Observations on the Biology and Anatomy of Myerslopiidae (Hemiptera, Membracoidea)

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Adults and nymphs of Mapuchea chilensis (Nielsen), from the poorly known family Myerslopiidae, were collected from the litter horizon of temperate forests and shrub bogs in southern Chile. The species apparently feeds on roots and creeping stems of angiosperms. Salivary sheaths of captive specimens terminated in vascular bundles. Indirect evidence suggests feeding on phloem sap. Both nymphs and adults are strong jumpers and both actively disperse, as evidenced by their capture in pan traps. The Malpighian tubules of this species produce no brochosomes and, unlike in most previously studied Membracoidea, comprise no specialized secretory segment. Each tubule comprises secretory cells scattered among excretory ones, a condition not previously known among Hemiptera.

1. Introduction

The family Myerslopiidae includes three genera, two in New Zealand and one in Chile, altogether containing 19 morphologically similar species [1]. Adult myerslopiids are stout, strongly sclerotized insects, 3 to 7.5 mm in length, with elytra-like, medially sealed tegmina, and without traces of hind wings underneath. Both adults and nymphs dwell cryptically on or near the ground, and their integument, sculptured with ridges and protuberances, is usually camouflaged with hardened soil (Figures 1(a)–1(h)). Members of the family have formerly been classified as a tribe within the leafhopper subfamily Ulopinae (Cicadellidae) but more recently were recognized as a sister lineage to the rest of Membracoidea (leafhoppers + treehoppers), which shares some plesiomorphic traits with cicadoids and cercopoids [2]. The disjunct range of the group has been interpreted as evidence of its considerable age and association with Gondwanaland [1, 2].

Until now the main sources of information on the myerslopiids biology were collecting records of the New Zealand species, the majority of which described the habitat as simply "moss" or "litter" [3]. This led Hamilton to assume that myerslopiids, like peloridiids, are "associated with moss rather than with higher plants." Bryophagy has not been known in Cicadomorpha and is exceptionally rare among other Auchenorrhyncha [4]. Other authors referred to myerslopiids as possible fungivores [1] or even predators [5]. The life history of myerslopiids thus merits clarification.

Also of interest is the activity of the Malpighian tubules of myerslopiids. Secretions of these organs play important roles in the biology of different groups of Cicadomorpha [6–8]. For example, cicadellids actively coat their integuments with water-repellent sheaths of brochosomes, proteinaceous products of specialized Malpighian tubule segments [9–12]. It has been argued that the production of brochosomes had evolved in the common ancestor of Membracoidea [13]. Because myerslopiids have been identified as being among the few leafhopper taxa lacking brochosomes [14], data on their Malpighian tubules are much needed.

An expedition to temperate Chile during the austral summer of 2013-2014 gave the author an opportunity to clarify the microhabitat of myerslopiids, observe behaviors of captive specimens, and preserve material for anatomical studies. A note on the myerslopiids karyotype and tests has already been published [15]. Here, I present ecological, behavioral, and anatomical observations to demonstrate that myerslopiids present no significant departures from cicadellids in their life history traits, but their Malpighian tubules have a different organization, not previously known among Hemiptera.
2. Methods

Collecting was done mostly by manual sorting of detritus on a white plastic sheet. At the Senda Darwin biological station, additional specimens were collected in water-filled white or yellow disposable plastic dishes placed on the ground as pan traps. Most of the collected specimens were kept live in ventilated plastic food containers on plant cuttings, moss, rotten wood, and detritus from the source microhabitat. Their behaviors were intermittently observed under a stereomicroscope.

Adults and nymphs collected at Senda Darwin were caged on cuttings of *Griselinia racemosa* roots, identified as a likely food source. To examine salivary sheaths, roots segments where the insects had been observed feeding were marked with a pen marker and then excised, fixed in 4% formaldehyde for 24 hours, and stored in 70% ethanol. These root chunks were subsequently embedded into paraffin, cut into 5 µm sections, and stained with hematoxylin and eosin.

The ultrastructure of the Malpighian tubules was examined in two adult females, one adult male, and two 3rd-instar nymphs. The specimens were preserved in 2.5% glutaraldehyde in PBS buffer, pH 7.4. The tubules were dissected out, stained with osmium tetroxide, embedded in Epon, and sectioned into 60 nm sections. The sections were poststained with uranyl acetate and lead citrate and examined on a JEM-1011 transmission electron microscope (JEOL Ltd., Japan).

3. Results

3.1. Collected Material. Chile: Huerquehue National Park, N of Lake Tinquilco, head of the Lagos trail, 39.155°S, 71.716°W, mature *Nothofagus* forest with *Chusquea* understory (Figure 2(a)), 20–25 Dec., 2013, 10 adults and 5 nymphs from leaf litter with *Hydrangea serratifolia*, *Dendroligotrichum dendroides*, and small ferns (Figure 2(b)); same site, 9–10 Feb., 2014, 11 adults and 1 nymph (2nd instar). Huerquehue National Park, sector Anticura, near park entrance, 200 m N of Road 215, 40.665°S, 72.174°W, 15–17 Jan., 2014, >25 adults and 5 nymphs (5th instar) from ca. 3 m² of leaf litter under a tree, with abundant *Hydrangea serratifolia* and some herbaceous plants (Figures 2(c)–2(e)); trail Princesa, 40.665°S, 72.172°W, 15 Jan., 2014, 3 nymphs (5th instar) from forest leaf litter along trail; trail Chile, 40.669°S, 72.170°W, 18 Jan., 2014, 2 adults and 1 nymph (5th instar) from forest leaf litter with *Blechnum*, *Hydrangea*, and *Dendroligotrichum*; trail Salto del Indio, mature *Nothofagus* forest, 19 Jan., 2014, 4 adults from leaf litter. Alerce Andino National Park, sector Sargazo, near park entrance, 41.508°S, 72.621°W, open slope near forest edge, 6 Jan., 2014, 1 nymph (5th instar) from short grass and moss under *Drimys*; the same site, *Drimys* forest with *Chusquea*, 8 Jan., 2014, 2 adults from litter with *Dendroligotrichum* and creeping vines. Chiloé, Senda Darwin biological station, forest, postfire bog with abundant shrubs (*Baccharis*, *Tepualia*, and *Gaultheria*), large ferns (*Blechnum* spp., *Gleichenia squamulosa*), and trees (Figures 2(f) and 2(g)), 41.88°S, 73.67°W, 11 Jan., 2014, 3 nymphs (2nd and 3rd instars) from inside the spongy mass of decomposing old fronds around “trunks” of large *Blechnum magellanicum*; the same site, 27 Dec., 2013–4 Jan., 2014, 6 nymphs (5th instar) from accumulation of litter and peat under *Podocarpus* and large ferns, with abundant *Griselinia racemosa* roots; the same site, 13 Jan., 2014, and 1–7 Feb., 2014, 5 adults and 6 nymphs (4th and 5th instars), captured in plastic pan traps (Figure 2(g)), mostly on rainy nights. Chiloé National Park, sector Cucao, shrub bog along the Lahuan trail (Figure 2(h)), 42.621°S, 74.107°W, 13 Jan., 2014, 1 nymph (5th instar) from peat with roots; the same site, 3 Feb., 2014, 2 adults from litter and detritus under large ferns and trees; sector Rancho Grande, 42.572°S, 74.075°W, 3 Feb., 2014, 1 nymph from moss, coll. V. Hartung.

The specimens varied in size, sculpturing, and coloration between and within the collecting sites but were all assigned to *Mapuchea chilensis* (Nielson). The type locality of that first South American myr松loid to be described is in the Anticura sector of the Puyehue NP [5]. Szwedo [16] made it the type of his genus *Mapuchea*, in which he described two additional species, each based on a single specimen: *M. burckhardtii*, from Bosque de Fray Jorge NP, which is the northernmost outpost of Valdivian temperate rain forests, and *M. hamiltoni* from the Huerquehue NP. The specimens I collected in the Huerquehue NP did not consistently differ from the specimens collected elsewhere.

3.2. Observations of Caged Insects. Due to their cryptic habitat and camouflage, the insects could not be observed in nature. Caged adults and nymphs survived indefinitely until killed. They walked slowly but most of the time clung motionlessly to plant stems or roots, occasionally expelling droplets of clear excrement, which was interpreted as a sign of feeding. The insects were not observed feeding or staying long on green plant parts but twice were observed feeding on damp rotten wood. When disturbed, both nymphs and adults jumped strongly. In one adult, a jumping distance of 50 cm was recorded. The jumps ended by crash-landing followed by brief immobility. Two captive 5th instar nymphs molted to the adult stage. One of these (Figure 1(c)) was closely watched for 9 hours following the molt, during which it remained inactive. No attempts to coat the integument with soil or plant debris were noticed in either of molted specimens during the days following the molt. Grooming, courting, and mating were not observed.

3.3. Salivary Sheaths. Seven salivary sheaths were tracked, all of them terminating in the vascular bundle of the rootlet. Among these, two penetrated into the xylem (Figures 3(a) and 3(b)), while the rest were difficult to interpret, terminating equivocally between the phloem and the xylem (Figures 3(c) and 3(d)) or being poorly preserved. One branched sheath was observed (Figure 3(a)).

3.4. Malpighian Tubules. The general arrangement of the digestive tract and four Malpighian tubules is as typical for Cicadomorpha (Figure 4). The shape of the Malpighian tubules is identical in adults and nymphs. Each tubule gradually widens towards the base but becomes constricted again for a short length before entering the filter chamber.
Figure 1: Mapuchea chilensis. ((a), (b), (c), and (d)) Specimens from the Huerquehue NP. (a) Scanning electron image of a female molted in captivity and not camouflaged with soil; the left tegmen is mangled. (b) The same specimen live, showing color pattern. (c) Freshly molted female. (d) Camouflaged specimen. (e) Specimen from the Puyehue NP. (f) Soil camouflage in combination with disruptive coloration of legs makes this specimen hard to see; note that the eyes remain clean. (g) 5th instar nymph. (h) 3rd instar nymph. All photos of live specimens were taken in captivity.
Figure 2: *Mapuchea chilensis* habitats (collecting sites). (a) *Nothofagus* forest N of the lake Tinquilco in Huerquehue NP. (b) The same forest floor with ferns and *Hydrangea* sp. (c) Puyehue NP near Anticura campground, leaf litter near a tree base, with abundant *Hydrangea serratifolia*. (d) The same, detailed view; note the absence of mosses and ferns. (e) The same, with litter removed to expose creeping stems of *H. serratifolia*; also note the abundant volcanic ash deposited upon the 2011 Puyehue-Cordón Caulle eruption. (f) Shrub bog at the biological station Senda Darwin on Chiloé. (g) The same, showing pan traps. (h) Shrub bog in Chiloe NP near Cucao.
Figure 3: Histological sections of *Griselina racemosa* roots with salivary sheaths of *Mapuchea chilensis*. (a) Branched sheath, with one branch penetrating into the xylem. (b) Sheath terminating in the outermost layer of the xylem. ((c) and (d)) Sheath terminating equivocally, between the phloem and the xylem.

In both nymphs and adults, the exposed parts of the tubules comprise two types of cells, excretory and secretory. In the thicker, more basal part of the tubule, secretory cells are interspersed among more numerous excretory cells. As the tubule narrows distally, the secretory cells gradually disappear; the most distal part seemingly comprises only excretory cells.

The excretory cells have an extensively developed labyrinth of basal membrane infoldings (Figures 5(a) and 5(b)). Their apical membrane bears a brush of long, simple, or lamellar microvilli, containing mitochondria, and forms intracellular canaliculi, some of them branching or forming flask-shaped cavities (Figures 5(b) and 5(c)). These cavities are also lined with microvilli or lamellae (Figure 5(d); compare with Figure 8 in [17]). The cytoplasm contains numerous conspicuous mitochondria and, in some cells, deposits of glycogen granules (Figure 5(d); compare with [18]).

The secretory cells (Figures 6(a)–6(h)) are smaller and lack apical microvilli and conspicuous mitochondria. They contain numerous secretory vacuoles filled with spherical or somewhat irregular dense granules, ca. 0.2 \( \mu \)m in diameter, with coarsely granulose surface (Figure 6(f)), and, occasionally, smaller particulate material. The 0.2 \( \mu \)m granules are also present in the tubule lumen (Figure 6(c)). The granules are formed in Golgi cisternae (Figures 6(g) and 6(h)). Most of the remaining cytoplasm is filled with cisternae of endoplasmic

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Figure 4: *Mapuchea chilensis* digestive tract with the Malpighian tubules and an isolated tubule (omitting the basal part entering the filter chamber). OE: oesophagus; FC: filter chamber; MG: midgut; MT: Malpighian tubules; and RT: rectum.

The exposed length of the tubule is convoluted and nodulose, resembling twisted rope, distally less so (Figure 4). The tubules form two pairs, the termini of each pair united in a loop attached to the rectum.
4. Discussion

4.1. Habitats and Hosts. *M. chilensis* was collected in temperate Chile from forests and shrub bogs. This is similar to the New Zealand myerslopiids, which also occur in both forested and open habitats [3]. *M. chilensis* dwells cryptically on the ground or within decomposing plant material. On bogs, where the litter horizon is not well defined, the insect can be found at considerable depths within the peat. Most of the microhabitats where *M. chilensis* was found contained roots or stems of angiosperms, which apparently served as the food source. At several forest locations in the Huerquehue, Puyehue, and Alerce Andino parks, the insects were found between creeping stems of the vine *Hydrangea serratifolia*. It grew abundantly at the site in Anticura where the largest numbers of *M. chilensis*, 30 specimens, were collected (Figures 2(c)–2(e)). In at least two sites on the bog at Senda Darwin (Figure 2(f)), *M. chilensis* appeared to be associated with surface roots of the shrub *Griselinia racemosa*, abundantly ramifying within the litter and peat. Interestingly, like myerslopiids, the genus *Griselinia* has a disjunct distribution between southern South America and New Zealand. Caged insects were observed feeding on both *G. racemosa* roots (Figures 3(a)–3(d)) and *H. serratifolia* stems. No single plant species was present at every collecting site, indicating that *M. chilensis* is not associated with one host, but all such sites contained angiosperms. Most of the previous records did not associate myerslopiids with angiosperms, although three New Zealand species have been collected, among other places, from *Celmisia* and mats of *Raoulia*, both of Asteraceae [3]. Ferns were present at most of the collecting sites (Figures 2(b) and 2(g)) but were absent from the Anticura site (Figures 2(c)–2(e)). Curiously, three nymphs were found within dead plant material coating the “trunks” of large ferns that microhabitat could potentially contain epiphytes. A single record of a New Zealand myerslopid from a fern has been listed [3].

Previous records consistently associated myerslopiids with mosses [3]; yet mosses were completely absent from the *Mapuchea*-rich site in Anticura (Figures 2(c)–2(e)). The mosses on the bogs of Chiloé (Figures 2(f)–2(h)) were intensively sampled, yielding abundant peloridiids ([19]; V. Hartung, personal communication), but only occasional individuals of *M. chilensis*. The latter species was consistently found on those same bogs in moss-free detritus accumulated under shrubs, large ferns, and trees.

4.2. Feeding Mode. Salivary sheaths revealed that the insects fed by tapping into the vascular tissue. This feeding mode is consistent with the presence of a filter chamber and general...
Figure 6: Secretory cells in the Malpighian tubules of *Mapucaea chilensis*. ((a)–(d)) Cells in the tubules of a 3rd instar nymph. (a) A single secretory cell (its nucleus marked by an asterisk) among excretory cells. (b) The same cell, closeup. (c) Contact between a secretory and an excretory cell; arrowheads point at the junction. Note secretory granules in the tubule lumen. (d) Secretory vacuoles with granules and additional particulate material. Arrowheads point at cisternae of endoplasmic reticulum. ((e)–(h)) Cells in the tubules of an adult. (e), (f) Secretory vacuoles variably loaded with granules. (g), (h) Golgi regions with developing granules. Im: tubule lumen.
structure of the gut and Malpighian tubules, similar to those in previously studied Cicadomorpha (Figure 4). However, the exact source of the ingested sap, phloem or xylem, could not be identified based on the sheaths because of the small size of the sample. Salivary sheaths mark not only feeding, but also probing behavior, thus requiring many observations in order to make inferences. For example, among the 389 examined salivary sheaths of *Philaenus spumarius* (L.) examined by Horsefield, only 60% terminated in the xylem [20].

Although some of the observed salivary sheaths terminated in the xylem (Figures 3(a) and 3(b)), the external morphology of myerslopiids indicates xylem-feeding to be unlikely. The low nutritional value of xylem sap requires xylem-feeders to intensify ingestion by increasing the volume of cibarial pump muscles [21]. Externally this is manifested in the frontoclypeus being disproportionately enlarged in all stages, but especially so in young instars. The head proportions of myerslopiids follow the opposite developmental trajectory, with the frontoclypeus becoming progressively more inflated towards the adult stage (Figures 1(a) and 1(h); also mentioned in [16]). Therefore, the most likely food source is phloem sap.

4.3. Locomotion and Other Behaviors. While adults jump in all the Cicadomorpha except cicadas, nymphal jumping is thought to have evolved in the ancestral membracoids and been subsequently lost in the lineage that includes several leafhopper subfamilies and treehoppers [13]. The evidence of jumping by both adult and immature *Mapuchea* is, therefore, not surprising. It is worth mentioning that the leafhopper subfamily Ulopinae, to which myerslopiids have been assigned in the past, contains *Ulopa* species with nonjumping nymphs.

The fact that adults and nymphs of *M. chilensis* were consistently captured in pan traps (Figure 2(g)) suggested that they emerged to the surface in order to disperse. The insects quickly drowned because their integument is not water-repellent. Climbing or accidentally falling from vegetation into these traps was unlikely, suggesting that the trapped insects had been moving around by hopping.

The origin of the characteristic soil crust of myerslopiids remains a mystery. Its consistent presence on the majority of collected specimens (Figures 1(d)–1(h)) suggests that it may be actively produced, but my limited observations did not discover any such behavior. Similar crusts have been reported for the leafhopper genera *Evansiola* China and *Paulianiana* Evans, formerly allied with myerslopiines within Ulopinae [22].

4.4. Malpighian Tubules. It has long been known that the Malpighian tubules of Membracoidea are unique among Hemiptera in having, in their middle or somewhat more distally, a glandular segment [23]. It is usually inflated throughout the life cycle, but in adults of some leafhoppers it becomes secondarily deflated. This segment has been observed in all the previously examined Cicadellidae (e.g., [23, 24]), Membracidae ([23, 24], Rakitov unpublished), and Melizoderidae (Rakitov, unpublished), but not in Aetalionidae (Rakitov, unpublished). The glandular segment consists entirely of specialized secretory cells and is flanked on either side with narrow, nodulose tubule segments consisting entirely of excretory cells [17]. In most adult cicadellids, the secretory cells produce brochosomes, proteinaceous particles of curious structure, which are released after molts and applied in special behaviors onto the integuments (reviewed in [11]). In some cicadellid subfamilies, the glandular segments secrete brochosomes starting from the 1st nymphal instar, while in others they produce other diverse products prior to switching, during the last nymphal instar, to production of brochosomes. These Malpighian tubule secretions of leafhopper nymphs include granules, tubules, or colloids [25]. Their chemistry and function are unknown, but some leafhopper nymphs apply these secretions onto their bodies [26]. Similar products have been found in the glandular regions of the immature and adult Membracidae and Melizoderidae (Rakitov, unpublished). It has been argued that treehoppers have evolved from within leafhoppers and quit secreting brochosomes secondarily [13].

Unlike in most previously studied Membracoidea, the Malpighian tubes of *M. chilensis* have no glandular segment composed of secretory cells (Figure 4). Instead, the tubes of both nymphs and adults have secretory cells interspersed among excretory cells along the length of tubule, except in the terminal region. The secretory cells produce simple granules, ca. 0.2 μm in diameter, which accumulate in large numbers in secretory vacuoles prior to being released into the lumen (Figures 6(a)–6(f)). This resembles the granular secretions produced in the Malpighian tubules of some cicadellid nymphs prior to switching to the production of brochosomes [25]. In both cases, the granules form in Golgi cisternae (Figures 6(g) and 6(h)), suggesting their proteinaceous nature. In the absence of chemical data, it is impossible to conclude whether the secretory products of *M. chilensis* are related to those of cicadellid nymphs, but this seems to be a possibility, given their structural similarity and the fact that *Myerslopiidae* and *Cicadellidae* are close relatives. The tubule excretory cells of *M. chilensis* (Figures 5(a)–5(d)) are identical to those of cicadellids [17] and represent a generic type common among insects. Therefore, the essential difference between *M. chilensis* and the previously studied Membracoidea concerns how the secretory cells are situated along the tubule: interspersed in the first case and forming a compact zone in the second.

Interspersed secretory cells, of unknown function, have been observed in the Malpighian tubules in other insect orders [27] but, to the best of my knowledge, not in Hemiptera. The closest living relatives of Membracoidea are spittlebugs (Cercopoidea) and cicadas (Cicadoidea), the three groups forming together the infraorder Cicadomorpha. During the nymphal stage, the Malpighian tubules of spittlebugs and cicadas produce secretions, spittle components in the first group [6, 7, 28–30] and the so-called anal liquid, of uncertain function, in the second [8]. In both groups, the tubules have a similar organization, representing a third type, different from that of either *M. chilensis* or other Membracoidea. Each has a
short inflated glandular basal segment, composed of secretory cells producing mucosubstances, and a long nodulose distal segment, composed of essentially excretory cells, with characteristic apical microvilli and intracellular canaliculi, which additionally produce proteinaceous granules [8, 28–31].

The basal parts of the tubes show no secretory modification in Membracoidea, including M. chilensis, but their more distal parts secrete proteinaceous substances in all Cicadomorpha, representing three ways (Figures 7(a)–7(c)) to distribute the excretory and secretory functions along the tubule: (1) cells homogenous, each combining the secretory and excretory functions (spittlebug and cicada nymphs); (2) secretory cells interspersed among excretory cells (M. chilensis); and (3) excretory and secretory cells segregated into separate segments (Cicadellidae and Membracidae).

Like in M. chilensis, the Malpighian tubules of Aeta-

ionidae have no morphologically distinct glandular region (Rakitov, unpublished), but their cell composition and ultrastructure have not yet been examined.

I have argued in the past that the secretory specializations of the Malpighian tubules together with the habit of applying their secretions onto the body, displayed by spittlebugs, cicadas, and membracoids, have a common evolutionary origin [8]. The fate of the tubule secretory products of Mapuchea is unknown; no postmolt behaviors similar to anointing of leafhoppers and treehoppers [26] was observed, but the paucity of observations makes conclusions premature.

Myerslopiidae merit further study in this regard, as they may represent the only recent group of Membracoidea which had separated prior to and thus may shed light on the origin of brochosomes and related behaviors of leaffoppers.

Conflict of Interests

The author declares that there is no conflict of interests regarding the publication of this paper.

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