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The life history of *Pseudometagea schwarzii*, with a discussion of the evolution of endoparasitism and koinobiosis in Eucharitidae and Perilampidae (Chalcidoidea)

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Abstract

The immature stages and behavior of *Pseudometagea schwarzii* (Ashmead) (Hymenoptera: Eucharitidae: Eucharitini) are described, and the presence of an endoparasitic planidium that undergoes growth-feeding in the larva of the host ant (*Lasius neoniger* Emery) is confirmed. Bayesian inference and parsimony ancestral state reconstruction are used to map the evolution of endoparasitism across the eucharitid-perilampid clade. Endoparasitism is proposed to have evolved independently three times within Eucharitidae, including once in *Pseudometagea* Ashmead, and at least twice in *Perilampus* Latreille. Endoparasitism is independent as an evolutionary trait from other life history traits such as differences in growth and development of the first-instar larva, hypermetamorphic larval morphology, and other biological traits, including koinobiosis.

Introduction

Eucharitidae and Perilampidae form a monophyletic group within Chalcidoidea (Hymenoptera) (Munro et al. 2011, Heraty et al. 2013). Females lay their eggs away from the larval food source, and the sclerotized first-instar larvae (planidia) are active in

finding their hosts (Clausen 1940a, b). Planidia attack the host larval stage, but do not develop further until the final host larval instar or prepupal stage is reached and histolysis during pupal formation begins (Smith 1912, Clausen 1941). The number of larval instars is variable within Perilampidae, with four larval instars reported for Chrysolampinae (Darling and Miller 1991), and three (Smith 1912, Clancy 1946) or four (Parker 1924, Tripp 1962, Darling and Roberts 1999) for Perilampinae. Only three instars have ever been reported for Eucharitidae (Wheeler 1907, Clausen 1923, 1928, 1940a, Ayre 1962, Heraty et al. 1993, Heraty 1994, 2000).

Development on the host pupa is nearly identical for all taxa, with the first and later instars developing as ectoparasitoids typically on the posterior ventral thoracic region of the host pupa (Smith 1912, Clausen 1940a, Darling and Miller 1991, Darling 1992, 1999). A secondarily endoparasitic late instar larva and pupa is known only in *Monacon* Waterston (Perilampidae) (Darling and Roberts 1999). As currently recognized, the Perilampidae (Chrysolampinae, Philomidinae and Perilampinae) are parasitoids of Coleoptera, Neuroptera, Diptera (Tachinidae) and Hymenoptera (Apoidea, Ichneumonoidea and Tenthredinoidea), and are paraphyletic with respect to the Eucharitidae (Darling 1992, Munro et al. 2011, Heraty et al. 2013, Murray et al. 2013). Although paraphyletic to Eucharitidae, no changes in the classification of Perilampidae have been proposed without a more comprehensive review of the entire superfamily (Heraty et al. 2013). Eucharitidae (Akapalinae, Gollumiellinae, Oraseminae and Eucharitinae) are monophyletic and, except for Akapalinae for which the hosts are unknown, are all parasitoids of ants (Murray et al. 2013).

Oviposition, host location and planidial behaviors vary across Eucharitidae and Perilampidae. Eggs of Chrysolampinae are deposited in the flower head or seed pod, and larvae move to the weevil host, where they remain as ectoparasitoids until host pupation (Askew 1980, Darling and Miller 1991). The oviposition behavior, means of accessing the host and behavior on the host larva is unknown for Philomidinae, but the planidia are ectoparasitoids on the pupae of halictid bees (Darling 1992). Perilampinae oviposit in close proximity to the host, with planidia either becoming ectoparasitoids (e.g., *Perilampus chrysopae* Crawford (Clancy 1946), *Steffanolampus salicetum* (Steffan) (Darling 1999) and *Monacon robertsi* Bouçek (Darling and Roberts 1999), or endoparasitoids when primary parasitoids of Diprionidae or hyperparasitoids of Ichneumonoidea and Tachinidae (e.g. *Perilampus hayalinus* Say, Smith 1912, Ford 1922, Tripp 1962). Perilampid planidia are cannibalistic as ectoparasitoids on the host pupa, and only one parasitoid develops per host pupa (Darling 1992). Within Eucharitidae, eggs are deposited more remotely from the host, and planidia use a variety of behaviors to gain access to the host ant larva (Clausen 1940b, 1941, Heraty 2000). Eucharitid planidia can be either endoparasitic or ectoparasitic on their host ant larvae. Cannibalistic behavior has not been observed, and in some Eucharitinae, multiple parasitoids can develop on a single host pupa (Clausen 1923, Heraty and Barber 1990, Lachaud and Pérez-Lachaud 2012). In Gollumiellinae and Oraseminae, planidia are endoparasitic on Formicinae (*Nylanderia*) and Myrmicinae, respectively (Wheeler 1907, Heraty et al. 1993, 2004, Heraty 1994). Both *Nylanderia* Emery and Myrmicinae lack a cocoon,

and development occurs on exposed pupae in the brood pile. In Eucharitinae, development takes place within the host cocoon of Ponerinae, Ectatomminae, Formicinae or Myrmeciinae (Heraty 2002, Lachaud and Pérez-Lachaud 2012, Murray et al. 2013, Torréns 2013). Planidia of Eucharitinae are all ectoparasitoids, with one exception – *Pseudometagea schwarzii* (Ashmead) was described by Ayre (1962) as having an internal planidium on larvae of *Lasius neoniger* Emery (Formicinae).

Both Eucharitidae and Perilampidae are koinobionts, that is, parasitoids that do not kill their host initially but instead transition through more than one host life history stage (Askew and Shaw 1986). Most koinobionts are internal parasitoids and therefore can more easily transition from one instar or life stage to another (Gauld and Hanson 1995). Very few switch their mode of parasitism once on their primary host. Endoparasitism by the planidia on the host larva is so far known only in some *Perilampus* (Perilampinae) and all known Gollumiellinae, Oraseminae and *Pseudometagea* (Eucharitinae). Because the endoparasitic planidia of Perilampidae do not feed, Darling and Roberts (1999) considered the term endoparasitoid inappropriate. However, at least in Eucharitidae, while in or on their larval host, planidia may exhibit sustenance feeding, at most becoming turgid, or may undergo what can be termed growth feeding in which the planida expand to several hundred times their original size (Wheeler 1907, Smith 1912, Tripp 1962). Therefore at least some feeding, and thus true endoparasitism, does takes place within Eucharitidae.

Hypermetamorphic larvae with both discrete morphologies and behaviors across different instars is uncommon within the Hymenoptera. Type I hypermetamorphism involves oviposition away from the food source and an active first-instar parasitoid (Pinto 2003). Both the terms triungulins (with legs) and planidia (without legs) have been used, but Pinto (2003) disputed the term triungulin (=three clawed) and lumped both forms under the category of planidium (= little or diminuitive wanderers). In contrast, Darling and Miller (1991) and Darling (1992) proposed that because of their unique structure and phylogenetic placement, the term planidium should be reserved for Perilampidae and Eucharitidae, with other [Type I] forms referred to as planidiaform. Type I hypermetamorphic larvae are rare, and appear to have evolved only twice in Hymenoptera, once in Ichneumonidae (Tripp 1961) and in a monophyletic Eucharitidae + Perilampidae (Heraty et al. 2013). For the Chalcidoidea that oviposit away from the host, not all of their larvae are hypermetamorphic (von Rosen 1956, Parnell 1963, Askew 1980). The planidia of Eucharitidae and Perilampidae share a suite of character states that is unique among insects and appear to have been derived only once within Chalcidoidea (Heraty and Darling 1984, Darling 1992, Munro et al. 2011, Heraty et al. 2013).

Both endoparasitism and koinobiosis are derived and likely independent traits within Hymenoptera (Askew and Shaw 1986, Gauld 1991, Whitfield 1992, Wahl and Sharkey 1993). Most koinobionts are endoparasites, and ectoparasitic koinobionts are rare (Gauld and Hanson 1995). However, both Perilampidae and Eucharitidae are koinobionts that are nearly always ectoparasitic in later instars, with oviposition away from the host and planidial larvae.

In this paper, we confirm the observation of endoparasitism within the genus *Pseudometagea*, and propose that endoparasitism may have developed at least five times within the perilampid-eucharitid lineage. We use these data to interpret the evolution of endoparasitism and koinobiosis within this specialized group of type I hypermetamorphic Chalcidoidea.

Materials and methods

Collections

A total of 46 colonies of *Lasius neoniger* were sampled from under stones along a roadside in the Ojibway Long Grass Prairie Nature Reserve, Windsor, Ontario, Canada (42°15'42.9"N, 83°04'02.2"W) from 2–4 June, 1982. No attempt was made to sample entire colonies, but instead only the larvae, cocoons and representative adult ants that could be readily aspirated. Collections were stored in 70% ethanol. Cocoons were dissected and the contents examined for parasitized ant larvae, prepupae, ant pupae or parasitoid pupae. Representative adults and larval stages of both *Pseudometagea* and *Lasius* were dried using hexamethyldisilazane (HMDS, Heraty and Hawks 1998), card-mounted, and deposited in the UC Riverside Entomology Research Museum under museum barcode numbers UCRC_ENT 00352406–31.

Endoparasitism trait mapping

Literature records for internal or external parasitism by planidia on their larval host are known for 27 species of Perilampidae (Chrysolampinae and Perilampinae) and Eucharitidae (Wheeler 1907, Smith 1912, Brues 1919, Ford 1922, Clausen 1923, 1928, 1940a, Wheeler and Wheeler 1937, Clancy 1946, Ayre 1962, Tripp 1962, Askew 1980, Laing and Heraty 1981, Heraty and Darling 1984, Darling and Miller 1991, Darling 1992, 1999, Heraty et al. 1993, Heraty 1994, 2000, 2002, Darling and Roberts 1999, Pérez-Lachaud et al. 2006, Carey et al. 2012, Lachaud and Pérez-Lachaud 2012, Torréns 2013) (cf. Table 1). We used Bayesian inference and parsimony methods to model the ancestral states and behavioral changes for the parasitoid larvae.

Exemplar trees for trait mapping were pruned from the Bayesian analysis of molecular data (18S, 28S, COI, COII) for 237 taxa by Murray et al. (2013). Except for *Perilampus*, larval behaviors are identical within genera. Thus, for those taxa with trait data for species that were not represented in the Murray et al. analysis, we matched these names to closely related species of the same genus (* on Fig. 11). BayesTraits Multistate v1.0 was used for ancestral host reconstruction in a Bayesian framework. Using a distribution of 10,000 trees, each chronogram was pruned from 237 taxa to 27 taxa, retaining the original topology and branch lengths of each phylogeny. For state reconstruction, an empirical Bayes approach was employed, where first the data were analyzed under maximum likelihood

Table 1. Taxa used in reduced analysis, with references to the mode of parasitism and taxon substitutes (grey) from the matrix. Voucher codes and Genebank accession numbers are included. Classification abbbreviations: PEC Chrysolampinae; PEP Perilampinae; EUG Gollumiellinae; EUO Oraseminae; EUP Eucharitinae: Psilocharitini; EUE Eucharitinae: Eucharitini.

Classif.	Reference taxon	planidial mode	Reference	Taxon in analysis	D_number	Genbank Accession Numbers			
						18S	28S D2-D5	COI	COII
PEC	*Chrysolampus sisimbryi*	ectoparasite	Darling and Miller 1991	*Chrysolampus sisimbryi*	D0970	JN623326	AY552188	KC008309	–
PEC	*Chrysolampus thenae*	ectoparasite	Askew 1979	*Chrysolampus* sp. (Australia)	D0160	JN623327	AY552185, JN624069	–	–
PEP	*Steffanolampus salicetum*	ectoparasite	Darling 1999	*Steffanolampus salicetum*	D0320	JN623354	AY552177, JN624088	KC008323	–
PEP	*Monacon robertsi*	ectoparasite	Darling and Roberts 1999	*Monacon robertsi*	D0318	JN623340	AY552181, JN624078	KC008315	–
PEP	*Perilampus chrysopae*	ectoparasite	Clancy 1946	*Chrysolampus* sp. (Australia)	D0113	JN623345	AY552178	KC008319	–
PEP	*Perilampus fulvicornis*	endoparasite	Laing and Heraty 1981	*Perilampus fulvicornis*	D0713	JN623342	JN623717, JN624080	KC008217	KC008489
PEP	*Perilampus hyalinus*	endoparasite	Smith 1912, Tripp 1962	*Perilampus hyalinus*	D0972	AY552257	AY552180	KC008318	–
EUG	*Gollumiella longipetiolata*	endoparasite	Heraty et al. 2004	*Gollumiella longipetiolata*	D0405c	JN623252	AY552191, JN624020	KC008166	KC008328
EUO	*Orasema costaricensis*	endoparasite	Wheeler and Wheeler 1937	*Orasema costaricensis*	D0342	AY672931	AY672931	–	–
EUO	*Orasema simulatrix*	endoparasite	Carey et al. 2012	*Orasema simulatrix*	D0422	JN623259	AY552206, JN624027	KC008181	–
EUO	*Orasema sixaolae*	endoparasite	Wheeler and Wheeler 1937	*Chrysolampus* sp. (Australia)	D2919	KC008506	KC008091, KC008142	KC008182	–
EUO	*Orasema viridis*	endoparasite	Heraty 2000	*Chrysolampus* sp. (Australia)	D0248	–	AY672955, KC008493	–	–
EUO	*Orasema xanthopus*	endoparasite	Heraty et al. 1993	*Orasema xanthopus*	D1090	KC008510	KC008093, KC008143	–	–
EUO	*Timioderus acuminatus*	endoparasite	Heraty 2000	*Chrysolampus* sp. (Australia)	D0116	JN623266	AY552195, JN624034	–	–
EUP	*Neolosbanus palgravei*	ectoparasite	Heraty 1994	*Neolosbanus palgravei*	D2845	–	KC008096, KC008146	–	KC008334
EUE	*Ancylotropus manipurensis*	ectoparasite	Clausen 1928	*Ancylotropus cariniscutis* (Malaysia)	D0701	–	KC008109	KC008186	KC008348
EUE	*Austeucharis fasciiventris*	ectoparasite	Brues 1919	*Austeucharis* sp. (Australia: NSW)	D0904	–	AY671806	KC008233	KC008412

Classif.	Reference taxon	planidial mode	Reference	Taxon in analysis	D_number	Genbank Accession Numbers				
						18S	28S D2-D5	COI	COII
EUE	*Chalcura affinis*	ectoparasite	Clausen 1940	*Chalcura* sp. (Australia: NT)	D0647	–	KC008099	KC008211	KC008387
EUE	*Dicoelothorax platycerus*	ectoparasite	Torréns and Heraty 2012	*Dicoelothorax platycerus*	D2512	–	KC008115, KC008149	–	KC008431
EUE	*Eucharis adscendens*	ectoparasite	Clausen 1940	*Eucharis adscendens*	D0729	JN623231	AY552229, JN624007	KC008189	KC008353
EUE	*Galearia bruchii*	ectoparasite	Torréns 2013	*Galearia bruchii*	D2491	KC008531	KC008117	–	–
EUE	*Kapala terminalis*	ectoparasite	Clausen 1940	*Kapala terminalis*	D1270	KC008561	AY671891	KC008290	KC008479
EUE	*Latina rugulosa*	ectoparasite	Torréns 2013	*Latina guriana* (Argentina: SA)	D1073b	AY552319	AY552242	KC008246	KC008433
EUE	*Pseudochalcura gibbosa*	ectoparasite	Heraty and Barber 1990	*Pseudochalcura gibbosa*	D0910	AY552295	AY552218	KC008199	KC008373
EUE	*Pseudometagea schwarzii*	endoparasite	Ayre 1962	*Pseudometagea schwarzii*	D0274	AY552292	AY552215	KC008188	KC008352
EUE	*Stilbula cyniformis*	ectoparasite	Clausen 1940	*Stilbula* sp. 1 (Nigeria)	D2692	AY552301	GQ331923, KC008495	–	KC008367
EUE	*Stilbula tenuicornis*	ectoparasite	Clausen 1923	*Stilbula* sp. 2 (Singapore)	D2837	KC008517	KC008097	KC008196	KC008368

analysis, and then these results informed the choice of Bayesian priors. For the Bayesian implementation, reversible-jump MCMC was used instead of specifying a model of character change. We designated a hyperprior on an exponential distribution, drawn from a uniform distribution on the interval 0 to 5 with a rate deviation of 15. 500 million generations were run, sampling every 50,000, with the first 100 million generations discarded as burn-in. Acceptance rates were considered as valid if they fell within a range of 0.2–0.4.

During analysis, BayesTraits picks one of the 10,000 trees randomly for each iteration and calculates state probabilities at designated nodes of interest. There are two nodes that are not present on all trees: Perilampinae + Eucharitidae, and Eucharitini excluding *Pseudometagea*. For trees without the node present, the probability of the ancestral states was set as uninformative at a 0.5 probability for each state. This incorporates uncertainty of the node in proportion to its appearance in the distribution of trees. Tracer v1.5.0 (Rambaut and Drummond 2007) was used to obtain the mean output value for at each node of interest.

Mesquite v2.73 (Maddison and Maddison 2010) was used for parsimony reconstruction of ancestral states by tracing the character on the maximum clade credibility tree from Murray et al. (2013).

Results

Fourteen (30.4%) of the 47 colonies of *L. neoniger* sampled were parasitized by *P. schwarzii*. From these parasitized colonies, we recovered 17 unparasitized mature ant larvae, 473 unparasitized ant pupae, and 115 parasitized cocoons. Of the parasitized cocoons, there were 15 mature ant larvae within the cocoon with an internal planidium of *P. schwarzii* (PS), 7 ant pupae with external PS planidia, 5 ant pupae with a second instar PS, 2 ant pupae with a third instar PS, and 86 PS pupae. Only one parasitoid per host larva or pupa was ever observed. Three larval instars were observed for *P. schwarzii*. The few mature larvae and numerous ant and parasitoid pupae suggest that sampling took place at the end of the overwintering generation of brood for that year. Numerous males of *P. schwarzii* flying over the ant colonies during collection also suggested a recent emergence. One female emerging from the nest entrance attracted several males that swarmed the female in a ball on the ground. The supposedly now mated female left the cluster of males and flew to the nearest host plant and began oviposition into flower heads of *Erigeron strigosus* Muhl. ex Willd. (Asteraceae).

The unfed planidium (Fig. 1), taken from these same collections, was described by Heraty and Darling (1984) and earlier by Ayre (1962). The feeding and later stages are herein described as follows. On mature larvae within the cocoon, the planidia were found completely embedded in the thoracic region of the host (pl, Figs 2, 5, 9, 10). All of the internal planidia were in the growth phase, with the body greatly swollen and the tergites reduced to well-separated bands (Figs 2, 5). The entrance hole (eh) was marked by a sclerotic ring (Fig. 9). After pupation of the host, the enlarged planidium became ectoparasitic (exposed by molted exuvium) and then relocated to the ventral region of the thorax behind
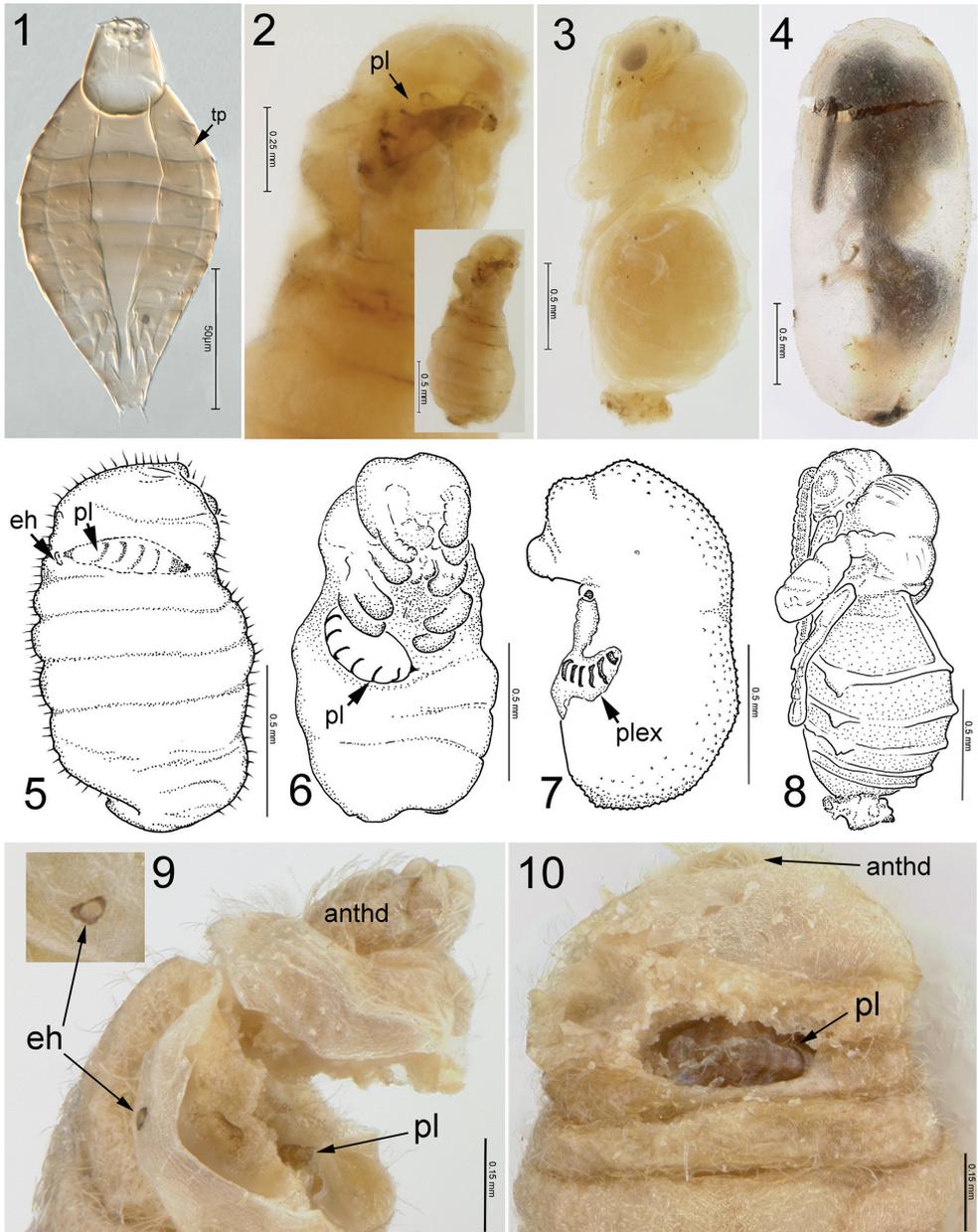
Figures 1–10. *Pseudometagea schwarzii*. **1** planidium (first-instar), tp = tergopleural line **2** *Lasius neoniger* larva with internal planidium (pl), inset is full size ant larva (from cocoon) **3** female pupa **4** adult male in process of opening cocoon **5** *Lasius* larva with planidium, eh= entrance hole **6** *Lasius* pupa with external planidium **7** third instar *Pseudometagea* with attached planidial exuvium (plex) **8** *Pseudometagea* pupa **9** ant larva with entrance hole and broken to show internal planidium (apical segments), anthd = ant head **10** ant larva with dissection showing fed (expanded) internal planidium.
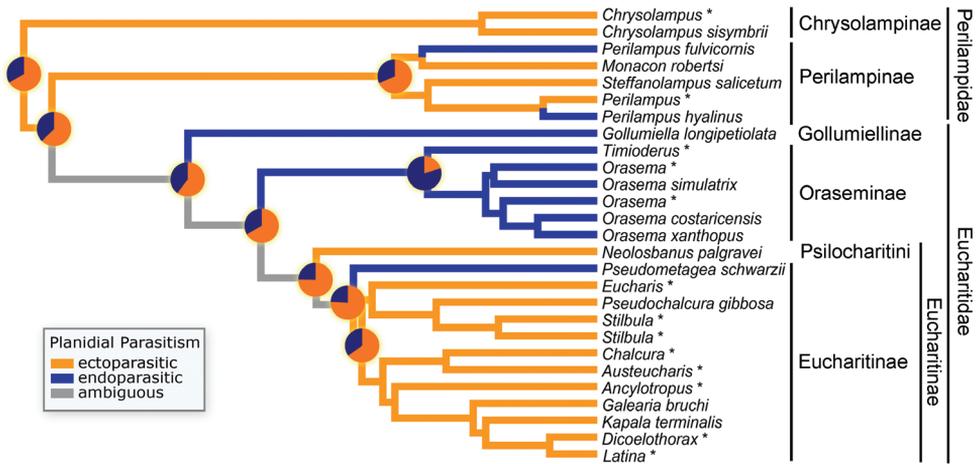
Figure 11. Ancestral character reconstruction of mode of planidial parasitism. Terminal taxa coded as either ectoparasitic (orange) or endoparasitic (blue). Branches of phylogeny colored according to parsimony reconstruction, with gray branches indicating ambiguity. Pie charts at selected nodes show probabilities of each state from the Bayesian analysis. Taxa were coded directly from life history records except where indicated by asterisks, when taxa were substituted for those included in the analyses of Murray et al. (2013) as detailed in Table 1.

the legs (Fig. 6). The third-instar had a swollen dorsal prothoracic region, a single thoracic spiracle and a weakly pustulate dorsal surface, and retained the cast planidial exuvium (plex) as observed for other Eucharitidae (Fig. 7). The pupa lacks any ocellar ornamentation (found in some Eucharitinae) or petiolar nodules (found in Oraseminae), but does have lateral bladder-like abdominal processes (found in some Eucharitinae) (Figs 3, 4, 8) (cf. Brues 1919, Heraty et al. 1993, Heraty 1994, 2002). Adults emerge from the cocoon by cutting a transverse slit in the cocoon prior to the wings becoming fully expanded (Fig. 4).

Ancestral states reconstruction for ectoparasitism and endoparasitism was done using BayesTraits (Bayesian inference) and Mesquite (parsimony) (Fig. 1). Relationships and branch lengths were based on Murray et al. (2013). The acceptance rate for the Bayesian analysis reached 0.227. Ectoparasitism is regarded as the ancestral trait for the perilampid-eucharitid lineage. The sister group relationship with this clade within Chalcidoidea is unclear (Munro et al. 2011, Heraty et al. 2013), but an ectoparasitic ancestor appears most likely given our selected taxa and using both Bayesian inference and parsimony (Fig. 11). Within Perilampinae, both *Perilampus hyalinus* and *P. fulvicornis* have internal planidia, whereas *P. chrysopae* and all other Perilampinae, are always external. In Eucharitidae, planidia shift to endoparasitism in *Gollumiella* Hedqvist (Gollumiellinae), all Oraseminae, and *Pseudometagea* (Eucharitinae). Although ambiguous under parsimony, BayesTraits suggests a slightly higher probability (>60%) for ectoparasitism by the planidium on the host larva being plesiomorphic, followed by five independent derivations of an endoparasitic planidium in Perilampinae + Eucharitidae (Fig. 11).

Discussion

Three types of feeding by planidia have been observed on the host larva. The planidia of Perilampidae are all considered as non-feeding (little to no expansion of body segments) while on or in the host larvae (Darling and Roberts 1999, Heraty et al. 2004). The endoparasitic planidia of Gollumiellinae and Oraseminae undergo sustenance feeding, at most becoming turgid with the tergites slightly separated (Heraty et al. 1993, Heraty 1994, 2000). Some ectoparasitic perilampid planidia also partake in limited host feeding, as evidenced by a slight expansion of the body and separation of the planidial tergites (Darling, pers. comm.). This sustenance-feeding by external planidia occurs in some Perilampinae and most Eucharitini (Eucharitinae) (Clausen 1928, 1940a, Heraty and Barber 1990, Lachaud and Pérez-Lachaud 2012 [note error in their fig. 2d, the expanded planidia is on an ant prepupa, not a larva; pers. comm. J.-P. Lachaud], Torréns and Heraty 2012, Torréns 2013). However, in *Neolosbanus* Heraty (Eucharitinae; Psilocharitini), the ectoparasitic planidia partakes in growth feeding and swells considerably while on the host larva, although not to their final immense size as occurs on the host pupa (Heraty 1994). Only the first instar larvae of *Stilbula tenuicornis* (Clausen) (Eucharitini) have been reported to growth feed and complete their development on the larval host (Clausen 1923). In *Pseudometagea*, planidia are not only endoparasitic, but they increase to their full swollen size while within the host larva (Figs 2, 5), and prior to their migration to the venter of the thorax of the host pupa (Fig. 6).

Our results confirm the report by Ayre (1962) that planidia of *P. schwarzii* are endoparasitic, and that they "burrowed into the ant larva in the dorsal or pleural region of the second or third thoracic segment". Ayre (1962) reported that planidia burrowed into the ant larva both prior to pupation and occasionally prior to the final host instar molt. Ayre (1962) also observed that the planidia overwintered and remain dormant within the host (presumably with minimal feeding) until the spring when they undergo a great increase in size "when the ant larvae commence feeding in the spring". From this, we interpret that growth feeding in the host larva occurs just prior to cocoon formation. This would agree with our observation of internal engorged planidia within host larvae in the cocoon (Figs 2, 5, 9, 10). The planidium of *P. schwarzii* attains full size while in the host larva. Such an advanced state prior to host pupation has only been observed for *Stilbula tenuicornis*, which apparently grew and moulted to the second instar while on the host larva, and then completed development on the host pupa (Clausen 1923, 1928). Eucharitinae can have expanded growth-feeding planidia while on the host larvae, but these reach at most only a third of the final first instar size (Torréns 2013). The development and feeding location of the enlarged planidium and later instars on the ventral region of the pupal thorax is otherwise similar across Eucharitidae and Perilampidae (Wheeler 1907, Clausen 1923, Darling and Miller 1991, Darling 1992, Heraty et al. 1993, Heraty 1994, 2000).

One of the important discoveries noted by Ayre (1962) is the presence of an endoparasitic first instar in *Pseudometagea*. In all other known Eucharitinae, the plan-

idium remains as an ectoparasite of both the larval and pupal host (Clausen 1923, Clausen 1928, Clausen 1940a, Heraty and Barber 1990, Heraty 1994, Lachaud and Pérez-Lachaud 2012, Torréns and Heraty 2012, Torréns 2013). Our results suggest that endoparasitism developed independently at least three times in Eucharitidae, and possibly at least twice in Perilampinae. Growth-feeding by the planidium on the host larva is not unique for *Pseudometagea*, and has been observed in both *Neolosbanus* and *Galearia* Brullè, which have external planidia. However, the degree of host feeding and attainment of a full size first instar by *Pseudometagea* within the host larva is unique.

The causes for developing an endoparasitic lifestyle in these taxa are unclear. For most koinobionts, endoparasitism avoids issues associated with detachment during host moults between instars. However, the majority of both Eucharitidae and Perilampidae are ectoparasitic during all life stages, and retain enough mobility to relocate and reattach to the host after each moult. In Perilampidae, endoparasitism is associated with hyperparasitism, with planidia entering the primary host and then locating and attacking the ichneumonid or tachinid parasitoid within (Smith 1912, Ford 1922, Tripp 1962). In Gollumiellinae and Oraseminae, the ant hosts lack a cocoon. Perhaps an endoparasitic lifestyle prevents detection of planidia on larvae in the open brood pile, although the same risks occur throughout the ant parasitic Eucharitidae with ectoparasitic planidia. As well, later development in both Gollumiellinae and Oraseminae occurs openly on the naked pupa. For *Orasema* Cameron attacking polymorphic Myrmicinae, possible control of larval growth through limited feeding by the internal planidia was suggested by Heraty (1990). However, *Pseudometagea* attacks a monomorphic ant host, and development of the later instars occurs within the host cocoon.

Potentially, endoparasitism could be associated with overwintering in the ant larval host.

Overwintering in the egg stage is known for some Eucharitinae (Clausen 1940b, Heraty and Barber 1990). Overwintering by planidia on their ant host has only been documented for *Pseudometagea* (Ayre 1962) and *Austeucharis* Bouçek (Eucharitinae; ectoparasitic and may overwinter on host larva or in cocoon) (Taylor et al. 1993). Overwintering as a planidium on the host may be more common in some groups such as *Orasema* that rely on the seasonal presence of their plant hosts and have no means of overwintering as adults.

Pseudometagea occupy a phylogenetically important position within the family as the sister group of the remaining Eucharitini (Fig. 11). Their planidia are endoparasitic and overwinter on the larvae of their host ant, and they have a growth phase of development while in the larval host. Endoparasitism in Perilampidae may be correlated with hyperparasitism. Within Eucharitidae, our results suggest that endoparasitism has developed multiple times (Fig. 11), with its appearance independent of other derived larval behaviors including growth feeding, association with ants that have a cocoon, and overwintering. The reasons for the derivation of each of these traits are unclear, and future studies of both eucharitid and ant biology are necessary to better understand the evolution of parasitoid developmental behaviors.

Acknowledgements

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