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# Morphology and life history of an ant parasitoid, *Psilocharis afra* (Hymenoptera: Eucharitidae)

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## Abstract

Eucharitidae (Hymenoptera) are specialized ant (Formicidae) parasitoids. As we begin to develop a better understanding of their phylogenetic relationships, it is critical to establish baselines for morphological and biological data. A morphological review and the first report of life history data for *Psilocharis afra* Heraty is provided based on new material from the Mpumalanga Province of South Africa. *Psilocharis* Heraty is included in Eucharitinae, but it is unclear whether it is the sister group of all other members of the subfamily, or sister group to *Neolosbanus* Girault in a monophyletic Psilocharitini, which would in turn be sister group to Eucharitini. The oviposition habits of *P. afra* differ from those of other Eucharitidae in that eggs are placed among trichomes under bracts at flower bases, instead of either being inserted into cavities formed in plant tissue by an enlarged ovipositor (as in Oraseminae and some *Neolosbanus*) or inserted into cavities in plant tissue, as in most Eucharitini. The egg and first-instar planidia larva are described, and adult morphology is discussed with reference to Eucharitidae and other parasitoid Hymenoptera.

**Key words:** Formicidae, parasitoid, behavior, morphology, planidium, first instar larva

## Introduction

Eucharitidae is the only family of insects in which all species with known biology are parasitoids of the immature stages of ants (Lachaud & Pérez-Lachaud 2012; Torréns 2013). Adults deposit their eggs outside of the nest into or on plant tissue, and the minute first-instar larvae make their way into the nest by some means of phoretic attachment to foraging ant workers (Ayre 1962; Clausen 1940a, 1940b). Within the ant nest, the eucharitid larva first attacks the larval stages of the ant as either an endoparasitoid or ectoparasitoid, but its development is only completed as an external parasitoid of the ant pupa (Heraty & Murray 2013).

Eucharitidae and Perilampidae together form a monophyletic group, with the three subfamilies of Perilampidae (Chrysolampinae, Philomidinae and Perilampinae) forming a grade to a monophyletic Eucharitidae (Heraty *et al.* 2013). Four subfamilies of Eucharitidae are currently recognized, Akapalinae (1 genus, Australia), Gollumiellinae (2 genera, Indo-Pacific region), Oraseminae (13 genera, worldwide) and Eucharitinae (48 genera, worldwide) (Burks *et al.* 2017; Heraty 2002; Heraty *et al.* 2013). The subfamily Eucharitinae is further subdivided into Eucharitini and Psilocharitini, with the latter consisting of two genera, *Psilocharis* Heraty and *Neolosbanus* Heraty (Heraty 1994, 2002). Psilocharitini are the sister group to Gollumiellinae + Eucharitinae based on morphology (Heraty 2002), but sister group only to Eucharitini whenever molecular data is included (Heraty *et al.* 2013; Heraty & Darling 2009; Heraty *et al.* 2004; Munro *et al.* 2011; Murray *et al.* 2013). With the exception of Psilocharitini, each higher-level group is monophyletic in both morphological and molecular analyses. While Psilocharitini are monophyletic in morphological analyses (Heraty 1994, 2002), and one combined analysis (Heraty *et al.* 2013), in other analyses based on molecular or combined data they are paraphyletic, with either *Psilocharis* (Heraty *et al.* 2004) or *Neolosbanus* (Heraty & Darling 2009; Munro *et al.* 2011, 2013) as the sister group to Eucharitini. More robust data sets, with more taxa and gene regions, continue to support *Psilocharis* as the sister group of *Neolosbanus* + Eucharitini, with a posterior probability of 96% reported by Murray *et al.* (2013).

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*Psilocharis* is intriguing both because of its uncertain placement at the base of Eucharitinae, and because it is the sister group of the only known eucharitid fossil, *Palaeocharis rex* Heraty & Darling, which is known from Baltic amber (~44 mya) (Heraty & Darling 2009). *Psilocharis* was then used as a major calibration point for estimating divergence times for Eucharitidae (Murray *et al.* 2013). The estimate of the crown age of *Psilocharis* ranges from 58–27 mya, whereas the divergence of *Neolosbanus* + Eucharitini ranges from 65–39 mya (Murray *et al.* 2013).

Previously, nothing was known of the biology or immature stages of *Psilocharis*. *Neolosbanus palgravei* (Girault) is an external parasitoid of *Hypoponera* Santschi (Ponerinae) and uses an expanded ovipositor to insert single eggs into leaf tissue (Heraty 1994). Other species of *Neolosbanus* have an acicular ovipositor, but their oviposition habits remain unknown. Most Eucharitini use an acicular ovipositor to oviposit into either fruit or a cavity within the plant tissue, and often deposit large numbers of eggs with each event (Clausen 1940b). Some derived Eucharitini can either oviposit onto the surface of leaves or can (rarely) insert small groups of eggs into plant tissue (Clausen 1940b; Torréns 2013), however this is not known in other eucharitid lineages. The oviposition behavior of *N. palgravei* is similar to that of Oraseminae, with both using an enlarged ovipositor to deposit one or a few eggs into leaf tissue (Heraty 2000), and Gollumiellinae, which use an enlarged ovipositor to insert only the tip of the egg into leaf tissue (Heraty *et al.* 2004). Outside of Eucharitidae, Perilampidae (Chrysolampinae and Perilampinae, unknown in Philomidinae) use an acicular ovipositor to either place their eggs onto the leaf surface, into pre-existing cavities, or into woody tissue (Clancy 1946; Darling & Miller 1991; Darling & Roberts 1999; Laing & Heraty 1981; Smith 1916). *Jambiya* Heraty & Darling (unplaced to subfamily) is the only perilampid with an enlarged ovipositor that might be suitable for oviposition into leaves (Heraty & Darling 2007). The fossil *Palaeocharis* Heraty & Darling appears to have an acicular ovipositor. *Psilocharis* sits at a transition point between Gollumiellinae and Oraseminae, both of which insert eggs into plant tissue, the majority of Eucharitini which drill into plant tissue to locate a cavity for egg deposition, and *Neolosbanus,* which has species that likely undertake both behaviors, based on having either an acicular or enlarged ovipositor.

*Neolosbanus* and Eucharitini only parasitize ants with cocoons (Ectatomminae, Formicinae, Myrmeciinae, Ponerinae), although some likely erroneous records exist for Myrmicinae (Heraty 2002; Lachaud & Pérez-Lachaud 2012). *Neolosbanus* and all Eucharitini except *Pseudometagea* Ashmead are also ectoparasitic as a first instar on the host larva (Heraty & Murray 2013). Gollumiellinae and Oraseminae only parasitize ants without cocoons (Formicinae and Myrmicinae), and all are endoparasitic in the first instar (Heraty 2000; Heraty *et al.* 2004; Heraty & Murray 2013). Again, *Psilocharis* sits at a transition point for parasitism of ants with or without cocoons and a shift in behavior from endoparasitism to ectoparasitism.

A large population of *P. afra* was found in South Africa that provided ample material for a morphological study of the genus for future comparison across Eucharitidae. This allowed us to explore and annotate the morphology of *Psilocharis*, and has also allowed us to explore some biological traits relevant in this pivotal genus. The ant host remains unknown.

## Materials and methods

Excavation of ant nests and observations on flower visitation by *Psilocharis afra* were carried out in 2006 and 2016 at a grassland seep site comprising Northern Escarpment Quartzite Sourveld, near Pinnacle Rock, situated 4.4km NE Graskop, 1500m, 24°54.444'S 30°51.694'E in Mpumalanga, South Africa. Numerous populations of flowering *Helichrysum nudifolium* (L.) and *Nidorella auriculata* DC (both Asteraceae) were present across the area with all stages of phenology present. The main study population was comprised of about 400 individual *Helichrysum* plants, 10% of which had young flower inflorescences, 30% with mature flowers optimal for *Psilocharis* visitation, and 60% with old flowers, which had dried out and opened up for wind dispersal of the seeds. This population grew on dark soil comprising a mixture of granite derived sand and damp humus. Populations growing on red dolerite derived soils at other sites about 15 km north of the study area had no *Psilocharis* present.

Samples were made by sweeping both adult wasps and ants, observing adults with flowers in Drosophila Culture Vials, and collecting ant nests and brood into 95% ethanol. Photographic documentation of live *Psilocharis* and ants, in situ at the study site, was accomplished using a hand-held Nikon D7000, with a AF-S Micro Nikkor 105 mm macro lens and Nikon slave flashes. *Psilocharis afra* are vouchered in the UCR Entomology Research Museum with individual identifiers UCRCENT00435203–268, 282–298, and in the Iziko South African Museum, Cape Town (SAMC) with catalogue numbers SAM-HYM-P064421-26; P080362. Ant vouchers were deposited at UCR with the voucher numbers UCRCENT00593–640, 647–668 and 468521–554, and in the Iziko South African Museum, Cape Town (SAMC) with catalogue numbers SAM-HYM-C028009-35; C028101-117. First-instar *P. afra* were vouchered on slides as UCRCENT00414330–336. Specimen UCRCENT184095 was sequenced for ribosomal 18S (JN623244) and 28S-D2-D3 (JN623657, JN624015) as part of Munro *et al.* (2011). Plant vouchers are retained by JMH.

Adult specimens were collected into 95% ethanol and dried using hexamethydisilazane (HMDS) following protocols outlined by Heraty & Hawks (1998), or using a Critical Point Drier (Leica EM CPD300). For SEM, dissections were made directly on SEM stubs, with specimens anchored with silver paint, and after dissection, dried hemolymph and other debris removed using a hooked minutien pin and a small portion of glue scratched from double-sided scotch tape. All SEM preparations were coated with gold/palladium alloy and examined with a Philips XL30_FEG SEM. Microscope slides were prepared from specimens digested with 10% KOH and mounted in Canada Balsam. First-instar larvae were digested in 10% KOH and directly slide mounted in Hoyer's Medium. Slide images were taken using a GT-Vision montage imaging system on a Leica DMRB compound microscope at UCR. Images of flowers, planidia, and whole mounted specimens were taken at the Iziko South African Museum in Cape Town, South Africa using a Leica LAS multi-stacking imaging system comprising a Leica® Z16 microscope with a Leica DFC450 Camera and 0.63× video objective attached. The imaging process, using an automated Z-stepper, was managed using the Leica Application Suite V 4.9 software installed on a desktop computer (diffused lighting was achieved using a Leica LED5000 HDI dome).

Terms and abbreviations follow Heraty *et al.* (2013). Abbreviations from that paper are not repeated here, but instead only new abbreviations or terms are listed in Table 1. All morphological terms have been vetted through the Hymenoptera Anatomy Ontology (HAO) Portal (http://api.hymao.org) (Yoder *et al.* 2010). Definitions available in the HAO can be searched by pasting the HAO numbers mentioned in the text into any web browser.

## Results and discussion

**Adult morphology.** The morphology of *Psilocharis afra* was discussed in its original description (Heraty 1994). The availability of new specimens enables this more detailed investigation (Figs 1–36, 42). We have extensively labeled the morphological structures to establish a standard terminology for both Eucharitidae and Chalcidoidea. Most structures are as discussed by Gibson (1997), Heraty (1994, 2002), and Heraty *et al.* (2013), and are not further discussed here. The extensive annotation is self-explanatory, with abbreviations defined in Table 1. Some additional structures are discussed here which have received little or no attention in previous literature.

The labrum (lbr) in *P. afra* is similar to those previously described from Eucharitidae (Darling 1988), but with tapering setae (Fig. 9). The epipharynx (epp) possesses a single pair of epipharyngeal setae (eps) near its center (Fig. 10). A four-digitate labrum is found in Gollumiellinae, most Oraseminae, *Neolosbanus* (Psilocharitini) and *Pseudometagea* (Eucharitini), and is considered plesiomorphic within Eucharitidae.

Eucharitidae possess spatulate bristles near the cutting surface of the posterior mandibular surface, here referred to as "basal mandibular bristle" (bmb) and "apical mandibular bristle" (amb) (Fig. 2). Similar bristles occur in *Eutrichosoma mirabile* Ashmead (Pteromalidae: Eutrichosomatinae), in which the basal mandibular bristle is spatulate but the apical one is not. Only scattered non-spatulate bristles are present on the posterior surface of the mandibles in *Akapala* Girault and all examined Perilampidae.

Each mandible contains a series of internal "rods" (Fig. 23: $mr_{1\text{-}3}$) that are associated with each of the mandibular teeth (3/2 formula). Bucher (1948) referred to these as skeletal rods, and correctly stated that they were a feature of all Chalcidoidea. Ronquist & Nordlander (1989) referred to them in Ibaliidae (Cynipoidea) as mandibular rods. The detailed morphology and potential function of these rods in Proctotrupomorpha has never been studied.

A row of setae overhangs the anteclypeus, emerging immediately dorsal to it. Heraty (1994) referred to these as the "anteclypeal setae" (Fig. 3: acs). They occur in other *Psilocharis* but are not known from any other genera.

**TABLE 1.** Morphological abbreviations.

| | | | |
|---|---|---|---|
| 1-2vf | 1st-2nd valvifers | dgs | digital spine |
| 3vu | 3rd valvula | dig | digitus |
| 3vv | third valvula | dor | dorsum |
| aa | anterior area of 2vf | dr$_1$ | dorsal ramus of 1vv |
| aap | aedeagal apodeme | drm | digital retractor muscle |
| ab | articulating bulb | dsd | dorsal scrobal depression |
| acr | acropleuron | dv (2vv) | dorsal (2nd) valvula |
| acs | antecostal sulcus | dvc | distal vertical conjunctiva of 2vf-3vf complex |
| acy | anteclypeus | epp | epipharynx |
| ads | anterodorsal swelling of petiole | eps | epipharyngeal seta |
| adv | annuli of dorsal valvula | esg | epistomal groove |
| aed | aedeagus | F1-9 | 1st-9th flagellomere |
| af | antennifer | f$_2$p-f$_3$p | meso- metafurcal pit |
| amb | apical mandibular bristle | f$_2$b | mesofurcal bridge |
| anl | anellus (F1 may also be used) | fm$_{1\text{-}3}$ | pro- meso- metafemur |
| anr | antennal rim | fra | frenal arm |
| apa | aedeagal apodeme | frn | frenum |
| apc | anterior transverse petiolar carina | fu$_2$-fu$_1$ | mesothoracic interfurcal muscle |
| asf | anterior section of dorsal flange of 2vf | gal | galea |
| aul | aulax of 1vv | glo | glossa |
| ax | axilla | gm | genital membrane |
| axl | axillula | ham | hamuli |
| bc | basal cell of fore wing | hp | horizontal plate of mesofurca |
| bcp | basiconic peg sensillum | hpf | horizontal plate of mesodiscriminal lamella |
| bmb | basal mandibular bristle | hwmc | median complex of hind wing |
| BR | basal ring | hyc | hypostomal carina |
| btc | basitarsal comb | hyh | hypopygial hairs |
| btn | basitarsal notch | irm | intrinsic retractor muscle |
| bts | basitarsomere | iva | intervalvifer articulation |
| C+Sc+R | costal vein + subcostal vein + radial vein of hind wing | ivp | intervolsellar process |
| cal | propodeal callus | lac | lacinia |
| cc | costal cell | lb | laminated bridge of ovipositor |
| cer | cercus | lbp | labial palp |
| clv | clava | lbr | labrum |
| cly | clypeus | lcl | lateral clypeal line |
| crd | cardo | lhp | lateral hair patch [of petiole] |
| cs | campaniform sensilla of stigmal vein | lhs | lateral hypopygial seta |
| cvpr | cervical prominence | lep$_2$ | lower mesepimeron |
| cx$_{1\text{-}3}$ | pro-, meso-, metacoxa | les$_2$ | lower mesepisternum |
| dc$_2$ | mesodiscrimen | les$_3$ | lower metepisternum |
| dfd | dorsolateral frontal depression | llm | lateral lobe of mesoscutum |

*...continued on the next page*

**TABLE 1.** (continued)

| | | | |
|---|---|---|---|
| lmd | left mandible | pop | postoccipital pit |
| lpa | lateral panel of axilla | ppm | posterior protractor muscle |
| M5-12 | muscles of female ovipositor | pps | pit corresponding to prospinasternal apodeme |
| mb | median bridge of second valvifers | pre | prepectus |
| mdl | marginal digit of labrum | prm | prementum |
| mfd | mesofemoral depression | prp | propodeum |
| mlb | marginal seta of labrum | ps$_1$ | apical parameral sensillum |
| mlm | median lobe of mesoscutum | ps$_2$ | subapical parameral sensillum |
| mps | multiporous plate sensillum | ps$_3$ | subbasal parameral sensillum |
| mr | mandibular rod | ps$_4$ | basal parameral sensillum |
| mrm | marginal rim of mesoscutellum | psa | prospinasternal apodeme |
| Ms | metasomal sterna | psp | placoid sensillum of pedicel |
| msd | median scrobal depression | pss | postspiracular sulcus |
| msl | marginal seta of labrum | ptp | posterior tentorial pit |
| msp | mesothoracic spiracle | R$_{1a}$ | hind wing radial process 1a |
| Mt | metasomal terga | rad | radicle |
| Mt$_{8+9}$- 2vfa | 1st syntergal-2nd valvifer muscle | ret | retinaculum |
| mtps | metapleural sulcus | rmd | right mandible |
| mts | metascutellum | s$_1$ | prosternum |
| mtsa | metascutellar arm | sas | subantennal sulcus |
| muc | mucro | Sc+R | hind wing subcostal + radial vein |
| mv | marginal vein | sca | supraclypeal area |
| mxp | maxillary palp | scd | (meso-) scutellar disc |
| no$_{1-3}$ | pro-, meso-, metanotum | scp | scape |
| not | notaulus | scr | antennal scrobe |
| occ | occiput | sct | mesoscutellum |
| ocv | oblique carina of ventral valvula | sdp | subocellar depression |
| pa | posterior area of 2vf | sfb | subforaminal bridge |
| paa | pars articularis | smv | submarginal vein |
| pap | postalar process | spa | sensillar patch |
| par | paramere | sss | scutoscutellar suture |
| ped | pedicel | sti | stipes |
| pet | petiole | stv | stigmal vein |
| pgp | postgenal pit | TI&II | fused 1st and 2nd metasomal terga |
| ph$_1$ | prophragma | tb$_{1-3}$ | pro- meso- metatibia |
| phb | phallobase | tbs$_{1-3}$ | pro- meso- metatibial spur |
| php | pharyngeal process | tgl | tegula |
| pl$_1$ | propleuron | tor | torulus |
| pla | planidium | tpl | tergopleural line |
| pmu | processus musculares | tps | transepimeral sulcus |
| pmv | postmarginal vein | tr$_{1-3}$ | pro- meso- metatarsus |
| pom | field of postoral microtrichia | tra1-3 | 1st pro- meso- metatarsomere |

*...continued on the next page*

**TABLE 1.** (continued)

| | |
|---|---|
| trb1-3 | 2nd pro- meso- metatarsomere |
| $trc_{1-3}$ | pro- meso- metatrochanter |
| tsa | transscutal impression |
| tss | transepisternal sulcus |
| tva | tergo-valvifer articulation |
| tvv | teeth (annuli) of ventral valvula |
| $uep_2$ | upper mesepimeron |
| $ues_2$ | upper mesepisternum |
| $ues_3$ | upper metepisternum |
| ven | venter |
| vp | ventral pores of scape |
| vtc | vertexal carina |
| vv (1vv) | ventral (1st) valvula |

The antennal scrobe (Fig. 1: scr) contains depressions (dsd: dorsal scrobal depressions, msd: median scrobal depression) that are apparently superficial features. These are not homologous to facial pits as referred to in the HAO because they do not correspond to apodemes and are not associated with landmarks mentioned in definitions of relevant comparable structures known from Ceraphronoidea and Platygastroidea (HAO_0000319, HAO_0000808). Similar depressions occur in *Psilocharis theocles* (Walker) and in some Oraseminae (Burks *et al.* 2018). The median scrobal depression (msd) is likely not homologous with a similar depression known from some Trigonoderini (Pteromalidae) (Heydon 1997).

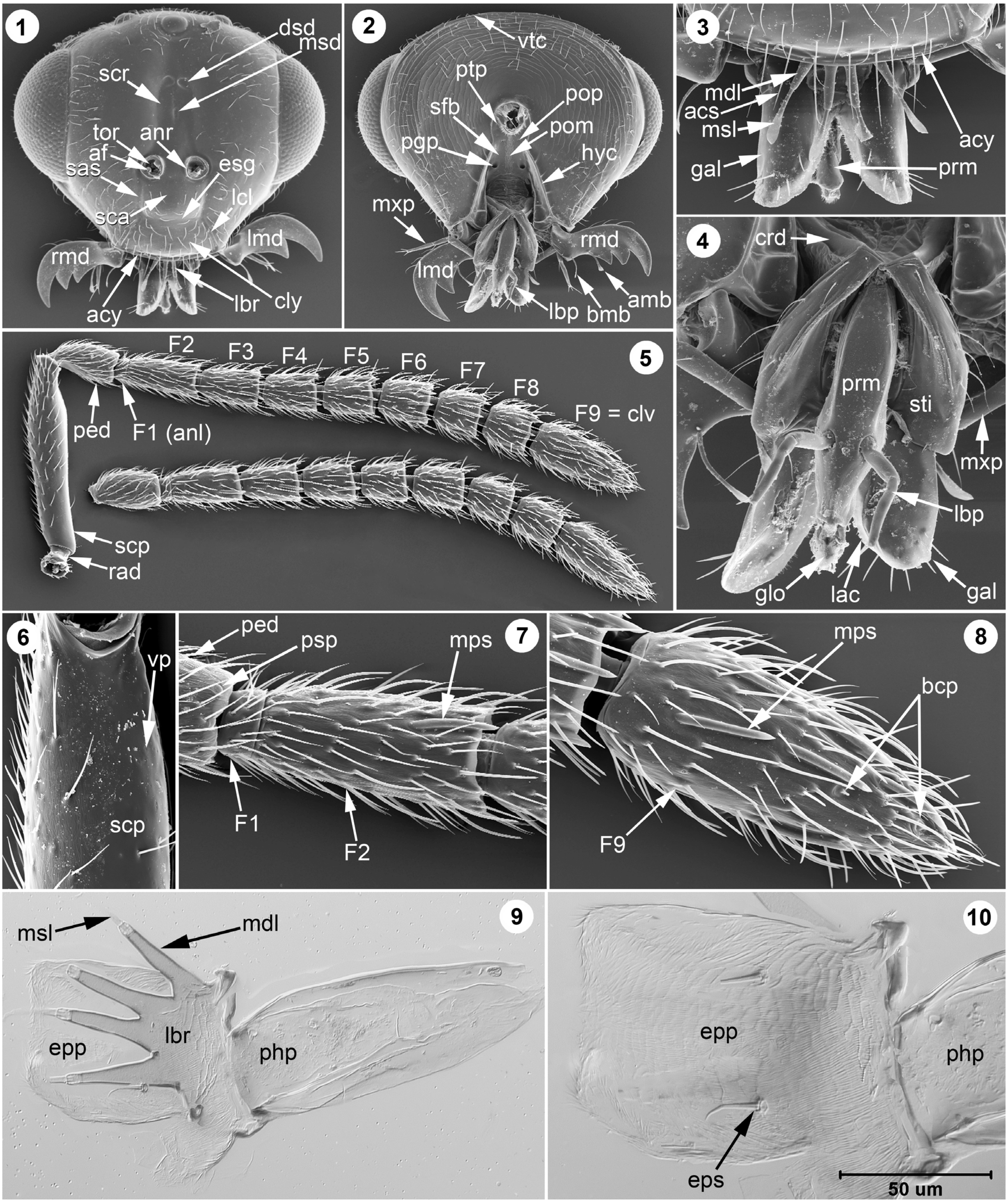
Structures associated with antennal attachment have received varying treatments in recent decades (Gibson 1997; Goulet & Huber 1993). We agree with the original use of the term "torulus" (Fig. 1: tor) as referring to a cavity (Kirby & Spence 1826: 366), and note that Kirby & Spence used the word *torus* in a different sense (=bed) from its currently more prevalent topological sense. The antennal rim (anr) is recognized as a structure encircling the torulus (Ronquist & Nordlander 1989). The antennifer (af) is the articular process on which the antenna pivots (MacGillivray 1923; Snodgrass 1942).

The scape in males has sensory pores on its ventral surface (Figs 6, 21: vp). Similar pores are found in Perilampidae, Gollumiellinae, only rarely in Oraseminae (undescribed species of *Orasema* Cameron), and some *Neolosbanus,* but are absent in Eucharitini; thus pores are likely plesiomorphic in Eucharitidae. Similar sensory structures are present in various Chalcidoidea (Heraty *et al.* 2013), but their shape and occurrence on a specialized ventral plaque is variable. The pedicel has a placoid sensillum (Fig. 7: psp). Basiconic peg sensilla (Fig. 8: bcp) occur very rarely in *P. afra*, as in other Eucharitidae and in Perilampidae, though they are present on the clava. We assume that their presence indicates remnants of an ancestral separation between clavomeres, noting that they occur at flagellomere apices in other Chalcidoidea (Heraty *et al.* 2013). The clava is composed of three fused segments, as evidenced by the three rows of multiporous plate sensilla (Fig. 8: mps), suggesting a total of 11 flagellomeres.

A single transverse carina is present across the vertex (Fig. 2: vtc). We prefer to use the term "vertexal carina" for this structure (Girault 1908). A similar carina has been called the "hyperoccipital carina" in Platygastroidea (Masner 1979), but this structure extends from one eye to another, whereas the carina in *P. afra* occurs entirely posterior to the eyes. Our primary goal in naming it here is to distinguish it from the occipital carina, which is a different structure that can co-occur with it.

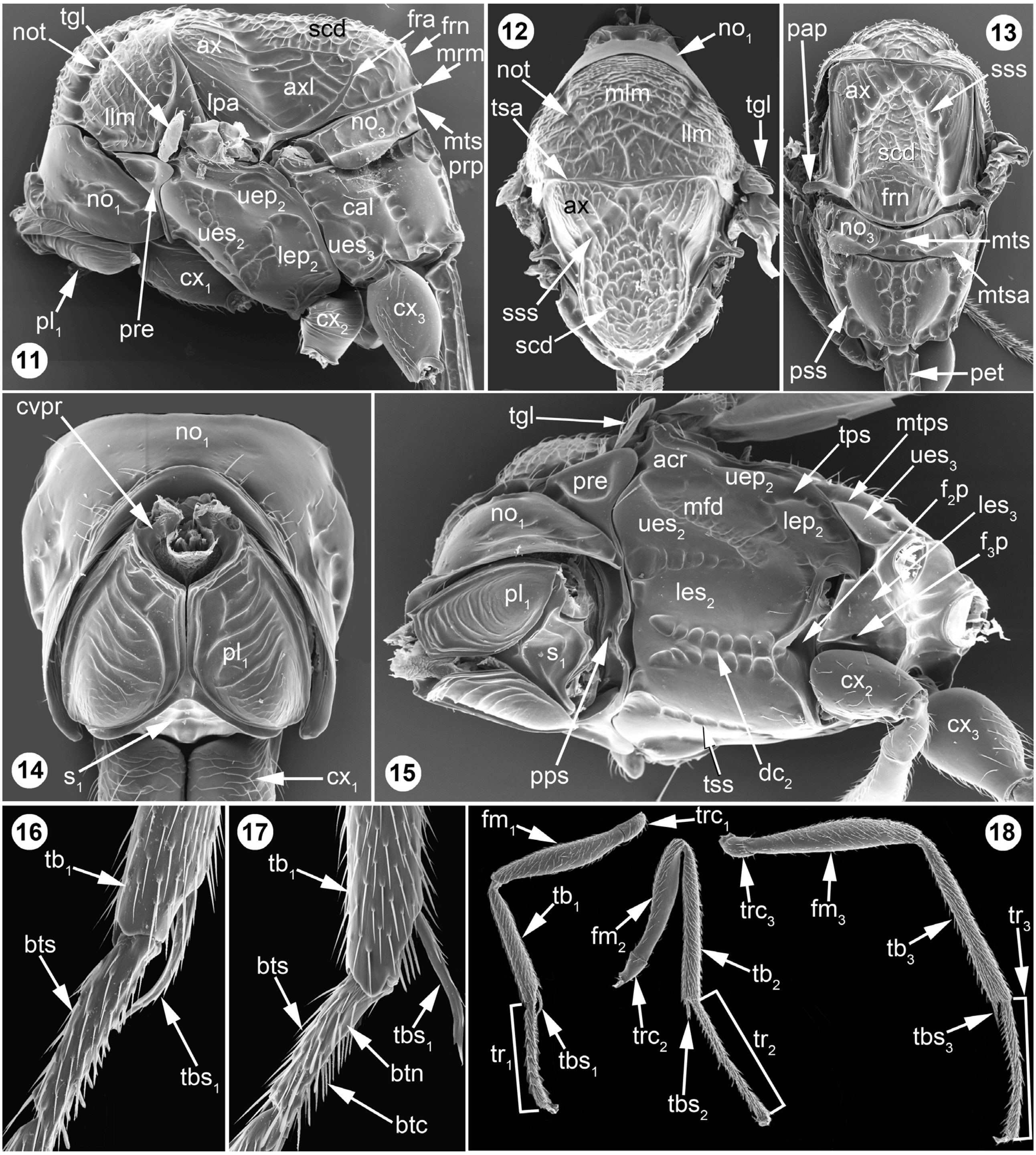
The "propleuron" of Apocrita has been called the proepisternum by many authors, including Snodgrass (1910b). This terminology ignores the fusion of the lateral cervical sclerites with the propleuron (Martin 1916), and little basis exists for determining the limits of a proepisternum in Apocrita. Matsuda (1970) referred to this structure as the "cervicopleuron," but this term has not entered common usage. We refer to this structure as the propleuron (Figs 11, 14, 15: $pl_1$). The cervical prominence (Fig. 14: cvpr) is recognized, as defined by Ronquist & Nordlander (1989). A pit is present on the ventral surface of the prepectus in *P. afra* (Fig. 15: pps), leading to an internal apodeme (Fig. 29: psa), and we follow Krogmann & Vilhelmsen (2006) in calling this the "prospinasternal apodeme."

While an epimeron is not generally indicated on the prothorax and metathorax in Chalcidoidea, it generally is indicated for the mesothorax in other Apocrita (Snodgrass 1910b). This is in conflict with Matsuda's (1970) statement that the mesepimeron is greatly reduced, and present at most as a narrow posterior strip in all higher Hymenoptera. Krogmann & Vilhelmsen (2006) and Vilhelmsen *et al.* (2010) instead found the mesopleural ridge to be unequivocally present in some chalcidoids as according to Snodgrass' indication, and we follow their interpretation here.



**FIGURES 1–10**. *Psilocharis afra*. **1**, head anterior, ♀; **2**, head posterior, ♀; **3**, mouthparts anterior, ♀; **4**, mouthparts posterior, ♀; **5**, antennae, lateral and medial, ♀; **6**, scape ventral, ♂; **7**, funicle base lateral, ♀; **8**, clava lateral, ♀; **9**, labral complex dorsal, ♀; **10**, epipharynx ventral, ♀.

The “scutellum” and “dorsellum” of most chalcidoid literature has sometimes been called the “mesoscutellum” and “metascutellum” in more recent literature (Krogmann & Vilhelmsen 2006), which more consistently reflects serial homology (Snodgrass 1910b). Typically in Chalcidoidea what is referred to as the scutellum is actually the (meso-) scutellar disc, as bounded by the axillae, axillulae and their sulci (if all are present), and the frenum, if present. The mesoscutellum refers to a combination of all of the above structures. Within Eucharitidae, separate use of the terms scutellar disc and frenum are preferred (Figs 11–13: scd, frn). We could find no indication of the scutellar disc sensilla, which are known to occur in both Gollumiellinae and Oraseminae (Romero & Heraty 2010).



**FIGURES 11–18**. *Psilocharis afra* ♀. **11**, mesosoma lateral; **12**, mesosoma dorsal; **13**, mesosoma posterior; **14**, propleural-complex ventral; **15**, mesosoma subventral; **16**, protibia and basitarsus medial; **17**, protibia and basitarsus lateral; **18**, pro-, mid- and metatibia.

The postalar process (Fig. 13: pap) has inconsistently been called the posterior notal wing process by many authors (history summarized by Ronquist & Nordlander 1989). It does not articulate directly with any axillary sclerite, but an apparent accessory sclerite closely approaches it (corresponding to the structur labeled as y in Snodgrass 1910a: fig. 26). The postalar process is continuous with the lateral surface of the axilla and with the posterior mesoscutellar rim.

The internal mesofurcal bridge (Fig. 30: $f_2b$) and associated musculature is the same as proposed by Heraty *et al.* (1997).

The term "frenal arm" (Fig. 11: fra) was used by Heraty *et al.* (2013) to reference a lateral area on the frenal line, useful as a landmark because it is visible in some species where the frenal line is not discernable medially. The Hymenoptera Anatomy Ontology (HAO_0001903) treats the frenal arm as synonymous with the "posterior bar of the mesoscutellum" *sensu* Karlsson & Ronquist (2012) under the concept "mesoscutellar arm." While we admit that a mesoscutellar arm (*sensu* HAO_0001903) could be reasoned to exist according to serial homology with the metascutellar arm *sensu* Vilhelmsen (2000a), this is not necessarily what is defined in that concept, and is not necessarily equivalent to the frenal arm. Vilhelmsen (2000a) labeled a posterior elevated area when establishing the concept of the scutellar arm as part of the metanotum (as a metascutellar arm), without reference to the mesoscutellum. The indication of a "posterior bar of the mesoscutellum" by Karlsson & Ronquist (2012) is of a raised surface along the posterior margin of the mesoscutellum in *Opius dissitus* Muesebeck (Braconidae), which may be synonymous with the posterior rim of the mesoscutellum. The posterior bar of the mesoscutellum is instead cited in HAO as synonymous with the frenal arm, which we regard as an anteromedial split from the mesoscutellar arm (which by our reckoning occurs lateral to this split and extends to the postalar process). Part of the reasoning behind the HAO's usage may stem from treating the frenum as separate from the mesoscutellar disc (Figs 11–13: scd), which could be defined as housing of the dorsomedian pulsatile organ in Hymenoptera (Krenn & Pass 1995; Karlsson & Ronquist 2012). However, we are not aware of any research addressing how the frenum would limit the pulsatile organ in certain species in which the frenal line is only a superficial structure that does not correspond to an internal frenal ridge (Krogmann & Vilhelmsen 2006). Furthermore, pulsatile organs were not found in *Encarsia formosa* Gahan (Aphelinidae), the sole chalcidoid examined in the study by Krenn and Pass (1995), and one that lacks a frenum. Therefore, we prefer to use the terminology established by Heraty *et al.* (2013) until the frenum is shown to correspond in all instances to a different concept.

The marginal rim of the mesoscutellum (Figs 11–13: mrm) co-occurs with a distinct frenum in *P. afra*. We consider it to represent the posteromedian part of the split in the mesoscutellar arm.

The metapectal-propodeal complex in Apocrita has been treated to several different terminology systems (Ferris 1940; Gibson 1997; Matsuda 1970; Ronquist & Nordlander 1989; Snodgrass 1910b, 1942). While the dorsal area of the metapectus is not separate from the propodeum in *P. afra*, some landmarks can be found. For instance, the metapleural wing process and lateral coxal articular process of the metapleuron are pleural structures that remain present and serve as landmarks indicating limits of the metepisternum (but not necessarily of the metapleuron). The lateral surface of the metepisternum can be called the upper metepisternum (Figs 11, 15: $ues_3$), and the ventral surface would therefore be the lower metepisternum (Fig. 15: $les_3$). The lower metepisternum was called the metasubpleuron by Ronquist & Nordlander (1989) and Karlsson and Ronquist (2012). We instead use terms consistent with our treatment of the mesopectus because we do not find clear indication of a subpleuron in chalcidoids.

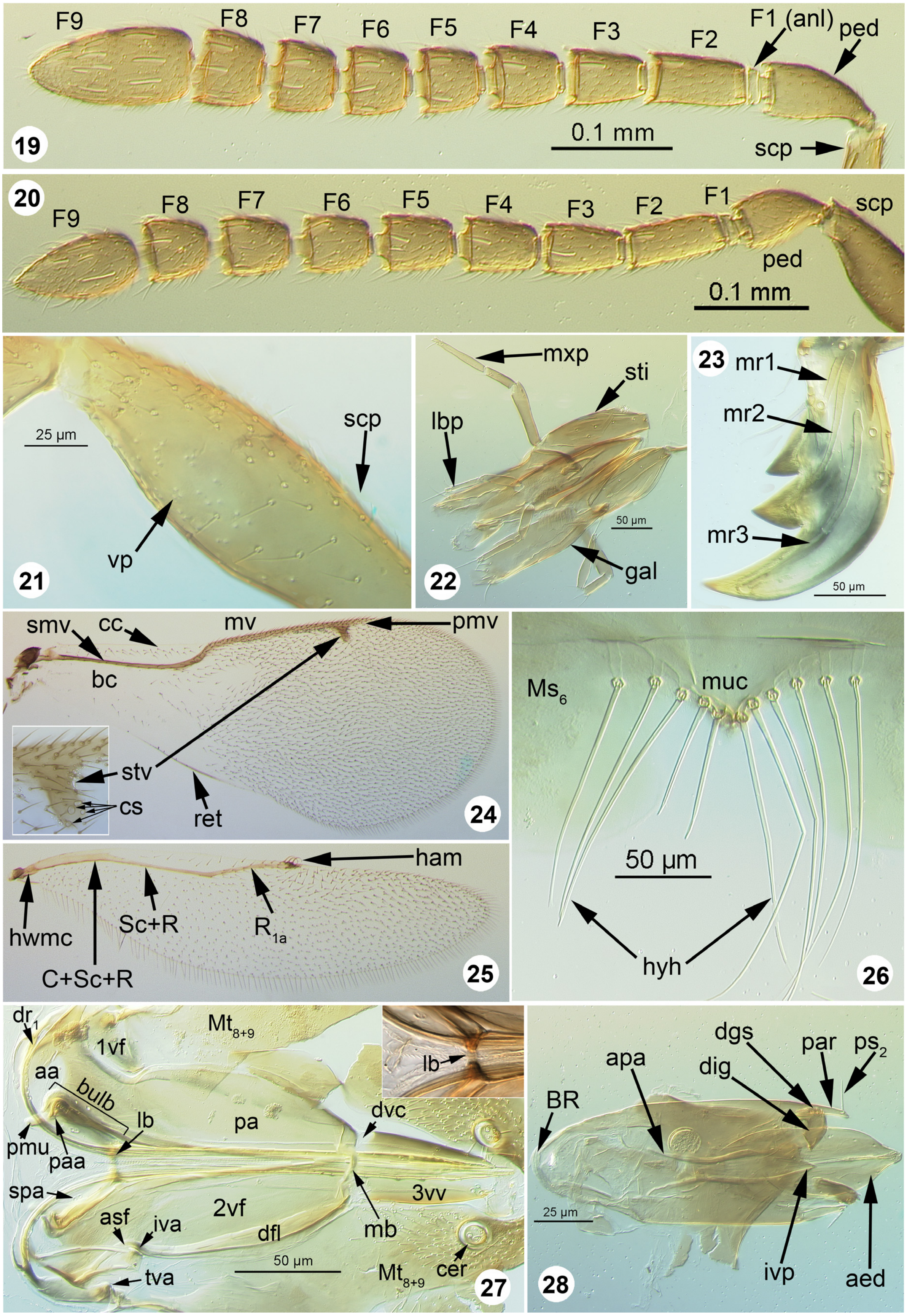
Characteristic of *Psilocharis*, the base of the petiole has the lateral margin carinate, but not the dorsal margin (Fig. 31). The petiole is elongate and the ventral apical margin articulates beneath the anterior margin of the first gastral sternite (Fig. 31). An antecostal sulcus across the first gastral sternite is absent. The hypopygium ($Ms_6$) in most species of *Psilocharis* has a distinctive transverse row of long hairs that encircle the base of the ovipositor (Figs 26, 31, 32).

The general structure of the ovipositor for Eucharitidae is as described and illustrated by Heraty & Quicke (2003), Heraty & Darling (2007), Heraty (2003), and Heraty *et al*. (2013), but here we try to harmonize our terminology for structures and muscles with Vilhelmsen (2000b) and Ernst *et al*. (2013). The articulating bulb in *Psilocharis* is an elongate structure composed proximally by the pars articularis (Fig 27: paa; 2nd valvifer in part) and the processus musculares (Fig 27: pmu; 2nd valvula in part). The bulbs are united across the median line posteriorly by a medially directed process connected to a transverse ligament (lb) (Fig. 27). The ligament is dorsal to the valvulae, and the bulb articulates distally with the shaft of the dorsal valvula (2vv). The connection across the bulbs is variable in Chalcidoidea (connection absent, laminated bridge or a sclerotized bar, character 223 of Heraty *et al*. 2013); this is the first report of medially directed processes. The third valvula (3vv) is separated from the second valvifer by an external suture and vertical conjunctiva (Figs 27, 32: dvc; character 46 of Heraty 1994 referred to as separation of gonostylus). In cross section, the ovipositor stylets of *Psilocharis* are similar to other Chalcidoidea with the dorsal valvula (2vv) dorsally cleft and overlapping (*cf.* Heraty & Quicke 2003). The sensillar patch (spa) of the anterior area of the second valvifer (2vf) has only two closely spaced sensilla (Fig. 27). Apically, the second valvifers are connected by a transverse, sclerotized median bridge (Figs 27, 34: mb; bridge of the inner plate (IPB) *sensu* Bucher 1948; transverse ridge of Heraty & Darling 2007; character 224 of Heraty *et al.* 2013). The first valvifer is the preferred term instead of gonangulum as applied by Heraty (2003) and Heraty & Darling (2007). The valvulae in *P. afra* are acicular. The dorsal (2nd) valvula (dv) has a set of oblique apical annuli, and basally articulates with the ventral (1st) valvula (vv) by the aulax (aul); the paired ventral valves have a series of blunt, relatively evenly spaced lateral teeth (tooth-shaped annuli) and a few scattered campaniform sensilla (Fig. 32).

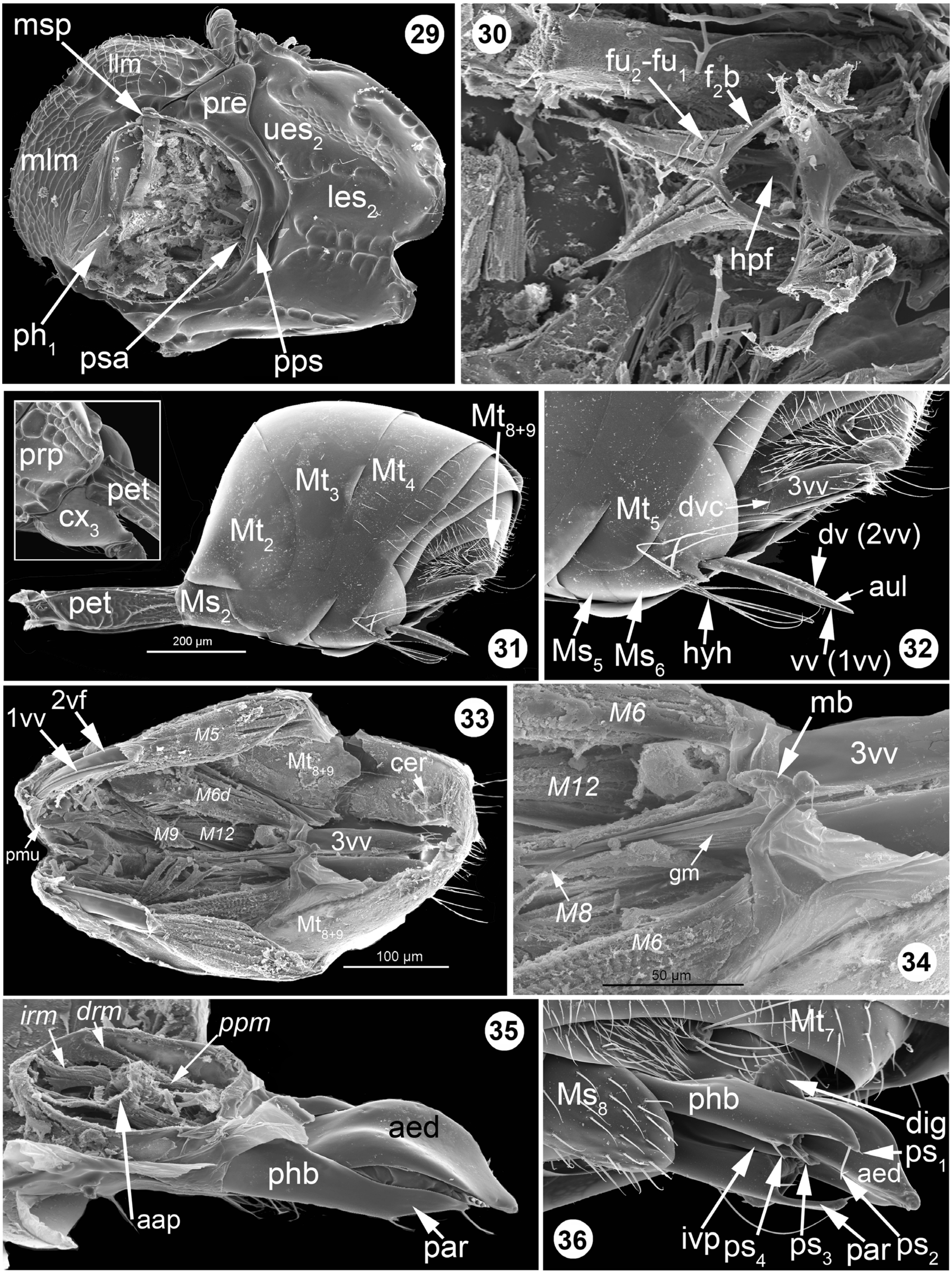
We were able to identify four muscle groups of the ovipositor (Fig. 33: *M5, M6d, M9, M12*). We follow Vilhelmsen (2000b) for naming of muscles. In *Psilocharis* and most other Chalcidoidea, $Mt_8$ and $Mt_9$ are fused, but we follow other studies that suggest that muscles connect to $Mt_8$ (T9 of Ernst *et al.* 2013). *M5* originates on the dorsal surface of $Mt_8$ and inserts on the anterior section of the dorsal flanges of the second valvifer (HAO_0001569). *M6d* originates on the anterior surface of $Mt_8$ and inserts on the posterior area of the second valvifer and in part onto the median bridge. Vilhelmsen (2000b) reports two pairs of ventral T9 ($Mt_8$)-second valvifer muscles (M6), with the dorsal pair connecting T9 with the "2nd valvifers, close to the median bridge". We find no evidence of the lower muscle (HAO_0001616), and *M6d* does not have a separate entry in the HAO. *M9* originates on the margin of the first valvifer and inserts on the genital membrane (cited as M8 in Ernst *et al.* 2013; HAO_001746). *M12* (cited as M9 in Ernst *et al.* 2013; HAO_0001815) originates on the posterior part of the 2nd valvifer and inserts on the processus muscularis (pmu) of the 2nd valvula.

Treatments of the male genitalia of Hymenoptera have employed an exceedingly large and confusing array of terms, in part because of differing theories of male genitalic origin in insects (Michener 1944, 1956; Snodgrass 1941, 1957), but also because of varying patterns of usage (Yoder *et al.* 2010). In this case, we choose Snodgrass' terms because they have been used often for Chalcidoidea (Bouček 1952; Dominichini 1953; Gibson 1997; Pinto 1992). Snodgrass (1941) used the word "caulis" as a near equivalent to his concept of the phallobase, to refer to the structure sometimes referred to as the "genital capsule" in chalcidoid literature (Pinto 1992). Snodgrass apparently used "caulis" only for cases when a basal ring, parameres, and volsellae (=digitus, Fig. 36: dig) were not recognizable as separate entities. We call this structure the phallobase (Figs 35, 36: phb) in *P. afra*. The parameres (Figs 28, 35, 36: par) each bear four apparent sensilla, but homology of these sensilla across Chalcidoidea (much less other Hymenoptera) is dubious. The apical parameral sensillum (Fig. 36: $ps_1$) is very small in *P. afra* and may not be the same as the structure found in Chalcididae (Delvare 1987). A longer subapical parameral sensillum (Fig. 36: $ps_2$) is also present, as well as basal and subbasal parameral sensilla (Fig. 36: $ps_3$, $ps_4$).

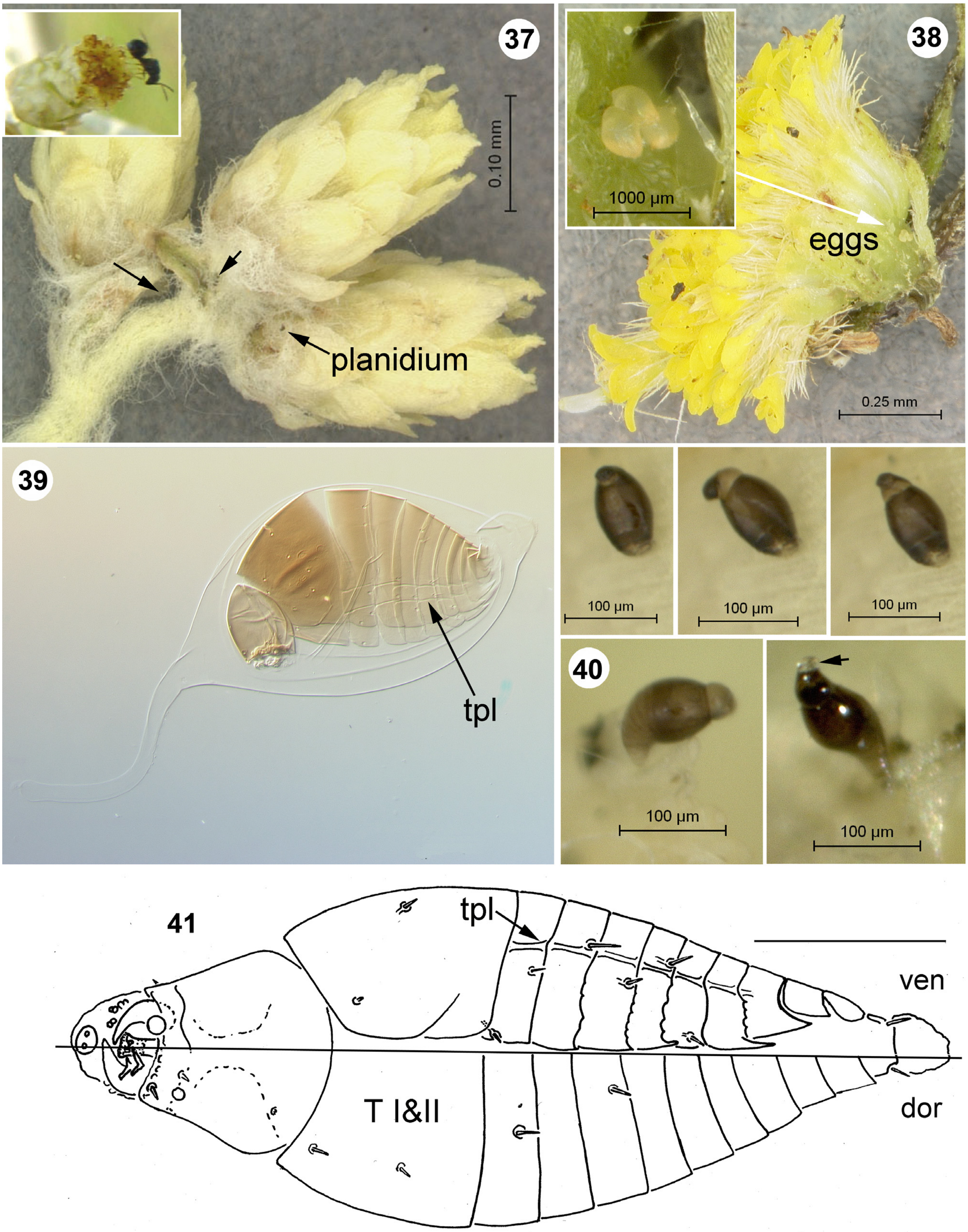
Musculature of the aedeagus in chalcidoids has been discussed by Sanger & King (1971) and Chiappini & Mazzoni (2000). Our observations most closely fit those of Sanger & King, whose designations we discuss along with those of Snodgrass (1941). The aedeagus in most Chalcidoidea is associated with a pair of long apodemes that extend into the phallobase. We document a pair of intrinsic retractor muscles (*irm*) that originate on the anterior margin of the phallobase and attach to the apex of the aedeagal apodeme (aap), and another pair of protractors (*ppm*) that originate on the posterior area of the phallobase (Fig. 35). These correspond to muscles 8 (retractor) and 10 (protractor) of Snodgrass (1941, fig. 6B). Muscles 2 (protractor) and 5 (retractor) of Sanger & King (1974, fig. 8) originate on what Snodgrass termed the "proximal ends of the parameral plate" and the "posterior mesal parts of the parameral plates", respectively. The proximal attachment of the retractor suggests that the cupula (=basal ring, HAO_0000238) in *Psilocharis* is reduced to at most the posterior dorsal rim, as illustrated for *Torymus* Dahlbom (Torymidae) (under its junior synonym *Callimome* Spinola, fig. 8L: BR, in Snodgrass 1941). The protractors cause extension of the aedeagus. The digitus repressor muscle (*drm*) on either side of the aedeagus likely corresponds to muscle 19 of Snodgrass (1941), and likely muscle 8 of Sanger & King (1974), which originates on the inner wall of the phallobase and attaches to the digitus.



**FIGURES 19–28**. *Psilocharis afra*. **19**, antenna lateral, ♀; **20**, antenna lateral, ♂; **21**, scape subventral, pedicel in upper left, ♂; **22**, labial complex, ♀; **23**, right mandible, ♀; **24**, fore wing, inset enlargement of stigmal vein, ♀; **25**, hind wing, ♀; **26**, hypopygium, ♀; **27**, ovipositor, inset is dorsal aspect of apical connection of articulating bulb; **28**, male genitalia.



**FIGURES 29–36**. *Psilocharis afra*. **29**, mesosoma subventral, pronotum and propleuron removed, ♀; **30**, mesofurcal complex dorsal view, ♀; **31**, metasoma lateral, inset dorsolateral of propodeum and petiole, ♀; **32**, apex of metasoma ventrolateral, ♀; **33**, dissected ovipositor complex dorsal; **34**, ovipositor complex enlargement showing sclerotized connecting bridge (mb) dorsolateral; **35**, dissected male genitalic complex, dorsolateral, showing both anterior and posterior paired longitudinal muscles attaching to the anterior apex of the aedeagal apodeme (aap); **36**, male genitalic complex, ventrolateral.



**FIGURES 37–41**. *Psilocharis afra*. **37**, planidia and eggs with developed planidia in trichome mass at base of flowers of *Helichrysum*, inset of female on flower head; **38**, eggs under involucral bracts of *Nidorella*, inset is enlargement of undeveloped eggs; **39**, egg with mature planidium; **40**, single planidium, resting in upper left with remaining images of movement after disturbance, arrow pointing to expanded postlabial complex; **41**, first-instar ventral (ven) and dorsal (dor).

## Biology and description of immatures

*Oviposition*. Small clusters of unhatched mature eggs (Figs 37, 38) containing developed planidia (Fig. 39), and emerged planidia (Fig. 40) were discovered among the woolly trichome mass at the base of the flowers of *Helichrysum nudifolium* (Fig. 37). A small cluster of three undeveloped eggs were found under one of the most basal bracts of a flower of *Nidorella auriculata* (Fig. 38). Eggs were not inserted into the plant tissue but instead were placed among the trichomes, and appeared to be have been placed under the bracts (no oviposition scar apparent). Eggs or planidia were not found in the flower heads; females were observed walking on the flower apex (Fig. 37 inset), possibly attracted to an exudate observed on the flower petals.

Placing eggs rather than using the ovipositor to cut through or into plant tissue before oviposition may be a common feature of the genus. In Monga State Forest in Australia in 2002 JMH found single eucharitid eggs placed into the curled edge of fronds of bracken fern. However, two species, a species of *Chalcura* Kirby (Eucharitinae) and *Psilocharis theocles* (Walker), were both common at the site and oviposition could not be attributed directly to either species, although this would be an unusual behavior for *Chalcura* (*cf.* Clausen 1940b, *C. deprivata* Walker oviposits into flower buds; JMH with unpublished records of *Chalcura* oviposition into flower buds of *Acacia* (Acacieae) and *Exocarpos* (Santalaceae)). Within Eucharitidae, placing eggs, as opposed to ovipositing into or through tissue, is known only in members of the Kapala Clade, which is a derived group of Eucharitini (Torréns & Heraty 2013; Torréns *et al.* 2016). Among outgroups, the behavior of placing eggs is somewhat similar to some species of *Perilampus* Latreille (Perilampinae) that oviposit onto the undersurface of leaves (Delvare 1987) or into the entrance holes of gall-forming Lepidoptera (Clausen 1940b; Laing & Heraty 1981).

*Egg* (Fig. 39). Smooth and fusiform with an elongate stalk that is slightly shorter than the egg body; pale yellowish-white when undeveloped (similar to other Eucharitinae and *Orasema* (Oraseminae)). The stalk is cylindrical and not apically enlarged as in some Eucharitinae (Heraty & Darling 1984; Torréns & Heraty 2013). An evenly cylindrical stalk occurs in both Oraseminae (*cf.* Burks *et al.* 2018) and *Neolosbanus* (Psilocharitini) (Heraty *et al.* 2004).

*First-instar larva* (Figs 39–41). The morphology and setal pattern of the planidium is typical for Eucharitinae (Clancy 1946): an oval prelabium, expandable postlabium (Fig. 40, arrow), posterior labial plate rectangular (not hatchet shaped), tergites I&II dorsally fused, tergopleural line present and separating the pleural and dorsal tergites (Figs 39, 41: tpl), and 12 tergites. The cranium has two pairs of small pleurostomal spines and two pairs of circular sensilla. The tergal setal pattern is four pairs on TI&II, 3 pairs on TIII, and pairs of 1, 2, 1 and 1 on the following tergites. Unusual for Eucharitinae, the posteromedial margins of TVI–VIII are only slightly produced and the posterior cerci are shorter than length of TXII. Larvae were observed to stand erect, resting with their head tucked downward. When the nearby area was disturbed by an insect pin the planidium would raise its head, extrude the labial complex (Fig. 40, arrow) and remain erect but move in a circular motion (captured in a series of images in Fig. 40; at rest in upper left image). Jumping by the planidia, as observed in several Eucharitinae (*cf.* Torréns 2013), was not observed. Both the dorsal fusion of tergites I&II and the tergopleural line are known for both Gollumiellinae and other Eucharitinae, but within Oraseminae the tergites are never fused, and a vague tergopleural line occurs only in one species related to *Orasema tolteca* Mann.

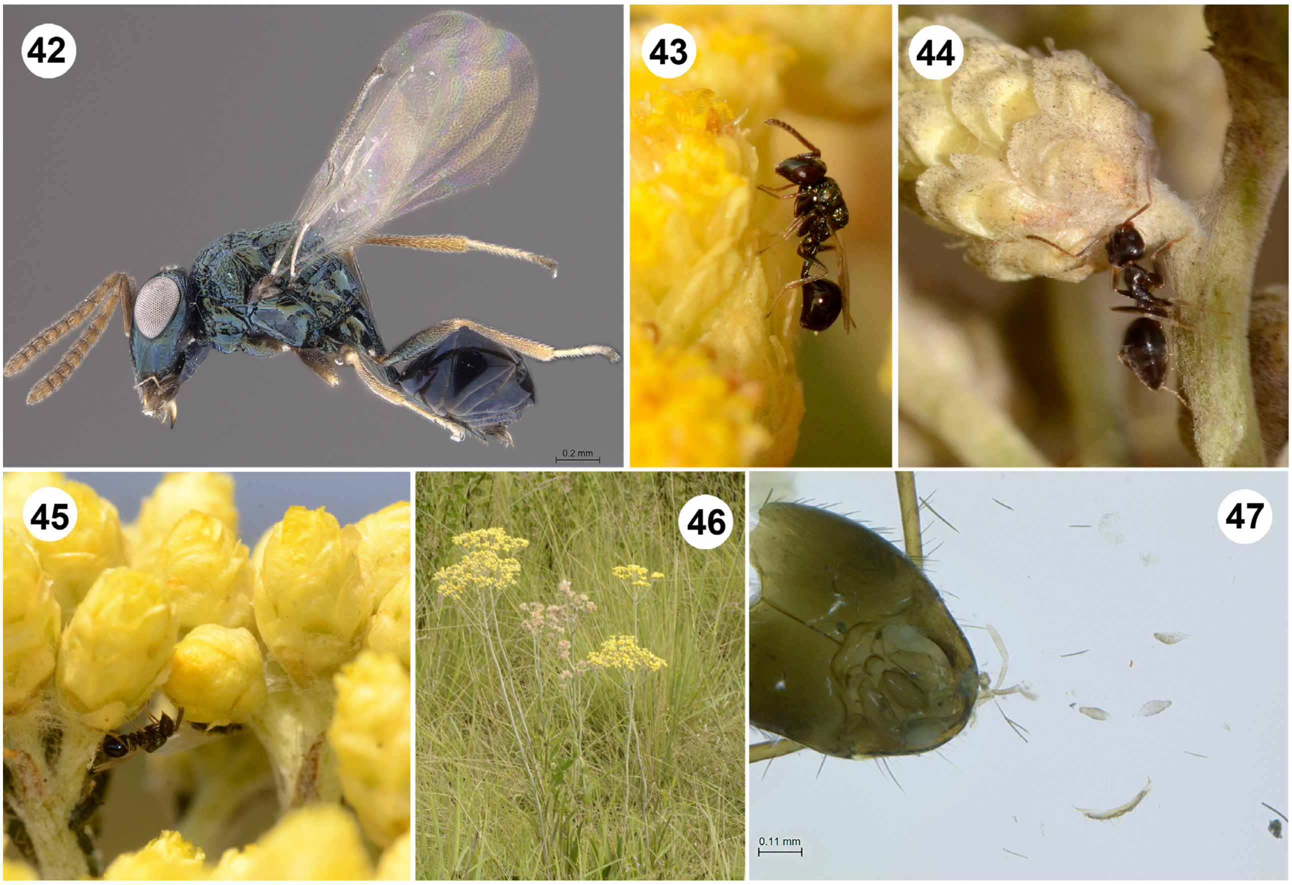
*Field Observations*. Both females and males of *Psilocharis afra* were observed visiting flowers of *Helichrysum nudifolium* (Figs 43, 45, 46) and less commonly *Nidorella auriculata*. They routinely patrolled the *Helichrysum* flower inflorescence spending time below the individual flower heads on the stems and bracts as well as walking on the top of the inflorescence where they were observed feeding at the florets by pushing their head down into the flower, presumably on nectar. Adults of *Psilocharis* were observed crawling up grass stems from near ground level at the base of a *Helichrysum* plant and taking flight to alight on the flower heads. Two ant nests were located at the base of this plant (nest 15: unidentified species of *Parasyscia* Emery; nest 16: *Nylanderia natalensis* Forel). The latter nest was situated in the root stock of the plant. No wasps were seen emerging from either nest entrance.

A specific observation on a female that landed on a flower head entailed her exploring multiple flower heads for a period of 9 minutes, constantly antennating while walking around at the bract base and flower peduncle, only rarely going onto the top of the exposed flower. She then selected two oviposition sites on adjacent flower heads. Each oviposition event took 30–40 seconds before she moved on to continue searching on other flower heads. She occasionally took flight for about 5 seconds before landing again. She also appeared to hop (short flight?) between closely adjacent flower heads within a single umbellifera. This activity was repeated with 6 oviposition events being observed over a period of about one hour, at the end of which she was captured and eaten by a crab spider, bringing the oviposition event to a premature end.

Numerous ant species were observed on the flower heads, often tending aphids. Flower visitation by *Psilocharis* was independent of whether ants and/or aphids were present and when present there was no deliberate interaction. Ant presence was not a precursor for oviposition. *Psilocharis* were, however, observed to fall prey to predators residing on the flower heads, such as Crab spiders (Thomisidae) and Jumping spiders (Salticidae). One wasp was observed being eaten by a salticid and 3 by thomisids.

Numerous copulation events were observed on flower heads of *Helichrysum nudifolium* in the bract area where females spent most of their time. Males were observed to mount females and remained in copula for about one minute, after which they dismounted and searched for additional females. Males fought each other for the opportunity to mate, particularly with males that were *in copula*. Competing males would physically try to dislodge a copulating male from his mate. It appeared that once a female was mated other attempts at copulation were unsuccessful. Males would mount a mated female, but within a couple of seconds would move off.

None of the planidia were observed to interact with a potential intermediate host. In species that are proposed to use an immature thrips (Thysanoptera) as an intermediate host, unattacked thrips and thrips with attached planidia are common in dissected flower heads (Johnson *et al.* 1986). No immature or mature thrips were observed within the flowers of the oviposition hosts. One immature and one adult thrips (Phlaeothripidae) were swept from the flowers but without attached planidia. The placement of the eggs at the base of the flowers makes any association with a thrips host unlikely.



**FIGURES 42–47**. **42**, *Psilocharis afra* ♂, habitus. **43**, *P. afra* ♂ on flower; **44**, *Nylanderia natalensis* at base of flower; **45**, *P. afra* ♀ ovipositing at base of flower; **46**, *Helichrysum nudifolium*; **47**, three planidia of *Gollumiella longipetiolata* squeezed from mouthparts of *Nylanderia* sp. in Malaysia.

Ants were collected both by sweeping the two identified host plants and by hand collection of ant colonies. Sweeping in 2006 yielded adults of three genera of Formicinae: *Camponotus* Mayr (7 individuals), *Plagiolepis* Mayr (11) and *Lepisiota* Santschi (17), and two of Myrmicinae: *Pristomyrmex* Mayr (1) and *Tetramorium* Mayr (1)

(Table 2). These foraging ants were examined externally and internally (within the head) for evidence of planidia, but none were found. Ant colonies sampled in 2006 and 2016 (Table 2) included two genera of Dorylinae: *Dorylus* Fabricius and *Parasycia* Emery (no brood); one genus of Dolichoderinae: *Technomyrmex* Mayr (2 colonies, pupae without cocoons); four genera of Formicinae: *Camponotus* (9 colonies, with cocoons), *Lepisiota* (10 colonies, pupae with cocoons), *Nylanderia* Emery (14 colonies, pupae without cocoons) and *Plagiolepis* (7 colonies, pupae with cocoons); seven genera of Myrmicinae: *Crematogaster* Lund (8 colonies, pupae without cocoons), *Monomorium* Mayr (2 colonies, pupae without cocoons), *Myrmicaria* Saunders (7 colonies, larvae but no pupae), *Carebara* Mayr (1 colony, pupae without cocoons), and *Pheidole* Westwood (10 colonies with numerous larvae and pupae without cocoons), *Solenopsis* Westwood (26 colonies, pupae without cocoons) and *Tetramorium* (15 colonies, without cocoons); and two genera of Ponerinae: *Bothroponera* Mayr (7 colonies, pupae with cocoons) and *Hypoponera* (9 colonies, with cocoons). No immatures of Eucharitidae were found in any of the ant colonies sampled despite sampling a broad time period in 2016. Of these ants, *Camponotus*, *Lepisiota*, *Myrmicaria, Bothroponera* and *Pristomyrmex* are likely too large to serve as hosts for *P. afra,* and *Solenopsis* and *Technomyrmex* are too small. Of the remaining ants sampled, none have been previously recorded as a eucharitine host. *Pheidole* is a potential host, but large numbers of brood were collected with no indication of active or previous parasitism, which among myrmicine hosts can be observed by deformed, fed-upon ant pupae remaining alive within in the nest (*cf.* Heraty & Darling 1984). *Nylanderia* is a host for *Gollumiella longipetiolata* Hedqvist (Heraty *et al*. 2004). Planidia of *G. longipetiolata* are collected by the adult ants and transferred to the brood in the infrabuccal pocket within the ant head, which was observed by dissecting the heads of adult ants (Fig. 47) from the ant collections made in Heraty *et al*. (2004). *Nylanderia* adults were common on the flowers and were found nesting at the base of the *Helichrysum* plants. Adults were observed probing the base of the flowers where eggs and planidia were observed (Fig. 44); however, none of the brood or adults had any evidence of planidial attachment.

**TABLE 2.** Ants collected either through field sweeping (f) or by excavating colonies (c) in 2006 or 2016, and whether or not their brood had cocoons at the Pinnacle Rock site near Graskop. An asterisk marks ants with brood that was too small to be a host of *Psilocharis*; an x marks presence of the ants in field sweeps.

| ant subfamily | ant species | February 2006f | February 2006c | February 2016cF | December 2016cD | with cocoon |
|---|---|---|---|---|---|---|
| Dolichoderinae | *Technomyrmex* sp. | | | 1 | 1 | no* |
| Dorylinae | *Dorylus* sp. | | | | 1 | no brood |
| Dorylinae | *Parasycia* sp. | | | 1 | | ? |
| Formicinae | *Camponotus n. irredux* | x | 1 | 7 | 1 | yes |
| Formicinae | *Lepisiota* sp. | x | 4 | 2 | 4 | yes |
| Formicinae | *Nylanderia natalensis* | | 1 | 10 | 3 | yes |
| Formicinae | *Plagiolepis* sp. | x | 2 | 1 | 3 | yes* |
| Myrmicinae | *Crematogaster* sp. | | 1 | 6 | 1 | no |
| Myrmicinae | *Monomorium* sp. | | | 1 | 1 | no |
| Myrmicinae | *Myrmicaria faurei* | | 1 | 6 | | no |
| Myrmicinae | *Carebara* sp. | | 1 | | | no |
| Myrmicinae | *Pheidole* spp. | | 2 | 5 | 3 | no |
| Myrmicinae | *Solenopsis* spp. | | | 12 | 14 | no* |
| Myrmicinae | *Tetramorium akermani* | | | 3 | | no |
| Myrmicinae | *Tetramorium bothae* | | | | 3 | no |
| Myrmicinae | *Tetramorium frigidum* | | | 4 | 1 | no |
| Myrmicinae | *Tetramorium* sp. | x | | | 1 | no |
| Ponerinae | *Bothroponera granosa* | | 1 | 4 | 2 | yes |
| Ponerinae | *Hypoponera* sp. | | | 7 | 2 | yes |
| Ponerinae | *Leptogenys intermedia* | | | 1 | 2 | yes |

## Conclusion

*Psilocharis* occupies a unique and somewhat ambiguous phylogenetic position within the family Eucharitidae. Morphological characters of both the first-instar larvae and adults are shared both with the more basal subfamilies Gollumiellinae and Oraseminae, and within Eucharitinae, both *Neolosbanus* (Psilocharitini) and the Eucharitini. Oviposition by placing eggs into tufts of trichomes or under bracts at the base of a flower head without penetrating the plant tissue is unique within the Eucharitinae, and is similar in behavior to the habits of chrysolampine parasitoids of weevils in flower heads (Darling & Miller 1991). The searching behavior of the planidia (resting in an upright position and showing activity when disturbed) is similar to other Eucharitidae and appears aimed at attaching to foraging ant hosts. Given the morphological similarity of adults to the fossil *Palaeocharis*, this perhaps gives us insights into the first behaviors of the family and ones that have been in place for over 40 million years. To better track evolutionary change in the ant parasitoids it is critical that we establish some of the key traits in both the ant host (subfamily and presence or absence of a cocoon) and behavior and morphology of the immature stages, especially whether the planidum is an ectoparasitoid or endoparasitoid on its larval host. While it is disheartening not to discover if the host of this critically placed taxon belongs to either Formicinae or Myrmicinae, hopefully our discussion of potential ant hosts can direct future investigators toward resolving the correct ant host relationships for this genus.

## Acknowledgements

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## References

Ayre, G.L. (1962) *Pseudometagea schwarzii* (Ashm.) (Eucharitidae: Hymenoptera), a parasite of *Lasius niger* Emery (Formicidae: Hymenoptera). *Canadian Journal of Zoology*, 40, 157–164.
https://doi.org/10.1139/z62-020

Bouček, Z. (1952) The first revision of the European species of the family Chalcididae (Hymenoptera). *Acta Entomologica Musei Nationalis Pragae*, 27, 1–108.

Bucher, G.E. (1948) The anatomy of *Monodontomerus dentipes* BOH., an entomophagous chalcid. *Canadian Journal of Research*, 26, 230–281.
https://doi.org/10.1139/cjr48d-020

Burks, R.A., Heraty, J.M., Dominguez, C. & Mottern, J.L. (2018) Complex diversity in a mainly tropical group of ant parasitoids: Revision of the *Orasema stramineipes* species group (Hymenoptera: Chalcidoidea: Eucharitidae). *Zootaxa*, 4401 (1), 1–107.
https://doi.org/10.11646/zootaxa.4401.1.1

Burks, R.A., Heraty, J.M., Mottern, J., Dominguez, K. & Heacox, S. (2017) Biting the bullet: revisionary notes on the Oraseminae of the Old World (Hymenoptera, Chalcidoidea, Eucharitidae). *Journal of Hymenoptera Research*, 55, 139–188.
https://doi.org/10.3897/jhr.55.11482

Chiappini, E. & Mazzone, E. (2000) Differing morphology and ultrastructure of the male copulatory apparatus in species-groups of *Anagrus* Haliday (Hymenoptera: Mymaridae). *Journal of Natural History*, 34, 1661–1676.
https://doi.org/10.1080/00222930050117549

Clancy, D.W. (1946) The insect parasites of the Chrysopidae (Neuroptera). *University of California Publications in Entomology*, 7, 403–496.

Clausen, C.P. (1940a) The immature stages of Eucharidae (Hymenoptera). *Journal of the Washington Academy of Sciences*, 30, 504–516.

Clausen, C.P. (1940b) The oviposition habits of the Eucharidae (Hymenoptera). *Proceedings of the Entomological Society of Washington*, 12, 504–516.

Darling, D.C. (1988) Comparative morphology of the labrum in Hymenoptera: the digitate labrum of Perilampidae and Eucharitidae (Chalcidoidea). *Canadian Journal of Zoology*, 66, 2811–2835.
https://doi.org/10.1139/z88-409

Darling, D.C. & Miller, T.D. (1991) Life history and larval morphology of *Chrysolampus* (Hymenoptera: Chalcidoidea: Chrysolampinae) in western North America. *Canadian Journal of Zoology*, 69, 2168–2177.
https://doi.org/10.1139/z91-303

Darling, D.C. & Roberts, H. (1999) Life history and larval morphology of *Monacon* (Hymenoptera: Perilampidae), parasitoids of ambrosia beetles (Coleoptera: Platypodidae). *Canadian Journal of Zoology*, 77, 1768–1782.
https://doi.org/10.1139/z99-155

Delvare, G. (1987) Some important morphological features of the Chalcidini (Hymenoptera: Chalcididae) and their implications in the classification of the tribe. *In*: Gupta, V.K. (Ed.), *Advances in Parasitic Hymenoptera Research: Proceedings of the Second Conference on the Taxonomy and Biology of Parasitic Hymenoptera*, *Gainsville*, *November 1987*. E. J. Brill, Leiden, pp. 25–64.

Dominichini, G. (1953) Studio sulla morfologia dell'addome degli Hymenoptera Chalcidoidea. *Bollettino di Zoologia Agraria e Bachicoltura*, 19, 183–298.

Ernst, A., Mikó, I. & Deans, A.R. (2013) Morphology and function of the ovipositor mechanism in Ceraphronoidea (Hymenoptera, Apocrita). *Journal of Hymenoptera Research*, 33, 25–61.
https://doi.org/10.3897/JHR.33.5204

Ferris, G.F. (1940) The myth of the thoracic sternites of insects. *Microentomology*, 5, 87–90.

Gibson, G.A.P. (1997) Chapter 2. Morphology and terminology. *In:* Gibson, G.A.P., Huber, J.T. & Woolley, J.B. (Eds.), *Annotated keys to the genera of Nearctic Chalcidoidea (Hymenoptera)*. National Research Council of Canada Research Press, Ottawa, 794 pp.

Girault, A.A. (1908) A monographic catalogue of the mymarid genus *Alaptus* Haliday, with descriptions of three new North American forms and of *Alaptus iceryae* Riley from type material. *Annals of the Entomological Society of America*, 1, 179–195.
https://doi.org/10.1093/aesa/1.3.179

Goulet, H. & Huber, J.T. (1993) *Hymenoptera of the World: an identification guide to families*: Agriculture Canada Research Branch, Ottawa, 668 pp.

Heraty, J.M. (1994) Classification and evolution of the Oraseminae in the Old World, with revisions of two closely related genera of Eucharitinae (Hymenoptera: Eucharitidae). *Life Sciences Contributions*, *Royal Ontario Museum*, 157, 1–174.
https://doi.org/10.5962/bhl.title.53489

Heraty, J.M. (2000) Phylogenetic relationships of Oraseminae (Hymenoptera: Eucharitidae). *Annals of the Entomological Society of America*, 93, 374–390.
https://doi.org/10.1603/0013-8746(2000)093[0374:PROOHE]2.0.CO;2

Heraty, J.M. (2002) A revision of the genera of Eucharitidae (Hymenoptera: Chalcidoidea) of the World. *Memoirs of the American Entomological Institute*, 68, 1–359.

Heraty, J.M. (2003) Three new species of *Gollumiella* Hedqvist (Hymenoptera: Eucharitidae). *Zootaxa*, 497 (1), 1–10.
https://doi.org/10.11646/zootaxa497.1.1

Heraty, J.M., Burks, B.D., Cruaud, A., Gibson, G., Liljeblad, J., Munro, J., Rasplus, J.-Y., Delvare, G., Janšta, P., Gumovsky, A. V., Huber, J.T., Woolley, J.B., Krogmann, L., Heydon, S., Polaszek, A., Schmidt, S., Darling, D.C., Gates, M.W., Mottern, J.L., Murray, E., Dal Molin, A., Triapitsyn, S., Baur, H., Pinto, J.D., van Noort, S., George, J. & Yoder, M. (2013) A phylogenetic analysis of the megadiverse Chalcidoidea (Hymenoptera). *Cladistics*, 29, 466–542.
https://doi.org/410.1111/cla.12006.

Heraty, J.M. & Darling, D.C. (1984) Comparative morphology of the planidial larvae of Eucharitidae and Perilampidae (Hymenoptera: Chalcidoidea). *Systematic Entomology*, 9, 309–328.
https://doi.org/10.1111/j.1365-3113.1984.tb00056.x

Heraty, J.M. & Darling, D.C. (2007) A new genus and species of Perilampidae (Hymenoptera: Chalcidoidea) with uncertain placement in the family. *Journal of the Entomological Society of Ontario*, 138, 33–47.

Heraty, J.M. & Darling, D.C. (2009) Fossil Eucharitidae and Perilampidae (Hymenoptera: Chalcidoidea) from Baltic Amber. *Zootaxa*, 2306, 1–16.

Heraty, J.M. & Hawks, D. (1998) Hexamethyldisilazane: A chemical alternative for drying insects. *Entomological News*, 109, 369–374.

Heraty, J.M., Hawks, D., Kostecki, J.S. & Carmichael, A.E. (2004) Phylogeny and behaviour of the Gollumiellinae, a new subfamily of the ant-parasitic Eucharitidae (Hymenoptera: Chalcidoidea). *Systematic Entomology*, 29, 544–559.
https://doi.org/10.1111/j.0307-6970.2004.00267.x

Heraty, J.M. & Murray, E. (2013) The life history of *Pseudometagea schwarzii*, with a discussion of the evolution of endoparasitism and koinobiosis in Eucharitidae and Perilampidae (Chalcidoidea). *Journal of Hymenoptera Research*, 35, 1–15.
https://doi.org/10.3897/JHR.35.6025

Heraty, J.M. & Quicke, D.L.J. (2003) Phylogenetic implications of ovipositor structure in Eucharitidae and Perilampidae (Hymenoptera: Chalcidoidea). *Journal of Natural History*, 37, 1751–1764.

https://doi.org/10.1080/00222930210130320

Heraty, J.M., Woolley, J.B. & Darling, D.C. (1997) Phylogenetic implications of the mesofurca in Chalcidoidea (Hymenoptera), with an emphasis on Aphelinidae. *Systematic Entomology*, 22, 45–65. https://doi.org/10.1046/j.1365-3113.1997.d01-26.x

Heydon, S. (1997) A review of the world genera of the Trigonoderini, with a revision of the species of North America north of Mexico (Hymenoptera: Pteromalidae). *Contributions of the American Entomological Institute*, 30, 1–84.

Johnson, J.B., Miller, T.D., Heraty, J.M. & Merickel, F.W. (1986) Observations on the biology of two species of *Orasema* (Hymenoptera: Eucharitidae). *Proceedings of the Entomological Society of Washington*, 88, 542–549.

Karlsson, D. & Ronquist, F. (2012) Skeletal morphology of *Opius dissitus* and *Biosteres carbonarius* (Hymenoptera: Braconidae), with a discussion of terminology. *PLoS One*, 7, e32573. https://doi.org/10.1371/journal.pone.0032573

Kirby, W. & Spence, W. (1826) *An Introduction to Entomology or, Elements of the Natural History of Insects. Vol. 3*. Longman, Rees, Orme, Brown and Green, London, 732 pp.

Krenn, H.W. & Pass, G. (1995) Morphological diversity and phylogenetic analysis of wing circulatory organs in insects, part II. Holometabola. *Zoology*, 98, 147–164.

Krogmann, L. & Vilhelmsen, L. (2006) Phylogenetic implications of the mesosomal skeleton in Chalcidoidea (Hymenoptera, Apocrita) – tree searches in a jungle of homoplasy. *Insect Systematics*, 20, 615–674. https://doi.org//10.1071/IS06012

Lachaud, J.P. & Pérez-Lachaud, G. (2012) Diversity of species and behavior of hymenopteran parasitoids of ants: A review. *Psyche*, 2012, 1–24. https://doi.org/10.1155/2012/134746

Laing, J.E. & Heraty, J.M. (1981) The parasite complex of the overwintering population of *Epiblema scudderiana* (Lepidoptera: Olethreutidae) in Southern Ontario. *Proceedings of the Entomological Society of Ontario*, 112, 59–67.

MacGillivray, A.D. (1923) *External Insect-Anatomy.* Scarab Company, Urbana, viii + 388 pp.

Martin, J. F. (1916) The thoracic and cervical sclerites of insects. *Annals of the Entomological Society of America*, 9, 38–83. https://doi.org/10.1093/aesa/9.1.35

Masner, L. (1979) The *variicornis*-group of *Gryon* Haliday (Hymenoptera: Scelionidae). *The Canadian Entomologist*, 111, 791–805. https:// doi.org/10.4039/Ent111791-7

Matsuda, R. (1970) Morphology and evolution of the insect thorax. *Memoirs of the Entomological Society of Canada*, 102, 1–431. https://doi.org/10.4039/entm10276fv

Michener, C.D. (1944) Comparative external morphology, phylogeny, and a classification of the bees (Hymenoptera). *Bulletin of the American Museum of Natural History*, 82, 151–326. https://doi.org/10.1111/j.1439-0469.1987.tb00594.x

Michener, C.D. (1956) Hymenoptera. *In*: Tuxen, S.L. (Ed.), *Taxonomist's Glossary of Genitalia in Insects*. S.H Service Agency, Copenhagen, pp. 359.

Munro, J.B., Heraty, J., Burks, R.A., Hawks, D., Mottern, J.L., Cruaud, A., Rasplus, J.-Y. & Janšta, P. (2011) A molecular phylogeny of the Chalcidoidea (Hymenoptera). *PLoS One*, 6, e27023. https://doi.org/10.1371/journal.pone.0027023

Murray, E., Carmichael, A.E. & Heraty, J.M. (2013) Ancient host shifts followed by host conservatism in a group of ant parasitoids. *Proceedings of the Royal Society, B*, 280, 1–9. [20130495] https://doi.org/10.1098/rspb.2013.0495

Pinto, J.D. (1992) Novel taxa of *Trichogramma* from the new world Tropics and Australia (Hymenoptera: Trichogrammatidae). *Journal of the New York Entomological Society*, 100, 621–633.

Romero, C.A. & Heraty, J.M. (2010) Ultrastructure of scutellar sensilla in *Aphytis melinus* (Hymenoptera: Aphelinidae) and morphological variation across Chalcidoidea. *Journal of Hymenoptera Research*, 19, 159–178.

Ronquist, F. & Nordlander, G. (1989) Skeletal morphology of an archaic cynipoid, *Ibalia rufipes* (Hymenoptera: Ibaliidae). *Entomologica scandinavica Supplement*, 33, 1–60.

Sanger, C. & King, P.E. (1971) Structure and function of the male genitalia in *Nasonia vitripennis* (Walker) (Hym.: Pteromalidae). *The Entomologist*, 104, 137–149.

Smith, H.S. (1916) The habit of leaf-oviposition among the parasitic Hymenoptera. *Psyche*, 24, 63–68. https://doi.org/10.1155/1917/52524

Snodgrass, R.E. (1910a) The anatomy of the honeybee. *U.S. Department of Agriculture*, *Bureau of Entomology Technical Series*, 18, 1–162. https://doi.org/10.1111/j.1365-3032.1985.tb00040.x

Snodgrass, R.E. (1910b) The thorax of Hymenoptera. *Proceedings of the United States National Museum*, 39, 37–91. https://doi.org/10.5479/si.00963801.39-1774.37

Snodgrass, R.E. (1941) The male genitalia of Hymenoptera. *Smithsonian Miscellaneous Collections*, 99, 1–86.

Snodgrass, R.E. (1942) The skeleto-musculature of the honey bee. *Smithsonian Miscellaneous Collections*, 103, 1–120.

Snodgrass, R.E. (1957) A revised interpretation of the external reproductive organs of male insects. *Smithsonian Miscellaneous*

*Collections*, 133, 1–60.

Torréns, J. (2013) A review of the biology of Eucharitidae (Hymenoptera: Chalcidoidea) from Argentina. *Psyche*, 2013 (926572), 1–14.
https://doi.org//10.1155/2013/926572

Torréns, J. & Heraty, J.M. (2013) A new genus of Eucharitidae (Hymenoptera: Chalcidoidea), with notes on life history and immature stages. *Zootaxa*, 3630 (2), 347–358.
https://doi.org/0.11646/zootaxa.3630.2.9

Torréns, J., Heraty, J.M., Murray, E. & Fidalgo, P. (2016) Biology and phylogenetic studies of *Lasiokapala* Ashmead (Chalcidoidea: Eucharitidae) with description of a new species from Argentina. *Systematic Entomology*, 41, 596–606.
https://doi.org/10.1111/syen.12176

Vilhelmsen, L. (2000a) Before the wasp-waist: comparative anatomy and phylogenetic implications of the skeleto-musculature of the thoraco-abdominal boundary region in basal Hymenoptera (Insecta). *Zoomorphology*, 119, 185–221.
https://doi.org/10.1007/PL00008493

Vilhelmsen, L. (2000b) The ovipositor apparatus of basal Hymenoptera (Insecta): phylogenetic implications and functional morphology. *Zoologica Scripta*, 29, 319–345.
https://doi.org/10.1046/j.1463-6409.2000.00046.x

Vilhelmsen, L., Mikó, I. & Krogmann, L. (2010) Beyond the wasp-waist: structural diversity and phylogenetic significance of the mesosoma in apocritan wasps (Insecta: Hymenoptera). *Zoological Journal of the Linnean Society*, 159, 22–194.
https://doi.org/10.1111/j.1096-3642.2009.00576.x

Yoder, M.J., Mikó, I., Seltmann, K.C., Bertone, M.A. & Deans, A.R. (2010) A gross anatomy ontology for Hymenoptera. *PLoS One*, 5, e15991.
https://doi.org/10.1371/journal.pone.0015991.g001