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Research Article

Morphology of the Preimaginal Stages of *Lasioptera donacis* Coutin (Diptera: Cecidomyiidae), a Candidate Biocontrol Agent for Giant Arundo Cane

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The larval stages of *Lasioptera donacis* Coutin consist of three instars which develop within the mesophyll of the leaf sheaths of *Arundo donax* (L.) (Poaceae). The larvae feed aggregatively on mycelia of an ambrosia fungus. The third instars are similar to other members of the genus except for a three-pronged spatula (typically two-pronged) and five lateral papillae (typically four) and with a nonbristled first instar. A related species, *L. arundinis* (Schiner) which breeds on fungus in *Phragmites* (Poaceae), also has a three-pronged spatula and five lateral papillae but has a bristled first instar. The third instar of *L. donacis* has a feeding and a nonfeeding prepupal stage. Papillae associated with the spatula are sensory organs, sensilla chaetica, sensilla trichodea, and sensilla ampullacea, perhaps related to extraoral digestion of the fungal mycelia. Pupation occurs in the host plant within a silken cocoon. Egression of the adult is through an escape hatch excavated by the third instar.

1. Introduction

The genus *Lasioptera* Meigen 1818 is defined taxonomically by structures on the postabdomen and ovipositor of the adult female [1]. These structures are mycangia, organs which function in the transport of fungal conidia. The gall midges of this genus are obligate symbionts of a fungus on which the larval stages feed [2, 3]. Females collect the conidia and oviposit them with the eggs into the host plant. The fungus is an opportunistic endophyte capable of exploiting living plant hosts. The fungus rewards its symbiont for providing transport to potential host plants by providing nourishment in the form of an ambrosia, a filamentous mycelial mass which fills the tunnels inhabited by the larvae [4]. In these cases of symbiosis the inoculation of the fungus into the plant by the insect has been documented for the Platypodidae (Coleoptera) [5], the Siricidae (Hymenoptera) [6], and the Cecidomyiidae (Diptera) [3, 7]. Coutin and Faivre-Amiot [8] first discovered and outlined the tritrophic relationships among this insect, *Lasioptera donacis* Coutin 2001, the fungus, and its host plant *Arundo donax* L. (Poaceae: Poales). These authors also described and illustrated the ovipositor with its mycangia on the adult female. The preimaginal stages have morphological features which adapt them to this resource niche and the objective of this study is to describe some of those adaptations.

General treatises on the morphology of larval cecidomyiids have been published by Otter [9], Möhn [10], Gagné [11–13], and Mamaev and Krivosheina [14]. The larval stages of the two most economically important cecidomyiids, the Hessian fly, *Mayetiola destructor* (Say 1817), and the sorghum midge, *Contarinia sorghicola* (Coquillett 1899), have been described in considerable detail primarily in the context of the damage to the host plant [15, 16]. Illustrations and descriptions of larval Lasiopteridi include Gagné et al. [17, 18] and Boe and Gagné [19]. The larvae of 20 species of *Lasioptera* were described in a taxonomic study by Möhn [10] which included the descriptions of new species based on larval specimens.

With few exceptions the larvae of *Lasioptera* spp. are narrowly host plant specific. Because *Arundo donax* (L.), the giant reed or carrizo cane, is an invasive plant that has spread into the riparian systems of the southern United States [20–22], *L. donacis* is under consideration as a potential biological control agent [23]. In that context, the basic biology of this insect is of some importance and the morphological adaptations of the immature stages indicate the specific means by which it exploits the fungus within its host plant. A related fungus feeding species, *Lasioptera arundinis* (Schiner 1854), breeds in a related host, the common reed, *Phragmites australis* (Cav.) Trin. (Poaceae), and hence has a life cycle and morphology similar to that of *L. donacis*. Thus, a secondary objective of our study was to ascertain the differences in larval morphology between *L. donacis* as they appear in the original description by Coutin [24] and observed by us, with those of *L. arundinis* as described by Möhn [10] and Rohfritsch [4].

2. Materials and Methods

2.1. Rearing of Lasioptera donacis. The late instars and pupae examined in this study were dissected from host plant material collected near Montpellier, France (43°40′N; 04°01′E), and Vandellos, Spain (41°01′N; 0°50′E). These were shipped by air courier to the USDA-APHIS Arthropod quarantine facility near Edinburg, TX, where a research colony is maintained. Eggs and first instar specimens were collected by inducing oviposition by colony adults placed in cages with potted Arundo plants in a greenhouse with temperatures maintained at 23° ± 3°C. Similarly, RH was targeted to 100% but actual recorded RH varied around a mean of 80%. Light cycle was natural. According to Coutin and Faivre-Amiot [8] *Lasioptera* females seek preexisting holes in the leaf-sheath, made by a chloropid, for oviposition. Thus, in order to rear *L. donacis* we artificially perforated the leaf sheathes of potted plants with a fine pair of forceps. In our laboratory colonies all egg masses found were in the mesophyll channels underlying these artificial perforations. The colony at Edinburg was first established in 2007 and continues to the present day.

2.2. Preparation of Specimens. For chaetotaxy some larval specimens were cleared and mounted following the KOH-Clove oil method of Gagné [12] while others were cleared with d-limonene (Histoclear, National Diagnostics, Atlanta GA). Some of the cleared specimens were stained with aceto-orcein or eosin to enhance visualization. Measurements of larvae and larval structures were made on a Leica MZ16 dissecting microscope with Leica application suite software. Photomicrographs of third instars were made on a Nikon Eclipse E400 microscope. For first and second instars whole specimens were mounted on slides using Permount (Fisher Scientific, Hampton NH) and photographed with a Nikon SMZ645 microscope. For internal organs larvae were mounted in paraffin, sectioned with a rotary microtome, and stained with methylene blue or eosin. Chaetotaxy follows Gagné [12] and Möhn [10]. Nomenclature for the sensory organs is that of Zacharuk and Shields [25].

3. Results

Lasioptera donacis has three larval instars that are passed entirely within the spongy mesophyll layer of the stem leaf sheaths of *Arundo donax*. The larvae are essentially colonial, feeding in clusters of about the same stage of development, and presumably from the same clutch of eggs, but at times in larger aggregations that can include two instars likely from successive ovipositions. Hodin and Riddiford [26] demonstrated that the nutritional content of the mycelium was a factor in the inducement of paedogenesis in fungus feeding midge larvae. Characterized by a fourth instar that lacks the spatula, paedogenesis has not been reported in *Lasioptera*, nor did we observe this phenomenon in *L. donacis*.

Figure 1: First instars with a mass of black yeasty mycelium (center) at approximately ten days after oviposition. White hyphae are extending out from the old egg clutch into the plant tissues.

Egg masses with fungal conidia were found in the medullary channels soon after exposure of the perforated plants to female midges. The first instars are free-living for at least several days after hatch because it typically requires about a week after inoculation for the dark mass of fungal mycelia to grow and accumulate (Figure 1). Invariably the second and early third instar larvae were found in channels of the medullary tissue, typically with the head end immersed in, and presumably feeding on, the dark yeasty mass of mycelia (Figure 2). The third instar has a feeding and a prepupal nonfeeding stage. At the end of the feeding stage the third instar spins a silken cocoon in which it will eventually pupate, still occupying the fungus filled channels of the leaf sheath mesophyll. The nonfeeding stage is found in the cocoon which is open at one end. Just prior to spinning the cocoon the larva cuts an escape hatch in the leaf wall from which the imago will eventually egress. It uses a sclerotized armature on the prothorax, common to almost all cecidomyiids, the “sternal spatula,” to excavate the emergence hole [26]. However, this hole does not open freely to the exterior; rather, a thin flap of epidermal plant tissue seals the hole. The larva then closes the cocoon by spinning a disc shaped, silken operculum. When the adult emerges from the escape hatch it simultaneously ecdyses from the pupal cuticle, characteristically leaving the pupal exuvium protruding from the exit hole in the leaf sheath (Figure 3).

Egg: typically dipteran in configuration: elongate, length about 0.5 mm, blunt at one end, tapering to a point at micropylar end. No respiratory horns visible. Color pinkish transparent. Observed in masses of 15 to 25 (Figure 4). Under

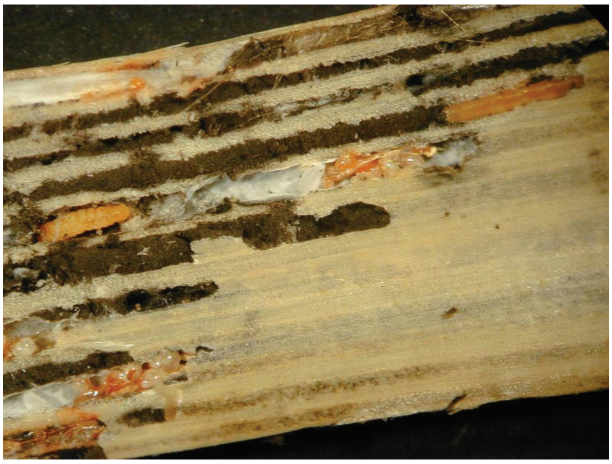


Figure 2: Third instars are found with the ambrosia fungus, a brown yeasty material, in the mesophyll layer of *Arundo donax*. Note the silken cocoons at center and upper left.

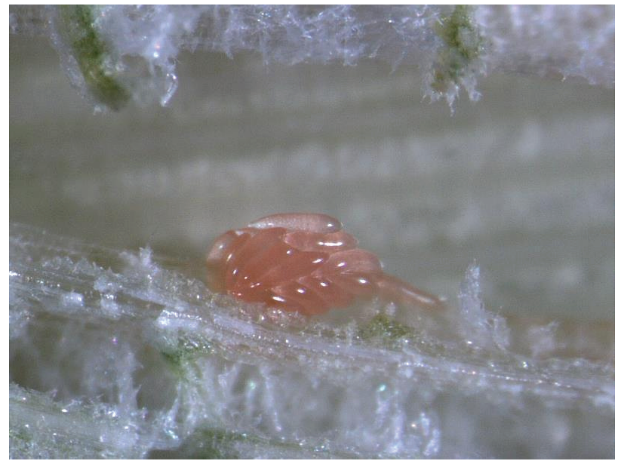


Figure 4: A clutch of eggs in a mesophyll cell of *Arundo donax*.

Figure 3: Pupal exuviae protruding from exit holes in the leaf sheath following emergence of the adults.

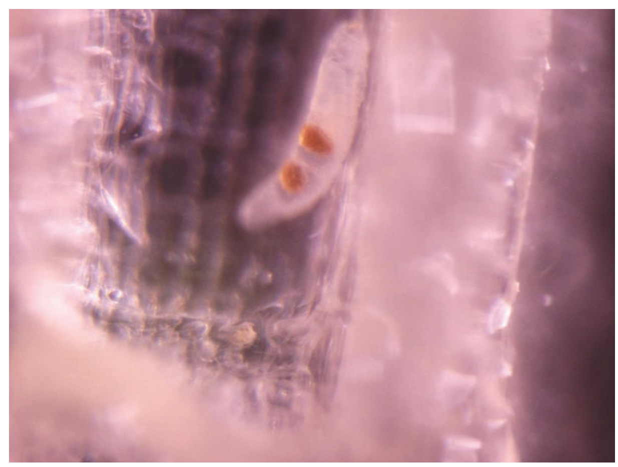


Figure 5: A recently hatched first instar. The orange spots are vacuoles of yolk carried over from the egg.

high magnification one can see within each egg yellowish vacuoles that are presumably yolk protein.

First instar: color pale sometimes pinkish, translucent, elongate fusiform in body shape; length 0.4–0.5 mm at eclosion. Two conspicuous orangish spots visible in abdomen at eclosion (Figure 5). These conspicuous spots may be the same vacuoles of yolk protein visible in the eggs. Soon after eclosion the material diffuses through the gut tract as the larva crawls away from the egg mass. Bolwig's organ, the subcuticular eyespots, is reddish and found between head capsule and prothorax (Figure 6). The cuticle is transparent and the head capsule, tentorium, and spiracles are nonsclerotized. The head capsule at this instar is almost as wide as the body segments. The antennae are orbicular with terminal nipple. The microverrucae of the cuticle are spinulate, particularly in the rows at the anterior margins of the body segments.

The first instar has a complement of haired papillae arrayed in number and position characteristic for the Lasiopteridi [1]: six dorsals on each thoracic segment and first eight abdominal segments; a pair of pleural papillae on each side; and a pair of hind-ventrals on all postcephalic segments except penultimate and terminal segments. Penultimate segment has four ventrals and terminal segment with eight terminal haired papillae, the hairs 3-4 μm long, about twice as long as papillus on which they are seated. The eighth-abdominal segment has larger pleural papillae, the hairs of which are 4.5–5.3 μm long (Figure 7). The first instar does not have the sternal spatula nor does it have discernible sternal or lateral papillae. The body (Figure 8) lacks the long (30 μm) bristles and numerous spines reported in *L. arundinis* [27].

Second instar: color pale yellowish, length 1.0–1.75 mm (n = 4). Second instar larvae do not have the sternal spatula. Head capsule distinctly smaller in proportion to the body segments compared to the first instar and with antennae more elongate (Figure 9). The cuticle is transparent and with backlighting under the compound microscope in each abdominal segment fat body is visible as three large darkened amorphous masses of tissue: one at middle and one on each side. Lateral

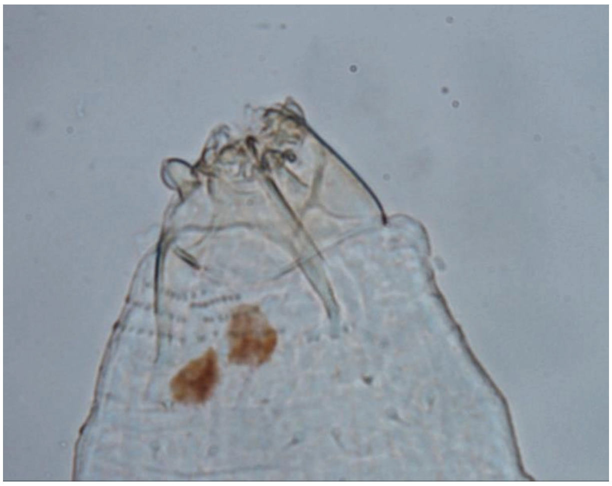


Figure 6: First instar, head and thoracic segments. The ovoid, red objects between the tentorial arms are Bolwig's organs. Note the rows of cuticular denticles.

Figure 8: The first instar does not have long mycangial bristles as reported in *Lasioptera arundinis*. Note that the head capsule is nearly as wide as the prothorax.

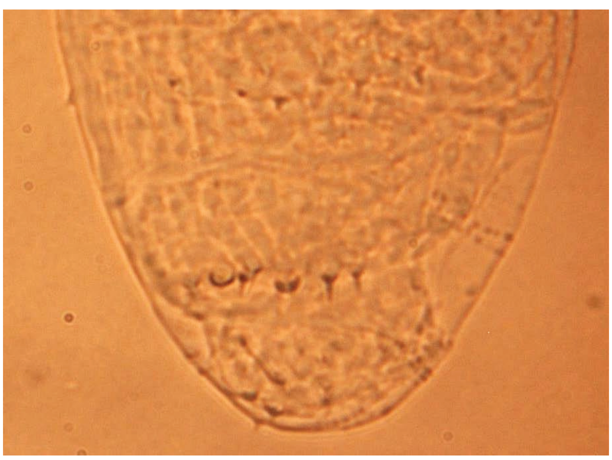


Figure 7: First instar, terminal abdominal segments showing haired papillae, the longest of which are those attendant to the spiracles.

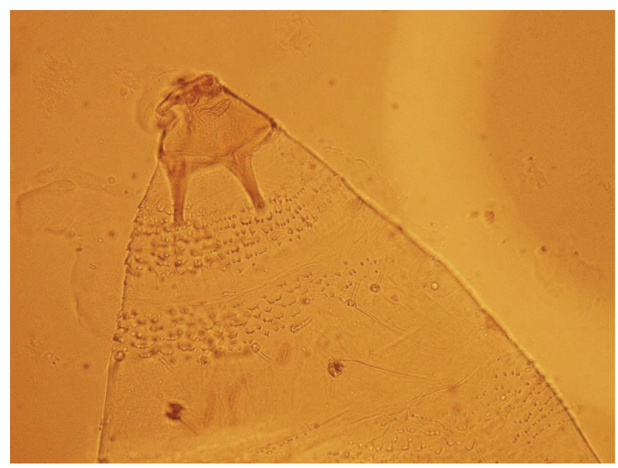


Figure 9: The head, neck, and prothorax (with spiracles) of the second instar. The spatula is absent. The dorsal and pleural papillae are setiform. The sternal and lateral papillae are absent.

papillae on thorax have 11–15 μm long bristles while those on eighth-abdominal segment are 16–19 μm long. Spiracles are present on prothorax and first eight abdominal segments.

Third instar: color orange in life fading to yellow in preserved specimens. Mean body length 3.06 ± 0.29 mm; range 2.66–3.71 mm ($n = 10$); width 0.57 ± 0.04; range: 0.51–0.65 mm ($n = 10$). Hemicephalic; body orthosomatic, asymmetrically fusiform, widest at mesothorax, somewhat dorso-ventrally compressed (Figure 10). In addition to the three thoracic segments and the nine abdominal segments, there is a pseudosegment, a neck or cervical segment between the head and prothorax. All body segments except neck segment and last two abdominal segments bulge laterally. Penultimate abdominal segment longest and ninth (terminal) segment shortest.

Spiracles of prothorax and eighth (penultimate)- abdominal segment prominent, turreted (Figure 11). Those on penultimate segment situated laterally in a plica (fold) anterior to the posterior intersegmental suture. Spiracles of remaining segments situated dorsolaterally, and though sessile, annular, and inconspicuous, apparently functional because they are connected to trachea. The tracheae are darkened terminally where attached to peritreme, particularly on sixth and seventh abdominal segments.

Body without macropapillae or projections. Also lacking the microdentition seen in first instars. Rather the epidermal surface is minutely verrucose (cuticular verrucae of Mamaev and Krivosheina [14]) on sides and dorsum of all segments, whereas on the ventral surface the microverrucae are confluent and arrayed in transverse lines, presumably as traction ridges. The head is small, retractile, prognathous, the capsule lightly-sclerotized, somewhat conical, anchored with prominent exocephalic tentorial arms. A pair of two segmented (pseudosegmented *cf* Solinas et al. [28]) antennae are present, situated subapically, one on each side of mouthparts. Pedicel

FIGURE 10: The spatulate third instar larva. Note that the head capsule is comparatively minute.

FIGURE 11: A turreted spiracle on the penultimate abdominal segment.

short, not longer than wide; scape acuminate, fusiform, elongate, length about 3x width. In some images the large placoid sensilla is visible on each scape (Figure 12). The mouthparts are rudimentary and consist of a two-sided labrum, the minute peg-like maxillae, and tiny mandibles. Small, linear, and sclerotized, the latter are aligned parallel to one another and to the sagittal (vertical) axis of the cranium (Figure 14).

The haired papillae are reduced in number from the first instars (Figure 13). The terminal segment has eight stout bristles in two rows of four ranging in length from 10 to 22 μm, the ental pairs longer (mean 14.1 ± 3.1 μm, $n = 12$). Setiform dorsal papillae in a row of six are present on pro- and mesothorax with two on eighth abdominal segments between the spiracles. Their length varies from 22 to 42 μm (mean 32.9 ± 5.5 μm, $n = 25$). All thoracic and first eight abdominal segments have a pair of hind ventrals. Prothorax, mesothorax, and eighth abdominal segments with a pair of pleural setae on each side, remaining segments with one on each side. Outer pleural seta of prothorax longest at 38–50 μm

FIGURE 12: Head of the third instar. Arrow points to the placoid sensilla on the scape of the antenna.

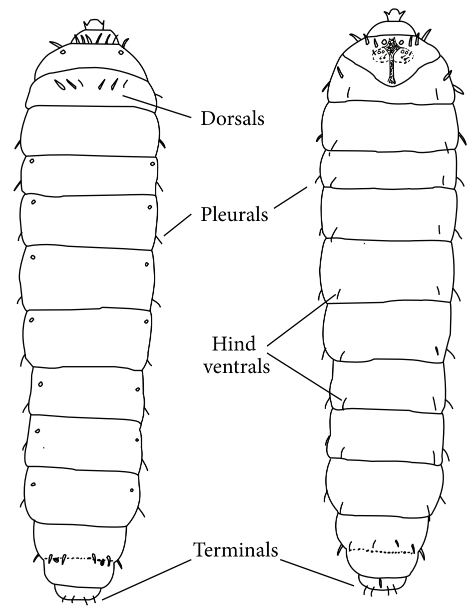


FIGURE 13: Diagrammatic position of setiform papillae on the third instar, dorsal on left, ventral on right.

(mean 41.1 ± 5.5 μm, $n = 10$), those on mesothorax next longest at 28–35 μm (32.5 ± 6.4, $n = 11$) with those on the metathorax and abdominal segments shortest at 12–17 μm (24.7 ± 8.3, $n = 9$). Hind ventrals are much smaller (mean 17.1 ± 5.3 μm, $n = 7$). Inner pleural papillae are haired on pro- and mesothorax; longer on prothorax (mean 32.5 ± 3.8 μm, $n = 4$) than on mesothorax (mean 22.3 ± 4.2 μm, $n = 7$) with those on eighth-abdominal segment even shorter at 18 μm. Metathorax and first seven abdominal segments are without dorsal or sternal haired papillae.

The number and position of setiform papillae in Möhn's [10] account of *Lasioptera arundinis* are somewhat problematic. He states that there are three pleural papillae on each side of the prothorax but that the third is often unhaired. We

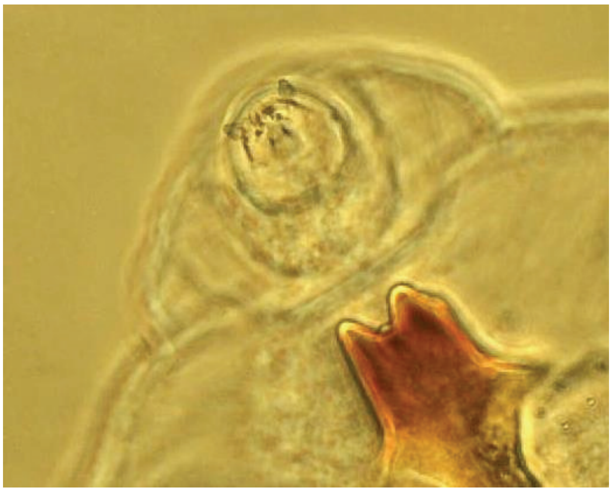


Figure 14: Ventral view of third instar head capsule showing rudimentary, paired mouthparts, and the three pronged spatula.

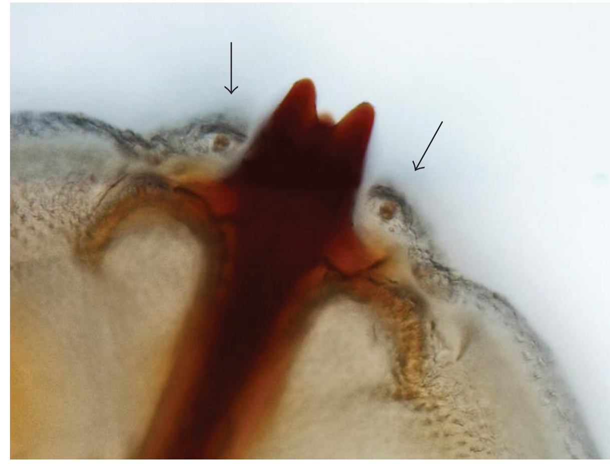


Figure 16: The three-pronged spatula is flanked by fleshy pads where the apodemes insert. The sternal papillae are seated between the spatula and the neck segment, one on each side (arrows).

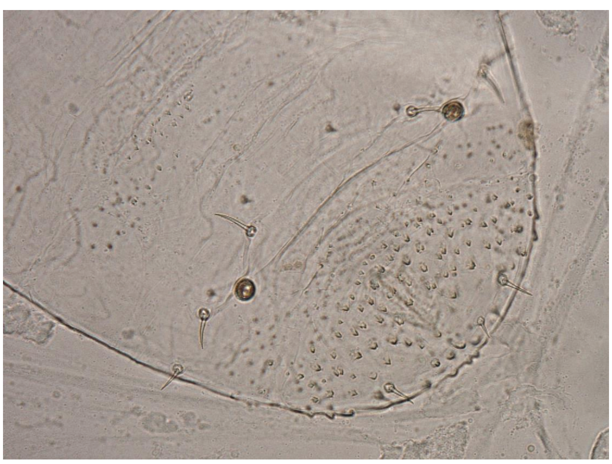


Figure 15: Setiform papillae flanking the spiracle on abdominal segment eight: one dorsal and two pleurals on each side.

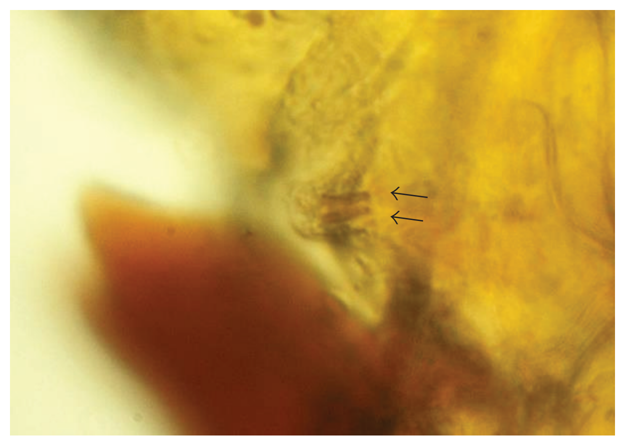


Figure 17: In profile the sternal papillae appear to have a pair of ducts (arrows) connecting to the paired sensory structures within its crater.

find only two on *L. donacis*. Möhn's figure of the abdominal segments depict a single pleural seta; yet his text description states there are two, and that is the number that we find in *L. donacis* as well (Figure 15).

The ventral side of the prothorax has the prominent sclerotized armature referred to as the sternal spatula [29] or breast plate [30], length 0.37 ± 0.04 mm ($n = 8$). Seated on and protruding from the midline it is about ten times longer than greatest width and widest anteriorly where it is anchored to the body by sclerotized lateral apophyses just posterior to the terminus (Figures 14 and 16). The anterior projection is strongly sclerotized, protruding from the body and bearing three forwardly directed teeth: two broad lateral teeth with a smaller middle tooth between and somewhat entad of the lateral teeth. A cross section through the spatula reveals that it is actually hollow. The caudal end of the spatula is expanded into a rounded base, the manubrium, which is embedded deeply in the cuticle. Internally there is a large V-shaped mass of muscle attached to the manubrium which inserts on the dorsum of the prothorax.

Attendant to each side of the anterior expansion of the spatula there is a fleshy pad bearing 3 + 2 lateral papillae. The three on the anterior margin are sclerotized and tubular or socket-shaped (Figure 18). The outermost of these bears a stout bristle, although in most specimens it is missing, presumably broken off from wear and tear. The two posterior, discal papillae are simple haired papillae (Figure 19). Located on the anterior portion of the prothorax ventrally, between the spatula and the head, there are two conspicuous crateriform structures, one on each side, referred to as the sternal papillae in the taxonomic literature although they are not papilliform (Figures 16 and 17).

Pupa: exarate, enclosed within a silken cocoon; capable of strong movement when disturbed. Body elongate, cylindrical; color off-white in early stages turning to orange later; wing pads black and with darkened patches of scales on the abdominal dorsum in fully mature, pharate specimens

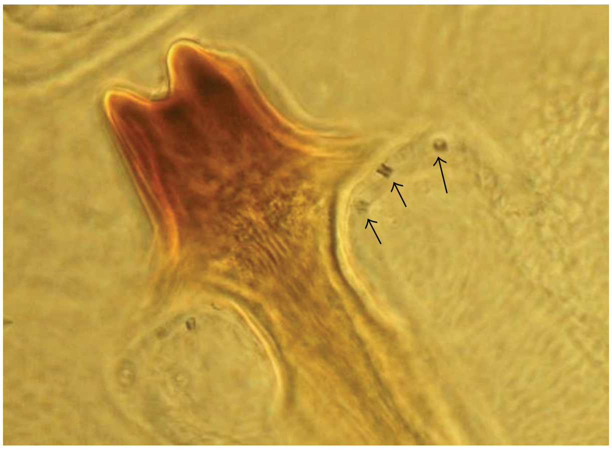


Figure 18: The fleshy pads attendant to the spatula have three sclerotized, socket-shaped papillae arrayed on their frontal margin (arrows).

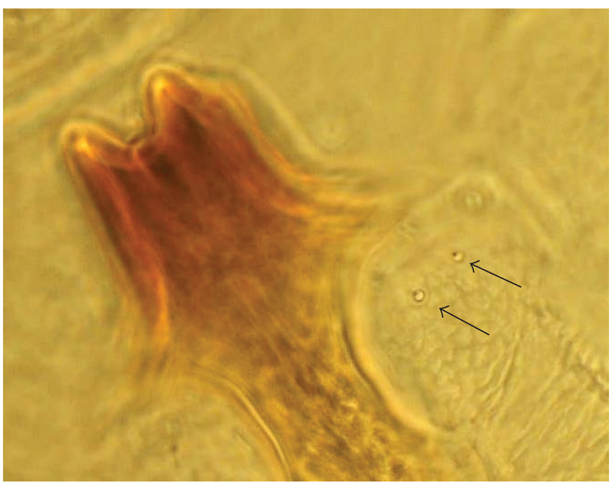


Figure 19: On the disc of each fleshy pad is a pair of haired papillae (arrows).

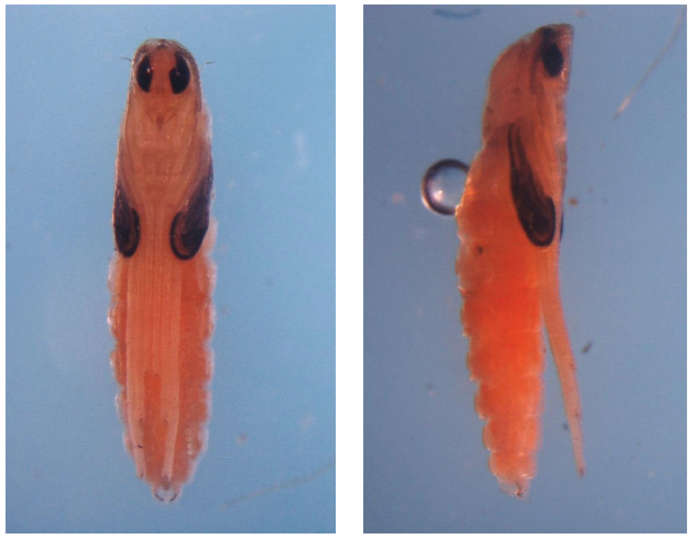
(Figures 20(a) and 20(b)). Abdominal segments without spines. Length: ranging from 2.2–3.0 mm (mean = 2.55 ± 0.24 mm, $n = 9$). Respiratory horns of thorax slender, tubular basally, filamentous apically. Leg sheaths nonoverlapping, unequal in length; metatarsi attaining abdominal terminus, mesotarsi nearly attaining abdominal segment VIII, protarsi attaining abdominal segment VI. Wing pads overlap metafemora. Beaded antennal sheathes overlap mesofemora and extend just past the base of the wing pads. Palp sheaths extend obliquely anterolaterad, reclining in the space between the eye and profemora. Frons without papillae, horns or ridges. Eyes entire, ocelli absent. Cephalic sclerite bearing a pair of prominent setae each inserted just behind the antennal base.

4. Discussion and Conclusions

The larvae of free-living cecidomyiids are variable with respect to the number and size of excrescences on the body surface as illustrated by Peterson [30], Mamaev and Krivosheina [14], and Gagné [11–13]. Such excrescences are typically absent in the endophytic species, including *Lasioptera*. On the other hand, the form of the body, elongate, cylindrical, and bulging at the thorax (Figure 10), is typical of stem-boring or tunnel inhabiting insect larvae from which one can infer that its morphology facilitates movement within a confined space.

The sternal spatula is a characteristic feature of third instar cecidomyiid larvae. Milne [31] found that soil inhabiting species use the spatula to excavate the pupal chamber in the soil. Mamaev and Krivosheina [14] state that phytophagous species use the spatula to perforate or enlarge perforations in plant tissue. Among cecidomyiid species variation occurs primarily in the shape of the apex which can have one, two, or three prongs [14]. *Lasioptera donacis* has a three-pronged spatula. Among the 20 European species of *Lasioptera* larvae described by Möhn [10], only *L. arundinis* has a three-pronged spatula, the others being cloven with two prongs. A species from Australia, *L. uncinata* Gagné also has a three-pronged spatula, but with the middle prong the longest and it has only four lateral papillae (five in *L. donacis* and *L. arundinis*). Also the host for *L. uncinata* is a non-graminaceous plant, *Melaleuca* spp. (Myrtaceae) [17]. According to Gagné [1] none of the New World *Lasioptera* spp. have grasses as the host. *Lasioptera arundinis* is the species most similar in morphology and ecology to *L. donacis*. Because the former exploits an endophytic fungus in a closely related grass species, common reed, *Phragmites australis* (Cav) Trin., *L. donacis* and *L. arundinis* may be closely related species. Or it may be convergence that they share important morphological features in the third instar including a three-pronged spatula and five lateral papillae. The difference between the first instars of *L. donacis* and *L. arundinis* is the presence of long bristles and spines in the latter which relates to a difference in their ecology. According to Rohfritsch [27] *L. arundinis* lays its eggs outside of the plant under the leaf sheathes of the side shoots. The bristles and spines enable the first instar to carry the fungal conidia which are then disseminated into the plant as the larva feeds on and penetrates the cortical parenchyma of the stem. Because *L. donacis* females lay their eggs along with the conidia into preexisting holes in the leaf sheath surrounding the main stem, the larvae of the latter species do not require such bristles. Also, according to Rohfritsch [4] the first instar larva of *L. arundinis* uses its mandibles to perforate the lamella and enter the pith of the stem. The first instar of *L. donacis* has minute, rudimentary mandibles.

In spite of the reduction in the size of the mouthparts, cecidomyiid larvae, in particular those of the economically important phytophagous species, are noted for the damage inflicted on the host plant. In the case of the Hessian fly, *Mayetiola destructor* (Say), and the sorghum midge, *Contarinia sorghicola* (Coquillet), the damage is evidently caused by the injection of digestive fluids rather than by the physical damage by the tiny mandibles [16, 32]. Extraoral digestion is the norm for cecidomyiids whether phytophagous, mycetophagous, or even predaceous [14]. For that purpose the salivary glands are typically enlarged [32]. Our sections of



(a) (b)

Figure 20: Late stage exarate pupa of *L. donacis*, ventral and side views.

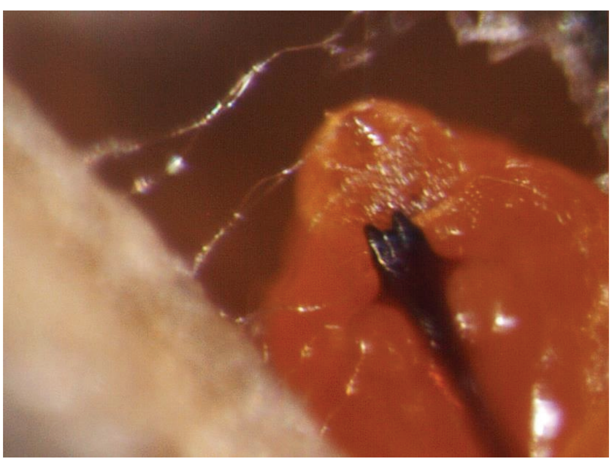


Figure 21: A prepupal third instar makes a cocoon with silk from the labial gland.

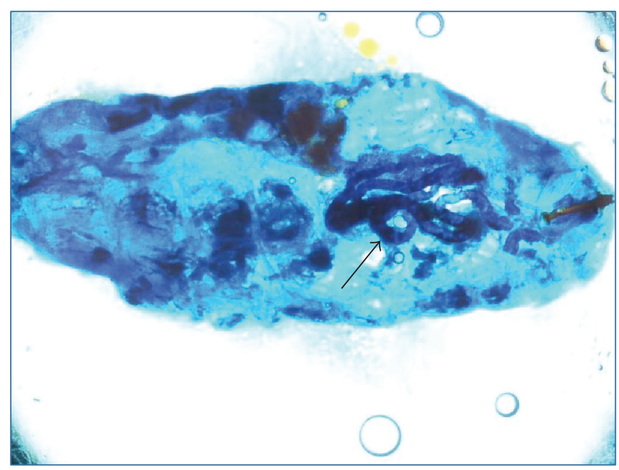


Figure 22: Longitudinal section of the late third instar shows the tubular salivary gland (arrow) associated with extraoral digestion and silk production.

L. donacis larvae show that the salivary gland dominates the internal thoracic space of the third instar (Figure 22). In cecidomyiids the glands have a dual function: producing digestive fluids during the feeding stage but also producing the silk for the cocoon (Figure 21) in the prepupal stage [33]. We presume that to be the case for *L. donacis* as well. Apart from having their heads directed at and into the fungal mycelium, we have not observed feeding directly in the *L. donacis* larvae, presumably because of their light aversion response. Our presumption of extraoral digestion is based on the rudimentary size of the mouthparts and the enlargement of the salivary glands, but also we have observed *vomitus* when the larvae are placed on filter paper. The lack of accumulations of frass in the colonies, of which residue is typically a conspicuous aspect of insect tunnels, may be a consequence of the mainly liquid diet. Rohfritsch [2] describes feeding by *L. arundinis* on the endophytic fungus and reports that in combination with extraoral digestion the larva uses the spatula to scrape and scratch the mycelium and then sucks in the solubilized predigested material. The juxtaposition of the spatula to the mouth opening in *L. donacis* suggests that the spatula serves as an extraoral masticating organ as well (Figure 23).

The number and distribution of the minute haired papillae of the first instar of *L. donacis* correspond to the chaetotaxy characteristic of the tribe Lasiopterini. In the later instars the number is reduced, especially on the abdomen, while those that persist have been transformed into sensory bristles. Whereas the socketed terminal bristles of the third instars are short and stiff (Figure 24), those on the thorax are long and flexile (Figure 25). In accordance with

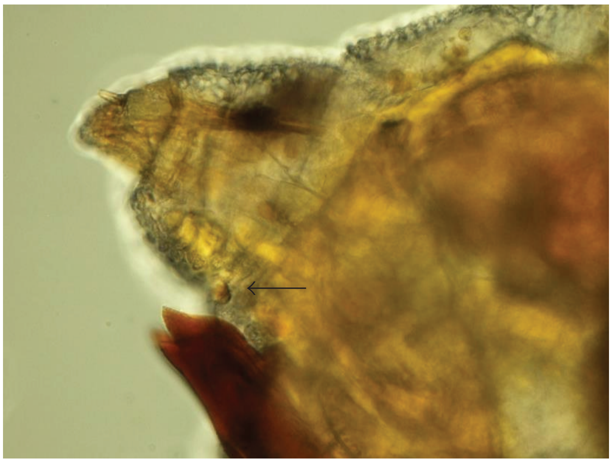


FIGURE 23: Lateral view of the prothoracic spatula. The head segment with antenna is to the far left. The head is seated in the collar segment within which the dark red Bolwig's organ can be seen (far right). On the prothorax between the spatula and the collar segment are the sternal papillae, one of which can be seen as a round, tan colored structure (arrow). When the collar segment inverts it brings the mouth into close juxtaposition to the spatula and the sternal papillae.

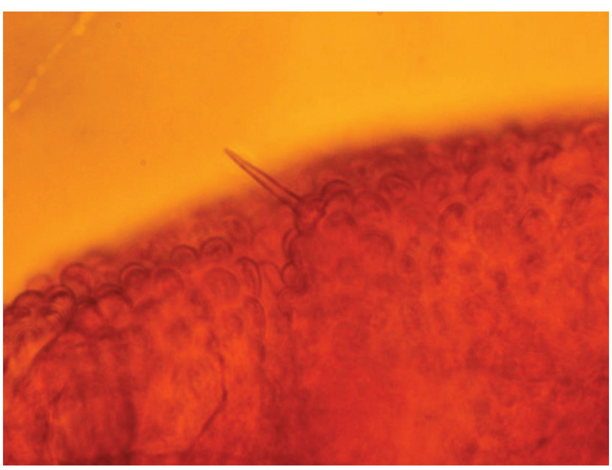


FIGURE 24: The terminal papillae of the posterior abdominal segments are stout socketed bristles and appear to be sensilla chaetica.

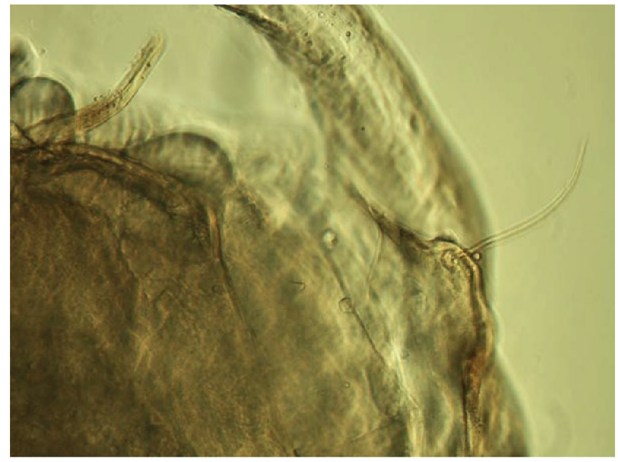


FIGURE 25: The pleural papillae on the prothorax have the form of flexile socketed setae, that is, sensilla trichodea.

FIGURE 26: Retracting the head into the neck segment juxtaposes the dorsal setae of the prothorax, three on each side ((a), (b), (c)) onto a fold collaring the head.

the nomenclature of Zacharuk and Shields [25] their structure suggests that the terminals are sensilla chaetica, which are tactile in function, while the thoracic bristles are sensilla trichodea, which are chemosensory as well as tactile. The dorsal bristles on the prothorax are arranged in a corona surrounding the head when it is retracted into the neck segment (Figure 26).

It strikes us that the role of the sensory organs in the endophytic species has been underappreciated. Bolwig's organ detects light [34] and is presumed to initiate escape and evasion behavior in the event the larvae is exposed. Skuhrava and Skuhravy [35] report that birds break into the cane to predate on the larvae. According to Solinas et al. [28] the gall-inhabiting cecidomyiid larvae have shorter antennae compared to the free living species and that is certainly the case with *L. donacis*. Whereas the antennae are small and the body is largely devoid of protuberances, the prothorax has conspicuous sensory papillae associated with the spatula. On either side of the frontal attachment of the spatula there is a fleshy pad which has the five lateral papillae. In most described species of *Lasioptera* there are four except in *L. arundinis* which also has five in a similar configuration to *L. donacis* as illustrated by Möhn [10]. Three are situated just behind the anterior margin and are sclerotized and tubular or socket-shaped. Behind them on the disc of the pad are two haired papillae. In the review of the sensory organs of larval insects by Zacharuk and Shields [25] there are no organs which are morphologically analogous to these structures. Being seated on the pads at the insertion of the spatula would suggest that their function may be proprioception related to the operation of the spatula in mastication. Among the postcephalic sensory organs proprioceptors such as

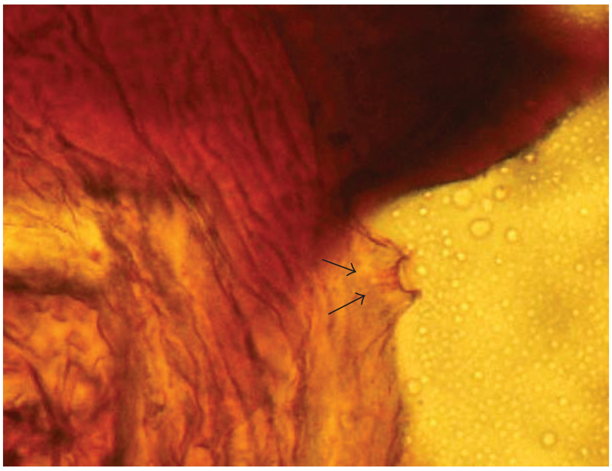


Figure 27: The sternal papillae in ectal view appear to be conical with paired structures internally (arrows), suggesting that they have a sensory function.

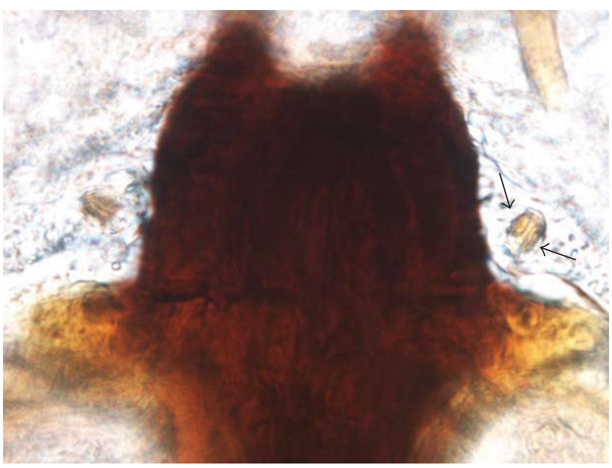


Figure 28: The paired structures within the sternal papillae are extrusible and may be sensory pegs (arrows).

the campaniform sensillae are common [36], but they are much smaller than these novel structures.

Even more conspicuous are the much larger sternal papillae, one on each side, seated on the ventral side of the prothorax between the spatula and the collar segment. In ectal view they appear to be conical with a pair of internal structures (Figure 27). In profile these structures appear to be connected to ducts (Figure 17). Moreover, the structures are eversible; that is, observed in living specimens they sometimes protrude from the central crater as pegs (Figure 28). This peg-in-pit arrangement in accordance with the system of Zacharuk and Shields [25] would be sensilla ampullacea which are chemosensory. The number of pegs varies among larval taxa, as many as seven in some Staphylinidae (Coleoptera) [37]. If they are two-pegged sensilla ampullacea the sternal papillae on the prothorax of *L. donacis* are perhaps gustatory in function, that is, an adaptation to extraoral digestion. The juxtaposition of these sensillae near the spatula would perhaps allow the larvae to "taste" the macerated mycelial mass as it feeds. They may thus be functionally analogous to the papilla sensilla found on the terminal organs of the head segment of house fly larva [38]. In the latter study the two pegs are referenced as "dendrites" exposed to the exterior and functioning as taste organs.

The reliance on an endophytic fungus for its food resource complicates but does not eliminate *Lasioptera donacis* as a candidate for biocontrol of the invasive weed, *Arundo donax*. However one can expect that a deeper understanding of the tritrophic relationships among the plant, the fungus, and the insect will be required before a successful program can be implemented.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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References

[1] R. J. Gagné, "A tribal and generic revision of the nearctic Lasiopteridi (Diptera: Cecidomyiidae)," *Annals of the Entomological Society of America*, vol. 62, no. 6, pp. 1348–1364, 1969.

[2] O. Rohfritsch, "A fungus associate gall midge, *Lasioptera arundinis* (Schiner) on its host plant, *Phragmites australis* (Cav.) Trin," *Bulletin de la Société Botanique de France. Lettres Botaniques*, vol. 139, pp. 45–59, 1992.

[3] A. Borkent and J. Bissett, "Gall midges (Diptera: Cecidomyiidae) are vectors of their fungal symbionts," *Symbiosis*, vol. 1, pp. 185–194, 1985.

[4] O. Rohfritsch, "Plants, gall midges, and fungi: a three-component system," *Entomologia Experimentalis et Applicata*, vol. 128, no. 1, pp. 208–216, 2008.

[5] T. C. Harrington, "Ecology and evolution of mycophagous bark beetles and their fungal partners," in *Ecological and Evolutionary Advances in Insect Fungal Associations*, F. E. Vega and M. Blackwell, Eds., pp. 257–291, Oxford University Press, London, UK, 2005.

[6] K. Graham, "Fungal—insect mutualism in trees and timber," *Annual Review of Entomology*, vol. 12, no. 1, pp. 105–126, 1967.

[7] K. V. Krishnamurthy, "Plant-insect-fungus association in some plant galls," *Proceedings: Animal Sciences*, vol. 93, no. 4, pp. 265–273, 1984.

[8] R. Coutin and A. Faivre-Amiot, "Interrelations ecologiques et symbiotiques complexes entre insectes et vegetaux, *Lasioptera donacis* n. sp. (Diptera: Cecidomyiidae) et *Arundo donax* L.," in *Congres National des Societes Savantes*, Sciences Fascia II, pp. 195–201, Perpignan, France, 1981.

[9] G. W. Otter, "On the morphology of the larvae of three species of Cecidomyidae (Diptera) from knapweed (*Centaurea*) flowers," *Transactions of the Royal Entomological Society of London*, vol. 87, no. 2, pp. 39–67, 1938.

[10] E. Möhn, "Cecidomyiidae (=Itoniidae)," in *Die Fliegen der Palaearctishen Region, Liefrung 274*, E. Lintner, Ed., pp. 97–160, Naquele and Obermiller, Stuttgart, Germany, 1988.

[11] R. J. Gagné, "Cecidomyiidae," in *Manual of Nearctic Diptera*, J. F. McAlpine, B. V. Petersen, G. E. Shewell, H. J. Teskey, J. R. Vockeroth, and D. M. Wood, Eds., pp. 257–292, Agriculture Canada Monograph, Ottawa, Canada, 1981.

[12] R. J. Gagné, *The Gall Midges of the Neotropical Region*, Cornell University Press, Ithaca, NY, USA, 1994.

[13] R. J. Gagné, "The gall midges (Diptera: Ceccdomyiidae) of hickories (Juglandaceae: *Carya*)," *Memoirs of the American Entomological Society*, vol. 48, pp. 1–147, 2008.

[14] B. M. Mamaev and N. P. Krivosheina, *The Larvae of the Gall Midges (Diptera: Cecidomyiidae): Comparative Morphology, Biology and Keys*, Balkema, Rotterdam, The Netherlands, 1993.

[15] R. J. Gagne and J. H. Hatchett, "Instars of the hessian fly (Diptera: Cecidomyiidae)," *Annals of the Entomological Society of America*, vol. 82, no. 1, pp. 73–79, 1989.

[16] R. S. Petralia, A. L. Wuensche, G. L. Teetes, and A. A. Sorensen, "External morphology of the mouthparts of larvae of sorghum midge, *Contarinia sorghicola*," *Annals of the Entomological Society of America*, vol. 72, no. 6, pp. 850–855, 1979.

[17] R. J. Gagné, J. K. Balciunas, and D. W. Burrows, "Six new species of gall midges (Diptera: Cecidomyiidae) from *Melaleuca* (Myrtaceae) in Australia," *Proceedings of the Entomological Society of Washington*, vol. 99, no. 2, pp. 312–334, 1997.

[18] R. J. Gagné, F. M. Kay, and T. A. Heard, "A new species of *Neolasioptera* (diptera: Cecidomyiidae) from *Parkinsonia* aculeata (leguminosae) in argentina for possible use in biological control in Australia, with a key to neotropical species of neolasioptera," *Zootaxa*, vol. 2866, pp. 61–68, 2011.

[19] A. Boe and R. J. Gagné, "A new species of gall midge (Diptera: Cecidomyiidae) infesting switchgrass in the northern Great Plains," *Bioenergy Research*, vol. 4, no. 2, pp. 77–84, 2011.

[20] A. M. Herrera and T. L. Dudley, "Reduction of riparian arthropod abundance and diversity as a consequence of giant reed (*Arundo donax*) invasion," *Biological Invasions*, vol. 5, no. 3, pp. 167–177, 2003.

[21] J. H. Everitt, C. Yang, and C. J. Deloach, "Remote sensing of giant reed with QuickBird satellite imagery," *Journal of Aquatic Plant Management*, vol. 43, pp. 81–85, 2005.

[22] C. Yang, J. H. Everitt, and J. A. Goolsby, "Using aerial photography for mapping giant reed infestations along the Texas-Mexico portion of the Rio Grande," *Invasive Plant Science and Management*, vol. 4, pp. 402–410, 2011.

[23] J. A. Goolsby and P. J. Moran, "Host range of *Tetramesa romana* Walker (Hymenoptera: Eurytomidae), a potential biological control of giant reed, *Arundo donax* L. in North America," *Biological Control*, vol. 49, no. 2, pp. 160–168, 2009.

[24] R. Coutin, "Une nouvelle cecidomyid des gaines foliaires de la Canne de Provence, *Lasioptera donacis* n. sp. (Diptera: Cecidomyiidae)," *Bulletin Societe Entomologique France*, vol. 106, pp. 105–108, 2001.

[25] R. Y. Zacharuk and V. D. Shields, "Sensilla of immature insects," *Annual Review of Entomology*, vol. 36, no. 1, pp. 331–354, 1991.

[26] J. Hodin and L. M. Riddiford, "Parallel alterations in the timing of ovarian ecdysone receptor and ultraspiracle expression characterize the independent evolution of larval reproduction in two species of gall midges (Diptera: Cecidomyiidae)," *Development Genes and Evolution*, vol. 210, no. 7, pp. 358–372, 2000.

[27] O. Rohfritsch, "Morphology and behavioral adaptations of the gall midge *Lasioptera arundinis* (Schiner) (Diptera: Cecidomyiidae) to collect and transport conidia of its fungal symbiont," *Tidschrift voor Entomologie*, vol. 140, pp. 59–66, 1997.

[28] M. Solinas, G. Nuzzaci, and N. Isidoro, "Antennal sensory structures and their ecological-behavioral meaning in Cecidomyiidae (Diptera) larvae," *Entomologica*, vol. 22, pp. 165–184, 1989.

[29] H. J. Teskey, "Morphology and terminology—Larvae," in *Manual of Nearctic Diptera, Vol. 1*, J. F. McAlpine, B. V. Peterson, G. E. Shewell, H. J. Teskey, J. R. Vockeroth, and D. M. Wood, Eds., Agriculture Canada Research Monograph no. 27, pp. 65–88, Biosystematics Research Institute, Ottawa, Canada, 1981.

[30] A. Peterson, *Larvae of Insects: An Introduction to Nearctic Species*, Edwardes Bros, Ann Arbor, Mich, USA, 1962.

[31] D. L. Milne, "The function of the sternal spatula in gall midges," *Proceedings of the Royal Entomological Society of London*, vol. 36, pp. 7–9, 1961.

[32] J. H. Hatchett, G. L. Kreitner, and R. J. Elzinga, "Larval mouthparts and feeding mechanism of the Hessian fly (Diptera: Cecidomyiidae)," *Annals of the Entomological Society of America*, vol. 83, no. 6, pp. 1137–1147, 1990.

[33] Y. Li, J. Wu, W. Cheng, W. Song, and X. Yuan, "Comparison of silk glands of diapause and non-diapause larval *Sitodiplosis mosellana*," *Journal of Insect Science*, vol. 12, no. 1, pp. 81–91, 2012.

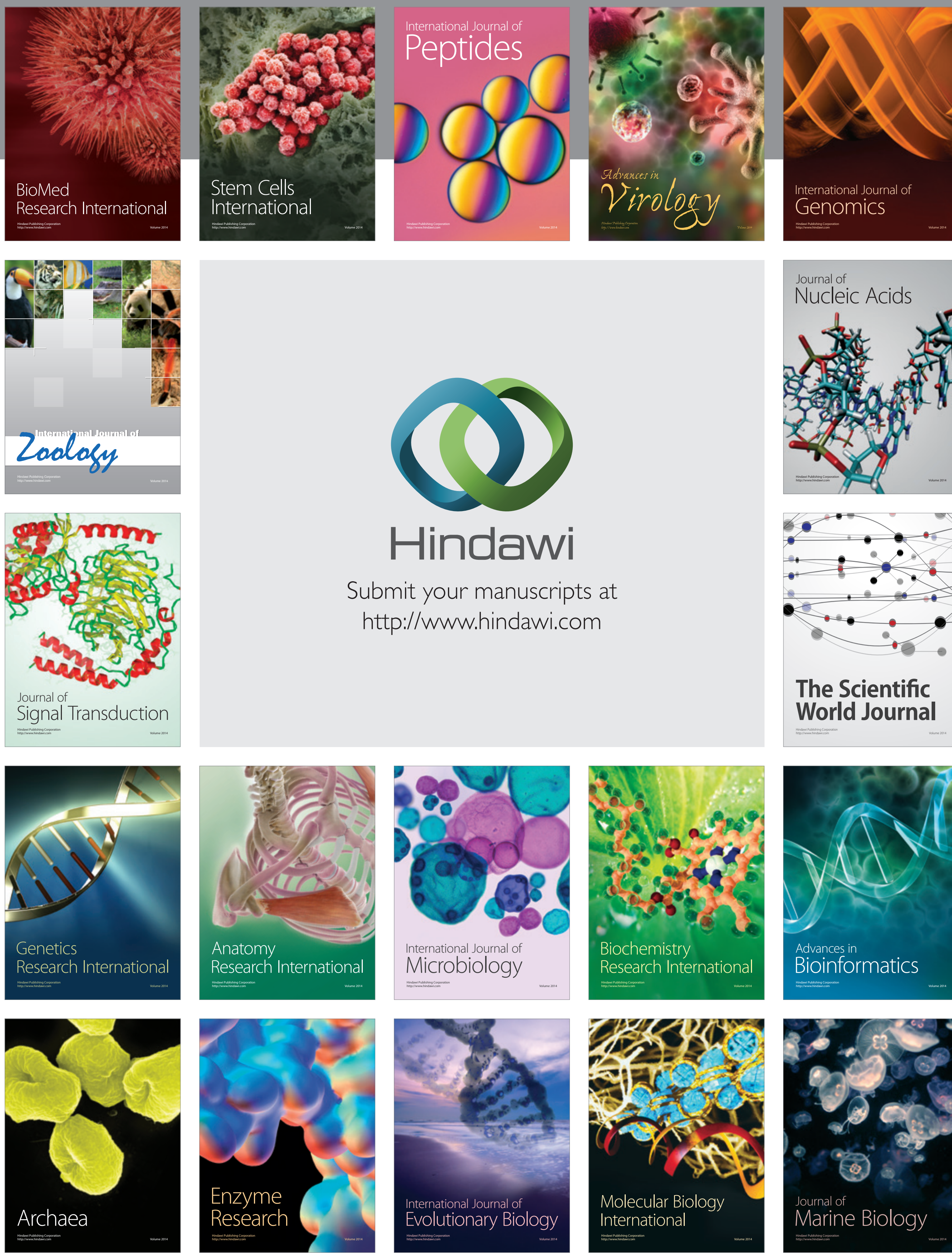
[34] N. Bolwig, *Senses and Sense Organs of the Anterior End of the House Fly Larvæ*, vol. 109 of *Videnskabelige Meddelelser fra Dansk Naturhistorisk Forening i Kjøbenhavn*, 1946.

[35] M. Skuhrava and V. Skuhravy, "Biology of gall midges on common reed in Czechoslovakia," in *Biology of Insect Galls*, J. Shorthouse and O. Rohfritsch, Eds., pp. 196–207, Academic Press, New York, NY, USA, 1992.

[36] T. De Filippis and A. C. R. Leite, "Scanning electron microscopy studies on the first-instar larva of *Dermatobia hominis*," *Medical and Veterinary Entomology*, vol. 11, no. 2, pp. 165–171, 1997.

[37] C. A. Skilbeck and M. Anderson, "The ultrastructure, morphology and distribution of sensilla on the antennae of the adult parasitoids *Aleochara bilineata* Gyll. and *Aleochara bipustulata* L. (Coleoptera: Staphylinidae)," *International Journal of Insect Morphology & Embryology*, vol. 25, no. 3, pp. 261–280, 1996.

[38] I.-W. Chu-Wang and R. C. Axtell, "Fine structure of the terminal organ of the house fly larva, *Musca domestica* L.," *Zeitschrift für Zellforschung und Mikroskopische Anatomie*, vol. 127, no. 3, pp. 287–305, 1972.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