: length 5.0–8.5 mm; width 2.0–2.5 mm. *Form* (Figure 23(d)): oblong rectangulate, about 3 times longer than broad. *Vestiture*: disc of cranium and pronotum vested with white setae, elytral disc vested with 1° setae, 2° setae absent. *Head* (Figures 15(a), 15(b), and 16(e)):

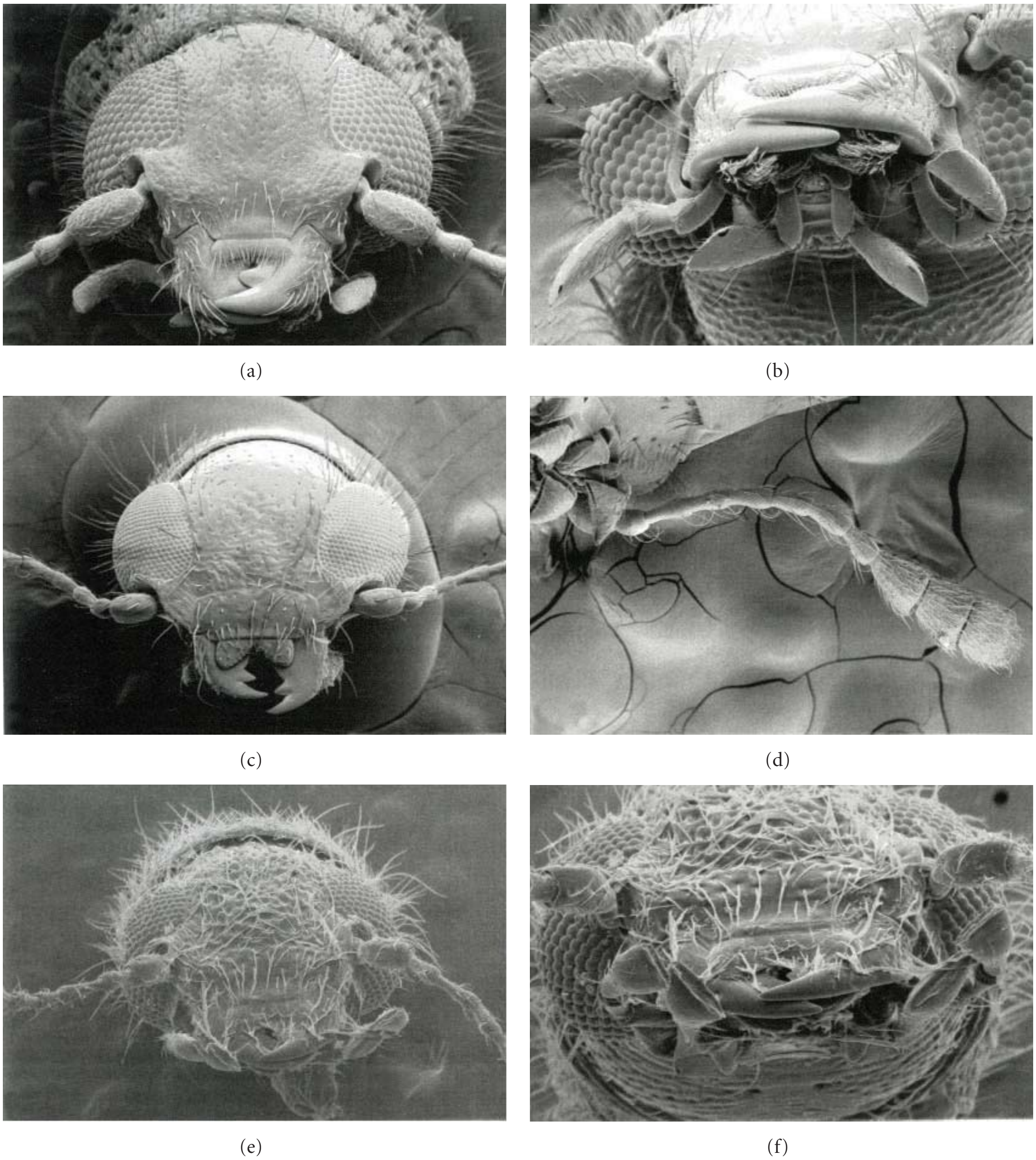


Figure 16: Various organs. (a, b) *Pylus fatuus* ((a) head, (b) mouthparts). (c, d) *Rhophaloclerus coquerelli* ((c) head, (d) antenna). (e, f) *Riotenerus fossipenneus* ((e) head, (f) mouthparts).

cranium quadrate, frons wider than width of eye, indented at middle and with large setiferous punctations that give cranium rugose appearance; gula (Figure 15(a)), large, trapezoidal, sutures oblique, gular processes widely separated, processes in form of two setiferous tubercles; labrum short, medial incision shallow, transverse tormal processes sinuous, confluent, epipharyngeal plate very small; mandible (Figure 15(e)), body stout, anterior, medial, and posterior dens well developed, penicillus well developed; maxilla (Figure 16(f)), laterolacinia present, terminal palpomere securiform; labium (Figure 16(f)), ligula not deeply incised, terminal palpomere securiform; eyes small, coarsely faceted, ocular notch small; antenna (Figures 15(h) and 21(e)), capitate, capitulum lax and narrow, scape about as long as combined length of pedicel and antennomere 3, funicular antennomeres filiform, capitular antennomeres narrow, antennomeres 9 and 10 subrectangular, antennomere 11 ovoid. *Thorax*: pronotum (Figures 15(c), and 15(k)), elongate, convex, side margins sinuous, sculptured with large round setiferous punctations, dorsolateral ridge extends from posterior angle to pronotal midline, surface coarsely punctated, prebasal fissure well developed, prointercoxal process not expanded distally; pronotal projections short; elytron sculptured with large spheroid asetiferous punctations, latter sub seriate, 1° setae always adjacent to asetiferous punctations, 2° setae absent, epipleural fold

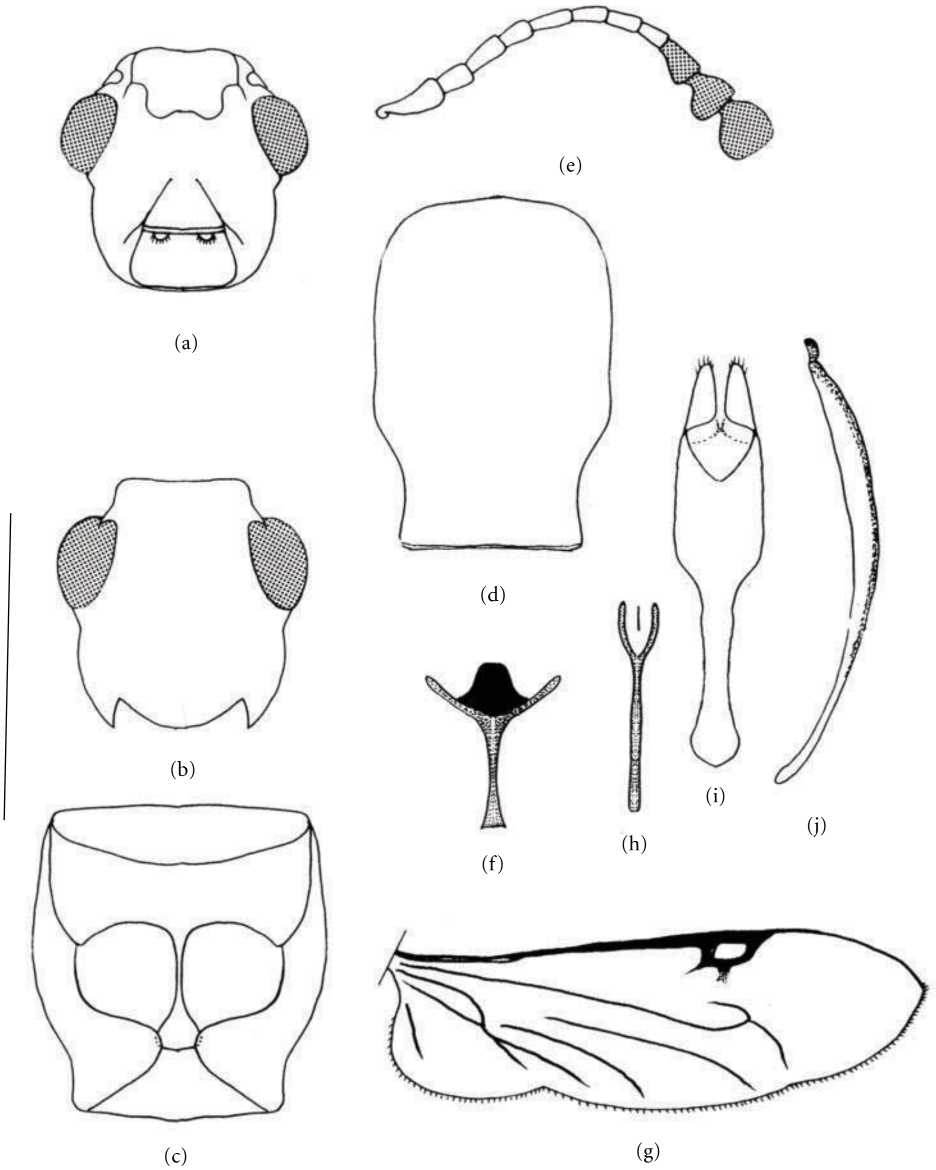


Figure 17: Various organs of *Tarsostenodes simulator*. (a, b) Head ((a) ventral, (b) dorsal). (c, d) Pronotum ((c) ventral, (d) dorsal). (e) Antenna. (f) Metendosternite. (g) Metathoracic wing. (h) Spiculum. (i) Tegmen. (j) Phallus.

laterally positioned, extended to elytral apex, anterior margin carinate; metathoracic wing (Figure 15(g)), wedge cell open; metendosternite (Figure 15(d)), with furcal lamina, furcal anterior plate diminutive, acuminate; legs, tibial spur formula 0-1-1, tarsal pulvillar formula 3-3-3, unguis without denticle. *Abdomen*: aedeagus (Figures 15(l), and 15(m)), shorter than length of abdomen, phallobase not lobate distally; phallic lateral plates very broad and uncinate distally; spicular plates small, triangular, acuminate, rarely, spicular apodemes not fused (Figure 15(f)), intraspicular plate rod shaped and very long; ovipositor, ventral and dorsal laminae unilobed, laminal rod present; distal margin of pygidium not incised, distal margin of male 6th sternite slightly incised. *Alimentary canal*: not studied. *Male mesodermal internal reproductive organs*: not studied. *Female mesodermal internal reproductive organs*: not studied.

Distribution. The members of this genus have been found only in Argentina.

Species Examined. Riotenerus fossipenne (Schenkling).

4.5.16. Tarsostenodes Blackburn (Figures 17(a)–17(j), 20(a), 20(b), and 23(f)). Tarsostenodes Blackburn [36]. Type species*: Tarsostenodes simulator* Blackburn [36]. By monotypy. Corporaal [5]. Kolibáč [6]. Bartlett [37].

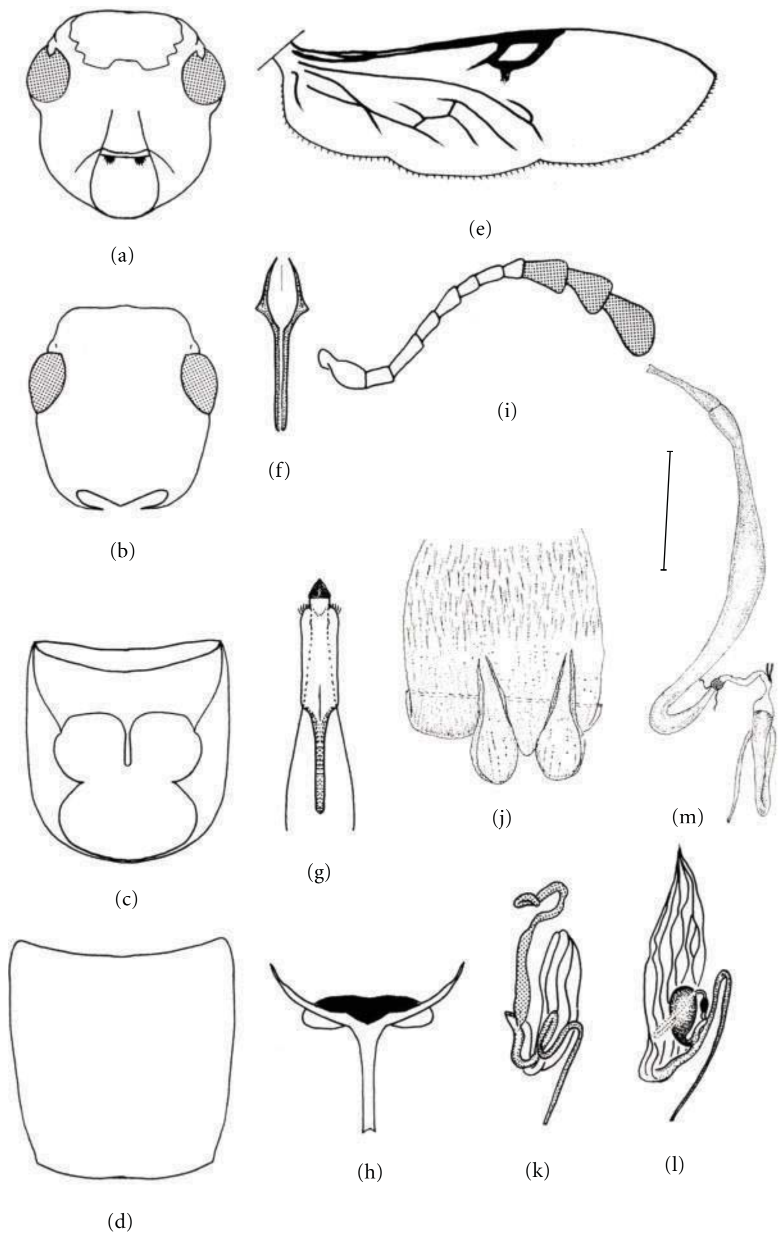


Figure 18: Various organs of *Tarsostenus univittatus*. (a, b) Head ((a) ventral, (b) dorsal). (c, d) Pronotum ((c) ventral, (d) dorsal). (e) Metathoracic wing. (f) Spiculum. (g) Aedeagus. (h) Metendosternite. (i) Antenna. (j) Stomodaeal valve (interior view). (k) Male mesodermal reproductive organs. (l) Female mesodermal reproductive organs. (m) Alimentary canal.

Synapotypic Characteristics. Procoxal cavity closed externally, pronotal projections wide, metathoracic wing cross veins rudimentary or missing, furcal lamina absent, epipleural fold narrow, elytra constricted at middle, phallobasic rod absent.

Diagnosis. The closure of the external aspects of the procoxal cavities (Figure 17(c)) will separate the members of this genus from any other within Tarsosteninae.

Description. Size: length 4.0–8.0 mm; width 1.0–2.0 mm. *Form* (Figure 23(f)): oblong, narrow rectangulate, about 4 times longer than broad. *Vestiture*: disc of cranium and pronotum densely vested with pale setae, elytral disc vested with 1° setae and shorter profusely distributed 2° setae. *Head* (Figures 17(a), 17(b), and 20(a)): cranium quadrate, frons much wider than width of eye, indented with large setiferous punctations; gula (Figure 17(a)), large, trapezoidal, sutures oblique, gular processes widely separated, processes in form of two setiferous tubercles; labrum short, deeply incised, transverse tormal processes not confluent, epipharyngeal plate very small; mandible, body long, anterior and medial dens well developed, posterior dens not well developed, penicillus well developed; maxilla (Figure 20(b)), laterolacinia present, terminal palpomere securiform; labium, ligula

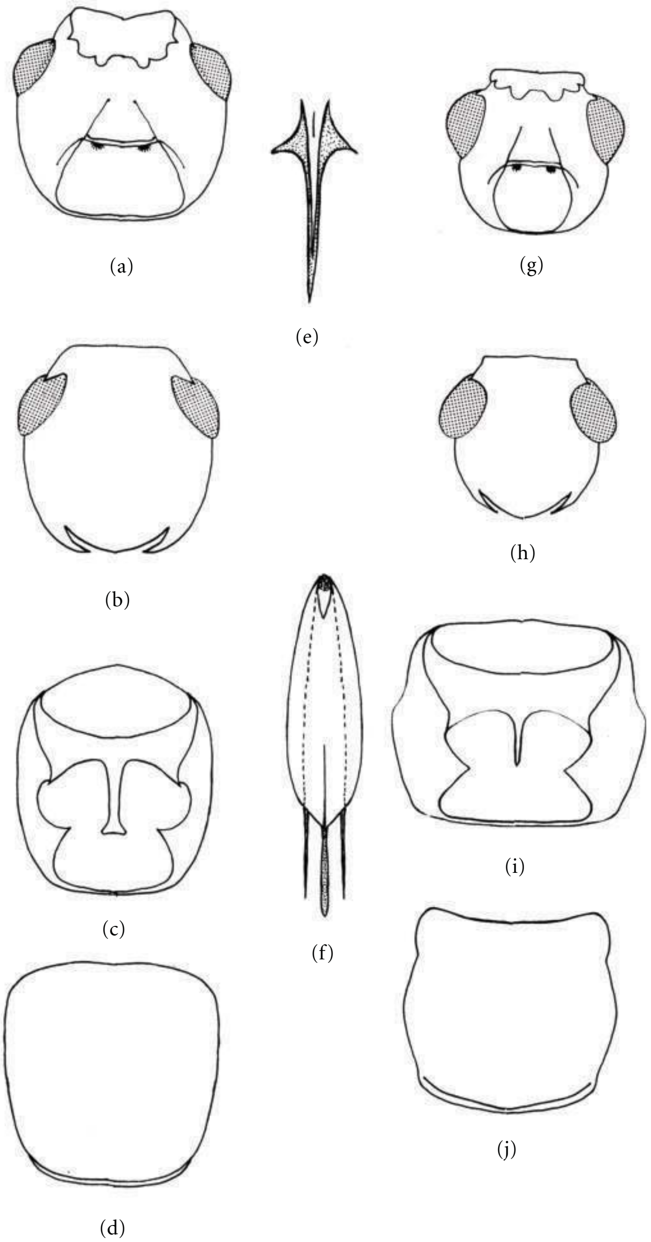
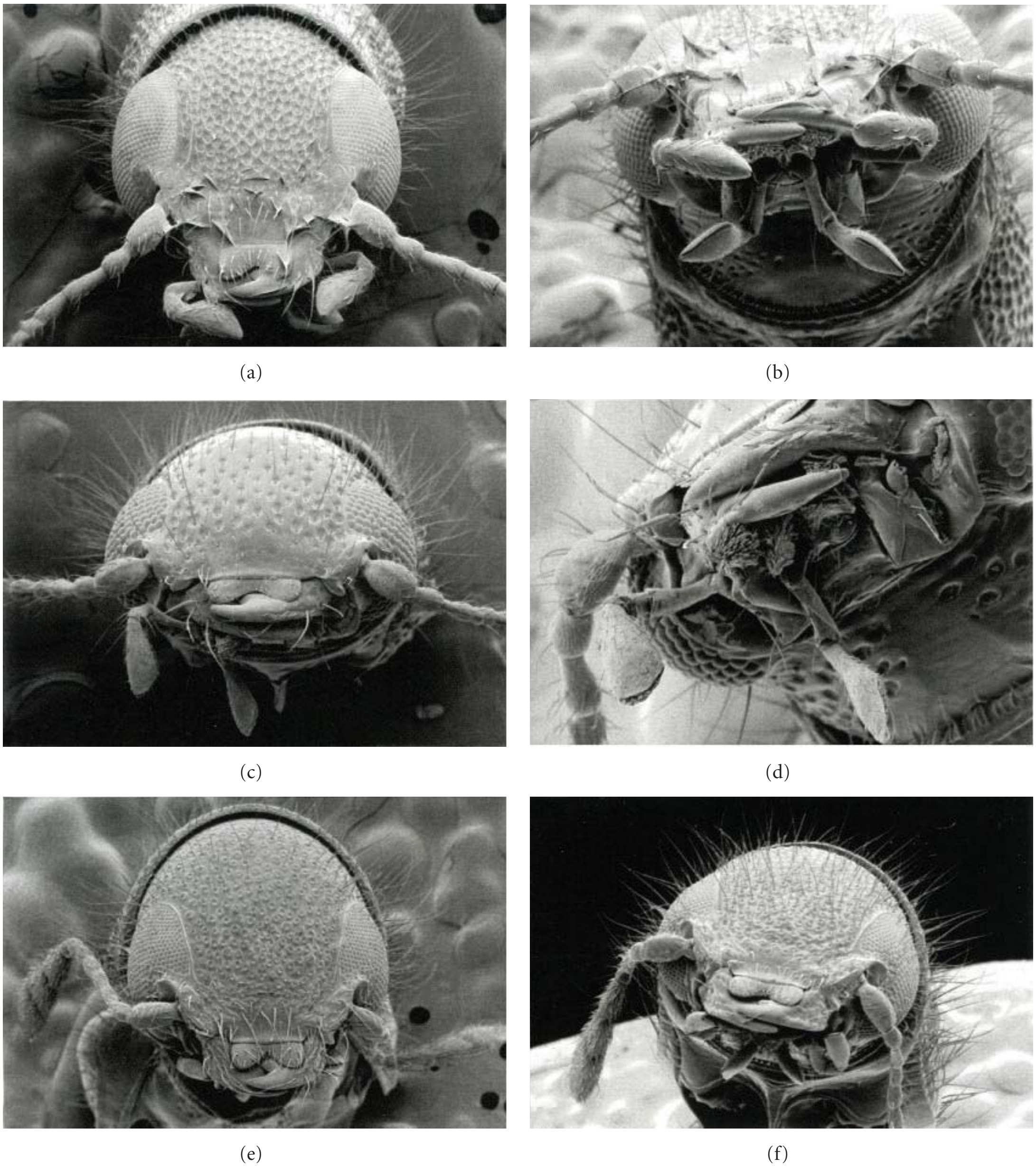


Figure 19: Various organs. (a)–(f) *Thriocera pectoralis*. (a, b) Head ((a) ventral, (b) dorsal). (c, d) Pronotum ((c) ventral, (d) dorsal). (e) Spiculum. (f) Aedeagus. (g)–(j) *Thriocerodes bifasciatus*. (g, h) Head ((g) ventral, (h) dorsal). (i) and (j) Pronotum ((i) ventral, (j) dorsal).

deeply incised, terminal palpomere securiform; eyes large, coarsely faceted, ocular notch large; antenna (Figure 17(e)), capitate, capitulum usually compact, rarely lax, scape about as long as combined length of pedicel and antennomere 3, funicular antennomeres filiform, capitular antennomeres expanded, antennomeres 9 and 10 expanded, antennomere 11 ovoid. *Thorax*: pronotum (Figures 17(c), and 17(d)), elongate, usually campaniform, rarely suboval, usually deeply constricted at base, disc indented with large setiferous punctations, interstitial spaces (spaces between punctations), very elevated, dorsolateral ridge extends from posterior angle to anterior midpoint, fractured by coarse punctations, sclerotized region above pronotal projection glabrous, prebasal fissure shallow, prointercoxal process very expanded distally, process confluent with pronotal projections (Figure 17(c)), latter particularly wide; elytron sculptured with deep asetiferous punctations, punctations seriate, rarely diminish in size to elytral apex, 1° setae always adjacent to asetiferous punctations, 2° setae densely distributed, interstitial spaces elevated, epipleural fold laterally positioned, very narrow to elytral apex, anterior margin not carinate; metathoracic wing (Figure 17(g)), cross veins rudimentary or missing; metendosternite (Figure 17(f)), without furcal lamina, furcal anterior plate prominent; legs, tibial spur formula 1-2-1, tarsal pulvillar formula 3-3-3, unguis without denticle. *Abdomen*: aedeagus (Figures 17(i), and 17(j)), shorter



(a) (b) (c) (d) (e) (f)

Figure 20: Various organs. (a, b) *Tarsostenodes simulator* ((a) head, (b) mouthparts). (c, d) *Tarsostenus univittatus* ((c) head, (d) mouthparts). (d, e) *Thriocera pectoralis* ((d) head, (e) mouthparts).

than length of abdomen, phallobase not reduced, lobate, lobes fimbriate; phallic lateral plates wide, phallobasic rod absent, phallic apex robust, spicular plates very narrow (Figure 17(h)), spicular apodemes fused, intraspicular plate rod shaped; ovipositor, ventral and dorsal laminae unilobed, laminal rod present; distal margin of pygidium not incised, distal margin of male 6th sternite slightly incised. *Alimentary canal*: not studied. *Male mesodermal internal reproductive organs*: not studied. *Female mesodermal internal reproductive organs*: spermathecal capsule tubular, well sclerotized, spermathecal gland attached to middle of spermathecal capsule.

Distribution. Known only from continental Australia and Lord Howe Island.

Species Examined. Tarsostenodes albonotatus Pic, *T. cribripennis* Schenkling, *T. guttulus* (White), *T. howensis* Bartlett, *T. leucogramma* Elston, *T. simulator* Blackburn, and four undescribed species.

4.5.17. Tarsostenosis Heller (Figure 23(e)). Tarsostenosis Heller [38]. Type species*: Tarsostenosis tricolor* Heller [38]. By monotypy. Corporaal [5].

Synapotypic Characteristics. Pronotum with linear glabrous elevations.

Diagnosis. Specimens belong to this genus if their pronotum shows a poorly developed lateral tubercle, the pronotum has

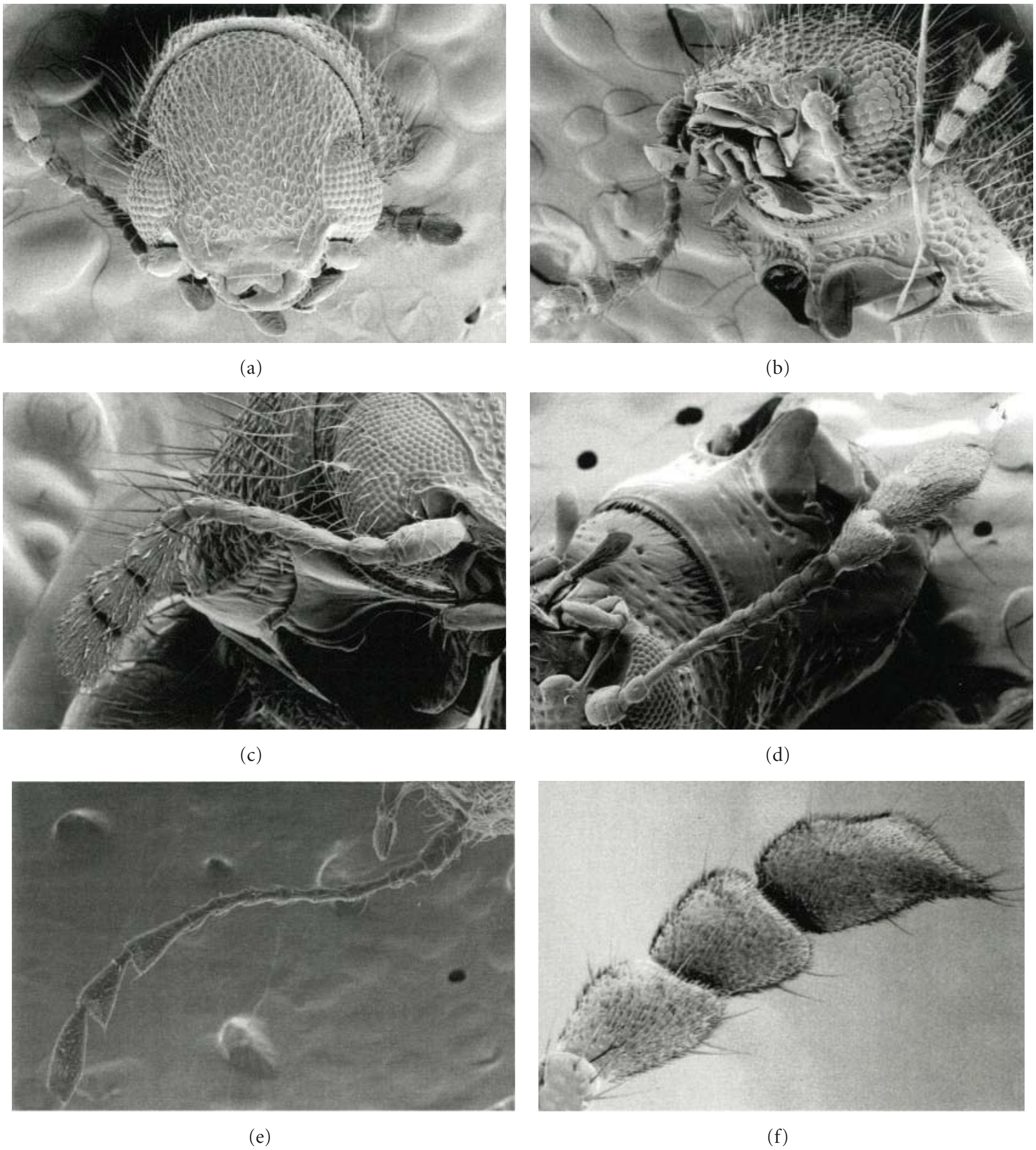

(a) (b) (c) (d) (e) (f)

Figure 21: Various organs. (a, b) *Thriocerodes bifasciatus* ((a) head, (b) mouthparts). (c) *Thriocera pectoralis* antenna. (d) *Tarsostenus univttatus* antenna. (e) *Riotenerus fossipenneus* antenna. (f) *Fallopylus pallipes* capitulum.

shallow linear glabrous elevations, and the elytra are devoid of 2° setae.

Description. Size: length 3.5–5.0 mm; width 1.0–1.5 mm. *Form* (Figure 23(e)): oblong, narrow rectangulate, about 4 times longer than broad. *Vestiture*: disc of cranium and sides of pronotum densely vested with pale setae, pronotal middle sparsely setose, elytral disc vested with 1° setae, 2° setae absent. *Head*: cranium quadrate, frons much wider than width of eye, indented with large setiferous punctations; gula large, trapezoidal, sutures oblique, gular processes widely separated, processes in form of two setiferous tubercles; labrum short, deeply incised, transverse tormal processes confluent, epipharyngeal plate very small; mandible, body short, anterior and medial dens well developed, posterior dens not well developed, penicillus well developed; maxilla, laterolacinia present, terminal palpomere securiform; labium, ligula deeply incised, terminal palpomere securiform; eyes small, coarsely faceted, ocular notch small; antenna, capitate, capitulum lax, scape about as long as combined length of pedicel and antennomere 3, funicular antennomeres filiform, capitular antennomeres slightly expanded, antennomeres 9 and 10 subtriangular, antennomere 11 ovoid. *Thorax*: pronotum quadrate, disc at sides indented with large setiferous punctations, disc at middle sparsely punctate, with linear glabrous elevations, dorsolateral ridge extends from posterior angle to anterior midpoint, fractured by coarse punctations, sclerotized region above pronotal projection not glabrous, prebasal fissure shallow, prointercoxal process not expanded distally, pronotal projections

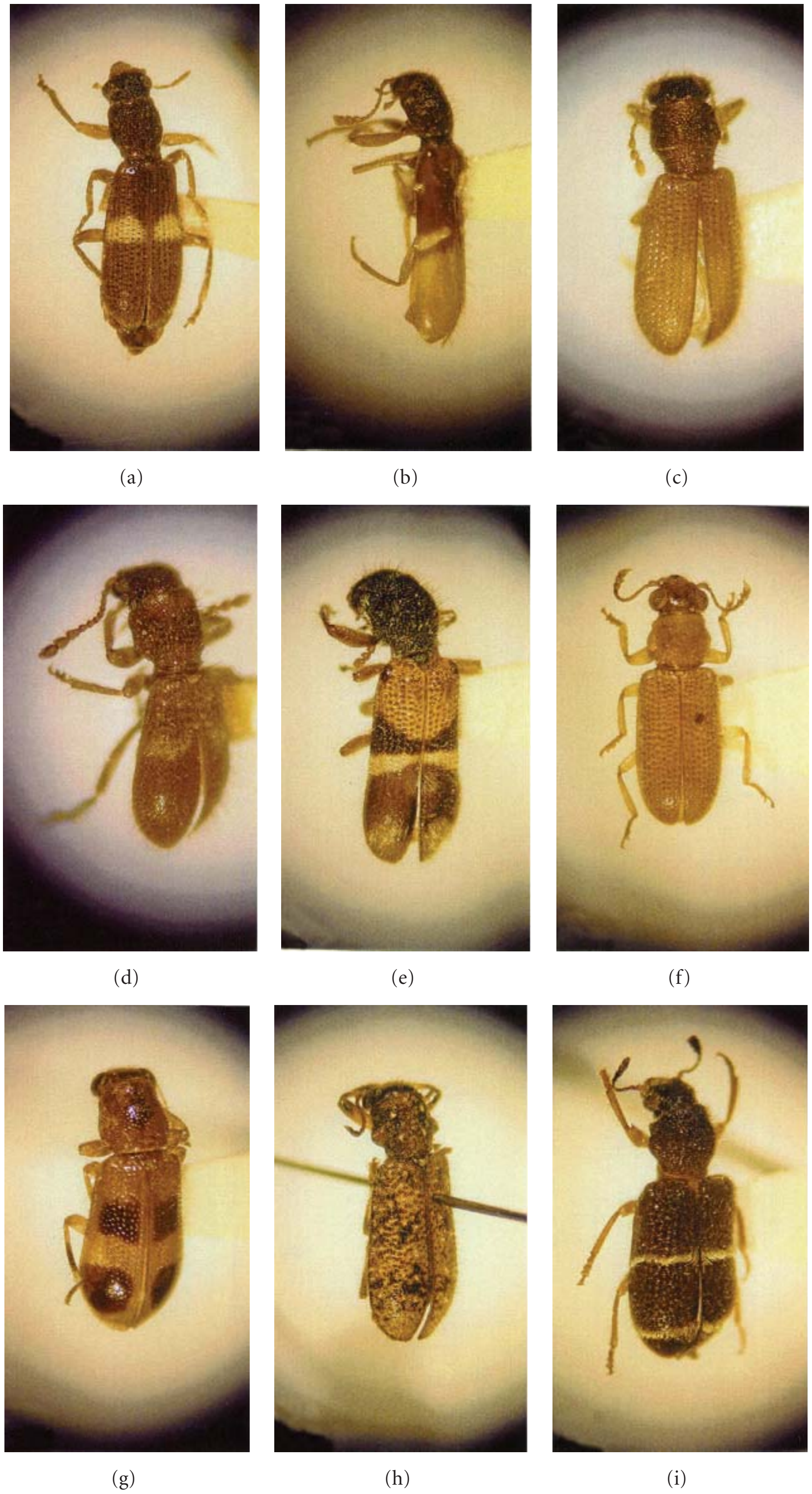


Figure 22: Habitus. (a) *Abiliella fasciata*. (b) *Agapetilus vietus*. (c) *Apopylus unumgarensis*. (d) *Apteropilo pictipes*. (e) *Blackburniella intricata*. (f) *Fallopylus pallipes*. (g) *Globoclava quadrimaculata*. (h) *Neopylus nahuelbutensis*. (i) *Parapylus bicinctus*.

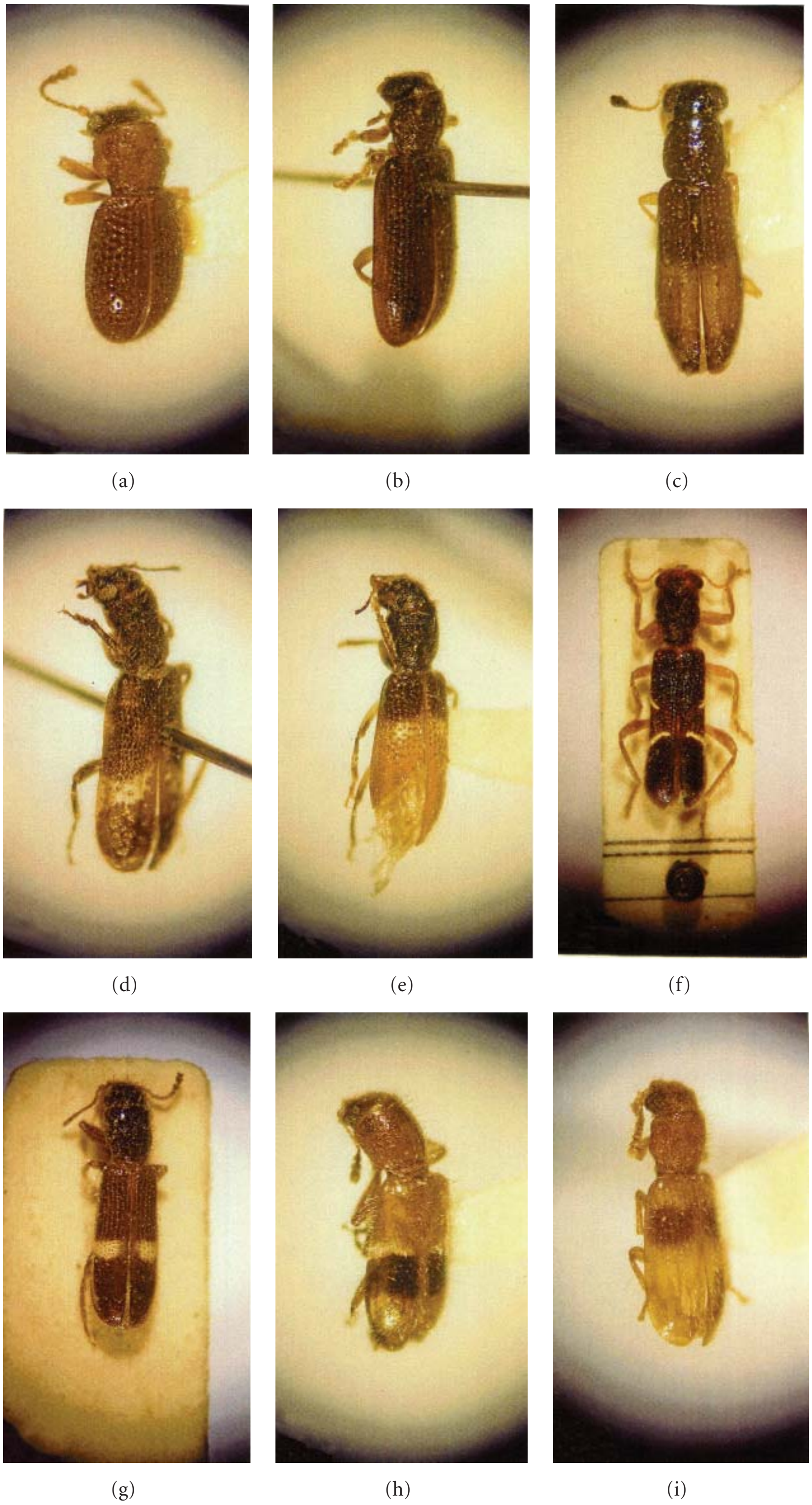

(a) (b) (c) (d) (e) (f) (g) (h) (i)

Figure 23: Habitus. (a) *Pseudopylus okei*. (b) *Pylus fatuus*. (c) *Rhophaloclerus coquerelii*. (d) *Riotenerus fossipennes*. (e) *Tarsostenosis tricolor*. (f) *Tarsostenodes simulator*. (g) *Tarsostenus univttatus*. (h) *Thriocera pectoralis*. (i) *Thriocerodes bifasciatus*.

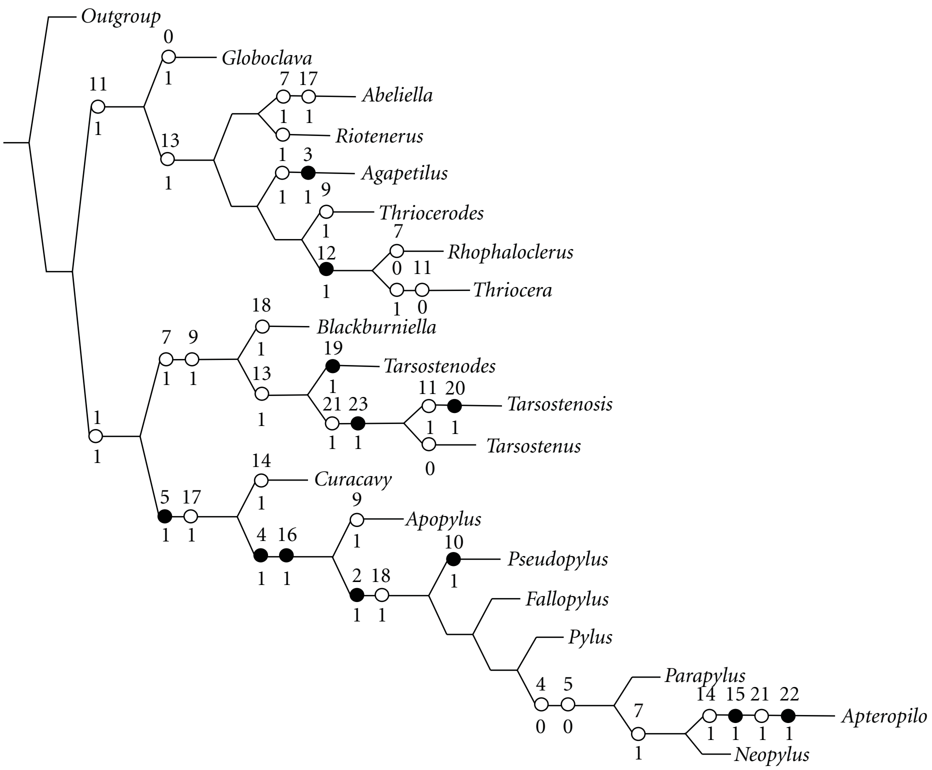


Figure 24: Phylogeny of genera of Tarsosteninae.

short; elytron sculptured with round asetiferous punctations, punctations seriate and extend to elytral distal two-thirds, 1° setae always adjacent to asetiferous punctations, 2° setae absent, interstitial spaces smooth, epipleural fold laterally positioned, very narrow to elytral apex, anterior margin carinate; metathoracic wing with open wedge cell; metendosternite with furcal lamina, furcal anterior plate shallow; legs, tibial spur formula 1-2-1, tarsal pulvillar formula 3-3-3, unguis without denticle. *Abdomen*: aedeagus shorter than length of abdomen, phallobase not reduced, not lobate but fimbriate; phallic lateral plates narrow, phallobasic rod linear, phallic apex robust, spicular plates narrow, slightly acuminate, spicular apodemes not fused, intraspicular plate rod shaped; ovipositor not examined. *Alimentary canal*: not studied. *Male mesodermal internal reproductive organs*: not studies. *Female mesodermal internal reproductive organs*: not studied.

Distribution. Species are known from Australia and New Caledonia.

Species Examined. Tarsostenosis hilaris (Westwood), (new combination), *T. tricolor* (Heller), and one undescribed species.

4.5.18. Tarsostenus Spinola (Figures 18(a)–18(m), 20(c), 20(d), 21(d), and 23(g)). Tarsostenus Spinola [12]. Type species: *Clerus univittatus* Rossi [39]. By monotypy. Corporaal [5]. Crowson [40]. Ekis and Gupta [41]. Matthews [22]. Opitz [42]. Kolibáč [6].

Synapotypic Characteristics. Aedeagus inverted, one pair of accessory gland.

Diagnosis. Specimens belong to this genus if their pronotum lacks a lateral tubercle, the dorsolateral ridge is present only in pronotal basal half, and the elytra are devoid of 2° setae.

Description. Size: length 3.5–8.0 mm; width 1.0–2.0 mm. *Form* (Figure 23(g)): oblong, narrow rectangulate, about 4 times longer than broad. *Vestiture*: disc of cranium and sides of pronotum densely vested with pale setae, pronotal middle sparsely setose, elytral disc vested with 1° setae, 2° setae absent. *Head* (Figures 18(a), 18(b), and 20(c)): cranium quadrate, frons much wider than width of eye, indented with large setiferous punctations; gula (Figure 18(a)), large, trapezoidal, sutures oblique, gular processes widely separated, processes in form of two setiferous tubercles; labrum short, deeply incised, transverse tormal processes confluent, epipharyngeal plate very small; mandible, body short, anterior and medial dens well developed, posterior dens not well developed, penicillus well developed; maxilla (Figure 20(d)), laterolacinia present, terminal palpomere subsecuriform; labium, ligula deeply incised, terminal palpomere subsecuriform; eyes small, coarsely faceted, ocular notch small;

antenna (Figure 18(i)), capitate, capitulum lax, scape about as long as combined length of pedicel and antennomere 3, funicular antennomeres filiform, capitular antennomeres slightly expanded, antennomeres 9 and 10 subtriangular, antennomere 11 ovoid. *Thorax*: pronotum (Figures 18(c) and 18(d)), quadrate, disc at sides indented with large setiferous punctations, disc at middle sparsely punctated, dorsolateral ridge extends from posterior angle to anterior midpoint, fractured by coarse punctations, sclerotized region above pronotal projection not glabrous, prebasal fissure shallow, prointercoxal process not expanded distally, pronotal projections short; elytron sculptured with round asetiferous punctations, punctations seriate and extend to elytral distal two-thirds, 1° setae always adjacent to asetiferous punctations, 2° setae absent, interstitial spaces smooth, epipleural fold laterally positioned, very narrow to elytral apex, anterior margin carinate; metathoracic wing (Figure 18(e)), wedge cell open; metendosternite (Figure 18(g)), with furcal lamina, furcal anterior plate shallow; legs, tibial spur formula 1-2-1, tarsal pulvillar formula 3-3-3, unguis without denticle. *Abdomen*: aedeagus (Figure 18(g)), shorter than length of abdomen, phallobase not reduced, lobate, lobes fimbriate; phallic lateral plates narrow, phallobasic rod linear, phallic apex robust, spicular plates narrow, slightly acuminate, spicular apodemes not fused (Figure 18(f)), intraspicular plate rod shaped; ovipositor, ventral and dorsal laminae unilobed, laminal rod present; distal margin of pygidium not incised, distal margin of male sixth sternite slightly incised. *Alimentary canal* (Figure 18(m)): no external evidence of ventricular crypts, four cryptonephridial malpighian tubules, stomodaeal valve comprised of four primary lobes, dorsal and ventral lobes reduced in size. *Male mesodermal internal reproductive organs* (Figure 18(k)): one pair of accessory gland. *Female mesodermal internal reproductive organs* (Figure 18(l)): spermathecal capsule ovoid, well sclerotized, spermathecal gland attached to apex of spermathecal capsule.

Distribution. *Tarsostenus univittatus* (Rossi) and *T. carus* (Newman) are cosmopolitan. Other species are known from Australia and New Caledonia.

Species Examined. *Tarsostenus carus* (Newman), *T. univittatus* (Rossi), and one undescribed species.

Notes. Kolibáč [43] presented a variety of illustrations about the organs of *T. univittatus* (Rossi).

4.5.19. Thriocera Gorham (Figures 19(a)–19(f), 20(e), 19(f), 21(c), and 23(h)). *Thriocera* Gorham [44]. Type species*:* *Corynetes pectoralis* Klug [45]. By original designation. Corporaal [5].

Synapotypic Characteristics. A uniquely derived characteristic has not been found.

Diagnosis. This is the only genus of Tarsosteninae which is oblong-short, lacks asetiferous punctations on the elytral disc, and has a 0-2-2 tibial spur formula.

Description. Size: length 3.6–7.0 mm; width 1.2–2.5 mm. *Form* (Figure 23(h)): oblong short rectangulate to narrow rectangulate, about 3 times longer than broad. *Vestiture*: disc of cranium and pronotum vested profusely with erect setae, elytral disc vested profusely with tall erect setae and shorter decumbent setae. *Head* (Figures 19(a), and 19(b)): cranium quadrate, frons much wider than width of eye, indented with very small setiferous punctations; gula (Figure 19(a)), large, trapezoidal, sutures oblique, gular processes widely separated, processes in form of two setiferous tubercles; labrum short, deeply incised, transverse tormal processes confluent, epipharyngeal plate very small; mandible, body short, anterior and medial dens well developed, posterior dens not well developed, penicillus well developed; maxilla (Figure 20(f)), laterolacinia absent, terminal palpomere subsecuriform; labium, ligula not incised, terminal palpomere subsecuriform; eyes small, finely faceted, ocular notch large; antenna (Figure 21(c)), capitate, capitulum compact, scape about as long as combined length of pedicel and antennomere 3, funicular antennomeres filiform, capitular antennomeres expanded, antennomeres 9 and 10 expanded, antennomere 11 ovoid. *Thorax*: pronotum (Figures 19(c) and 19(d)), quadrate, disc indented profusely with small setiferous punctations, lateral margins variously convex, dorsolateral ridge extends from posterior angle to anterior angle, not fractured and with smooth surface, sclerotized region above pronotal projection glabrous, prebasal fissure shallow, prointercoxal process expanded distally; pronotal projections short; elytron sculptured with numerous setiferous punctations, punctations not seriate, basal uncus variously developed, with or without elongate setae, epipleural fold laterally positioned, narrowed abruptly narrowed at elytral posterior two-thirds, anterior margin carinate; metathoracic wing with wedge cell open; metendosternite with furcal lamina, furcal anterior plate not prominent; legs, tibial spur formula 0-2-2, tarsal pulvillar formula 3-3-3, unguis with denticle. *Abdomen*: aedeagus (Figure 19(f)), shorter than length of abdomen, phallobase not reduced, not lobate, not fimbriate; phallic lateral plates narrow, phallobasic rod linear, phallic apex globose, spicular plates triangular and acuminate, spicular apodemes fused in posterior third (Figure 19(e)), intraspicular plate rod shaped; ovipositor, ventral and dorsal laminae unilobed, laminal rod present; distal margin of pygidium not incised, distal margin of male sixth sternite slightly incised. *Alimentary canal*: not studied. *Male mesodermal internal reproductive organs*: not studied. *Female mesodermal internal reproductive organs*: not studied.

Distribution. Known only from Africa.

Species Examined. *Thriocera pectoralis* (Klug) and 12 other species which may or not be described.

4.5.20. Thriocerodes Wolcott & Dybas (Figures 19(g)–19(j), 21(a), 21(b), and 23(i)). *Thriocerodes* Wolcott & Dybas [46]. Type species*:* *Incorynetes bifasciatus* Pic [47]. Designation by Kolibáč [6]. Corporaal [5].

Synapotypic Characteristics. Phallic apex digitiform, CuA vein oblique.

Diagnosis. Blackburniella Chapin, *Fallopylus* gen. nov., *Tarsostenodes* Blackburn, *Tarsostenus* Spinola, and *Thriocerodes* Wolcott & Dybas are characterized by having a 1-2-1 tibial spur formula. Specimens of *Thriocerodes* may be distinguished from specimens of the other aforementioned genera by having a transverse pronotum.

Description. Size: length 3.0–6.0 mm; width 1.0–2.0 mm. *Form* (Figure 23(i)): oblong short rectangulate, about 3 times longer than broad. *Vestiture*: disc of cranium and pronotum vested profusely with pale setae, elytral disc with serially arranged asetose punctations and with 1° setae and shorter serially arranged 2° setae. *Head* (Figures 19(g), 19(h), and 21(a)): cranium quadrate, frons much wider than width of eye, indented with very small setiferous punctations; gula (Figure 19(g)), large, trapezoidal, sutures oblique, gular processes widely separated, processes in form of two setiferous tubercles; labrum short, not deeply incised, transverse tormal processes confluent, epipharyngeal plate very small; mandible, body short, anterior and medial dens well developed, posterior dens not well developed, penicillus well developed; maxilla (Figure 21(b)), laterolacinia present, terminal palpomere subsecuriform; labium, ligula incised, terminal palpomere subsecuriform; eyes large, coarsely faceted, ocular notch large; antenna, capitate, capitulum lax, scape about as long as combined length of pedicel and antennomere 3, funicular antennomeres subfiliform, capitular antennomeres slightly expanded, antennomeres 9 and 10 expanded, antennomere 11 subquadrate. *Thorax*: pronotum (Figures 19(i) and 19(j)), subquadrate, lateral margins with median tubercle, dorsolateral ridge extends from posterior angle to anterior angle, sclerotized region above pronotal projection glabrous, prebasal fissure shallow, prointercoxal process not expanded distally; pronotal projections short; elytron sculptured with serially arranged asetiferous punctations, epipleural fold laterally positioned, narrowed to elytral apex, anterior margin carinate; metathoracic wing with wedge cell open, CuA vein oblique; metendosternite with furcal lamina, furcal anterior plate prominent; legs, tibial spur formula 1-2-1, tarsal pulvillar formula 3-3-3, unguis without denticle. *Abdomen*: aedeagus shorter than length of abdomen, phallobase not reduced, lobate, not fimbriate; phallic lateral plates narrow, phallobasic rod linear, phallic apex digitiform, spicular plates triangular, not acuminate, spicular apodemes fused or not, intraspicular plate rod shaped; ovipositor, ventral and dorsal laminae unilobed, laminal rod present; distal margin of pygidium not incised, distal margin of male sixth sternite slightly incised. *Alimentary canal*: not studied. *Male mesodermal internal reproductive organs*: not studied. *Female mesodermal internal reproductive organs*: spermathecal capsule well sclerotized, spermatheca gland attached to subapex of spermathecal gland, saccular bursa copulatrix well developed.

Distribution. This genus is known from Australia.

Species Examined. Thriocerodes bifasciatus (Pic), *T. bipartitus* (Pic), *T. corporaali* Wolcott & Dybas, *T. pygmaeus* (Blackburn), *T. pyloides* Kolibáč, *T. rolciki* Kolibáč, and four undescribed species.

Notes. Kolibáč [6] provides illustrations of species of *Thriocerodes*.

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Review Article

Parasitoid Guilds of *Agrilus* Woodborers (Coleoptera: Buprestidae): Their Diversity and Potential for Use in Biological Control

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Literature studies in North America (US and Canada), Europe, and Asia (particularly Russia, China, Japan, and the Korean peninsula) were reviewed to identify parasitoid guilds associated with *Agrilus* woodborers. There are at least 12 species of hymenopteran parasitoids attacking eggs of *Agrilus* beetles and 56 species (36 genera), attacking *Agrilus* larvae infesting various host plants in North America, Asia, and Europe. While most of the egg parasitoids (9 species) belong to the family Encyrtidae, a majority of the larval parasitoids are members of five families: Braconidae (24 species/11 genera), Eulophidae (8 species/4 genera), Ichneumonidae (10 species/9 genera), and Eupelmidae (6 species/5 genera). The highest rate of *Agrilus* egg parasitism (>50%) was exerted by encyrtid wasps (4 species) in North America, Asia, and Europe. In contrast, the highest rate of *Agrilus* larval parasitism (>50%) was caused by species in two genera of braconids: *Atanycolus* (North America) and *Spathius* (Asia), and one eulophid genus, *Tetrastichus* (Asia and Europe). Reported rate of *Agrilus* larval parasitism ichneumonids was frequent in North America, but generally low (<1%). Potential for success in biological control of emerald ash borer (*Agrilus planipennis* Fairmaire) in the USA with North American native parasitoids and old-association Asian parasitoids is discussed.

1. Introduction

Agrilus is the largest genus within the family Buprestidae (Coleoptera), with nearly 3,000 described species worldwide [1]. Generally, *Agrilus* spp. only attack angiosperms and do not develop in conifers [2]. Moreover, they tend to be specialists, most species being confined to a single genus or species of host plant. While most *Agrilus* species are not considered to be serious pests of agriculture or forests, at least two species have recently become seriously damaging in forests in their newly invaded areas in North America: the emerald ash borer (EAB), *A. planipennis* Fairmaire, and the gold spotted oak borer (GSOB), *A. auroguttatus* Shaefer. EAB was accidentally introduced to Michigan in late 1990s from its native range (northeast Asia, in parts of China, Russia, and Korea) possibly via wooden crates or pallets for cargo shipment [3]; it has since spread to 14 additional US states and two Canadian provinces and killed millions of North American ash (*Fraxinus* spp.) since its detection in 2002 [4, 5]. By contrast, GSOB is native to the oak forests of southwestern Arizona, and while its damage to oak trees in its invaded range has been on a smaller scale, it has killed more than 25,000 oaks in the oak savannahs of California since first discovered there in 2002 [6–8]. A few other exotic *Agrilus* species have also been recently detected in the United States (e.g., soapberry borer—*A. prionurus* Chevrolat in Texas [9]) and Canada (e.g., European oak borer—*A. sulcicollis* Lacordaire in Ontario [10]). Although some of the recently detected, exotic *Agrilus* species have not become as widespread or damaging as EAB and GSOB, the pest status of *Agrilus* borers as a whole along with other woodborers appears to have increased in recent years [11].

Table 1: Summary of online database search for *Agrilus* and associated parasitoids.

Search database	Years searched	*Agrilus* hits	Search hits combined with parasitoid (parasite, natural enemy, or biological control)
Agricola	1970-date	302	38
BioAbstracts (BIOS)	1926-date	507	43
Biological and Agricultural Index Plus	1983-date	30	2
Biological Sciences Set	1982-date	273	23
CSA Illumina		265	22
CAB Abstracts	1985-date	354	94
ISI Web of Science	1900-date	211	25
	No duplicates[a]		−105
	Total publications	1,942	142

[a] We did not review all 1,942 to exclude duplicates.

Management for the invasive (exotic) *Agrilus* woodborers (EAB and GSOB) in the United States initially focused on attempted eradication but changed to integration of several approaches when eradication failed to reduce the pests' populations in infested areas and slow spread of the pests to the noninfested areas [12, 13]. In some cases, control methods being used include delimitation of infested areas, regulatory restriction of movement of pest-infested wood or plant materials, insecticide treatment or physical destruction of infested trees [12–14], and biological control via introduction and release of natural enemies collected from pests' native ranges [7, 15–18]. Although none of these approaches individually is adequate, biological control, which relies on self-propagating and dispersing natural enemies, has potential to reduce invasive pest populations, particularly in forests [19–21].

Agrilus adults normally lay their eggs under loose bark or in crevices of host plant tissues and rarely cause significant damage; in contrast, *Agrilus* larvae typically bore into the living tissue (stems, trunks, branches, or roots) of their host plants, interrupt the translocation of water and nutrients as they feed, and can kill plants within one or a few years of infestation (e.g., EAB [22]; GSOB [6]; *A. prionurus* [9]). In their native habitats, *Agrilus* populations are generally suppressed by a diverse group of natural enemies and/or host tree resistance and only occasionally become serious pests. However, when introduced into ecosystems where host plants lack coevolutionary resistance, or where appropriate specialized natural enemies are absent, they can become severe pests. The recent invasions of North America by EAB from northeast Asia and GSOB from southwestern Arizona are excellent examples of this. For example, EAB is considered a sporadic pest of ash stands in its native range in Asia [23–26] but has become a serious pest threatening the existence of North American ash trees since it was accidentally introduced there [22]. Similar observations have been made for GSOB in its home range. Field studies in Asia found that a complex of natural enemies (primarily parasitoids) and host plant resistance by Asian ash trees appear to be the factors responsible for suppressing EAB populations and preventing them from frequently causing ash mortalities [15, 19].

Deliberate efforts have been recently undertaken in the United States to achieve biological control of EAB and GSOB through introduction of natural enemies (parasitoids) from the native ranges of these pests [7, 17]. These classical biological control efforts for EAB have led to the discovery and introduction of several egg and larval parasitoids that have the potential to establish and suppress the pests' populations in the newly introduced regions [19–21, 27]. Similar programs for GSOB commenced in 2010 and are too immature to reach tentative conclusions about natural enemy diversity and impacts. In reviewing the literature, we found that many groups of parasitoids and other natural enemies have reported attacking *Agrilus* beetles. An overview of the composition of the parasitoid guild attacking this group of woodborers will contribute to the current and future development of biological control programs to manage these pests, particularly those *Agrilus* that have invaded new regions or environments. In the present study, we first review the diversity of natural enemy complexes in particular, hymenopteran parasitoid guilds associated with egg and larval stages of *Agrilus* species, and then discuss the potential of those parasitoids for use as agents of classical biological control against this group of pests.

2. Literature Reviewed

We searched seven major online data bases using the key word "*Agrilus*" either alone or in combination with any of the key words "parasitoid," "parasite", "natural enemy", or "biological control" to locate relevant literature. Databases examined were (1) Agricola, (2) BioAbstracts (BIOS), (3) Biological Sciences Set, (4) Biological and Agricultural Index Plus, (5) CSA Illumina, (6) CAB Abstract, and (7) ISI Web of Sciences set. The key word "*Agrilus*" alone resulted in 1942 articles (Table 1), of which 142 articles remained when combined with "parasitoid or parasite, natural enemies, or biological control." It must be noted that database searches concluded in March 2011. For this paper, we included only

Table 2: Parasitoid guilds associated with *Agrilus* woodborers in North America and Asia.

Parasitoid guilds	Order: Family	Species	Recorded *Agrilus* host	Habitat	Native range in distribution	Level of parasitism	Reference sources
Egg parasitoids	Hym: Aphelinidae	*Ablerus sp.*	*A. anxius*	Birch trees	Northeastern USA/Canada	<0.2%	[28]
	Hym: Encyrtidae	*Avetianella* sp.	*A. anxius*; *A. subcinctus*	Birch trees; ash trees	Northeastern USA/Canada	<3.5%	[28, 34]
		Coccidencyrtus sp.	*A. liragus*	Poplar trees	Northeastern USA/Canada	∼55%	[35]
		Ooencyrtus erionotae	*A. sexsignatus*	Eucalyptus trees	Southeast Asia (Philippines)	32–57%	[31, 32]
		Ooencyrtus sp.	*A. anxius*	Birch trees	Northeastern USA/Canada	<2.4%	[28]
		Oobius agrili	*A. planipennis*	Ash trees	China/northeast China-Jilin province	>50%	[15, 36]
		Oobius agrili	*A. planipennis*	Ash trees	United States/Michigan	Not reported	[19]
		Oobius zahaikevitshi	*A. viridis and A. planipennis*	Hazelnut and ash trees, resp.	Northern Italy/Russian	8 –58%	[37, 38]
		Orianos brazai	*A. sexsignatus*	Eucalyptus trees	Southeast Asia (Philippines)	0–47%	[39]
		Signichorini tribe	*A. anxius*	Birch trees	Northeastern USA/Canada	<1%	[28]
		Ptinobius magniflcus	*A. ruficollis*	Raspberry, Blackberry, Dewberry	North America	Not reported	[40, 41]
	Hym: Signiphoridae	*Thysanus* sp.	*A. liragus*	Poplar trees	Northeastern USA/Canada	∼12%	[35]
	Hym: Eulophidae	*Pediobius* sp.	*A. planipennis*	Ash trees	United States/Michigan	Not reported	[42]
Larval Parasitoids	Hym: Braconidae	*Atanycolus charus*	*A. anxius* and *A. liragus*	Birch and poplar trees	Northeastern USA/Canada	0.3–52%	[29, 35]
		Atanycolus cappaerti	*A. planipennis;* *A. liragus and A. bilineatus*	Ash trees; poplar and chestnut trees	Northeastern USA/Canada	9–71%	[33]
		Atanycolus disputabilis	*A. planipennis* and other North American native woodborers	Oak trees	Northeastern USA/Canada	<1%	[43]
		Atanycolus simplex	*A. planipennis;* *A. liragus and A. bilineatus*	Ash trees; poplar and chestnut trees	Northeastern USA/Canada	<1%	[35, 44]
		Atanycolus hicorie	*A. planipennis and* other native *Agrilus* woodborers	Ash trees	Northeastern USA/Canada	<2%	[45, 46]
		Atanycolus nigropopyga	*A. planipennis* and other North American native woodborers	Ash trees	Northeastern US/Canada	<3%	JJD (unpublished)

Table 2: Continued.

Parasitoid guilds	Order: Family	Species	Recorded *Agrilus* host	Habitat	Native range in distribution	Level of parasitism	Reference sources
		Atanycolus picipes	*A. planipennis*	Ash trees	Vladivostok, Russia	<5%	JJD (unpublished), [25]
		Doryctes farthus	*A. anxius* and *A. liragus*	Birch and poplar trees	Northeastern US/Canada	<0.1%	[44]
		Doryctes rufipes	*A. anxius* and *A. liragus*	Birch and poplar trees	Northeastern USA/Canada	<0.1%	[44]
		Doryctes atripes	*A. anxius*	Birch tree	Northeastern USA/Canada	<0.1%	[44]
		Iphiaulaz impostor	*A. biguttatus*	Poplar trees	Europe	∼13%	[47]
		Leluthia astigma	*A. planipennis; A. difficilis* and other *Agrilus* spp.	Ash trees; honey locust trees	USA	∼2.1%	[48, 49]
		Spathius agrili	*A. planipennis*	Ash trees	China	60–90%	[50–56]
		Spathius agrili	*A. planipennis*	Ash trees	USA/Michigan	Not reported	[21, 57]
		Spathius agrilivorus	*A. planipennis*	Ash trees	Vladivostok, Russia	∼64%	JJD (unpublished), [25]
		Spathius curvicaudis	*A. biguttatus*	Oak trees	Europe	∼25%	[47, 58]
		Spathius floridanus	*A. planipennis* and other North American native woodborers	Ash trees	USA	<0.5%	JJD (unpublished)
		Spathius laflammei	*A. planipennis* and other North American native woodborers	Ash trees	USA	<1%	JJD (unpublished)
		Spathius simillimus	*A. anxius and A. liragus/; A. planipennis*	Birch and poplar tree; ash trees	USA/Canada	<0.5%	[18]
		Wroughtonia (Helconidea) ligator	*A. anxius, A. liragus and A. bilineatus*	Birch, poplar and chestnut trees	northeastern USA/Canada	<1%	[29, 44]
		Ecphylus sp.	*A. subcinctus*	Ash trees	USA	Not reported	[34]
		Heterospilus sp.	*A. subcinctus*	Ash trees	USA	Not reported	[34]
		Pareucorystes varinervis	*A. viridis*	Hazelnut	Europe/Russia	Not reported	[59]
		Monogonogastra agrili	*A. arcuatus*	Hickory, pecan	North America	Not reported	[60]
		Microbracon xanthostigmus	*A. ruficollis*	Raspberry, blackberry, dewberry	North America	Not reported	[40, 41]
	Hym: Chalcididae	*Phasgonophora sulcata*	*A. anxius, A. bilineatus and A. liragus; A. planipennis*	Birch, chestnut and poplar tree; ash trees	USA/Canada	2–20%	[28–30, 35]

Table 2: Continued.

Parasitoid guilds	Order: Family	Species	Recorded *Agrilus* host	Habitat	Native range in distribution	Level of parasitism	Reference sources
	Hym: Eulophidae	*Tetrastichus nr.rugglesi*	*A. anxius and A. liragus; A. planipennis*	Birch and poplar trees; ash trees	USA/Canada	<0.1%	[29, 35]
		Tetrastichus heeringi	*A. sinuatus and A.aurichalceus*	Pear trees and raspberries, resp.	Europe	55–75%	[61–63]
		Tetrastichus heeringi	*A. ribesi*	Black current	Europe	Not reported	[64]
		Tetrastichus sp.	*A. sexignatus*	Eucalyptus trees	Southeast Asia (Philippines)	2–50%	[31, 32]
		Tetrastichus planipennisi	*A. planipennis*	Ash trees	Northeastern China/Russian Far East	22–40%	[15, 65, 66]
		Tetrastichus planipennisi	*A. planipennis*	Ash trees	USA Michigan	0.80%	[27, 57, 67]
		Baryscapus agrilorum	*A. aurichalceus*	Raspberry	Europe/Hungary	Not reported	[62, 68]
		near Hadrotrichodes	*A. subcinctus*	Ash trees	USA	Not reported	[34]
		Entodon epicharis	*A. surorovi/*	Poplar	China	Not reported	[69]
		Entodon zanara	*A. surorovi*	Poplar	China	Not reported	[69]
	Hym: Eupelmidae	*Balcha indica*	*A. planipennis* and other Asia and North American woodborers	Ash trees	Southeast Asia/North America	<4%	[70–72]
		Calosota elongata	*A. auroguttatus*	Oak trees	USA/Mexico	15%	[6]
		Eupelmus pini	*A. planipennis*	Ash trees and weevils	North America	<0.2%	[70]
		Metapelma sp.	*A. subcinctus*	Ash trees	USA	Not reported	[34]
		Calosota agrili	*A. salicis*	Willow	Europe/Poland and Russia	Not reported	[73, 74]
		Pentacladia hatayensis	*Agrilus* sp.	Fig	Turkey	Not reported	[75]
	Hym: Eurytomidae	*Bephratoides agrili*	*A. anxius*	Birch trees	North America	<1%	[29]
		Eurytoma rosae	*A. rubicola* and *A. bilineatus*	Rose and chestnut trees		Not reported	
		Eurytoma sp.	*A. anxius*	Birch trees	North America	<1%	[29]
		Eurytoma sp.	*A. subcinctus*	Ash trees	North America	Not reported	[34]
	Hym: Ichneumonidae	*Cunocephalus sp.*	*A. planipennis*	Ash trees—to be confirmed	North America	<0.2%	[70]
		Dolichomitus messorperlongus	*A. anxius* and *A. liragus*	Birch trees	North America	<0.4%	[29]
		Dolichomitus vitticrus	*A. planipennis* and other native woodborers	Ash trees	North America	<0.2%	[70]
		Ephialtes sp.	*A. anxius* and *A. liragus*	Birch trees	North America	<0.4%	[35]

Table 2: Continued.

Parasitoid guilds	Order: Family	Species	Recorded *Agrilus* host	Habitat	Native range in distribution	Level of parasitism	Reference sources
		Glypta sp.	*A. anxius*	Birch trees	North America	<1%	[44]
		Ichneumon sp.	*A. anxius*	Birch trees	North America	<1%	[44]
		Olesicampe sp.	*A. anxius*	Birch trees	North America	<1%	[44]
		Unknown spp.	*A. suvorovi-populneus*	Poplar trees	Europe/Hungary	4-5%	[76]
		Orthizema sp.	*A. anxius;* *A. planipennis*	Birch trees; ash trees	North America	<1%	[44, 70]
		Pimploterus sp.	*A. anxius*	Birch trees	North America	<1%	[44]
		Labena apicaulis	*A. arcuatus*	Hickory, pecan	North America	Not reported	[60]
	Hym: Stephanidae	*Foenatopus sp.*	*A. sexsignatus*	Eucalyptus trees	Southeast Asia (Philippines)	2–50%	[31, 32]
	Hym: Pteromalidae	*Zatropus* sp.	*A. arcuatus*	Hickory, pecan	North America	Not reported	[60]
		Oodera sp.	*A. subcinctus*	Ash trees	USA	Not reported	[34]
	Hym: Bethylidae	*Sclerodermus pupariae*	*A. planipennis*	Ash trees	China	Not reported	[77]

those original research articles that provide information on parasitoid identity at the family, genus, or species levels (Table 2).

In addition to searching databases, we contacted colleagues who work on invasive EAB and GSOB beetles and their biological control in the United States and Canada for information on parasitoid guilds of these species. All relevant studies were read and analyzed for mention of *Agrilus* species, associated parasitoids, known host associations, host plants, and geographic distributions. If available, parasitism rates by each group, guild, or species of parasitoids were noted.

3. Results and Discussion

At the genus level, the guilds of egg and larval parasitoids of *Agrilus* species were similar in North America, Europe, and Asia. While several families of North American parasitoids (including Braconidae, Chalcididae, Ichneumonidae, and Eupelmidae) are capable of utilizing larvae of the newly introduced emerald ash borer (*A. planipennis*) as a novel host, some Asiatic species of parasitoids appear to be more specific and only utilize Asian *Agrilus* species as hosts. From the geographic distribution point of view, it appears that there is more diversity in the parasitoid complex associated with *Agrilus* beetles in North American than in Asia and Europe. However, this geographic difference in parasitoid diversity may actually reflect different levels of research activities on the subject. For example, the invasion of North America by EAB has certainly resulted in much more research activities on the parasitoid complex of this group of woodborers in North America.

There are at least 12 species of hymenopteran parasitoids that attack eggs of *Agrilus* beetles and 56 parasitoid species that attack *Agrilus* larvae in various plants in North America, Asia, or Europe (Table 2). While most of these egg parasitoids (9 species) belong to the family Encyrtidae, a majority of the larval parasitoids are members of five families: Braconidae (24 species/11 genera), Eulophidae (8 species/4 genera), Ichneumonidae (10 species/9 genera), and Eupelmidae (6 species/5 genera). One species of larval parasitoid (*Phasgnophora sulcata* Westwood) (Chalcididae) is frequently associated with native *Agrilus* woodborers in North America [28–30]. In addition, there is one larval parasitoid (*Foenatopus* sp.) in the family Stephanidae that was reported attacking *A. sexsignatus* (Fisher) infesting Eucalyptus trees in southeast Asia [31, 32].

The highest rates of *Agrilus* egg parasitism (>50%) occurred with four species of encyrtid wasps reported in North America, Asia, and Europe (Table 2). In contrast, the highest rates of *Agrilus* larval parasitism (>50%) were caused by two groups of braconid wasps: *Atanycolus* spp. (in North America) and *Spathius* spp. (in Asia), and three species of eulophid wasps (in Asia and Europe). Although ichneumonid wasps were frequently reported attacking *Agrilus* woodborers in North America, the reported rate of parasitism was very low (<1%) for all the ichneumonid species.

It is interesting to note that several species of North American native parasitoids, *Atanycolus* spp., *Spathius floridanus* Ashmead, *S. laflammei* Provancher, *S. simillimus* Ashmead, *Phasgonophora sulcata* Westwood, and one accidentally introduced Asiatic wasp *Balcha indica* (Mani and Kaul), have been recently reported attacking the invasive emerald ash borer. One group of native parasitoids, *Atanycolus* spp., has recently become the dominant mortality factor associated with emerald ash borer, attacking >50% of *A. planipennis* larvae at some forest sites in Michigan (USA) [19, 33]. The potential of both the native (new-association) parasitoids and the introduced (old-association) parasitoids (e.g., *Oobius agrili* Zhang and Huang, *Tetrastichus planipennisi* Yang, and *Spathius agrili* Yang) for biological control of EAB, in the USA, needs further investigation.

A diverse group of hymenopteran parasitoids attacks eggs and larvae of *Agrilus* woodborers in North America, Asia, and Europe. Literature review of this genus, in regards to its parasitoid guild, has interest due to the introduction of two species in North America (GSOB and EAB). In biological control, parasitoid species of invasive pests are often introduced from the land of origin, if proved to be safe (not become a pest themselves). In addition, new-association parasitoids that inhabit the region prior to the pest introduction sometimes exert pressure on this newly arrived pest and offer opportunity for research and augmentation of indigenous parasitoid populations. Our literature review has provided documentation of research activities for 12 egg parasitoid species and 56 larval parasitoid species. These parasitoids are identified from 19 species of *Agrilus*, a small representation of almost 3000 described, that attack 18 recorded plant types (13 hardwoods, 5 shrubs). Being a diverse genus, these results show a wealth of research opportunities for further work on *Agrilus* parasitoids worldwide. Nearly two thirds (64.3%) of the literature found was published after year 2000. Twenty-seven of 83 entries (32.5%), in Table 2, reference *A. planipennis*. These findings are results of EAB postdetection in 2002.

Although *Agrilus* species are relatively host specific, because of larvae's concealed nature, early stages and damage are difficult to assess and take much effort to obtain. This has implications on finding and identifying parasitoid complexes for biocontrol and may be a reason for so little literature. Of those parasitoid species found in association with EAB, some are ectoparasitoids and known to attack woodborers in different families (e.g., Cerambycidae). While data from the current literature do not show any particular relationship between host specificity and mode of parasitization (endo- versus ectoparasitoids), further research is needed to investigate such relationship.

Some species occur on multiple *Agrilus* spp., such as egg parasitoid *Oobius zahaikevitshi*. *Atanycolus cappaerti* is known to attack *A. planipennis*, *A. liragus*, and *A. bilineatus*, while *Leluthia astigma* attacks *A. planipennis*, *A. difficilis*, and other *Agrilus* spp. These may provide better access of parasitoids where poplar, chestnut, honey locust, and ash occur together.

The parasitoid guild of *Agrilus* in China, Russia, and North America and EAB distribution may provide species for introduction or augmentation. Though most of the parasitism rates are low (<10%), a few worthy candidates not yet used for introduction or augmentation include egg parasitoid, *O. zahaikevitshi* from Russia, and larval parasitoids *Atanycolus cappaerti, Spathius agrilovorus*, and *Spathius floridanus*. These species in the USA have not yet been reared in large numbers, and further studies on rearing methods need pursuing. It appears also that braconids and eulophids have provided the best potential for biological control, and the number of studies the last five years bear this out. It also indicates that species size and morphology (ovipositor length) for accessing the host from outside the host plant are important for success.

Finally, parasitoid work in biological control efforts often lack taxonomic expertise to provide accurate identifications. Some of these newly known parasitoid species are not well understood. Egg parasitoids are often disregarded due to size and inaccessibility of host eggs. These hamper ongoing biological control of invasive or cyclic native pest populations. A concluding question is should work be done now on conspecifics that have the potential to be invasive (e.g., *A. coxalis* attacks oaks in Mexico—California has a history of acquiring pests from MX, could *A. coxalis* be another threat to CA's besieged oaks forests?).

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Research Article

The Dark Side of the Light Show: Predators of Fireflies in the Great Smoky Mountains

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In the Great Smoky Mountains of East Tennessee, the Light Show is a popular seasonal attraction created by thousands of courting male *Photinus carolinus* fireflies (Coleoptera: Lampyridae) that flash in synchrony to locate females. This study was undertaken to provide a temporal snapshot of whether invertebrate predators are active within these dense and conspicuous firefly breeding aggregations. In addition, we examined whether female *Photuris* fireflies, which are specialist predators on other fireflies, show any feeding preferences within the diverse local firefly fauna. A field survey revealed a surprisingly diverse suite of generalist insectivores feeding on fireflies within *P. carolinus* breeding aggregations. In addition, laboratory studies revealed major differences in prey consumption rates when *Photuris* predators were given access to several lampyrid taxa. This suite of generalist and specialist predators appears to create a complex selective landscape that is predicted to be a powerful force shaping the evolution of firefly defenses.

1. Introduction

Animals with conspicuous courtship displays that breed in dense aggregations are expected to be targeted by many predators [1, 2]. Fireflies (Coleoptera: Lampyridae), however, have a reputation for being distasteful to many potential predators [3, 4]. Several lampyrid taxa have been shown to contain chemicals that confer protection against generalist insectivores such as birds, spiders, and ants [5–7]. While lists have published tallying instances of observed predation on various fireflies [3, 8], no study has described the predator guild active within a firefly breeding aggregation at a single location and season.

In North America, *Photuris* fireflies are specialist predators that eavesdrop on the courtship signals of other fireflies [9–12]. *Photuris* females have been shown to be voracious predators of certain *Photinus* fireflies [3, 13, 14], from which they sequester defensive compounds known as lucibufagins [6]. Lloyd [3, 10] reviewed numerous field observations of *Photuris* females preying upon several firefly species. Eisner et al. [6] reported lab studies in which 6 *Photuris* females each ate 2 *Photinus ignitus* males, and a study by Gronquist et al. [15] found that 5 *Photuris* females each ate 3 *Lucidota atra* fireflies, a diurnally active species that also contains lucibufagins. To date, however, no systematic study has been made of the feeding proclivities of these predatory *Photuris* fireflies.

The Great Smoky Mountains in East Tennessee host a diverse and abundant lampyrid fauna, including both diurnal and nocturnal species [16, 17]. Among these are *Photinus carolinus*, a species in which thousands of males gather in dense aggregations and flash synchronously to locate females [18, 19]. In the Great Smoky Mountains National Park (GSMNP), this phenomenon is popularly known as the Light Show. During their 2-week mating season in June, these fireflies attract close to 30,000 park visitors. Such aggregations might be expected to attract many predators as well. Faust [19] reported that *P. carolinus* males were often caught in webs of Araneidae spiders, and harvestmen (Opiliones: Phalangiidae) was found carrying dead *P. carolinus*.

Another abundant nocturnal firefly, *Phausis reticulata*, is also active in this rich alluvial montane habitat at the same time of night. Commonly known as blue ghost fireflies, these males fly slowly over the forest floor emitting a blue-green flickering glow. However, to date there has been no systematic survey describing the common predators of these two firefly species which are so popular with park visitors.

This study was conducted during the *P. carolinus* mating season with the goal to survey invertebrate predators of *P. carolinus* and *Phausis reticulata* adults and also to determine whether specialist *Photuris* predators differentially prey on various firefly taxa.

2. Methods

2.1. Field Surveys of Firefly Predators. Field observations were conducted at GSMNP by walking along a ~4 km path through *P. carolinus* breeding aggregations from 2000 to 2400 h during the peak display season (4–19 June 2011). Our surveys were conducted in Sevier Co. at Elkmont, Tennessee (35°39′13″N, 83°34′50″W), although this species is found throughout the park in second growth hardwood forests at about 750 m elevation [19]. Male courtship signals in *P. carolinus* consist of flash trains containing 4–8 pulses given at 0.5 sec intervals, followed by 6–9 sec of darkness; females respond to male advertisements by emitting a doublet flash approximately 3 sec following final pulse in a male's flash train [19]. We detected predation by looking along the ground and on vegetation for the distress flashes given by *P. carolinus*; these distress flashes consist of consistent, rhythmic single flashes repeated every 1.5–3 sec [19] and are easily distinguished from firefly courtship flashes. We also looked for continuous stationary glows emitted from the light organ of injured fireflies. Whenever predator-prey interactions were observed, they were recorded and photographed (Sony Cybershot DSC-T20). Prey captured by orb-weaving spiders was monitored by counting firefly and other captured prey nightly in webs at ~2400 h, toward the end of the *P. carolinus* flight period. Since webs were less likely to contain glowing prey towards the end of the firefly season, web surveys were made with spotlights.

Similar observations focusing on invertebrate predators of nocturnal fireflies were also made in other areas of GSMNP. Birds and other potential diurnal predators were not covered by our surveys, as nocturnal fireflies such as *P. carolinus* disperse during the day to rest on or under vegetation, and thus their interactions with diurnal predators are quite difficult to observe. Similarly, it was logistically impossible to include bat predators in our field survey.

2.2. Laboratory Tests of Photuris Feeding Preferences. While most adult fireflies do not feed, some *Photuris* females are specialist nocturnal predators that hunt *Photinus* males using a combination of stalking, aerial hawking, and aggressive mimicry of prey females [9, 10, 13, 20]. To determine whether predaceous *Photuris* females show preferences among males of different lampyrid taxa, we conducted laboratory trials using as prey several different firefly species that overlapped spatially and/or temporally with *Photuris* spp. Because *Photuris* is a taxonomically problematic group currently in need of revision, it is not possible to provide definitive species identifications for these predators. These females included *Photuris hebes* and *P. lucicrescens*, while others were in the *Photuris versicolor* complex (J. E. Lloyd, *personal communication):* here we refer to them collectively as *Photuris*.

All fireflies were kept on a 14 : 10 light cycle (this was shifted from natural by 9 h). Predatory *Photuris* females were housed individually in 1-quart (14 cm height × 10 cm diameter) plastic containers with damp paper towel and a silk plant, and prey was added at dusk. Because prey could move about and avoid attacks, this experimental setup provided considerably more natural conditions than the 9 cm petri dishes assays that have previously been used in lab studies of *Photuris* predation [6, 15]. *Photuris* behavior was observed for the first hour under blue light (many lampyrids show reduced retinal sensitivity for these wavelengths [21]), and trials were checked periodically for 24 h. These laboratory trials were conducted between 6 and 21 June 2011. Most prey was offered in pair-choice trials, which allowed us to test several species during their short breeding seasons. Some prey was offered in single-choice trials: 4 (of 8) *Phausis reticulata*, and 8 (of 40) *Photinus pyralis*.

Because they are lampyrid specialists, none of the 11 *Photuris* females we tested consumed any of the "palatable" prey we offered them in these experiments (these included *Tribolium* beetle larvae, as well as various flies, click beetles, grasshoppers, and bugs that were collected from the field). We therefore confirmed that *Photuris* predators were hungry, following trials in which no prey was eaten, by offering them prey shown to be highly desirable (*P. carolinus* or *L. atra*) in our preliminary experiments.

3. Results

3.1. Field Surveys of Nocturnal Firefly Predators. Several predators were found actively hunting in the midst of *P. carolinus* mating aggregations (Figure 1). Orb-weaving spiders including *Cyclosa conica* (Figure 1(a)) and *Neoscona arabesca* constructed webs at dusk that captured mainly *P. carolinus* males, which constituted 72% of prey items; on a single night one web contained 7 *P. carolinus* males. In addition, 2 *Phausis reticulata* males (blue ghost fireflies) along with 1 *P. carolinus* female were found trapped in webs. Thus, of the 25 total prey items found in these webs, the vast majority were male fireflies. Many of the males continued to flash rhythmically after they were wrapped in silk. We also noticed that fireflies were often positioned at the center of spider webs, although we did not quantify how often this occurred.

Remains were collected the next morning below marked web locations, and these silk-wrapped fragments suggested active predation on *P. carolinus* by orb-weavers. Some *P. carolinus* males had been partially consumed, while others were largely intact but had a large puncture wound at the anterior corner of their wing cover.

Additional predators included several *Leiobunum* spp. harvestmen (Opiliones: Phalangiidae) that we observed feeding on *P. carolinus* males. One had captured a newly eclosed *Photuris* which struggled unsuccessfully to escape (Figure 1(b)). In addition to preying on live fireflies, harvestmen were also observed feeding on silk-wrapped fireflies that they apparently scavenged from beneath the webs of orb-weaving spiders. An assassin bug, *Zelus luridus* (Hemiptera: Reduvidae), was found perched on a hickory tree leaf ~10 ft off the ground, feeding on a *P. carolinus* male that

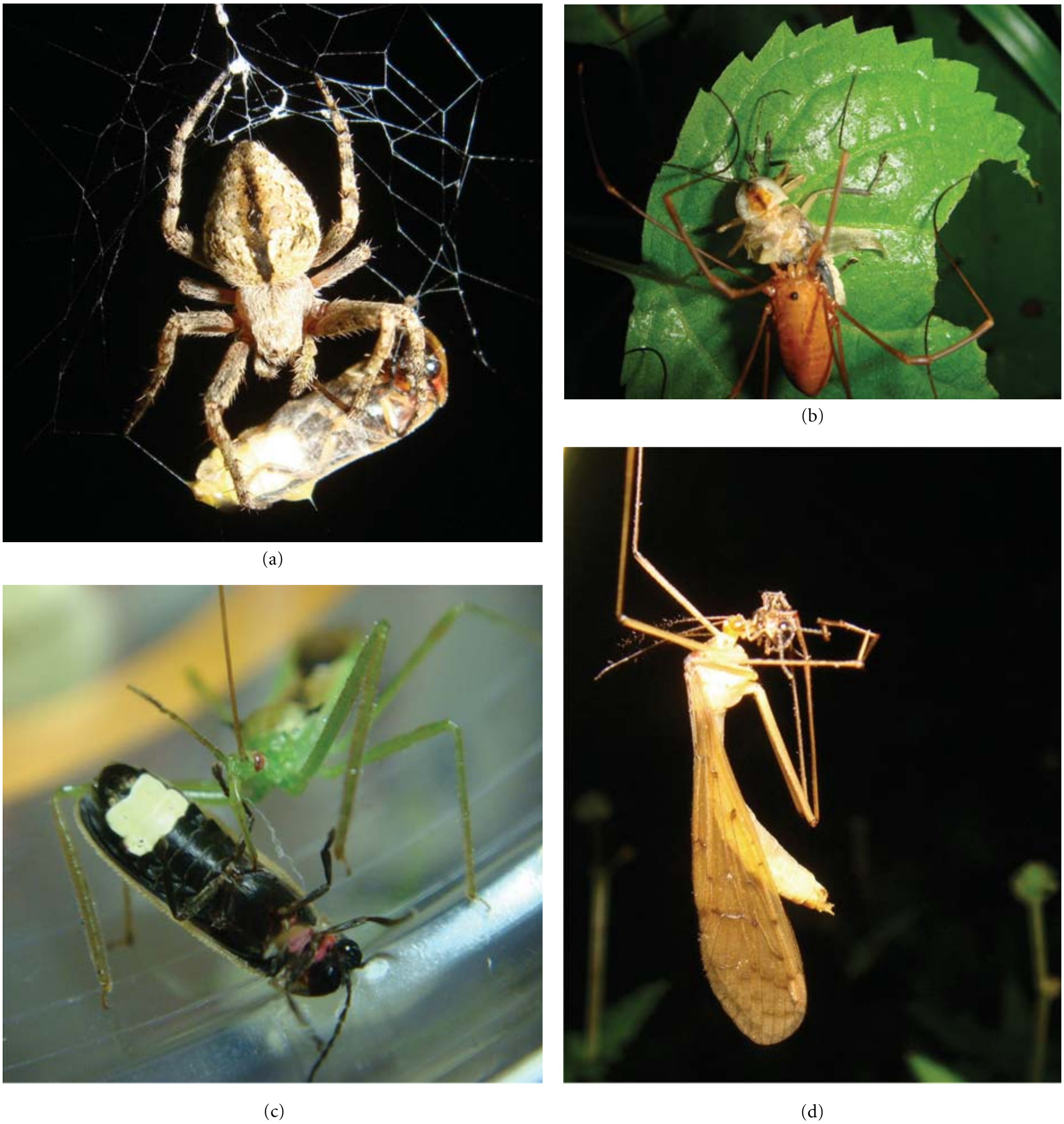
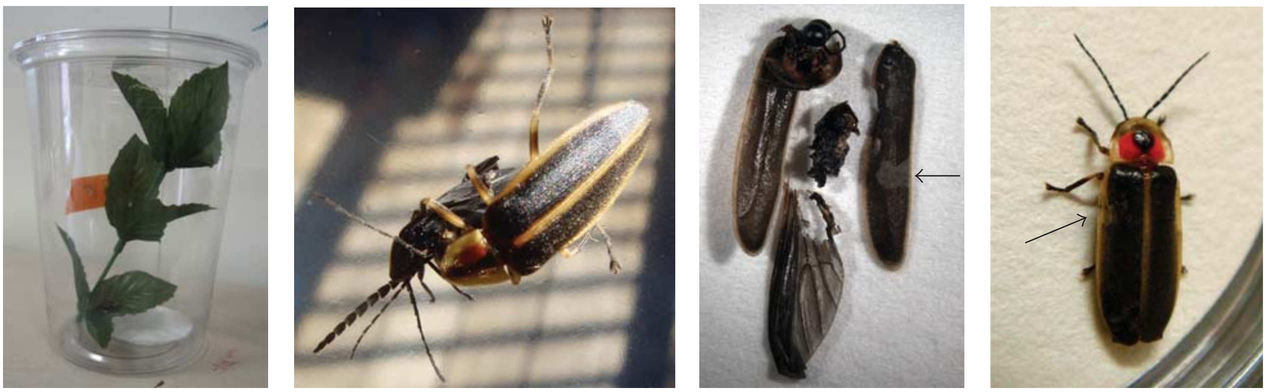


Figure 1: Some invertebrate predators of fireflies in the Great Smoky Mountains (photos by R. De Cock). (a) Orb-weaving spider (Araneidae) *Cyclosa conica* attacking a *Photinus* male that has been caught and wrapped. (b) Harvestman *Leiobunum* spp. (Opiliones: Phalangiidae) attacking a newly eclosed *Photuris* firefly. (c) *Zelus luridus* assassin bug (Hemiptera: Reduviidae) feeding on a male *Photinus carolinus*. (d) *Bittacus* spp. hangingfly (Mecoptera: Bittacidae) consuming a male *Phausis reticulata*.

was flashing periodically. When brought into the lab, this bug recommenced feeding on the same *P. carolinus* male by piercing the intersegmental membrane between the fireflies' thorax and abdomen with its proboscis (Figure 1(d)). Two small *Theridion* spp. cobweb spiders were each found eating a *Phausis* male wrapped in silk. In addition, two *Bittacus* spp. hangingflies (Mecoptera: Bittacidae) were each found consuming still-glowing *Phausis* males (Figure 1(d)).

When we surveyed four webs at the end of the *P. carolinus* flight season, we found only a single nonfirefly prey item at the study site. However, two webs were found nearby that each contained a single firefly (one web captured a *P. carolinus* male and the other a *Phausis reticulata* male). Thus, web capture efficiency appeared to be dependent on firefly population density, with fewer captures as firefly abundance declined.

3.2. Laboratory Tests of Photuris Feeding Preferences. All *Photuris* females fed readily under these experimental conditions (Figures 2(a) and 2(b)); one predator consumed 8 out the 11



(a) (b) (c) (d)

Figure 2: Laboratory tests of *Photuris* feeding preferences choice. (a) Trial setup with single *Photuris* spp. female in 1-quart container with artificial plant. (b) Female *Photuris* attacking an *L. atra* firefly. (c) Remnants of a male *Photinus carolinus* (arrow indicates dried hemolymph on the elytra). (d) A surviving male *Photinus pyralis* (arrow indicates puncture wound on left elytra).

prey she was offered over 7 days of testing. During the light photoperiod both predators and prey generally rested on the upper or lower surface of artificial leaves or sides of the container. Within 1 hr of dark phase, fireflies started walking, flashing, and occasionally flying. When prey fireflies were contacted by the *Photuris* female, they rapidly withdrew or dropped to the bottom of the container. During a predator attack, the *Photuris* female grasped the firefly with her front legs and then bit into the prey, often between the elytral shoulder and the pronotum, with her mandibles. Prey reflex bleeding (originally described by Blum and Sannasi [22] for *Photinus pyralis*) was often observed. When they were bitten, both *P. pyralis* and *P. carolinus* males released copious amounts of thick white fluid; we often observed that this fluid rapidly coagulated into a sticky mass that coated the predator's mouthparts. Although this appeared to temporarily prevent the *Photuris* female from continuing her attack, under laboratory conditions most predators eventually returned to continue feeding on the wounded prey. Notably, *Phausis reticulata* males did not exhibit reflex bleeding although they typically showed prolonged thanatosis. After 24 hours, prey that had been successfully attacked had been reduced to scattered bits of exoskeleton, including pronotum, eyes, elytrae, and wings (Figure 2(c)).

We found marked differences in consumption rates when various firefly species were offered to captive *Photuris* females (Figure 3). In three *Photinus* species, *P. carolinus*, *P. macdermotti*, *and P. marginellus*, 60–76% of males were eaten within 24 h; in contrast, only 12.5% of *Photinus pyralis* males were eaten. Microscopic examination of the surviving *P. pyralis* males revealed that many had been attacked, as bite marks and dried blood were seen on their pronotum or elytra (Figure 2(d)). Most *Phausis reticulata* also remained uneaten over 24 h, although again close examination of the surviving males revealed bite marks on their elytra or abdomen. We also tested two diurnally active species of *Lucidota* fireflies, *L. atra* and *L. punctata*, both of which were readily consumed by *Photuris* females (Figure 3).

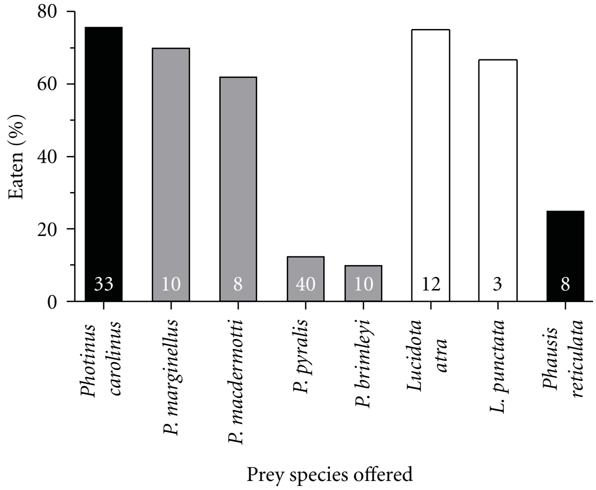


Figure 3: Differences among firefly taxa in the percentage of individuals consumed by predatory *Photuris* femmes fatales during 24 h laboratory trials. Sample sizes (number of trials) are shown within bars, and shading indicates the activity period of each species (black: fully nocturnal; grey: dusk-active; white: diurnal).

We incidentally noted very few parasitoids among the fireflies collected for this study: about 5% of *Photuris* females appeared to die from tachinid parasitism (*Strongygaster triangulifera* Loew), and less than 5% of prey (all species combined) died due to phorids (*Apocephalus antennatus* Malloch).

4. Discussion

Previous reviews have described the general diversity of firefly predators [3, 4]. Our study focused on providing a temporal snapshot of predation upon Smokies fireflies during the brief but explosive breeding season of the synchronous firefly, *Photinus carolinus*. Our field survey revealed a surprisingly diverse suite of predators feeding on fireflies within these aggregations, including the first record of hangingflies (Mecoptera) eating fireflies, here *Phausis reticulata*. Our results confirm that harvestmen are firefly predators as well as scavengers. This supports previous observations by Lloyd [3], who also noted that harvestmen (including *Leiobunum*) ate live fireflies (*Photuris* spp. and *Photinus scintillans*) on three occasions. It is not known whether these opilionid arachnids are able to visually detect light-emitting prey, but the capacity to orient toward light has been demonstrated for two cave-dwelling harvestmen that feed exclusively on the bioluminescent dipteran, *Arachnocampa luminosa* [23]. Our observation of predation on *Photinus carolinus* fireflies by the reduviid bug *Zelus luridus* confirms Lloyd's [3] reports of *Zelus spp.* attacking a female *Photuris congener*, and an unsuccessful attack by another reduviid on a male *Pteroptyx* firefly. Spiders and other invertebrates have also been reported to consume various Japanese fireflies [8]. It seems likely that such predators use a combination of substrate vibrations and visual cues to detect fireflies walking on leaf litter or vegetation, as shown for lycosid spiders [24].

Orb-weaving spiders are a notable source of male-biased mortality for fireflies, as they mainly capture flying males that are searching for females [3]. We found this to be especially true for the males of two fully nocturnal fireflies, *P. carolinus* and *Phausis reticulata*. While diurnal (*Lucidota atra*, *L. punctata*) and dusk-flying fireflies (*Photinus macdermotti* and *P. marginellus*) were also active at this site, these species were less susceptible to predation by nocturnal orb-weavers because their webs were constructed after dark and then dismantled before the next morning. Our field survey also supports previous observations that once fireflies are captured in a web, many continue to glow or flash rhythmically [3, 8, 25]. Previous authors have suggested that this behavior acts as a bioluminescent lure to attract additional prey to the web; so it would repay further investigation to determine whether and how spiders are able to induce their prey's bioluminescence. We also noticed that captured fireflies were quite often positioned at the center of webs, which might also serve to maximize a spider's chance of capturing additional prey that had been attracted.

Consumption of *Photinus carolinus* and *Phausis reticulata* by these diverse generalist predators remains somewhat surprising because firefly taxa have been shown to contain a variety of defensive steroidal pyrones collectively known as lucibufagins (LBGs). First isolated from *Photinus ignitus* and *P. marginellus* [5], LBGs have also been found in *P. pyralis* [26] and *Lucidota atra* [15], as well as in larvae of the European glow-worm, *Lampyris noctiluca* [27]. LBG deters predation by at least two generalist predators: *Hylocichla* thrushes [5] and *Phidippus* jumping spiders [6]. As *Photinus carolinus* and *Phausis reticulata* have not yet been examined, it is possible that these species lack chemical defenses. Alternatively, it may be that the suite of generalist predators active within these breeding aggregations is able to circumvent firefly chemical defenses.

Our lab results confirm previous field observations indicating that nocturnally active *Photuris* females are specialist predators upon other fireflies[9]. Eisner et al. [6] demonstrated that *Photuris* fireflies are incapable of producing LBG on their own but rather must rely on acquiring these compounds from their prey to gain protection against their own predators. Thus, we expect *Photuris* predation to select for very different defensive strategies than those that might be effective against generalist insectivores.

Although our results indicate that *Photuris* females readily consume a broad range of lampyrid prey, including males of the synchronous species *Photinus carolinus*, firefly taxa differed markedly in their susceptibility to predation by *Photuris* fireflies. What factors might account for such differences? It might be predicted that those prey species whose activity period overlaps with the fully nocturnal *Photuris* would show reduced susceptibility. However, observed *Photuris* predation rates did not follow this prediction: low consumption rates were seen for the fully nocturnal blue ghost firefly, *Phausis reticulata*, but also for two dusk-active fireflies, *Photinus pyralis* and *P. brimleyi*. In addition, *Photuris* females readily consumed some dusk-active, some fully nocturnal, and two diurnal species.

Another reasonable prediction is that *Photuris* consumption rates might be positively correlated with LBG content across lampyrid taxa. Unfortunately, this cannot currently be tested because the defensive chemistry of most firefly taxa remains unexamined. However, two firefly species shown in our study to be highly palatable are known to contain LBG: *Photinus marginellus* [5] and *Lucidota atra,* [15]. Thus, these species and others such as the synchronous firefly *Photinus carolinus* could be especially targeted by *Photuris* predators that are seeking to obtain LBG.

Several explanations may be considered for the very low *Photuris* predation rates we observed on three firefly species. Many firefly taxa exhibit reflex bleeding when disturbed, emitting droplets of hemolymph from their elytra and pronotum [22]. The released hemolymph rapidly coagulates, and this lampyrid bloodbath has previously been shown to deter predation by ants [7, 8, 22]. Our observations indicate that reflex bleeding may also help some fireflies escape predation by *Photuris* females, as we observed predators that were incapitated when their mouthparts became coated by sticky, coagulated blood. *Photinus pyralis* males are presumably desirable prey as they contain LBG [26], and they might use such copious reflex bleeding to gain mechanical protection against *Photuris* predators. The same may be true for *Photinus brimleyi*, although its defensive chemistry is unknown. Strong selection is expected for a prey's ability to glue shut a predator's mouthparts, as under natural conditions this would almost certainly allow the prey to escape. An alternate explanation is that additional chemical deterrents, or different and perhaps less desirable forms of LBG [26], make these particular *Photinus* species less attractive as prey for *Photuris*. Finally, in spite of their lack of reflex bleeding, *Phausis reticulata* males were often attacked yet not eaten. This suggests that these blue ghost fireflies also may have additional chemical deterrents and/or may lack the particular LBG required by *Photuris* females.

In summary, this temporal snapshot of predators active within Smokies firefly aggregations has revealed a surprisingly diverse suite of generalist insectivores. In addition, laboratory studies in which specialist *Photuris* predators were given access to several lampyrid taxa revealed major differences in prey consumption rates. The predator-prey interactions described here suggest that the evolution of firefly defenses occurs within a complex selective landscape involving both generalist and specialist predators. Testing evolutionary hypotheses concerning firefly chemical defenses and their effectiveness against both types of predators should prove a powerful approach for future investigations.

Additional Information

See Supplementary Material available online at doi: 10.1155/2012/634027. It is a short video that illustrates common predators on fireflies and shows attacks by predatory *Photuris* fireflies.

Acknowledgments

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Research Article

Effect of Air Humidity on Sex Ratio and Development of Ladybird *Harmonia axyridis* (Coleoptera: Coccinellidae)

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Length of development of larvae and pupae of the invasive alien ladybird beetle *Harmonia axyridis*, their survival rates, sex ratio, and fresh mass of the emerged adults were measured at three contrasting levels of relative air humidity: 30, 60, and 90%, 25°C and photoperiod 16L : 8D. Overall sex ratio was 51%, but there was a strong trend for higher proportion of males at low humidity and higher proportion of females at high humidity. Survival rate, larval developmental time, and adult mass were all differently influenced by air humidity depending on the food type. In individuals fed with aphid *Acyrthosiphon pisum* there was a trend for better survival, shorter development, and higher mass gained at higher humidity. These trends were opposite or nonsignificant in individuals fed with frozen eggs of moth *Ephestia kuehniella*.

1. Introduction

The multicoloured Asian lady beetle or harlequin ladybird *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) is native to eastern temperate Asia. It has a long history of use as a classical biological control agent of aphids and coccids in North America and has been deliberately introduced and has subsequently spread in four continents at a very fast rate during the last 23 years [1]. A CLIMEX model has been set up [2] to show the potential geographic distribution of *H. axyridis* that has been subsequently largely proven by new records. Besides several environmental temperature parameters and conditions for diapause, the model takes into account moisture parameters for the prediction of successful establishment in new areas. However, there are some regions where the prediction was not accurate. In Greece, *H. axyridis* was temporally established on orange trees heavily infested with aphids in 1993-1994 [3], but failed to survive in following years despite continued releases [4]. Another case of a failure in establishment of *H. axyridis* was observed [5] on the Azores islands. According to these studies, the released populations failed to establish due to ecological factors such as the maladaptation to the local conditions, mainly high temperature during diapause, and functional diversity saturation. Anyway, a conspicuous environmental trait that differs in these two regions from those where *H. axyridis* was successful is air humidity: very low in Greece and very high in Azores.

Among ambient factors controlled in laboratory experiments measuring development time and other life history traits of ectotherms, temperature and photoperiod are the most commonly and best studied, while moisture, for example, as relative air humidity, is often neglected: either not properly controlled or set to one value (e.g., [6]). More attention attracted moisture of substrate in soil arthropods [7] and of food or controlled atmosphere in stored product insects [8].

Developmental time of *Ophraella communa* (Coleoptera: Chrysomelidae) at different stages shortened along with the increasing relative air humidity (60%, 75%, and 90% RHs).

Table 1: Survival of developmental stages of *Harmonia axyridis* reared on two diets and in three levels of relative air humidity at 25°C and 16L : 8D photoperiod. *N*: initial number of second instar larvae, S3, S4, P and A: percentage survival to particular larval instar, pupal, or adult stage relative to the initial number of individuals, M: sex ratio (percentage of males). Chi-square tests with their probabilities were calculated for three-dimensional contingency table (food × humidity × survival/sex).

Food	RH %	*N*	S3 %	S4 %	P %	A %	M %
Acyrthosiphon	30	100	74	55	26	24	71
Acyrthosiphon	60	80	84	70	36	36	48
Acyrthosiphon	90	90	83	67	36	34	39
Ephestia	30	50	84	72	60	58	62
Ephestia	60	50	82	70	50	50	52
Ephestia	90	40	75	60	28	28	18
χ^2 ($d = 2$)			6.59	9.22	23.58	24.25	18.21
P			0.037	0.010	$<10^{-4}$	$<10^{-4}$	10^{-4}

The survival rates during the egg, larva, and entire immature stage were significantly higher at 75% RH and 90% RH than at 60% RH [9]. Low RH (20%) prolonged developmental duration of eggs and larvae reduced egg hatching and larval survival and reduced the body mass and body length of pine caterpillar, *Dendrolimus tabulaeformis* (Lepidoptera: Lasiocampidae), compared to 40%, 60%, and 80% RH [10]. As vapour pressure deficit decreased from 2.8 to 0.009 kPa, median life expectancy increased from 1.1 to 9.0 days for plum curculio *Conotrachelus nenuphar* (Coleoptera: Curculionidae) without food supply [11]. Complete development of *Ahasverus advena* (Coleoptera: Silvanidae) at 70% RH took 67, 58, and 48 days at 20, 22.5, and 25°C, respectively, while it was shorter (46, 31, and 26 days) at 90% RH [12].

The present study shows the effect of ambient humidity on some life history parameters of the ladybird *H. axyridis* that might play role in different establishment ability during its invasion to new areas.

2. Material and Methods

Adult beetles of the colour form *succinea* were collected outdoors and placed in laboratory, temperature 20°C, photoperiod 16L : 8D, and RH 60%, and fed with aphids *Acyrthosiphon pisum*. Eggs were removed from parents and placed to the experimental incubators at 25°C, 16L : 8D, and three contrasting levels of relative humidity: 30, 60, and 90%. They were checked daily for hatching. Neonate larvae were fed either with aphids *Acyrthosiphon pisum* or frozen eggs of *Ephestia kuehniella*. After moulting to the second instar, larvae were placed in groups of ten into 0.5 L glass jars covered with nylon mesh to provide air flow. They were continuously fed with either diet and provided with water in a vial that enabled limited evaporation. The food of larvae was offered in abundance to minimize their cannibalism. Survival and time of moulting to subsequent larval instars, pupa, and adult were recorded daily to calculate development time. Developmental time of individual instars was based on all individuals that survived to that particular instar even if they subsequently died. Adults were sexed one day after emergence, and fresh mass was recorded on electronic balances with precision 0.1 mg.

Low humidity (30%) was achieved by placing dishes with sodium hydroxide to the incubator; moderate and high humidity was made thanks to small or large evaporation surface of tissue dipped to dish with tap water. Temperature and humidity were measured with electronic equipment placed in the incubator among the jars with beetles and recorded daily. Precize environmental conditions measured twice daily in the three experimental incubators were (mean ± SD) 24.9 ± 0.1°C and 29 ± 6% RH, 24.9 ± 0.2°C and 59 ± 5% RH, and 25.0 ± 0.2°C and 87 ± 1% RH with no difference between morning and evening.

3. Results

3.1. Survival. Overall, survival of larvae from the second to the third instar (S3) was 80%, to the fourth instar, it was 65%, to pupa 37%, and to adult 36%. Individual treatments (food and humidity) slightly differed from each other (Table 1) in survival to the third and fourth larval instars, but there was much better survival to pupa and adult in individuals fed with *Ephestia* eggs than those fed *A. pisum* (χ^2 ($d = 1$) = 9.35, $P = 0.0022$). Survival to pupa and adult on *Ephestia* eggs was lower at 90% RH than in the two lower humidities (χ^2 ($d = 2$) = 8.71, $P = 0.013$). Survival to pupa and adult on *A. pisum* was lower at 30% RH than in the two higher humidities but this difference was not significant (χ^2 ($d = 2$) = 3.81, $P = 0.15$).

3.2. Sex Ratio. Overall, sex ratio in newly emerged adults was 51%, but there was a significant (χ^2 ($d = 2$) = 10.06, $P = 0.0065$) trend for higher proportion of males at low humidity and higher proportion of females at high humidity in both food treatments (Table 1).

3.3. Developmental Time. Development time of the first instar was two days except in larvae fed with *Acyrthosiphon* at 30% RH, where it was slightly but significantly prolonged (two-way ANOVA, $F_{2,404} = 4.76$, $P = 0.009$) (Table 2). There were opposite trends in the development time of the second instar, shortening with increasing humidity in larvae fed with

Table 2: Developmental time (days) and adult fresh mass (mg) of *Harmonia axyridis* reared on two diets and in three levels of relative air humidities at 25°C and 16L : 8D photoperiod. D1 to D4, DL and DP: development time of particular larval instar, entire larval stage, and pupa, FMM: fresh mass of males, FMF: fresh mass of females. Means that do not differ significantly are marked with the same letter in each column.

Food	RH %	D1 days	D2 days	D3 days	D4 days	DL days	DP days	FMM mg	FMF mg
Acyrthosiphon	30	2.1 a	3.6 a	3.4 bc	7.5 a	15.1	5.0	18.2	20.7
Acyrthosiphon	60	2.0 b	3.3 ab	3.5 bc	6.2 b	14.1	5.0	18.3	25.1
Acyrthosiphon	90	2.0 b	3.1 b	3.1 c	6.4 ab	13.5	5.0	19.2	25.4
Ephestia	30	2.0 b	3.1 ab	3.8 ab	6.3 b	14.6	5.0	19.4	23.0
Ephestia	60	2.0 b	3.3 ab	4.0 a	6.9 ab	15.4	5.0	19.4	23.5
Ephestia	90	2.0 b	3.5 ab	3.1 c	7.3 a	14.3	5.0	14.5	21.8

Acyrthosiphon and prolonging with increasing humidity in larvae fed with *Ephestia* (two-way ANOVA, $F_{2,323} = 4.01$, $P = 0.019$). In the third instar, there was high effect of humidity (one-way ANOVA, $F_2 = 13.4$, $P = 3.10^{-6}$) with shorter development at high humidity and a separate effect of food (one-way ANOVA, $F_1 = 10.4$, $P = 0.001$) with longer development time in larvae fed with *Ephestia* eggs than with *A. pisum*. In the fourth instar, there were again opposite trends in the development time ($F_{2,147} = 5.4$, $P = 0.005$) with shortening of time with increasing humidity in larvae fed with *Acyrthosiphon* and vice versa. When analyzing the entire larval development, the opposite trends were still present but not significant (two-way ANOVA, $F_{2,147} = 2.56$, $P = 0.08$); no difference accountable to either food, humidity, or their interaction was found. Pupal development time was identical in all treatments (two-way ANOVA, $F_{2,143} = 0.08$, $P = 0.92$).

3.4. Body Size. There was a trend for increasing adult body mass with increasing humidity in larvae fed with *A. pisum* but not in those fed with *Ephestia* eggs but generally, there was overall no significant difference (two-way ANOVA, $F_{2,143} = 2.37$, $P = 0.097$). When males and females were included in the analysis of variance, the interaction (opposite trends) of food and humidity became significant (three-way ANOVA, $F_{2,143} = 4.06$, $P = 0.02$). Females were much heavier (23.8 mg) than males (18.8 mg) in all treatments (three-way ANOVA, $F_1 = 40.6$, $P < 10^{-6}$) but the differences were greater at 90%. The ratio of body mass of females over males was 1.26.

4. Discussion

4.1. Survival. Since we started the experiment with second instar larvae, and there is often high mortality in the first instar, our data are not fully comparable to others. Anyway, our survival rates are much lower than those of Berkvens et al. [13]. They reared each larva individually, while we started with groups of ten. Cannibalism rate is high in this ladybird species and accounted for a part of the mortality, although we have not precise data. At least, the cannibalism recorded in this study did not occur due to the lack of food. Earlier, we observed increased larval cannibalism when larvae did not have an access to liquid water for drinking, so we expected higher mortality at low humidity which was not confirmed.

4.2. Developmental Time. In a previous study [14], at 26°C on a diet of *Acyrthosiphon pisum*, the mean duration of each stage was as follows: egg 2.8 days, first instar 2.5 days, second instar 1.5 days, third instar 1.8 days, fourth instar 4.4 days, and pupa 4.5 days. In our experiments, the development was longer for the second through the fourth larval instars (3.3, 3.5, and 6.8 days). Similarly, we measured complete larval development time (12.7 days) on *A. pisum* diet in a previous study [15] which was shorter than the time measured here (14.5 days). The total developmental time was 20.5 days in *succinea* morph whose parents were wild-caught in Belgium and larvae fed with *A. pisum* at 23°C [16], while in our study, the total development time was estimated as 22.5 days (providing egg stage was 3 days). Part of the difference might be explained by molesting of the satiated larvae by aphids crawling over the container. In some of the previous studies, aphids were provided with their host plants and thus did not disturb resting larvae. In the present study, plants were not included because they would increase the humidity in containers, and aphids were walking all the time over the containers, disturbed resting satiated larvae which on turn also walked around the container resulting in energy expenditure and subsequently longer development.

There was generally no significant difference in the development time between the "natural" food, aphid *A. pisum* and supplement food, and frozen eggs of the flour moth *E. kuehniella*. Similarly, Berkvens et al. [16] found similar developmental times when feeding several morphs or populations by these two foods.

4.3. Body Size. While higher temperature shortens the development, it often decreases adult weight. Ladybird larvae reared at higher temperatures produced smaller adults than larvae reared at lower temperatures [17]. Among our treatments, there were cases when shorter developmental time resulted in larger adults but also vice versa. Fresh body mass was smaller in our experiments than in those of Berkvens et al. [16] (about 35 mg) for both foods.

4.4. Interaction between Food Type and Humidity. Three important life history parameters, that is, survival rate, larval developmental time, and adult mass were all differently influenced by diverse ambient humidity depending on the food type. In individuals fed with aphid *A. pisum*, there was a trend for better survival, shorter development, and higher mass gained at higher humidity. These trends were opposite or nonsignificant in individuals fed with eggs of *E. kuehniella*. Such a difference might be explained by the food quality. While aphids survived longer and in a better condition at high air humidity when they were without their food plant, moth eggs became soon mouldy and rejected at the highest humidity. It seems that the effect of different humidities was mainly mediated through the food and did not strongly influence the life parameters directly. Since *H. axyridis* performed comparatively well in all levels of humidity, it seems that it is not a strong single limiting factor for the further spread of this alien ladybird species to new areas.

4.5. Sex Ratio. The only measured parameter that showed a consistent trend regardless of food treatment was the sex ratio. It was close to 1 : 1 (0.5) at the medium humidity, while there were more males at low humidity and more females at high humidity. It emerged as differential larval mortality, maybe different rates of larval cannibalism. The only similar case found was the sex ratio of *Attagenus fasciatus* (Coleoptera: Dermestidae) which was male biased at 30°C at 40 and 60% RH, but not at 80% RH [18].

From the evolutionary perspective, sex ratio biased towards females is better, resulting in higher population growth, so that areas with high humidity might be colonized more quickly than those with low air humidity.

5. Conclusion

The effect of air humidity is context dependent. In our study, we used only one constant experimental temperature and constant humidity, while in the field, these factors fluctuate on a daily basis. The effect might be different in the field where plant material or other resources provide water for drinking or higher-humidity shelter in generally dry conditions or exposure to sun may generally reduce high humidity. The only report from the field conditions was a negative correlation between *H. axyridis* abundance and air relative humidity (70–90%) in noncitric plants in Brazil [19]. We recommend to monitor the sex ratio in field populations in regions with contrasting humidity although they must be checked for the occurrence of male killing bacteria [20].

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Research Article

Competition for Aphid Prey between Different Lady Beetle Species in a Laboratory Arena

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Direct competition for aphid prey (Hemiptera: Aphididae) was evaluated between and among several lady beetle species (Coleoptera: Coccinellidae). The behavior of three native (*Coccinella trifasciata, Coleomegilla maculata,* and *Hippodamia convergens*) and four nonnative (*Coccinella septempunctata*, *Harmonia axyridis*, *Hippodamia variegata,* and *Propylea quatuordecimpunctata*) lady beetles was observed in laboratory arenas. The beetles were kept alone, paired with conspecifics or paired with heterospecifics, and presented with potato aphids (*Macrosiphum euphorbiae*). *Harmonia axyridis* was the most successful aphid predator in our study, being able to find aphids more quickly and consume more of them compared to most other lady beetle species. It was also by far the most aggressive of the tested species. *Coccinella septempunctata, C. trifasciata,* and *C. maculata* generally followed *H. axyridis* in aphid consumption. Prey discovery, consumption, and aggressive behaviors were dependent on which species were present in the arena. Except for the generally superior *H. axyridis*, there was no obvious dominance hierarchy among the other tested species and no dichotomy between the native and non-native species. Asymmetric interactions between lady beetle species may affect their abilities to coexist in the same habitat.

1. Introduction

Lady beetles comprise an ecologically and economically important group of insects that are also charismatic and well known to the general public [1, 2]. Understanding intraguild interactions among lady beetle species is important both for their conservation and for their maximum utilization as biological control agents. For example, the establishment of nonnative lady beetle species often coincides with declines in native lady beetle abundances [3–9] and has been implicated in having profound effects on the populations of pestiferous prey [4, 9, 10].

Competition is often assumed when predatory species consuming the same prey species are found in the same area [11]. Persistent species that share prey and an evolutionary history are often considered to have achieved a compromise over time, allowing them to coexist by differentially exploiting the same prey species [12, 13]; for example, by foraging at different times [14]. When species consuming the same prey are newly brought together, the ability of each to acquire the same necessary resources may allow for their coexistence [15, 16]. Intraguild predation, however, does not mean that a sufficient share goes to each predator [6, 17–19]. Consumption by a more efficient predator may eventually result in the competitive exclusion of the less efficient predator [16, 20].

Most comparative studies of different lady beetle species have either dealt with their relative abundances in the field [3–9, 21] or focused on intraguild predation [3, 6, 7, 17, 22–32]. The recent spread of *Harmonia axyridis* (Pallas) outside of its native range has been the impetus for a number of additional behavioral comparisons [33]. *Harmonia axyridis* has been shown to outcompete other lady beetle species in evaluations of intraguild predation [17, 24, 31], prey utilization [6], pathogen tolerance [34], and in the acquisition of prey tended by aggressive ants [35]. Relatively little research effort has been dedicated to competition for prey items among lady beetle species. In an extensive field survey, Finlayson et al. [21] documented native and nonnative lady beetle species occurring together in a variety of habitats throughout Maine. A series of experiments [35, 36, and this study] were then conducted to compare behavior between different species. In the present study, we investigated behavior of seven lady beetle species competing for prey in a laboratory arena. We hypothesized that recently introduced species that share habitats with the native species [21], but appear to replace them over time [9], are more aggressive aphid predators.

2. Materials and Methods

2.1. Study Species. Aphidophagous lady beetle species, which were known to be abundant in Maine and were found together in the same habitats [21, 36], were chosen for the present study. Three species are native: the three-banded lady beetle *Coccinella trifasciata perplexa* Mulsant, the twelve-spotted lady beetle *Coleomegilla maculata lengi* Timberlake, and the convergent lady beetle *Hippodamia convergens* Guérin. The native range of *C. trifasciata* is north from New Jersey to Labrador and west to California and Alaska [37]. *Coleomegilla maculata* is native to eastern North America from Georgia to Ontario, and west to Texas and Minnesota [37]. The range of *H. convergens* extends from British Columbia and Ontario to South and Central America and the Antilles [37].

The nonnative lady beetles used in the present study were the seven-spotted lady beetle *Coccinella septempunctata* (L.), the multicolored Asian lady beetle *Harmonia axyridis* (Pallas), the variegated lady beetle *Hippodamia variegata* (Goeze), and the fourteen-spotted lady beetle *Propylea quatuordecimpunctata* (L.). *Harmonia axyridis* is native to Central and Eastern Asia [33, 38]. The other three species are of Palearctic origin [39, 40]. All were inadvertently or intentionally introduced into North America. *Coccinella septempunctata* has been established in the eastern United States since 1979 [41]. *Harmonia axyridis* was first documented as established in North America in 1988 [42, 43] and now occurs throughout much of the continental United States [33]. *Hippodamia variegata* is widespread throughout northeastern North America [44–49]. In Maine, *P. quatuordecimpunctata* was first documented in 1988 in Aroostook, Penobscot, and Kennebec Counties, where it is believed to have expanded its range from populations in Quebec dating to1968 [50].

The potato aphid, *Macrosiphum euphorbiae* (Thomas), served as the prey. *Macrosiphum euphorbiae* is common in Maine and native throughout North America [51]. It is known to feed on over 200 plant species, including potato, apple, aster, and rose [51] and is a common prey item for many lady beetle species [2, 37, 52].

2.2. Insect Origins and Maintenance. Lady beetles were collected 48–72 hours before the initiation of each trial and were provided with water, but no food, for 48 hours before trials began. Beetles were collected in Orono, Maine (44.8835°N, 68.6721°W) from a variety of habitats: mixed shrub (*Solidago* sp., *Rubus* sp., *Prunus* sp., *Rosa* sp., *Cornus sericea*, and *Alnus* sp.), apple (*Malus* sp.), grain (*Hordeum* sp. and *Avena* sp.), mixed organic crops (*Solanum lycopersicon, Allium* sp., *Brassica* sp., *Pisum* sp., and *Phaseolus* sp.), and field (*Phleum pratense, Trifolium* sp., *Cirsium* sp., *Vicia* sp., and *Fragaria* sp.). Potato aphids were obtained from a colony maintained in our laboratory. The colony was originally founded from aphids collected in Presque Isle, Maine (46.6528°N, 68.0109°W) from potato (*Solanum tuberosum*, Family: Solanaceae) fields and then maintained on excised potato foliage in the laboratory. Until they were used in trials, lady beetles and aphid colonies were housed separately in ventilated, 0.95 L ball glass jars (Jarden Home Brands, Inc., Daleville, IN, USA) held within Percival I-33VL Intellus environmental chambers (Percival Scientific, Inc., Perry, IA, USA) at 16 (light) : 8 (dark) hour photoperiod. The temperature was maintained at 20 ± 1°C during both the photophase and scotophase. Trials were conducted from May 16 to September 8, 2006.

2.3. Competition Trials with Paired Lady Beetles. Each trial took place in an observation arena under a clear, ventilated plastic container (8.9-cm diameter and 9.5-cm height), which was turned upside down and placed inside the bottom of a Petri dish. For each container, a cut potato leaf was placed in a small plastic vial with water. Using a paintbrush, 4 adult wingless aphids were placed on the upper surface of the leaf. Aphid number was chosen based on a previous study [36] in which lady beetles consumed between 5.33 ± 0.4271 (*P. quatuordecimpunctata*) and 9.17 ± 0.2039 (*H. axyridis*) adult potato aphids in a 24-hour period. Therefore, we believe that four aphids provided an adequate, but not overabundant, food supply. The vial containing the vegetation and aphids was then placed in an upright position inside the observation arena. Adult lady beetles were transferred to a different observation arena by allowing each lady beetle to crawl on to the tip of a paintbrush and then onto the interior of the arena. After a 10-minute period of adjustment, the cover holding the lady beetle(s) was switched with the cover under which the vial holding the leaf and aphids was housed, simultaneously exposing the lady beetle(s) to the aphids. Trials were conducted for 45 minutes. Time to prey discovery (of the first aphid), number of prey consumed by each beetle (documented to 0.25 aphid when the entire aphid was not consumed), and behavior (as a count of aggression delivered and received by each beetle in each trial) were recorded. The following behaviors were considered aggressive: chasing, grasping, biting, climbing upon, and attempting to or successfully stealing prey. Ten trials were conducted in random order, with individuals of each species and with pairs of all combinations of each species, including conspecific pairings.

2.4. Prey Consumption and Discovery Time by Single Lady Beetles. To serve as a comparison with the paired trials described above, aphid consumption and time to prey discovery was also documented in trials with single lady beetles. These trials were conducted following the same protocol as described above, but with one individual introduced in each arena. Ten trials were conducted with each of the seven lady beetle species.

Table 1: Mean (± SE) aphid consumption (number of aphids), prey discovery time (minutes), and aggression delivered (number of occurrences) by seven lady beetle species during laboratory trials. The data were pooled for all trials conducted with a given species (see text for details). Means in each column followed by the same letter are not significantly different from each other (Tukey's HSD tests, $P < 0.05$). Nonnative species are printed in bold font.

	Aphid consumption			Aggression delivered	Prey discovery time	
	Alone	Same species	Other species	Other species	Same species	Other species
C. trifasciata	1.30 ± 0.34b	1.55 ± 0.21ab	1.78 ± 0.17ab	0.22 ± 0.05b	15.95 ± 3.11ab	16.47 ± 2.02b
C. maculata	1.60 ± 0.37ab	1.55 ± 0.20ab	1.42 ± 0.16bcd	0.23 ± 0.06b	20.30 ± 2.75a	17.80 ± 2.01b
H. convergens	1.20 ± 0.29b	1.35 ± 0.20ab	1.30 ± 0.14bcd	0.20 ± 0.05b	18.40 ± 3.07a	19.18 ± 2.18b
C. septempunctata	1.70 ± 0.42ab	1.50 ± 0.28ab	1.48 ± 0.17abc	0.13 ± 0.04b	18.70 ± 3.75a	20.80 ± 2.33ab
H. axyridis	2.70 ± 0.30a	1.95 ± 0.23a	2.10 ± 0.17a	0.57 ± 0.06a	6.35 ± 1.47b	13.23 ± 1.99b
H. variegata	0.70 ± 0.26b	0.75 ± 0.12ab	0.84 ± 0.12d	0.13 ± 0.04b	24.90 ± 3.33a	28.13 ± 2.13a
P. quatuordecimpunctata	1.10 ± 0.23b	1.03 ± 0.13b	0.94 ± 0.11cd	0.33 ± 0.06b	17.85 ± 3.39a	20 ± 2.18 ab
N	10	20	60	60	20	60
P	0.0146	0.0122	<0.0001	<0.0001	0.0002	<0.0001
F	2.90	2.85	5.99	6.27	4.76	5.56
DF	6, 63	6, 133	6, 413	6, 413	6, 133	4, 413

2.5. Measurements of Lady Beetle Weight and Size. Because differences in predator size have been used in some studies to explain differences in competition [6, 17, 53, 54], the weight and volume of 20 lady beetles of each species were documented. The weight of each beetle was determined to the 0.0001 gram using an electronic Ohaus Adventurer Balance AR2140 (Ohaus Corp., Pine Brook, NJ, USA). Width, length, and height were measured using a ruler mounted in the eyepiece of a Stereoscopic Zoom Microscope SMZ800 (Nikon Instruments Inc., Melville, NY, USA) at 10x magnification. Volume was estimated by multiplying width (across the pronotum, dorsal side), length (from the frons of the head to the end of the elytra, dorsal side), and height (the greatest height below the elytra, laterally).

2.6. Statistical Analyses. The Wilk-Shapiro test (PROC UNIVARIATE; SAS Institute, Inc. 2002) was used to test data normality. Data were transformed using rank transformations [55]. Untransformed data were used to calculate the means and standard errors reported in this paper.

Behavioral data were analyzed using one-way ANOVAs followed by Tukey's HSD tests (PROC GLM, SAS Institute, Inc. 2002). First, we compared the overall differences among the species for beetles that were held alone, paired with conspecifics, and paired with heterospecifics (all species other than the species of interest pooled together). Lady beetle species were used as the main effect (Table 1). Secondly, we tested the effects of the competition context (beetle held alone, paired with conspecifics, or paired individually with each of the heterospecific species) separately for each lady beetle species. Competition contexts were used as the main effect (Tables 2–4). Aphid consumption, prey discovery time, aggression received, and aggression delivered were used as dependent variables in both analyses.

Table 2: Number of aphids (mean ± SE) consumed by *C. trifasciata* and *C. maculata* in different competition contexts (see text for details). Means in each column followed by the same letter are not significantly different from each other (Tukey's HSD tests, $P < 0.05$). Nonnative species are printed in bold font.

Competition context	*C. trifasciata*	*C. maculata*
Alone	1.30 ± 0.34ab	1.60 ± 0.37ab
C. trifasciata	1.70 ± 0.34ab	0.40 ± 0.22b
*C. trifasciata**	1.40 ± 0.27ab	N/A
C. maculata	2.60 ± 0.37ab	1.60 ± 0.31ab
*C. maculata**	N/A	1.50 ± 0.27ab
C. septempunctata	1.00 ± 0.42b	1.80 ± 0.36a
H. axyridis	1.30 ± 0.37ab	1.00 ± 0.27ab
H. convergens	2.60 ± 0.31a	1.55 ± 0.26ab
H. variegata	1.20 ± 0.36ab	1.95 ± 0.51a
P. quatuordecimpunctata	2.00 ± 0.39ab	1.80 ± 0.42ab
P	0.0073	0.0262
F	2.87	2.33
DF	8, 81	8, 81

* When beetles were paired with conspecifics, the data are listed separately for each beetle in the pair.

Correlation analysis (PROC CORR; SAS Institute Inc. 2002) was used to test associations between aphid consumption, prey discovery time, aggression delivered, and aggression received. The analyses were conducted both within each species (e.g., correlation between aphid consumption and prey discovery time for *H. axyridis*), as well as between the two paired species (e.g., correlation between aphid consumption by *H. axyridis* and *C. septempunctata*) or the two

Table 3: Number of aggression events (mean ± SE) delivered by *H. axyridis* and *H. variegata* in different competition contexts (see text for details). Means in each column followed by the same letter are not significantly different from each other (Tukey's HSD tests, $P < 0.05$). Nonnative species are printed in bold font.

Competition context	***H. axyridis***	***H. variegata***
H. axyridis	0.10 ± 0.10b	0.50± 0.17a
H. axyridis*	0.10 ± 0.10b	N/A
C. maculata	0.60 ± 0.16ab	0.10 ± 0.10b
C. septempunctata	0.10 ± 0.10b	0.00 ± 0.00b
C. trifasciata	0.80 ± 0.13a	0.00 ± 0.00b
H. convergens	0.70 ± 0.15ab	0.00 ± 0.00b
H. variegata	0.50 ± 0.17ab	0.00 ± 0.00b
H. variegata*	N/A	0.00 ± 0.00b
P. quatuordecimpunctata	0.70 ± 0.16ab	0.20 ± 0.13ab
P	0.0003	<0.0001
F	4.70	4.72
DF	7, 72	7, 72

*When beetles were paired with conspecifics, the data are listed separately for each beetle in the pair.

individuals of the same species in case of conspecific trials. Most of the correlations between aphid consumption and prey discovery time were statistically significant. Therefore, for the ease of interpretation, their results are reported separately (Table 5) from statistically significant comparisons between all other combinations of variables (Table 6).

Weights and volumes of different lady beetle species were compared using one-way ANOVA (PROC GLM, SAS Institute, Inc. 2002). Means were separated by Tukey's HSD tests.

3. Results

Aphid consumption was significantly different among the species whether the beetles were held alone, paired with conspecifics, or paired with heterospecifics (Table 1). *Harmonia axyridis* generally consumed the most aphids, while *P. quatuordecimpunctata* and *H. variegata* consumed the least. Also, *H. axyridis* was the most aggressive species towards other lady beetles when held with heterospecifics (Table 1). No difference in delivered aggression was detected among the species paired with conspecifics (d.f. = 6, 133, $F = 2.07$, $P = 0.1544$). The overall amount of received aggression was similar among the tested species ($P > 0.15$).

Prey discovery time did not differ among species when the beetles were held alone (d.f. = 6, 63, $F = 1.01$, $P = 0.4273$). However, in the presence of conspecifics, *H. axyridis* found aphids quicker compared to the other species (Table 1). In the trials with heterospecifics, *H. variegata* discovered prey slower than all other species except *C. septempunctata* and *P. quatuordecimpunctata* (Table 1).

Competition context affected aphid consumption for two of the tested lady beetle species (Table 2). *Coccinella trifasciata* consumed fewer aphids when paired with *C. septempunctata* than when paired with *H. convergens*, while *C. maculata* consumed fewer aphids when paired with *C. trifasciata* than when paired with *C. septempunctata* or *H. variegata*. Prey discovery time did not vary within any of the tested species regardless of the competition context ($P > 0.2$).

Harmonia axyridis exhibited significantly more aggression towards *C. trifasciata* than towards the other lady beetle species (Table 3). Interestingly, *H. variegata,* which was a rather peaceful species in our trials, significantly increased its level of aggression when paired with *H. axyridis* (Table 3). *Coccinella trifasciata*, *H. convergens*, *H. variegata*, and *P. quatuordecimpunctata* received different amounts of aggression from different lady beetle species (Table 4). A statistically significant difference was also detected for *C. maculata*, but the effect was relatively weak, inconsistent, and its biological significance is uncertain (Table 4). Beetles from all five aforementioned species received more aggression from *H. axyridis* compared to at least one other species with which they were paired. *Hippodamia variegata* also received as much aggression from *P. quatuordecimpunctata* as from *H. axyridis* (Table 4).

Not surprisingly, aphid consumption was negatively correlated with prey discovery time (Table 5). In other words, the beetles that found their prey the most quickly consumed the most. The only exceptions were *C. trifasciata* paired with *C. maculata, H. convergens* paired with *H. axyridis,* and *H. axyridis* paired with *P. quatuordecimpunctata.* Correlation coefficients were marginally significant for *C. maculata* paired with *H. axyridis, H. axyridis* paired with *C. trifasciata*, and *P. quatuordecimpunctata* paired with *C. maculata* (Table 5).

Correlation analyses also revealed a number of strong relationships between other measured parameters (Table 6). In six trials, aphid consumption by one species was negatively correlated with aphid consumption by the other species confined in the same arena. Similarly, there were three cases of negative correlations between prey discovery times by two beetles in a pair. In five comparisons, aphid consumption by one species was positively correlated with prey discovery time by the other species. Aggressive behavior increased aphid consumption for *C. maculata* when paired with *C. trifasciata*, and for *H. convergens* when paired with *H. axyridis*. However, prey discovery time for *C. maculata* increased with increased aggression against *C. septempunctata.* Receiving aggression from *P. quatuordecimpunctata* significantly decreased aphid consumption by *C. septempunctata*. Similarly, prey discovery time for three aphid species increased as they received more aggression from another beetle in the pair (Table 6).

Coccinella septempunctata was the largest of the species tested, closely followed by *H. axyridis* (Table 7). *Hippodamia variegata* was the smallest.

4. Discussion

Results of the present study suggest the existence of asymmetric competitive interactions among the tested lady beetle species. There were significant differences in aphid consumption and prey discovery times among the species, and numerous occasions of aggressive encounters among the beetles confined in the observation arenas. The nature and strength

Table 4: Number of aggression events (mean ± SE) received by *C. trifasciata, C. maculata, H. convergens, H. variegata*, and *P. quatuordecimpunctata* in different competition contexts (see text for details). Means in each column followed by the same letter are not significantly different from each other (Tukey's HSD tests, $P < 0.05$). Nonnative species are printed in bold font.

	C. trifasciata	*C. maculata*	*H. convergens*	***H. variegata***	***P.*** *quatordecim-punctata*
C. trifasciata	0.20 ± 0.13b	0.30 ± 0.15ab	0.20 ± 0.13ab	0.10 ± 0.10b	0.30 ± 0.15ab
*C. trifasciata**	0.30 ± 0.15ab	N/A	N/A	N/A	N/A
C. maculata	0.20 ± 0.13b	0.00 ± 0.00b	0.20 ± 0.13ab	0.30 ± 0.15ab	0.10 ± 0.10b
*C. maculata**	N/A	0.10 ± 0.10ab	N/A	N/A	N/A
C. septempunctata	0.20 ± 0.13b	0.10 ± 0.10ab	0.30 ± 0.15ab	0.00 ± 0.00b	0.10 ± 0.10b
H. axyridis	0.80 ± 0.13a	0.60 ± 0.16a	0.70 ± 0.15a	0.50 ± 0.17a	0.70 ± 0.15a
H. convergens	0.00 ± 0.00b	0.30 ± 0.15ab	0.10 ± 0.10b	0.20 ± 0.13ab	0.20 ± 0.13ab
*H. convergens**	N/A	N/A	0.10 ± 0.10b	N/A	N/A
H. variegata	0.00 ± 0.00b	0.10 ± 0.10ab	0.00 ± 0.00b	0.00 ± 0.00b	0.20 ± 0.13ab
H. variegata*	N/A	N/A	N/A	0.00 ± 0.00b	N/A
P. quatuordecimpunctata	0.40 ± 0.16ab	0.20 ± 0.13ab	0.30 ± 0.15ab	0.50 ± 0.17a	0.10 ± 0.10b
P. quatuordecimpunctata*	N/A	N/A	N/A	N/A	0.10 ± 0.10b
P	0.0012	0.0298	0.0132	0.0028	0.0170
F	3.89	1.79	2.39	3.22	2.88
DF	7, 72	7, 72	7, 72	7, 72	7, 72

* When beetles were paired with conspecifics, the data are listed separately for each beetle in the pair.

Table 5: Correlations between aphid consumption and prey discovery time for single and paired lady beetles in trials ($N = 10$). Each row represents the relationship between aphid consumption and prey discovery time for the species in the left column when it was alone or paired with the species in the first row of the table. Ct: *Coccinella trifasciata*, Cm: *Coleomegilla maculata*, Hc: *Hippodamia convergens*, Cs: *Coccinella septempunctata*, Ha: *Harmonia axyridis*, Hv: *Hippodamia variegata*, Pq: *Propylea quatuordecimpunctata*. Nonnative species are printed in bold font.

		Alone	Ct	Cm	Hc	**Cs**	**Ha**	**Hv**	**Pq**
Ct	*r*	−0.8698	−0.7745	−0.3644	−0.8675	−0.8541	−0.7642	−0.9107	−0.7571
	P	0.0011	<0.0001	0.3005	0.0011	0.0017	0.0101	0.0002	0.0112
Cm	*r*	−0.9524	−0.7942	−0.8559	−0.9011	−0.6469	−0.6235	−0.8016	−0.7745
	P	<0.0001	0.0061	<0.0001	0.0004	0.0432	0.0541	0.0053	0.0085
Hc	*r*	−0.7994	−0.8708	−0.8199	−0.9091	−0.9039	−0.5518	−0.9431	−0.9184
	P	0.0055	0.0010	0.0037	<0.0001	0.0003	0.0982	<0.0001	0.0002
Cs	*r*	−0.8420	−0.8009	−0.8193	−0.8701	−0.8735	−0.9240	−0.9066	−0.8609
	P	0.0022	0.0054	0.0037	0.0011	<0.0001	0.0001	0.0003	0.0014
Ha	*r*	−0.9389	−0.6010	−0.7980	−0.8140	−0.6836	−0.7743	−0.9708	−0.2439
	P	<0.0001	0.0661	0.0057	0.0042	0.0293	<0.0001	<0.0001	0.4970
Hv	*r*	−0.9447	−0.7891	−0.8894	−0.9322	−0.7487	−0.8316	−0.8647	−0.8033
	P	<0.0001	0.0067	0.0006	<0.0001	0.0127	0.0029	<0.0001	0.0051
Pq	*r*	−0.8818	−0.8734	−0.6182	−0.7900	−0.8852	−0.6361	−0.8284	−0.7502
	P	0.0011	0.0010	0.0568	0.0065	0.0007	0.0480	0.0031	0.0001

of the observed interactions varied depending on the species involved.

Harmonia axyridis was the most successful aphid predator in our study, being able to find aphids quicker and consume more of them compared to most other lady beetle species. Furthermore, *H. axyridis* was by far the most aggressive of the tested species. These observations are consistent with a number of studies that have documented the superior competitive abilities of *H. axyridis* among lady beetle species [6, 17, 24, 26, 28, 31, 56, 57]. A superior competitive ability of invasive species to utilize resources over native species has been also documented in numerous other systems [58–61].

Interestingly, it took about twice as long for *H. axyridis* to find aphids when paired with heterospecifics than when paired with conspecifics (Table 1). It is possible that attacking heterospecifics distracted them from searching for aphids. Indeed, *H. axyridis* attacked heterospecifics 5–8 times more often than conspecifics (Table 3) although the differences were not always statistically significant. The aphid consumption data suggest that such a strategy paid off. Similarly,

Table 6: Additional significant correlations between aphid consumption, prey discovery time, aggression delivered, and aggression received by lady beetles in trials ($N = 10$). Nonnative species are printed in bold font.

Correlation between:	And:		
Aphid consumption	Aphid consumption	*r*	*P*
C. septempunctata	*C. trifasciata*	−0.9049	0.0002
C. trifasciata	*H. convergens*	−0.7356	0.0127
C. maculata	***H. axyridis***	−0.7098	0.0112
C. septempunctata	*H. convergens*	−0.8195	0.0053
H. axyridis	*H. convergens*	−0.9133	0.0003
H. axyridis	***P. quatuordecimpunctata***	−0.8497	0.0020
Prey discovery time	Prey discovery time	*r*	*P*
C. septempunctata	*C. trifasciata*	−0.7653	0.0085
C. septempunctata	*H. convergens*	−0.8138	0.0030
H. convergens	***P. quatuordecimpunctata***	−0.7001	0.0143
Aphid consumption	Prey discovery time	*r*	*P*
C. trifasciata	***C. septempunctata***	0.8350	0.0017
C. septempunctata	*H. convergens*	0.7069	0.0002
C. septempunctata	*C. trifasciata*	0.7665	0.0112
H. convergens	***C. septempunctata***	0.8344	0.0022
P. quatuordecimpunctata	***H. variegata***	0.7107	0.0088
Aphid consumption	Aggression delivered towards	*r*	*P*
C. maculata	*C. trifasciata*	0.7994	0.0063
H. convergens	***H. axyridis***	0.7327	0.0029
Aphid consumption	Aggression received from	*r*	*P*
C. septempunctata	***P. quatuordecimpunctata***	−0.7812	0.0080
Prey discovery time	Aggression delivered towards	*r*	*P*
C. maculata	***C. septempunctata***	0.9225	<0.0001
Prey discovery time	Aggression received from	*r*	*P*
C. maculata	***C. septempunctata***	0.8511	0.0017
H. convergens	*C. maculata*	0.8370	0.0002
C. septempunctata	***P. quatuordecimpunctata***	0.8392	0.0028
Aggression delivered by	or Aggression received by	*r*	*P*
C. trifasciata	*C. trifasciata*	0.7003	0.0004

Michaud [6] found *H. axyridis* to be a highly evolved interspecific competitor in the Florida citrus ecosystem.

Harmonia axyridis data generally agree with our hypothesis that recently introduced lady beetle species that replace native species over time are more aggressive aphid predators. However, we did not observe the same situation for the other three nonnative species. There was no distinct dichotomy between supposedly more aggressive nonnative species and supposedly more docile native species. Also, except for the generally superior *H. axyridis*, there was no obvious dominance hierarchy among the other tested species.

The native lady beetle species used in the current study, *C. maculata*, *C. trifasciata*, and *H. convergens* are currently numerous in Maine [21]. Native species, *Coccinella transversoguttata* (Brown) and *Hippodamia tredecimpunctata tibialis* (Say), that have experienced declines in abundance since nonnative lady beetle introductions [9] were excluded because they were not easily found in numbers sufficient for testing [21]. It would be interesting and valuable to pair native species once numerous in Maine with both the now common nonnative species and the native species that still persist.

Among the species tested, *C. septempunctata*, *C. trifasciata* and *C. maculata* generally followed *H. axyridis* in aphid consumption. *Coccinella septempunctata* and *H. axyridis* were also the heaviest and largest species among the seven species tested (Table 7). Despite *C. septempunctata*'s large size and being among the species consuming the most aphids, *C. septempunctata* generally did not deliver or receive more aggression than other species. Larger lady beetle species have been shown to be competitively favored over smaller ones [6, 17, 53, 54], possibly because they are able to consume more due to their larger size, or perhaps because their size is advantageous in direct fighting. *Coccinella septempunctata* has also been documented to deter aggression by ants

Table 7: Mean (± SE) weight (mg) and volume (mm^3) of lady beetle species ($N = 20$) used in laboratory trials. Means in each column with the same letter are not significantly different (Tukey's HSD tests, $P < 0.05$). Nonnative species are printed in bold font.

	Weight	Volume
C. trifasciata	1.04 ± 0.0007c	20.41 ± 1.2005d
C. maculata	0.91 ± 0.0008c	15.10 ± 0.8356de
H. convergens	0.87 ± 0.0009c	32.43 ± 1.8409c
C. septempunctata	2.25 ± 0.0017a	78.87 ± 2.6835a
H. axyridis	1.68 ± 0.0015b	66.30 ± 2.4081b
H. variegata	0.40 ± 0.0004d	8.64 ± 0.5435e
P. quatuordecimpunctata	0.63 ± 0.0005dc	12.87 ± 0.8090e
P	<0.0001	<0.0001
F	38.63	280.85
DF	6, 133	6, 133

chemically by producing a defensive alkaloid and bleeding reflectively [62, 63]. It is possible that chemical defense is also used by *C. septempunctata* to prevent aggression from other coccinellids.

It is worth noting that *H. axyridis*, *C. septempunctata*, *H. convergens*, *H. variegata*, and *P. quatuordecimpunctata* showed no difference in aphid consumption and prey discovery time whether they were kept alone or paired with any other species tested in the study, including conspecifics (data not shown). Perhaps if a given species is an efficient predator that can find and consume aphids quickly, its ability to acquire prey may not be significantly hindered by the presence of other lady beetles. Prey consumption by *C. trifasciata* and *C. maculata*, on the other hand, differed depending on which species they were paired with.

Significant negative correlations between the numbers of aphids consumed and prey discovery times in paired trials (Table 6) confirm the existence of competitive interactions. Furthermore, we detected a number of significant positive correlations between the number of aphids consumed by one beetle in a pair and prey discovery time by the other beetle in the pair. In other words, the longer it took a beetle to discover the prey, the more aphids its competitor could consume.

Increased aggression delivered by *C. maculata* and *H. convergens* (Table 6) was correlated with increased aphid consumption by those species in trials with *C. trifasciata* and *H. axyridis*, respectively. In those cases, aggression may have helped deter other species from consuming prey. On the contrary, increased aggression by *C. maculata* was correlated with its own increased prey discovery time, suggesting that it was distracted from foraging.

Receiving aggression from *P. quatuordecimpunctata* increased prey discovery time and decreased aphid consumption for *C. septempunctata* (Table 6). Similarly, prey discovery time increased for *H. convergens* with the increase in aggression it received from *C. maculata*, and for *C. septempunctata* with the increased aggression it received from *P. quatuordecimpunctata*. In a conspecific pairing of *C. trifasciata*, aggression received by one conspecific was correlated with the aggression it delivered, meaning that aggressive interactions were not one sided, but equally met by the other conspecific.

Overall, our results confirm that behavioral interactions between different lady beetle species affect their ability to secure prey items, with *H. axyridis* generally having a competitive advantage over the other species. Our study was conducted in a relatively simple setting of a laboratory arena with a limited number of aphids. Furthermore, prey choice was limited to a single aphid species. Increased environmental complexity, including variations in prey species and their abundances (including relative abundances of winged and wingless morphs), may modify competitive abilities of and interactions between certain species. Nevertheless, our findings support the idea that behavioral differences in prey discovery, consumption, and intraguild aggressiveness may, in part, lead to reductions in native lady beetle species following the establishment of *H. axyridis.*

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Research Article

A New Lycid Genus from the Dominican Amber (Insecta, Coleoptera, Lycidae, Leptolycinae, Leptolycini)

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A new fossil genus, *Electropteron* **gen.n.**, and a new species, *E. avus* **sp.n.**, are described from the Dominican Amber. *Electropteron avus* **gen.n.**, **sp.n.**, appears to be related to some of the extant Great Antillean lycids and is the first fossil taxon from the subfamily Leptolycinae.

1. Introduction

No taxa of the family Lycidae have so far been known from the ambers of the New World. All previously described amber lycids come from the Baltic Amber and all belong to the subfamily Erotinae [1–3], although Klebs [4] signaled, also from the Baltic Amber, a representative of *Lygistopterus* Mulsant, 1838 (Calochrominae).

The first Dominican Amber lycid, a well preserved and clearly observable male specimen, turned out to represent a new genus and a new species, apparently very close to some of the recent Leptolycinae from Hispaniola and Puerto Rico. Its gender is easily defined by the characteristic structure of the terminal abdominal segments, with the elongate, pointed at apex ultimate sternite enveloped laterally by a tergite. The description of the new taxon is given below.

2. Description

2.1. Electropteron ***gen.n.***

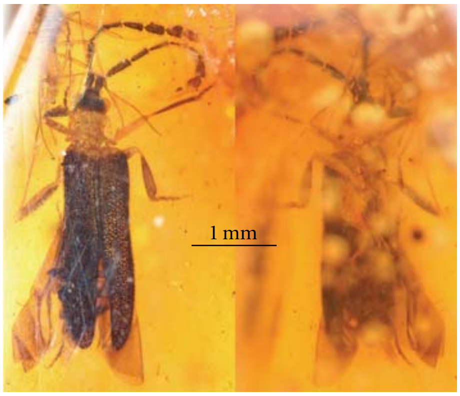
2.1.1. Type Species: Electropteron Avus ***sp.n.***

Description. ***Adult male***. Alate, slender, elongate (Figure 1(a)). Head subquadrate, slightly narrowed behind eyes. Fastigium right-angled. Eyes relatively small, spherical. Maxillary palps slender, with ultimate palpomere pointed distally. Gula prominent. Antennal prominence conspicuous, antennal sockets approximate. Antenna 11-segmented, moderately long, slightly widening distally; antennomeres 4–11 flattened, with slightly uneven edges, antennomeres 2 and 3 short, transverse, subequal in length (Figure 2(a)); pubescence on antennomeres 4–11 relatively short and erect (Figures 1(a) and 1(b)).

Pronotum small, ca. 6 times shorter than elytra, transverse, trapezoidal, with obscure median impression in posterior third; posterior angles produced laterally (Figure 1(a)). Prosternum short, V-shaped (Figures 1(b), 2(b)). Thoracic spiracles small, not projecting beyond coxae. Mesoventrite transverse, short. Mesonotum with rather prominent, elongate scutellum (Figure 1(a)). Elytra long, narrowing, and dehiscent distally, covering abdomen, except genital capsule, with two noticeable primary costae (presumably, costae 2 and 4); interstices irregularly areolate; short and erect elytral pubescence uniform (Figure 1(a)). Metaventrite transverse, with acute posterior angles; discrimen complete, attaining to mesosternum.

Pro- and mesocoxae elongate; metacoxae approximate; angle between metacoxae ca. 90°. Legs slender; trochanters elongate, but considerably shorter than femurs, cylindrical, connected to femora distally; femurs and tibiae flattened, tibiae straight, widened distally; tarsomeres 1–4 narrow, without plantar pads; all claws simple. Ultimate sternite and tergite elongate, pointed at apex (Figures 1(a), 1(b), and 2(c)).

Female. Unknown.



(a) (b)

Figure 1: General view of *Electropteron avus* **gen.n.**, **sp.n.**, holotype male: (a) dorsally; (b) same, ventrally.

Etymology. The name of the genus is derived from "electron" and "pteron," the Greek for "amber" and "wing." Gender neuter.

Diagnosis. Electropteron **gen.n.** appears to be related to the extant genus *Tainopteron* Kazantsev, 2009, from Puerto Rico [5], but is distinguishable by the flattened and distally slightly widening antennomeres 4–11 (Figure 2(a)), less transverse pronotum and more elongate elytra completely covering the folded wings (Figure 1(a)). The new genus is different from *Leptolycus* Leng et Mutchler, 1922, another Greater Antillean extant endemic [5], by the flattened antennomeres 4–11, their short pubescence, transverse pronotum with explanate sides, and short V-shaped prosternum (Figures 1(a), 1(b), and 2(b)). On the other hand, *Electropteron* **gen.n.** is somewhat similar to *Ceratoprion* Gorham, 1884, distributed in the highlands of Central America and the Ands south to Ecuador, differing by the nonserrate antennomeres 4–11 and their erect pubescence and by the absence of the median longitudinal pronotal carina.

2.2. *Electropteron avus* **sp.n.** *Figures 1(a)–2(c)*

2.2.1. Material. Holotype, Male, specimen no. 09155/2198988208, Dominican Amber, Oligocene (Insect Centre, Moscow).

Description. ***Male.*** Dark brown to black; antennomere 11, pronotum, scutellum, elytra proximally, at scutellar level, meso- and metaventrites, genital capsule, coxae, trochanters, and femurs yellowish.

Head with deep impression behind antennal prominence. Eyes small, interocular dorsal distance over 2 times greater than eye radius. Antennae attaining to elytral middle, with antennomere 3 subequal in length to pedicel (antennomere 2) and 5.5 times shorter than antennomere 4 (Figures 1(a)–2(a)).

Pronotum transverse, ca. 1.5 times as wide as long, slightly narrowing anteriorly, with almost straight anterior margin, noticeable anterior, and small acute posterior angles. Scutellum parallel-sided and medially emarginate at apex (Figure 1(a)).

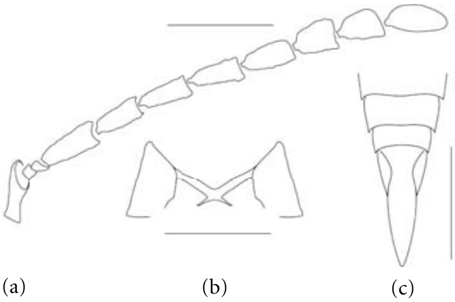

(a) (b) (c)

Figure 2: Details of *Electropteron avus* **gen.n.**, **sp.n.**, holotype male: (a) antenna; (b) prosternum; (c) apex of abdomen, ventrally. Scale: 0.5 mm.

Elytra elongate, 3.3 times as long as wide at humeri, narrowing distally, dehiscent in distal two fifths, with two primary costae reaching their apices and costa 1 noticeable in proximal fourth (Figure 1(a)).

Legs are relatively short, tibiae subequal in length to femurs (Figures 1(a), 1(b)).

Length (from anterior head margin to end of abdomen): 3.3 mm. Width (humerally): 0.7 mm.

Female. Unknown.

Etymology. The name of the new species is derived from "avus," the Latin noun for "grandfather," alluding to its hypothetic ancestry to some of the extant Greater Antillean lycids.

Diagnosis. Electropteron avus **sp.n.**, the only known representative of the genus, is easily distinguishable from the described extant lycids, as well as from the Baltic Amber lycid taxa, by the generic characters.

3. Discussion

Electropteron avus **gen.n.**, **sp.n.**, which is evidently close to some of the recent Leptolycini from Hispaniola and Puerto Rico, is tentatively attributed to the same tribe, although the tribe itself with the unusually wide range of morphologies of its members [5–7] may well prove to represent several independent lineages. The tribe Leptolycini is confined to Central America, Greater Antilles, and mostly northern part of South America. It is one of those enigmatic groups of net-winged beetles where females are not known and the pupa phase is presumably absent in female development [8]. The discovery of a representative of this group in the ca. 30-million-year-old Dominican Amber, actually in the area of the current distribution of its close relatives, gives further clues for the reconstruction of the history and phylogenetics of the family.

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Review Article

Adult Diapause in Coleoptera

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Recent studies dealing with adult (reproductive) diapause in the Coleoptera are reviewed, as a kind of supplement to the classic compendia. In the first two sections, the general characteristics of adult diapause are described and principal terms explained. Original articles dealing with 19 species from nine coleopteran families (Coccinellidae, Chrysomelidae, Bruchidae, Curculionidae, Carabidae, Silphidae, Scolytidae, Scarabaeidae, and Endomychidae) are reviewed. Finally attempts are made at generalisations from the papers reviewed, and hypotheses on diapause evolution are inferred. A polyphenic character of diapause is a prominent feature in *C. septempunctata* and *L. decemlineata*, but has been found also in other Coleoptera and in insects generally and often generates voltinism heterogeneity within populations.

1. Introduction

Adult diapause is the most common form of diapause in Coleoptera. It occurs in about 90% of beetle species [1], belonging mostly to the families of Coccinellidae, Chrysomelidae, and Curculionidae, and partly also Carabidae (the so-called carabid "autumn breeders" diapause as larvae). Another insect order with a high incidence of species entering diapause in the adult stage, is the Heteroptera with about 70% species. The lowest incidence of adult diapause is among species in the orders Lepidoptera and Hymenoptera (about 5% each).

2. General Remarks on Adult/Reproductive Diapause

To save space in discussions of individual species and avoid repetition, we describe the common characteristics here. (For more details see [1, 2].)

Diapause is an adaptive arrestment of development that helps synchronize active stages with suitable environmental conditions and so increase survival potential during unfavourable periods of the year. Insects that diapause as adults, the larvae and the young adults, develop when the food resources are present. For the stressful period without food (often lasting many months) adults prepare in time by accumulating reserves (lipids, glycogen, proteins) and substances needed for resistance to future hazardous changes of environmental conditions. To begin early enough before the start of the dangerous period, diapause is induced by signals heralding the arrival of the unfavourable season; usually the cue is photoperiodic. Short (decreasing) day length serves as a signal of approaching winter and induces winter diapause (hibernation). In contrast, long-day photoperiods announce summer and induce estivation/summer diapause. Temperature and other environmental conditions act during the sensitive stage in concert with photoperiod in diapause induction or aversion (= prevention).

Such regulation is typical for facultative diapause that can, but need not, be entered in each generation. Quite often, however, the genetically fixed propensity is so strong that diapause, may be obligatory, is entered under any environmental conditions. Usually a population is not genetically homogeneous: both tendencies may be mixed, as we will see below, for example, in *Coccinella septempunctata* or *Leptinotarsa decemlineata*.

To terminate diapause, the insect has to go through diapause development, that is, horotelic processes of physiogenesis that often proceed best at temperatures in the range of 5–10°C, but cold is not always a prerequisite for the resumption of development [3]. In many cases, diapause may be terminated by tachytelic processes of termination that is due to some environmental stimuli, such as a temperature increase or rainfall in case of summer diapause.

For quite a long period diapause research was focused only on hibernation/winter diapause, as the traditional view equated diapause with resistance to freezing. Estivation/summer diapause was long neglected, although it is rather common [4]. Quite often the terms hibernation versus estivation do not respond well to timing of seasons in the field: winter diapause may begin as early as in midsummer, as we will see below in several ladybird species.

Also, while hibernation lasts until spring, its diapause phase in temperate insect populations dwindles into postdiapause quiescence around the winter solstice. In this phase the morphological development of insects is arrested only directly by low temperature (or absence of food), and in spring (or by transfer to suitable laboratory conditions) morphological development is resumed.

The most conspicuous feature of adult diapause (often termed reproductive diapause) is the suppression of reproductive functions: maturing of ovaries and male accessory glands, and mostly also mating activity. Endocrinological regulatory pathways in adult diapause begin in the neurosecretory cells of brain whose axons terminate in corpora cardiaca. The pathway continues in another endocrinological organ, the corpora allata, where juvenile hormone is produced that regulates the activity of reproductive organs. In adults destined for diapause, food consumed is not used for maturation of gonads but for accumulating reserves. Thus the ovaries consist of threadlike ovarioles, so hidden in the much-enlarged fat body that they are sometimes difficult to find in dissection.

The above general traits were revealed in several coleopteran species that were studied in detail for many decades and published in series of papers, such as the studies on *Leptinotarsa decemlineata* and *Coccinella septempunctata.* The classic papers are not reviewed here as they are reviewed in the above-mentioned compendia [1, 2]. Here we focus on more recent findings, mostly those published in the last two decades. It may be warned that the basic paradigm of diapause, built in the previous century, had not yet been broken.

3. Coccinellidae

3.1. Coccinella septempunctata L. Among ladybirds, adult diapause has evidently been most studied in the originally Palaearctic species *Coccinella septempunctata*, the seven spot, that has in two recent decades invaded the Nearctic region and attracted attention of researchers there (e.g., [5]). Both in Europe and USA *C. septempunctata* has been found heterogeneous as to the induction of diapause (see Section 1).

In Bohemia (50°N, Western Czech Republic), the population in the autumn consists of two fractions. Although in some years aggregations of both sexes of dormant *C. septempunctata* may be found in their hibernation quarters in grass tussocks from early August onwards, one can also find actively feeding coccinellids on vegetation with aphids (often on different weeds, such as *Carduus* spp. and Daucaceae) for the whole of September and into early October [6]. The physiological condition of these two fractions was determined by dissection immediately after sampling and after rearing. Whereas the alimentary canal in the dormant beetles was empty of food and there were no traces of vitellinization in the ovaries, the digestive tract was full of food in more than half of the active adults sampled and 13–20% of females possessed vitellinized oocytes or even eggs. The difference between the dormant and active beetles became striking when they were reared for three weeks under long days, at 19–22.5°C with plentiful aphid food; the ovarioles of about 85–90% of dormant females remained without any vitellinization, while about 90% of the females collected on plants possessed vitellinized oocytes after rearing [6].

Dissections in mid-July of females collected outdoors a fortnight after adult emergence indicated a strong tendency to univoltinism: 84–93% of the females, entered diapause. The offspring of overwintered adults (F 1) also displayed a high incidence of diapause despite rearing under long-day conditions of 16L : 8D or 18L : 6D with a surplus of suitable prey. When such experiments were repeated in five years the incidence of diapause in F 1 fluctuated between 60 and 90%. A gradual decrease in diapause incidence across generations suggests selection against a propensity for obligatory diapause under long days [6].

A dominant effect of photoperiod and its modification by temperature was documented in samples from the selected lines. Under long days diapause was prevented in most females and 87–96% females reproduced in spite of low temperatures (18 or 18.5°C, resp.). Under short days of 12L : 12D or 8L : 16D, diapause incidence was high (85–94%) at low temperature (about 18°C), but low at high temperature: at temperatures alternating between 24-25°C (night) and 27-28°C (day) only 10% entered diapause.

In central Europe and the Paris region (France), the progress of diapause development in *C. septempunctata* was monitored by transfers of adults from the field to the laboratory at 25°C. Diapause was completed in December-January, whereupon it was replaced by postdiapause quiescence that lasted until spring when, under the influence of temperature increasing above around 12°C, the adults dispersed from hibernation sites to localities with aphids, where they fed and reproduced [6].

Whereas field observations indicate a univoltine cycle in central Greece, a tendency to multivoltinism was documented in *C. septempunctata* in this region, when four subsequent generations were reared in modified outdoor conditions [7]. The conditions were improved by shading of the rearing cages from direct insolation and continually providing surplus of suitable aphid prey.

3.2. Ceratomegilla undecimnotata (Schneider). Similar to *H. convergens*, the relative role of food and photoperiod in diapause regulation in *C. undecimnotata* is not yet clear enough, although the share of food/prey appears important.

The earlier studies on this Palaearctic species were undertaken in France and Czech Republic [6]. Detailed laboratory and field studies in central Greece [8] widened our knowledge of diapause in this species. The authors dissected the females of *C. undecimnotata* that were sampled from the field in mid-June. About 40–50% of nonreproductive females were recorded in the plain, while most females (70–100% in different years) were at the same time immature in aggregations on mountain summits, where they remained until spring. This should indicate a univoltine cycle. When, however, the beetles were provided with a surplus of aphids outdoors (under shading, but natural photoperiod), five subsequent generations were produced. With about 30% of immature females in the first three generations, such diapause incidence was not far from the field records from the plain. These data indicate that *C. undecimnotata* populations from central Greece are heterogeneous as regards the induction of diapause. These findings are similar to *C. septempunctata* and *H. convergens*.

Samples from mountain tops were regularly transferred to laboratory (25°C, long day 16L : 8D, prey surplus). Females were activated under these conditions by tachytelic processes and laid eggs after a gradually shortened preoviposition period (92 d in July, 63 d in August, 20 d in September). This decrease in diapause intensity demonstrated the progress of diapause development by horotelic processes in the field.

All these results, similar to the French and Czech findings [6], indicate that *C. undecimnotata* is a long-day insect (long day is diapause preventing and not diapause inducing as assumed in the recent paper [8]). (The generic name *Hippodamia* used in [8] is not correct.)

3.3. Hippodamia convergens Guerin. In this common Nearctic aphidophagous ladybird, several possibilities of diapause induction were proposed by Hagen [9] for the plains of Northern California—and not much has been added later to his hypothesis. Originally, before the installation of irrigation systems, most individuals had an (obligatory ?) univoltine cycle (with a complex migration to mountains where enormous quantities of beetles aggregate for overwintering and are collected for biocontrol purposes). Later the Californian ladybird populations changed to multivoltine cycle, due to the high abundance of introduced aphids and are induced to diapause by photoperiod and temperature. However, diapause can still be nutritionally induced in a part of the population [9, 10]. In the upper coastal plain of South Carolina, diapause in *H. convergens* is terminated in December/January after the transfer to temperatures >15.5°C, despite short days 12L : 12D [11].

We need to know much more about the combined action of food and other factors in this species. Such studies have evidently begun with the analyses of the role of nutritional factors (nonaphid protein rich alternative food) in the arid conditions. In the Great Plains region in the central USA *H. convergens* is normally bivoltine, with obligate winter hibernation and facultative summer estivation which creates the possibility for additional generations when conditions permit. Various cases of nutritional regulation of reproductive diapause were analysed in females of *H. convergens* in these populations [12, 13]. The importance of drinking sap on sunflower in the summer months in West Kansas was examined. Sunflower petioles and pollen as well as lepidopteran eggs were provided to the beetles collected in early June. While these females did not oviposit in the absence of protein food, feeding on eggs of *Ephestia kuehniella* followed by pollen enabled 66% of the females to lay viable eggs at a low rate of 6.6 eggs/day. The females, transferred on 14 August to essential aphid food (*Schizaphis graminum*), laid six times more eggs.

These experiments stressed the adaptive role of the life cycle in *H. convergens* in that it enables survival during arid summer conditions when there is a shortage of the essential food, aphids. In the absence of protein-rich food, the 1st generation can enter diapause. Another tactic could be to wait in a state of lowered metabolism (but less lowered than in diapause) for the reappearance of essential aphid food, relying meanwhile on alternate foods. Then a switch to intensive egg laying can be quick, as was shown by a short oviposition delay of only 4 or 6–9 days on essential prey [12, 13].

3.4. Harmonia axyridis (Pallas). After the very early Russian studies from Asia (see [6]) only two Japanese papers were published, dealing with diapause of this coccinellid from east Asia—before its invasion to America and Europe. In Japan this bivoltine long-day insect hibernates in diapause [14, 15] and uses the polyol myo-inositol to increase its cold-hardiness [15].

After its arrival in Europe this invasive species was studied in South-Eastern France, Northern Italy, and Belgium and has become the most studied coccinellid. Facultative diapause of the multivoltine strain is induced by short-day photoperiod 12L : 12D at 23°C and lasted 1–3 months; eggs of *Ephestia kuehniella* were used as a suitable alternative food [16–19].

4. Chrysomelidae

4.1. Leptinotarsa decemlineata (Say). It was probably the first insect model for the detailed experimental study of adult diapause. Thanks to the intensive research by the team of Professor Jan de Wilde, Wageningen, The Netherlands, particularly the physiological/endocrinological aspects of diapause have been intensively investigated since the late 1950's [20]. These studies are reviewed in the important compendia [1, 2] and in the introduction of a paper by de Wilde's followers [21]. Research on the prolonged diapause in *L. decemlineata* and its dependence of soil types was focused by the team of Professor Raisa Ushatinskaya, Moscow, Russia [22].

The main facts on diapause regulation from the classic Dutch studies will be given here to make the reading of more recent studies below more easy. Diapause is induced by short-day photoperiod: 10L : 14D at 25°C have been used in Wageningen and 16L : 8D was the long-day photoperiod. Both larvae and adults are sensitive to induction [23]. 20–30% of beetles enter diapause under any photoperiod; thus a propensity to obligatory univoltinism is indicated, similar to the case of *C. septempunctata*, discussed above. Diapause development in *L. decemlineata* progressed well under any of three temperatures, 4, 12, and also 25°C, that is, it does not need period of low temperatures for its completion (similar to quite a number of other insect species [3]). At 4°C mortality was high (15% after 3 mo, 50–70% after 6-7 mo), while it was <10% at 12 and 25°C. Diapause development was faster at 25 than 12°C, and 50% of females spontaneously emerged from soil after 14 and 21 wk, respectively. Sensitivity to photoperiod is retained during diapause development at least to February: at this sensitive period, diapause can be terminated by only three long days. The females lose photoperiodic response for at least 5 wk after the completion of diapause development, but the responsiveness is restored 3 wk after diapause in a part of the population (recurrent photoperiodic response, discussed in Section 12). However, photoperiodic sensitivity is never lost completely, even after the completion of diapause it affects the rate of vitellogenesis and ovarian maturation.

Because of the importance of the Colorado potato beetle as pest, the primary insect defoliator of solanaceous crops in North America and Eurasia [24], research continues on different aspects. Flight incidence and duration in relation to mating was recorded by flight mills [25]. Mating has a pronounced effect on flight activity decreasing it in females, evidently because migration and reproduction interfere with each other, and increasing it in males—they may thus mate with females from different localities.

Oviposition and burrowing behavior (as contrasting characteristics of nondiapause versus diapause, resp.) were compared in 1st generation females along a 5° latitudinal gradient, in six populations from North Dakota and Minnesota, USA, and Manitoba, Canada. Four locations were sampled in the Red River Valley region (between 49°49′N and 47°00′N) and two in east central Minnesota (45°20′N and 44°44′N). Different incidence of oviposition was recorded under long days in the RRV region (0-1%) and in ECM samples (9–15%). The authors conclude that *L. decemlineata* has the capability of becoming adapted to local environmental drivers, while retaining intrapopulation variability [26].

Some Colorado potato beetles enter prolonged (>1 yr) dormancy, an event quite common in adults dormant in the soil, such as some curculionids discussed below. This phenomenon was studied earlier in Russian populations and those from Western United States, where a very high incidence (22%) was recorded. M. J. Tauber and C. A. Tauber [24] studied its frequency in the Upstate New York in a 10-yr field study and recorded an average 2.04 (0–72)% in 12,607 beetles. They explain this relatively low incidence in North Eastern United States by the late arrival of *L. decemlineata* after the introduction of cultivated solanaceous crops. In Western United States, in contrast, the Colorado potato beetle commonly occurs on wild solanaceous host plants in drought-prone habitats.

Both the effect of age of potato foliage and temperature are important in the prediapause beetles [27]. The adults consumed older foliage at a faster rate, particularly at the higher temperature of 17°C (compared with 11.5°C) and consumed 45% higher weight of leaves. It is assumed that there is a fixed requirement of accumulated reserves to achieve prediapause satiation. If the food is less rich in needed substances, larger amounts have to be consumed.

In populations from Central Europe 70–80% of reproducing females develop under >15 h day length, while under <14 h day length all beetles enter diapause [28]. In experiments the photoperiod of 12L : 12D was used as short day, and 18L : 6D as long day. The index of food conversion was 5.4 under long days, but 7.2 and 11.9 under short days (at 20 and 25°C, resp.). Pupae were smaller under long days due to a greater loss of biomass during the prepupal stage that was almost twice as long as under short days.

The functional state of flight muscles was assessed by staining with commercially available (Sigma-Aldrich) tetrazolium salts; the color develops due to reduction of a colourless salt by mitochondrial enzymes [29].

The research on diapause of *L. decemlineata* continues also in the recent molecular biology age. In a study of gene expression patterns during the first 20 postemergence days in beetles programmed for diapause (at 8L : 16D, 24°C), that is, in prediapause phase, oxygen consumption was measured in this period. The respiration rate increased from 0.4 mL/g/h on day 1 to 1.1 mL/g/h on day 4, and after a plateau between days 4 and 7 the oxygen consumption decreased to 0.08 mL/g/h on day 15. The CO_2 production followed the same curve, with an additional conspicuous peak on day 7. Among the clones of genes isolated, elevated levels of expression of the glycine-rich transcripts (that function in structural support of insect cuticle) persisted for four days longer in diapause-programmed beetles, compared with nondiapause adults. The differentially regulated genes were downregulated between days 13 and 20, that is, at the end of prediapause when the metabolic rate was already much decreased [30].

The series of papers by Yocum and coauthors has continued by a recent one [31]. Prediapause and diapause phases of development are well marked by expression of genes in laboratory reared adults. However, it is much less clear in field collected adults, evidently due to the polyphenic character of diapause, mentioned earlier. The authors conclude that this property contributes to the status of *L. decemlineata* as a "superpest" of potatoes [31]. This characteristic is similar to that in *C. septempunctata*, where also the plastic character of adult diapause is obviously associated with the "success" of the species [32].

4.2. *Colaphellus bowringi Baly.*

A complex analysis of diapause regulation was conducted by Professor Xue and coauthors in a series of recent papers. The cabbage beetle, *C. bowringi,* is a pest of cruciferous vegetables in mountain areas of Jiangxi Province, China. There are four generations per year, one in spring and three in autumn. The beetle estivates and hibernates as adult in the soil. A life-cycle polymorphism was reported by Xue and Zhang 20 yrs earlier (for an English summary of that paper published in Chinese, see [33]). Although the adults enter diapause at the same time, they differ much in diapause duration (several months–two yrs) and thus they expressed heterogeneous voltinism. Without regard to diapause induction and duration, the post-diapause beetles emerge from soil either between late

February and early April, or between mid-August and early October.

C. bowringi is a short-day species (i.e., long days induce diapause), but the photoperiodic response is strongly affected by temperature. High temperatures enhance the diapause-averting effects of short days and suppress the diapause-inducing effects of long days. Diapause incidence is 100% at <20°C at any photoperiod. Photoperiod plays a relatively small role in diapause induction; short days can prevent diapause only at temperatures above 20°C. The mechanisms ruling the complex seasonal life-cycle in *C. bowringi* are well explained by experimental results [33]. It is probably the first documented case of summer diapause induction by low temperature instead of high temperature. Diapause is entered by early-emerging individuals in April. The authors suggest that the photoperiodic and temperature controls of diapause induction have a different genetic basis.

Experiments on the effect of thermoperiods on diapause induction in *C. bowringi* showed again the importance of temperature, particularly during the photophase [34].

Other detailed experiments documented an important effect of host plants on diapause incidence in *C. bowringi* [35]. The highest incidence of diapause was caused by feeding on radish (*Raphanus sativus*) and the dark green variety of Chinese cabbage: the lowest incidence was obtained by feeding on the yellow-green variety of Chinese cabbage with thin leaves. Most adults entered diapause on mature and aged leaves. Diapause incidence was affected by host plants only within a certain range of photoperiods and temperatures; it was best manifested at 25°C and 13L : 11D. Regardless of host plants, all adults entered diapause at 20°C or at 16L : 8D, as indicated in the earlier papers.

There is no negative tradeoff between diapause duration and several parameters of performance in adults after diapause: the body weight, longevity, and fecundity of beetles with the longer diapause duration of 21 mo were higher than those with the shorter duration of 5, 11, and 17 mo [36].

Crossing a high diapause strain with a laboratory selected nondiapause strain showed that diapause capability is inherited in an incomplete dominant manner; maternal inheritance of diapause induction is stronger than paternal inheritance [37].

4.3. Zygogramma bicolorata Pallister. This chrysomelid was successfully introduced to Jammu and Kashmir, India for biological control of carrot weed, *Parthenium hysterophorus* L. Adults enter diapause from August to December with a peak in late November. They burrow into soil and are dormant about 1–3 cm bellow the surface. The incidence of burrowing adults increases with soil moisture and is higher in silty soil (47%) than in sandy soil (24%). Diapause is facultative as nondiapausing adults breed in winter under laboratory conditions. The beetles become active in March and, after having defoliated their host plants in an area, they disperse and need not be introduced to other areas. By treating the newly emerged beetles with human insulin (5 : g) the incidence of diapause was lowered and the fecundity increased [38].

In a population from Jabalpur, India, 64% of beetles entered diapause at 26°C and photoperiod was not important. Storage of females at 10°C for 6 mo did not lower their fecundity [39].

4.4. Plagiodera versicolora Laicharting. This is a species with facultative diapause that feeds on several species of willows. Experimental populations from the region of the river Ishikari (43°N, Hokkaido, Japan) had both univoltine and bivoltine life-cycles and were most abundant on *Salix sachalinensis* Fr.Smidt.

All females entered diapause at 10L : 14D, but a rather high incidence also was recorded at 16L : 8D (68% with a range of 40–100%) [40]. These are evidently results from rearing beetles on leaves of mixed quality, as only 10% diapause was reported in the 1st generation reared on 2–22 July on young leaves [41]. Diapause induced under short days of 10L : 14D at 22°C was terminated by long days of 16L : 8D at 22°C [42].

Later the effect of photoperiod and temperature (16L : 8D and 20°C in the laboratory) was experimentally isolated from the effect of seasonally changing quality of host-plant leaves [41]. While the abiotic laboratory conditions were kept constant, the leaves of *S. sachalinensis* were collected in the field and thus gradually more mature leaves were provided. The reproductive parameters declined in the 2nd and 3rd generations, in comparison with the 1st generation. Diapause incidence increased from 10 to 60%, the preoviposition period increased from about 9 to 16%, and the fecundity during the first 10 days of the egg laying period decreased from about 50 to 18 eggs per female. The authors thus documented the effect of host plant age and suggested that the combination of both day length and host-plant conditions cuing diapause is adaptive [41].

4.5. Galerucella calmariensis L. It was introduced to the United States for the biological control of purple loosestrife (*Lythrum salicaria* L.). The adults undergo a facultative reproductive diapause (the paper's abstract mentions obligatory diapause by mistake) during summer, autumn, and winter. Diapause can be averted by long days of 16L : 8D and induced by 8L : 16D. Adults are responsive to diapause-inducing photoperiods. The authors failed to isolate the cultures efficiently from insolation with white tissue tents as the natural photoperiod produced a seasonal effect; in early summer the ovaries matured better [43].

4.6. Crioceris sp. This undescribed chrysomelid species was studied in the Western Cape Province, South Africa (34°35′S) as a promising biocontrol agent of bridal creeper (*Asparagus asparagoides* (L.) W. Wight) with the intention to introduce it to Australia.

The majority of fully developed adults remain inside cocoons in soil for various periods of summer diapause. Field observations suggest that rainfall might be the cue for termination of diapause or dormancy. The effect of wetting was demonstrated in the laboratory. Only 29% ($n = 135$) adults emerged from dry cocoons at 20°C within 76 days.

This proportion was substantially increased by wetting, and even more by repeated wetting.

No research addressed the mechanism of diapause regulation by physical and biotic environmental factors, although larvae were reared successfully to pupation in soil at 15 and 20°C [44].

5. Bruchidae

5.1. Callosobruchus subinnotatus (PIC). It is a major pest of stored bambara groundnut, *Vigna subterranea* (L.) Verdcourt in sub-Saharan West Africa. Adult polymorphism was described in this bruchid, similar to that of some other species of the family, particularly *C. maculatus* (Fabricius), that was the model insect for a series of classic ecological studies by Professor Syunro Utida from Kyoto University, Japan, in the years 1954–1981. The terms for the two polyphenic forms, used in the earlier *C. maculatus* studies, were also used here although they do not seem very adequate: "active" and normal adults. While the normal adults have a high fecundity, low longevity, and lower tendency to dispersal, the "active" phase shows opposite qualities. Dissections reveal immature ovaries and male gonads, so we might consider this suspension of reproduction an adult diapause or at least a diapause-like phenomenon. Although high population density was suggested in several Utida's articles to be the factor responsible for the development of "active" form, no attempts have been made to address this influence in *C. subinnotatus* [45].

Another congeneric bruchid, *Callosobruchus rhodesianus* (Pic), suffering from strong competition by *C. maculatus* on cowpea, *Vigna unguiculata* (Walp.) in Togo, Africa, reproductive diapause was recorded [46] in a part of population.

5.2. Bruchidius dorsalis Fahraeus. This multivoltine seed-eater occurs in Central and Southern Japan. Females oviposit on seedpods of the Japanese honey locust, *Gleditsia japonica.* Newly matured seeds are available from August to autumn, but the females may use also dry, hardened seeds; thus host seeds can be utilized almost the whole year.

In contrast to most insect species, in warmer regions *B. dorsalis* enters diapause in different developmental stages: final larval instars and adults. Even nondiapausing early instars may overwinter [47]. In Sagamihara (35°34′N) 3 to 4 generations develop per year. Some autumnal adults produce the new generation before winter, while another part of the population overwinters before spring reproduction. Diapause is induced by short days and the first five days after adult emergence are sensitive to diapause-inducing factors.. Diapause incidence was higher and the critical photophase longer in cooler regions [48, 49].

6. Curculionidae

6.1. Curculio nucum (L.). This specialist of hazelnut trees has an obligate 2-yr cycle in France (45°46′N, 420 m a.s.l.) with one larval diapause in winter, pupation and ecdysis of adults (in soil) the next summer, and in the 2nd winter adult diapause. In spring the overwintered adults emerge from the soil in April and appear on trees from May to early July [50]. Early emergence from the soil enables females to oviposit in nuts before they fully harden. As they cannot penetrate the mature nut, they must oviposit before July.

Females lose about 8.5% of their weight during overwintering, but their lipid content does not decrease. Thus the authors suppose that females use lipids accumulated during the larval stage for egg production and obtain other nutrients from adult feeding. Evolutionary forces triggering the obligate 2-yr cycle are discussed [50].

6.2. Exapion ulicis (Forster). This univoltine species consumes the seed of gorse, *Ulex europaeus*, that has peak fruiting in spring and was introduced to some countries for its biological control. Adults lay eggs in spring into young green pods where the larvae develop adults feed on leaves and flowers of gorse and then diapause in autumn and winter. In winter the beetles stay on branches and are able to resist cold. In Brittany, France, the species was studied together with *E. lemovicinum* (Hoffmann), a species that overwinters in the larval stage, to understand its cold-hardiness. *E. ulicis* adults are freezing intolerant, but exhibit a low supercooling point of −17°C. The regulation of diapause induction and termination was not studied [51].

7. Carabidae

7.1. Nebria salina Fairmaire and Laboulbene. This species is common in unproductive habitats, such as sand dunes and upland grasslands. This short-day insect is an autumn breeder that enters a summer diapause. Females require at least two months of exposure to short-day photoperiods of <12L : 12D. Under long days of 18L : 6D the ovaries do not mature. Still shorter days of 6L : 18D stimulate better growth of ovaries. The males matured after two months irrespective of photoperiod.

In the field (Hamsterley, County Durham, UK) the main activity of *N. salina* was concentrated in September [52]. Thus, the life cycle of this species resembles the congeneric autumn breeder *Nebria brevicollis*, where diapause was studied in the 80's by Hengeveld and Loreau (quoted in Telfer and Butterfield [52]).

7.2. Carabus yaconinus. When the authors transferred this spring breeder from the field to laboratory experimental photoperiods, the beetles showed a long-day photoperiodic response in autumn and early winter. In the course of winter the response was gradually lost, so that in late April the ovaries of females matured both in short and long days [53]. However, in summer the photoperiodic response resumed again. Thus *C. yaconinus* appears to be another case of recurrent photoperiodic response that was revealed for the first time in a pentatomid bug *Aelia acuminata* [54] and recorded later also in *L. decemlineata* and *C. septempunctata* (see above).

8. Silphidae

8.1. Nicrophorus nepalensis. This subtropical short-day breeding carrion beetle that occurs in Taiwan (24°45′N, 745 m a.s.l.) is active mainly in early spring (February–May) and also in autumn (October-November). Reproduction is best promoted at 20°C and a photoperiod of 12.5L : 11.5D, but only in the presence of carrion, whereupon oviposition starts after 2 weeks. At a lower temperature of 15°C and 11L : 13D, maturation is slower, so that the oviposition begins after about 9 weeks. In contrast, longer days (14L : 10D) prevent oviposition at 25°C, but enable oviposition of 45–50% of females at 20°C. Summer diapause is an efficient adaptation as the development of offspring on carrion in summer would suffer from competition with more quickly developing dipteran larvae on quickly decomposing dead animals [55].

9. Scolytidae

9.1. Ips typographus L. The number of generations varies in different countries, similar to other insect species. Flight parameters were studied in young beetles from five populations: one in Denmark (56°51′N) and four in Sweden (between 57°40′N and 62°51′N). The flight propensity of beetles that emerged in the period 34–80 days was tested and those flying less than 100 s (“non-fliers”) were found by dissection to be in diapause with undeveloped ovaries and large fat reserves. The frequency of such beetles increased with increasing latitude from 35 to 70%. While “fliers” migrate to find a breeding site, diapausing “non-fliers” often overwinter on the ground beneath the brood tree [56].

In a Central European population, diapause was induced by <16L : 8D at 20°C and the critical photoperiod (50% diapause incidence) was 14.7L : 9.3D. Temperature of >23°C prevented diapause even at 12L : 12D. Neither gonads nor flight muscles matured in diapausing adults. Overwintering adults, shown to be in diapause by their response to photoperiod, reproduced at the long-day photoperiod of 18L : 6D but not at 12L : 12D when transferred in October from the forest to laboratory 20°C [57].

10. Scarabaeidae

10.1. Dasylepida ishigakiensis Niijima and Kinoshita. This “white grub” is a serious pest of sugarcane on Okinawa Islands, Japan. It is the only scarabaeid beetle in which the regulation of adult diapause has been studied. Both larvae and adults of this subtropical beetle undergo diapause in a semivoltine (2-yr) life cycle. Adults emerge from pupae in the soil and stay there for about two months. This delayed emergence from the soil cannot be related to synchronization with food, because the adults have degenerated mouthparts and do not feed. The beetles begin to leave the soil in late autumn when cooler temperatures are favorable for mating. Sexual maturation in the laboratory is suppressed at temperatures of 25–30°C, but proceeds well at 15–20°C. Photoperiod does not seem to act in diapause regulation [58].

11. Endomychidae

11.1. Stenotarsus subtilis (=rotundus) Arrow. S. subtilis provides a case of tropical diapause that was studied in Panama [59, 60]. This beetle forms large aggregations for a long dormancy, comprising 6 months of the wet season and 4 months of the dry season. Breeding sites, food, and diapause-inducing factors all remain unknown. Experiments with beetles collected from aggregations revealed the role of environmental factors in diapause development. Although in Panama (9°N) the difference between the longest and shortest day is only 1 h, increasing day length from March onward stimulates weak development of corpora allata, primary oocytes, and flight muscles that had remained resorbed for about 6 months. Mating and dispersal coincide with the onset of rains in late April. Two-month exposures to contrasting humidities revealed that higher humidity also stimulates development of the aforementioned organs [59, 60].

12. Concluding Remarks

It is almost impossible to make general conclusions from the recent data given above on the diapause of beetles. There are at least two obstacles: (1) diapause is a seasonal adaptation (see the Introduction) and it apparently evolved independently in individual species under specific environmental selective pressures that ultimately result in a genetic basis. Diapause of individual species/populations is thus intrinsically diversified—and inherently resists generalization. (2) The conditions for reasonable comparisons are further aggravated by diverse research protocols that have been employed by individual researchers either arbitrarily or under various technical constraints. Even in the case of currently studied species (that often are economically important), such as some chrysomelids, curculionids, or coccinellids, research analyses have often remained incomplete. This is still even more true for fragmentary studies some of which are included here only to show the wide range of records.

In spite of these difficulties, we may try to deduce some general features (that may apply to other insect orders as well). No particular break of the classic paradigms was made in the recent papers. They rather were further corroborated and extended. The most general, and the most studied, is the signaling function of photoperiod, often modified by effects of temperature and food that announces seasonal transitions with astronomical precision. In particular populations, photoperiodic response is always adapted to geographic latitude, as has been shown above best in *L. decemlineata* populations originating from locations separated by about 5° latitude. In some common and widely studied species, such as the Nearctic coccinellid *H. convergens*, the evidence of the photoperiodic regulation has still remained rather scarce.

An effect of food quality implicated in several of the discussed species. The difference of old versus young leaves is well documented in *L. decemlineata* and *C. bowringi*; the effect of alternative food (e.g., pollen) versus essential aphid species has been studied in detail in *C. septempunctata* [6] and *H. convergens* [12, 13]. In the ladybird *Ceratomegilla undecimnotata* (Schneider) the physiological age of host plant can even act through the aphid prey, as reported in the 70's see [61] (see also) [6].

The effect of population density has remained rather neglected in the beetles discussed here. The exceptions are early articles on the bruchid *C. maculatus*, where the effect was recorded long ago by Professor Utida, and the observations on *C. subinnotatus*.

As mentioned above, diapause adaptations are very plastic in response to selection. Similar to changes in photoperiod, beetles can adapt quite quickly to environmental changes associated with changes in food supply. Thus, for example, introduction of irrigation in arid areas led to the establishment of prey in two coccinellids, *H. convergens* in California and *Chilocorus bipustulatus* L. in Israel, and thereafter to changes in their life cycles [6].

It is worthwhile to speculate about a hierarchy of individual factors governing diapause regulation. The basic driver is usually photoperiodic response to the precise annual astronomical repetition of day length. We may assume that less rigid reactions to less predictable environmental changes in food availability and quality, and other factors such as temperature, humidity, and population density, can be superimposed. The archetypal nutritive factor is "prepared to enter the game" in the case of unpredictable events affecting prey abundance—and this is facilitated by phenotypic plasticity.

Polyphenic character of diapause is a very important feature in *C. septempunctata* and *L. decemlineata*, but it has been found also in other Coleoptera and in insects generally: populations are heterogeneous as to voltinism tendencies. For populations with mixed uni- and polyvoltine tendencies we might envisage a scenario which combines plasticity with resilience. One aspect of the life-cycle strategies is the "safety" ("insurance") factor of the univoltine trait which is permanently perpetuated in the gene pool and maintained (i.e., not selected out) despite the frequent momentary occurrence of conditions favourable for the production of an additional generation, since these transitory conditions are unreliable in the long run. However, polygenes facilitate population responses to changes in the environment. If there is a promising improvement they may "open the gate" for intermittent multivoltine development, that may be more or less appropriate to capitalize on transitory environmental improvement. The system remains resilient because the univoltine trait is maintained quite intensively. This scenario is adequate for *C. septempunctata* and perhaps also *L. decemlineata* living in temperate regions/climate. In different climatic areas, the regulation of voltinism can differ.

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Research Article

Host Plant and Leaf-Age Preference of *Luprops tristis* (Coleoptera: Tenebrionidae: Lagriinae: Lupropini): A Home Invading Nuisance Pest in Rubber Plantation Belts

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Massive seasonal invasion by the litter-dwelling beetle *Luprops tristis*, into residential buildings prior to monsoon rains, and their prolonged state of dormancy render them a very serious nuisance pest in rubber plantations in the Western Ghats in southern India. Feeding preferences of *L. tristis* towards leaf litter of seven trees co-occurring in rubber plantations, cashew (*Anacardium occidentale*), mango (*Mangifera indica*), jackfruit (*Artocarpus heterophyllus*), wild jack (*Artocarpus hirsutus*), cocoa (*Theobroma cacao*), cassia (*Cassia fistula*), sapota (*Manilkara zapota*) and rubber (*Hevea brasiliensis*) were analyzed with no-choice and multiple-choice leaf disc tests. Results showed that *L. tristis* is a generalist feeder with a defined pattern of preference, with the leaf litter of rubber being the most preferred followed by those of jackfruit and cocoa. Tender leaves were preferred over mature leaves except for cocoa and sapota. Equal preference towards tender and mature cocoa leaves, presence of patches of cocoa plantations and the scarce distribution of other host plants in rubber plantation belts leads to the proposal that in the absence of tender and mature rubber leaves, cocoa becomes the major host plant of *L. tristis*.

1. Introduction

Seasonal mass invasion of a litter-dwelling detritivorous beetle, *Luprops tristis* (Fabricius, 1801) (Coleoptera: Tenebrionidae: Lagriinae: Lupropini), numbering 0.5–4 million per residential buildings prior to the onset of south west monsoon showers and subsequent aggregation in prolonged state of dormancy (Figure 1) render them a very serious nuisance pest in rubber plantation tracts in the Western Ghats in southern India [1]. Litter stands of rubber tree [*Hevea brasiliensis,* (Willd. ex Adr. De Jus) Müll. Arg. 1865] are the breeding and feeding habitat for *L. tristis*, with prematurely abscised leaves as the most preferred food resource, and a synchronized life cycle with the leaf phenology of rubber [2]. Their near absence in natural forests in contrast to exceptionally high abundance in rubber plantation litter established that rubber is the major host plant of the insect in the region [3–5]. Prevention of premature leaf fall of rubber may regulate the population build up of *L. tristis* in rubber plantations [2, 5]. However, their presence in the leaf litter of trees namely, cashew (*Anacardium occidentale,* Linnaeus 1753), mango (*Mangifera indica,* Linnaeus 1753), jackfruit (*Artocarpus heterophyllus,* Lamarck 1789), wild jack (*Artocarpus hirsutus,* Lamarck 1789), cocoa (*Theobroma cacao,* Linnaeus 1753), cassia (*Cassia fistula,* Linnaeus 1753), and sapota (*Manilkara zapota,* Linnaeus 1753) co-occurring in rubber belts (personal observations, first author) led to the hypothesis that *L. tristis* may also feed on the leaf litter of these plants. Hence, it is essential to determine the feeding preference of *L. tristis* on these potential alternate host plants before attempting control by prevention of premature leaf fall in rubber plantations.

We propose that (i) *L. tristis* is a specialist feeder on rubber litter, and (ii) does not feed on the leaf litter of cashew, mango, jackfruit, wild jack, cocoa, cassia and sapota plants. Data generated is expected to contribute towards adoption of control strategies to prevent the possible spread of *L. tristis* to nonrubber plantation belts where plantations of potential host plants are prevalent.

2. Materials and Methods

2.1. Study Organism. *Luprops tristis* (Fabricius, 1801) is generally regarded as an inconspicuous litter-dwelling detritivore but for their exceptionally high abundance in the rubber plantation litter stands across the moist south Western Ghats [1]. They are regionally referred to as *"Mupli vandu"* in Central and South Kerala and *"Ola prani," "Ola chathan,"* or *"Otteruma"* in North Kerala in South India. No data exists on its ancestral host or about alternate host plants as it remained as a minor darkling beetle species of least importance, till it became a nuisance pest in residential buildings with the spread of rubber plantations in the moist western slopes of the Western Ghats during 1960–1970 period [6].

2.2. Host Plants. Plants with which *L. tristis* associated are as follows.

Mango (*Mangifera indica*: *Anacardiaceae*: Sapindales) is an evergreen tree, indigenous to the Indian subcontinent. Although an evergreen tree, large quantities of old leaves are shed during summer vegetative flush. The leaf-flushing period can have one to five flushing events with the whole canopy flushing in synchrony or in patches [7]. The most common native variety, *Nattumavu*, in the rubber belts was selected for the study.

Cashew (*Anacardium occidentale*: *Anacardiaceae:* Sapindales) was originally spread from Brazil by the Portuguese and is widely grown for cashew kernels popularly known as "cashew nuts" [8, 9]. Although an evergreen tree, large quantities of old leaves are shed during presummer period prior to flowering (first author, personal observations).

Jackfruit (*Artocarpus heterophyllus: Moraceae*: Rosales) is a tall evergreen tree with spreading canopy. Although an evergreen tree, large quantities of old leaves are shed during summer vegetative flush. It is a common tree in the rubber belts as farmers use its fruits and seeds as a food item, leaves for fodder, and stem for timber [10].

Wild jack (*Artocarpus hirsutus*: *Moraceae*: Rosales) is a tall evergreen tree species that is endemic to the Western Ghats [11]. Large quantities of old leaves are shed during summer vegetative flush. It is a common tree in the rubber belts as planters allow a few trees to grow in the midst of rubber plantations due to the high commercial value of its wood and its taller canopy which do not interfere with growth of rubber plants.

Rubber (*Hevea brasiliensis*: *Euphorbiaceae*: Malpighiales) is a deciduous tree with a major annual leaf shedding during December, leaf flush in January, and flowering in February. Rubber plantations of about half a million hectares are present along the western slopes of the Western Ghats in the South Indian state of Kerala [2].

Cocoa (*Theobroma cacao*: *Malvaceae*: Malvales) is an evergreen tree native to Central America and South America. It was introduced as a crop plant into many tropical African and Asian countries for cocoa seeds which are used to make cocoa powder and chocolate [12]. It is a common tree in the rubber plantation belts as it grows well in the under storey of rubber in the region.

Sapota (*Manilkara zapota*: *Zapotaceae*: Ericales) is an evergreen tree native to Southern Mexico, Central America and the Caribbean [13]. It is grown in the front yards of residential buildings in the rubber belts for fruits as well as a shade tree.

Cassia (*Cassia fistula*: *Fabaceae*: Fabales) is a deciduous tree of deciduous forests ranging from tropical thorn to moist through subtropical thorn to moist forest zones and is a native of India. It produces yellow flowers in drooping racemes, making it an extremely showy tree in bloom with only flowers and no leaves [14]. It is a common ornamental tree in the surroundings of residential buildings as its flowers are considered as an auspicious first sight at the crack of dawn on the day of *Vishu*, a new year festival celebrated in the region [15]. Leaf shedding occurs during December–February period and flowering during March-April period.

2.3. Experiment Setup. The present investigation was carried out during March 2010 using test insects and host plant leaves collected from the vicinity of a rubber plantation in the Devagiri College campus, located 6 km east from the Malabar Coast at Calicut (11°15_N, 75°50_E), in the Kerala state of India.

To ensure uniformity of age at the beginning of the experiment, teneral adults were collected based on their brownish white body color [1] from rubber plantation litter in the college hostel premises. Collected beetles were reared in clay vessels placed in an environmental chamber and fed with a mixture of diced tender and mature leaves of all eight leaf types for 10 days to reduce the possible effect of leaf quality variations on growth rate and feeding preference. Beetles were deprived of food for 24 hrs before starting the feeding experiments.

Food preferences were analyzed with multiple choice and no choice leaf disc tests in the second week of March 2010 on successive days. Tender leaves of the eight potential plants were collected from the branches of the same height during February-March 2010 and senescent leaves during November 2009 to February 2010 period. Freshly sprouted leaves of five days of age were categorized as tender and the leaves turning yellowish brown prior to the onset of annual leaf shedding as senescent. Collected leaves were kept frozen in plastic bags, and undamaged leaves were used for analysis.

Leaf discs (400 mm^2) of each leaf type were cut and were individually marked with stapler pins (one stapler pin on leaf type 1; 2 pins parallel to each other on leaf type 2; 2 pins crosswise on leaf type 3; 3 pins parallel to each other on leaf type 4, and so on) that enabled their identification. One leaf disc of each leaf type was placed inside a clay vessel (9 cm diameter × 5 cm height) with a distance of 5 mm between the leaf discs for multiple-choice leaf disc tests, and a single leaf disc for no-choice leaf disc tests. Fifteen replicates of each experiment were conducted for tender and senescent leaves separately. In both, no-choice and multiple-choice experiments, three teneral beetles were introduced into the centre of the vessel and were allowed to feed for 24 hrs, (8 am to 8 am). Leaf area consumed was estimated using a 1 mm^2 mesh size-reticulated paper glued on a glass slide.

Table 1: Quantity (mm^2) of leaves consumed (mean ± SD) by *L. tristis* in multiple and no-choice experiment tests.

Leaf type	Tender leaves		Senescent leaves	
	No choice	Multiple choice	No choice	Multiple choice
Cashew	9.60 ± 7.84	1.93 ± 2.80	2.37 ± 3.94	0.31 ± 0.51
Cassia	6.37 ± 10.84	1.05 ± 2.59	0.92 ± 1.50	0.13 ± 023
Cocoa	76.93 ± 56.68	20.56 ± 43.95	4.13 ± 5.47	4.00 ± 4.80
Jackfruit	125.33 ± 83.61	41.66 ± 67.32	7.37 ± 7.72	0.47 ± 0.49
Mango	3.93 ± 4.91	0.13 ± 0.3	1.77 ± 2.15	0.38 ± 0.47
Rubber	103.03 ± 85.41	84.13 ± 66.78	48.40 ± 42.54	44.43 ± 34.59
Sapota	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Wild jack	13.13 ± 8.80	6.83 ± 5.86	1.30 ± 0.96	0.65 ± 0.67

Table 2: Two-way ANOVA for feeding preference of *L. tristis* with respect to the leaf type and leaf age in no-choice and multiple-choice experiment tests.

	No-choice				
	SS	df	MS	*F*	*P* value
Leaf type	1,4733.44	7	210.49	45.79	<.05
Leaf age	269.63	1	269.63	58.66	<.05
Leaf type × Leaf age	340.26	7	48.61	10.57	<.05
Error	1,029.62	224.00	4.60		
	Multiple-choice				
	SS	df	MS	*F*	*P* value
Leaf type	1,182.14	7	168.88	42.09	<.05
Leaf age	71.62	1	71.62	17.85	<.05
Leaf type × Leaf age	134.28	7	19.18	4.78	<.05
Error	898.73	224.00	4.01		

Table 3: One-way ANOVA for the quantity of tender and senescent leaves consumed by *L. tristis* in no-choice and multiple-choice experiment tests.

Leaf type	No-choice test		Multiple-choice test	
	F	*P*	*F*	*P*
Cashew	14.45	0	5.16	.03
Cassia	5.15	.03	7.55	.01
Cocoa	0.4	.53	2.23	.15
Jack fruit	34.35	0	9.09	.01
Mango	3.62	.05	3.55	.05
Rubber	3.64	.05	4.45	.04
Wild jack	46.17	0	23.33	0

Amount of leaf disc consumed during the tests was estimated by subtracting the unconsumed area from the initial area of 400 mm^2.

2.4. Data Analysis. Significance levels of the variation in the quantity of leaf consumed among the leaf types and leaf ages were assessed with two-way ANOVA and pairwise differences among leaf types with Tukey-Kramer post hoc tests (*t*-tests). Significance level of the variation in the quantity of leaf consumed between the tender and mature leaves of each leaf type were assessed with one-way ANOVA. The preference hierarchy was reached by ranking the eight leaf types based on the significance level of the pair wise treatments for each host plant. All analyses were done following square root transformation of the data [16]. Significance was determined at $P < .05$. All statistical analyses were performed by using Minitab 16 Academic Software for windows [17].

3. Results

Variation in the quantity consumed by *L. tristis* among the eight leaf types and between the leaf ages, in both no-choice and multiple-choice experiments, was recorded (Tables 1, 2, 3 and 4).

3.1. Senescent Leaves. L. tristis consumed more senescent rubber leaves than all other leaf types in both no-choice and multiple-choice experiments (Tables 1, 4 and 5). No feeding was recorded on sapota.

3.2. Tender Leaves

No-Choice Experiments. Equal quantity of tender leaves of rubber and jackfruit were consumed (Tables 1, 4 and 5). Rubber and jackfruit were preferred over other six leaf types (cocoa, cashew, mango, wild jack, cassia and sapota). No feeding was recorded on sapota.

Table 4: Tukey multiple comparisons (t-test) of the variation in the feeding preference of *L. tristis* towards tender and senescent leaves of different leaf types in no-choice and multiple-choice experiment tests.

Leaf types	Tender leaves		Senescent leaves	
	No choice	Multiple choice	No choice	Multiple choice
Rubber/Cashew	0	0	0	0
Rubber/Mango	0	0	0	0
Rubber/Jackfruit	0.95	0	0	0
Rubber/Wild jack	0	0	0	0
Rubber/Cocoa	0	0	0	0
Rubber/Cassia	0	0	0	0
Rubber/Sapota	0	0	0	0
Cashew/Mango	0.94	0.98	1	1
Cashew/Jackfruit	0	0.01	0.41	1
Cashew/Wild jack	1	0.84	1	1
Cashew/Cocoa	0.97	0.34	0.97	0.2
Cashew/Cassia	0.97	1	1	0.2
Cashew/Sapota	0.08	0.95	0.56	0.99
Mango/Jackfruit	0	0	0.37	1
Mango/Wildjack	0.63	0.27	1	1
Mango/Cocoa	1	0.04	0.96	0.25
Mango/Cassia	1	1	1	0.25
Mango/Sapota	0.65	1	0.6	0.99
Jackfruit/Wild jack	0	0.33	0.39	1
Jackfruit/Cocoa	0	0.83	0.95	0.33
Jackfruit/Cassia	0	0	0.11	0.33
Jackfruit/Sapota	0	0	0	0.97
Wild jack/Cocoa	0.71	0.99	0.97	0.45
Wild jack/Cassia	0.71	0.54	1	0.45
Wild jack/Sapota	0.01	0.19	0.58	0.92
Cocoa/Cassia	1	0.13	0.72	1
Cocoa/Sapota	0.56	0.02	0.08	0.03
Cassia/Sapota	0.56	1	0.92	0.03

Multiple-Choice Experiments. More quantity of tender rubber leaves were consumed than all other seven leaf types (cashew, mango, jackfruit, wild jack, cocoa, cassia and sapota) (Tables 1, 4 and 5). Jack fruit leaves were preferred over four leaf types (cashew, mango, golden shower and sapota) and cocoa over two leaf types (mango and sapota). No feeding was recorded on sapota.

3.3. Comparison of the Quantity of Tender and Senescent Leaves Consumed

No-Choice Experiments. Comparison of the tender and senescent leaves consumed revealed that no difference for cocoa and sapota leaves. More tender leaves were consumed for rubber, jackfruit, cashew, mango, wild jack and cassia (Tables 1 and 3).

Table 5: Preference hierarchy of *L. tristis* to various leaf types and leaf ages.

Leaf type	Tender		Senescent	
	No-choice	Multiple-choice	No-choice	Multiple-choice
Cashew	2	5	2	2
Cassia	2	5	2	2
Cocoa	2	3	2	2
Jackfruit	1	2	2	2
Mango	2	6	2	2
Rubber	1	1	1	1
Sapota	3	7	2	2
Wild Jack	2	4	2	2

Multiple-Choice Experiments. More quantity of tender leaves of rubber, jackfruit, wild jack, cashew, mango and cassia leaves were consumed, and no difference was noticeable for cocoa and sapota (Tables 1 and 3).

4. Discussion

4.1. Feeding Preference towards Tender and Senescent Leaves. Preference for tender leaves of most host plants except cocoa highlights the importance of leaf age in determining the food selection and food preference of *L. tristis*. Reasons for the preference towards tender leaves of most host plants, preference hierarchy in its food selection and non-differentiation of mature and tender leaves of cocoa, and non-feeding on sapota are not understood. High nutritional value could be the reason for the high preference towards tender leaves [18, 19]. Analysis of leaf physical and chemical traits is necessary to reach conclusions. Seasonal availability of tender rubber leaves by way of leaf disease-mediated premature fall and the distinct feeding preference of *L. tristis* on tender rubber leaves is cited as the reason for the high abundance of *L. tristis* in rubber plantations [5], and it is expected that control of premature leaf fall may lead to decline in the population buildup of *L. tristis* in rubber plantation belts. However, feeding on the tender leaves of other trees common in rubber plantation belts indicates that *L. tristis* has alternate leaf resources in the absence of rubber leaves. Analysis of reproductive performance of *L. tristis* on alternate host plants, survival experiments, oviposition, and larval feeding preferences are necessary to reach conclusions about the implications of present findings. Among the various alternate host plants, feeding pattern on cocoa requires special attention. In addition to the equal preference of *L. tristis* towards its mature and tender leaves, periodical pruning off tender shoots by the farmers leads to frequent tender leaf availability in cocoa plantations. How tender leaf availability of cocoa facilitates the population buildup of *L. tristis* needs to be ascertained. Spotting of *L. tristis* in cocoa plantations, equal preference towards tender and mature cocoa leaves and presence of patches of cocoa plantations and the scarce distribution of other host plants in rubber belts leads to the proposal that in the absence of

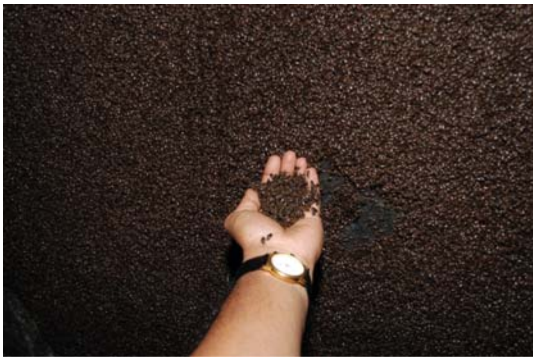

Figure 1: Aggregated beetles on the wall of a residential building.

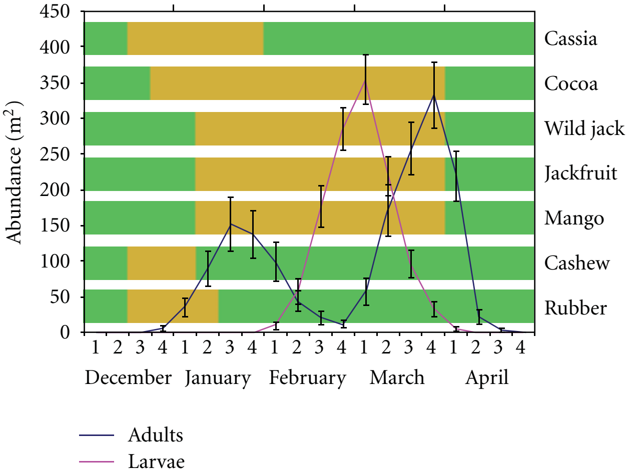

Figure 2: Foliage phenology of the host plants and population buildup of *L. tristis* in litter habitat during post-rainy breeding and feeding period (abundance data of *L. tristis* based on [2]; yellow colour depicts peak leaf fall period of evergreen plants and annual leaf shedding period of deciduous plants; blue coloured line depicts adults and pink coloured line larvae).

tender and mature rubber leaves, cocoa becomes the major host plant of *L. tristis* more than other host plants.

4.2. Host Plants of L. tristis and Implications. First experimental confirmation that *Luprops tristis* is a generalist and not a specialist on rubber and that it has distinct preference hierarchy in its food selection with rubber becoming the most preferred host plant species followed by jackfruit and cocoa is reached at. It raises the question whether showing inclination to feed up on three plants is enough to consider the beetle as a generalist? However, record of *L. tristis* in non-rubber belts in South East India before the introduction of rubber plantations in the moist south west India [20–22] indicates that rubber is not the sole host plant or ancestral host plant of *L. tristis*.

Selection of jackfruit and cocoa as the second most preferred food choice indicates that rubber plantation litter-centered efforts to control *L. tristis* such as premature leaf fall prevention in rubber plantations alone may not be effective as *L. tristis* may switch over to alternate host plants. Presence of *L. tristis* in the litter stands of cocoa, jackfruit, wild jack, cashew, mango and cassia (first author, personal observations) indicates that postdormancy beetles that arise from the 8-9 month long dormancy with the onset of post monsoon dry conditions ([2]; Figure 2) might be taking a transitory shelter and sustain on the tender and senescent leaves of cocoa and tender leaves of other host plants till annual litter fall followed by premature leaf fall occurs in rubber plantations.

Based on the host plant selection behavior of polyphagous insects [19, 23] and the phenology of *L. tristis* in rubber belts [5], preference towards rubber is attributed to two possibilities. Rubber is the most high-quality host plant species among the listed host plants. Feeding on other leaf types, even when rubber leaves were available during multiple-choice tests, logically raises question about the possible reasons for feeding on mixed diets and the advantages it provides. These observations reiterate the need for survival experiments and analysis of reproductive performance of *L. tristis* on each one of these host plants to reach conclusions. Secondly, literature on host plant selection of phytophagous insects revealed that concentrating on a particular species enables information about the environment to be processed more efficiently, increases the rate of host plant location/utilization [24–28], processes more information about that species by the females, and detects the variation in the quality of individual plants more efficiently [19, 29]. However, with overspecialization of *L. tristis* on the seasonally available leaves of the deciduous host plant, rubber would have affected its survival chances whereas generalist feeding behavior provides access to a greater resource base, a more nutritionally balanced diet [18, 30–32]. Hence, feeding on mixed diets of different evergreen host plants (generalist feeding behavior) may be an adaptive strategy of *L. tristis* to avoid overspecialization on a deciduous host plant (rubber) with highly seasonal leaf shedding behavior. However, evidence from plant-feeding insects showed that early experience in feeding on one type of resource may make it easier to exploit the same resource later in life or influence host choices later in development [33–35]. Hence, it is possible that the high degree of preference expressed for rubber could partly reflect pre-experiment feeding on this plant and associated induction of host preferences.

4.3. Predictions. High preference of *L. tristis* towards rubber leaves, presence of alternate host plants in rubber belts and earlier data on its wide prevalence in rubber plantations, high reproductive potential of *L. tristis,* and the possession of defensive gland secretions that deters the natural predators in litter habitat and aggregation sites [36, 37] indicate that prospects of further increase of *L. tristis* across the rubber belts is a certainty. Although *L. tristis* is recorded from Sri Lanka, South India, and Nepal [22], it is not recorded as a nuisance pest in these regions. Selection of rubber followed by jackfruit and cocoa as the most favored host plant and non-differentiation between tender and mature cocoa leaves indicates that *L. tristis* has high potential to become a nuisance pest in the new rubber plantation belts coming up in non-traditional rubber belts in the Indian subcontinent as well as in the cocoa plantation belts in South India.

4.4. Implications for Pest Management. Findings from the study have important bearing on the pest management strategies to be adopted for *L. tristis*.

(1) Present study establishes that *L. tristis* is a generalist feeder and not a specialist on rubber, and it has distinct preference hierarchy in its food selection with rubber becoming the most preferred host plant species followed by jackfruit and cocoa.

(2) Selection of jackfruit and cocoa as the preferred food choice after rubber, and its feeding on alternate host plants indicates that field stands-based efforts for control of *L. tristis* should consider the leaf litter accumulated around the alternate host plants.

(3) Non-differentiation of tender and mature cocoa leaves, shows that in the absence of rubber leaves cocoa plants become the major host plant of *L. tristis* than the less abundant jackfruit trees whose fallen tender leaves may not be available in sufficient quantity. Hence, it is likely that availability of cocoa litter might have contributed towards population build up of *L. tristis* in regions where intercropping of cocoa and rubber is practiced.

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