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The occurrence and phylogenetic implications of the ovipositor clip within the Figitidae (Insecta: Hymenoptera: Cynipoidea)

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Abstract

The presence of the ovipositor clip is surveyed throughout Figitidae. This morphological structure is postulated to restrain the parasitoid host during oviposition. All Figitinae and Eucoilinae that attack semi-concealed dipterous hosts were found to possess the clip. Figitids that attack fully concealed hosts all lacked the ovipositor clip. It is hypothesized here that two subfamilies, the Anacharitinae and Aspicerinae, attack fully exposed hosts yet lack the clip in order to quickly oviposit and prevent fighting with the host. Mapping of the presence/absence of the ovipositor clip on to two competing phylogenies suggests this structure evolved in parallel in Figitinae and Eucoilinae. The evolution of the ovipositor clip is probably responsible for the successful colonization of semi-concealed dipterous larvae by Eucoilinae.

Keywords: *Koinobiont, morphology, parasitoid.*

Introduction

The structure and function of the ovipositor within Hymenoptera have been cited as factors leading to the evolutionary success of the order (Gauld and Bolton 1988). Parasitic Hymenoptera display a variety of morphological adaptations of the ovipositor that are linked to the type of host attacked (Quicke et al. 1999). In order to better understand the evolution of parasitic forms of Hymenoptera, several studies have focused on ovipositor morphology (Scudder 1961; Austin and Browning 1981; Fergusson 1988; Quicke et al. 1992, 1994, 1999; Ronquist 1995; Heraty and Quicke 2003).

The Cynipoidea possess a unique ovipositor morphology in that the first and second ovipositor valves are coiled around the base of the ovipositor, along a vertical plane, within a laterally flattened metasoma (Ronquist and Nordlander 1989; Ronquist 1999). Some Figitidae and Cynipidae are further characterized by a basal 180° twist of the ovipositor valves. This condition (absent in figitid Anacharitinae and Charipinae) results in the paired valves assuming a dorsal position and the fused valves a ventral position when exerted from the metasoma (Ronquist 1999). The Figitidae *sensu* Ronquist (1999) are further

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characterized by all members possessing a point of weakness at the base of the third valva that allows ventral flexion of the ovipositor during oviposition (Ronquist 1999).

The focus of this paper is on another unique morphological feature of the ovipositor possessed by some Figitidae, the ovipositor clip (van Lenteren et al. 1998, 1999). This feature was described after studies on the oviposition behaviour of the eucoiline *Leptopilina heterotoma* (Thomson) revealed that certain naive female wasps were unable to remove their ovipositor in the early stages of oviposition if the host larva (*Drosophila* sp.) attempted to escape parasitization (Samson-Bosthuizen et al. 1974; van Lenteren et al. 1998). The result was the dragging of the female wasp behind the escaping larvae, through the fly colony growth medium. If the wasp did not perish from this ordeal, the ovipositor was eventually removed followed by several hours of the wasp cleaning herself (van Lenteren et al. 1998). The authors of that paper hypothesized that some physical means was preventing the removal of the ovipositor from the would-be host, and that older females learned from experience how to successfully use this structure to prevent the escape of potential host larva. In a follow-up paper, van Lenteren et al. (1999) elaborated on the structure and function of the ovipositor clip possessed by *Leptopilina heterotoma* and *L. bouvardi* (Barbotin, Carton, and Kelner-Pillout).

Van Lenteren et al. (1998) used light microscopy, scanning electron microscopy (SEM), and transmission electron microscopy (TEM) to reveal a concavity on the ventral surface of the fused valve on the ovipositor of *Leptopilina heterotoma*. The concavity was covered by a flexible lobe whose distal free end interfaced with a row of teeth (cf. van Lenteren et al. 1998, Figure 1). Freezing of ovipositing *L. heterotoma*, followed by SEM, revealed that the host cuticle was pinched in this clip (cf. van Lenteren et al. 1998, Figure 2) and the term "ovipositor clip" was coined to explain the structure. So far as known, this was the first and only description of a physical structure of a hymenopteran ovipositor used for host restraint (Quicke 1997; van Lenteren et al. 1998; Quicke et al. 1999).

Prior to this work, the only known figitid to possess the ovipositor clip is *Leptopilina heterotoma*, although Fergusson (1988) and Ronquist (1995) both mention the presence of a notch-like structure on several figitine and eucoiline taxa. Therefore, a much broader survey of the figitid fauna was necessary to examine the presence of the clip across Figitidae. The phylogeny of Figitidae in Buffington et al. (forthcoming) is used to map the presence/absence of the clip across the Figitidae, allowing for a better understanding of both the evolution of the ovipositor clip and of host preference across the family.

Materials and methods

Ovipositor terminology follows Ronquist and Nordlander (1989); other terminology follows Fontal-Cazalla et al. (2002) and van Lenteren et al. (1998).

Taxon sampling

All major clades of Figitidae recovered by Buffington et al. (2007) were sampled in this study except for Pycnostigminae and Parnipinae, for a total of 40 genera (one species per genus) classified in seven subfamilies. A list of all species examined is in Appendix 1. Additionally, clades that are particularly species rich (e.g. Eucoilinae) were sampled more thoroughly than less speciose clades (e.g. Aspicerinae). All voucher specimens are deposited in the Texas A&M University Insect Collection (College Station, TX) or in the Entomology Research Museum, UC Riverside (Riverside, CA); slide specimens

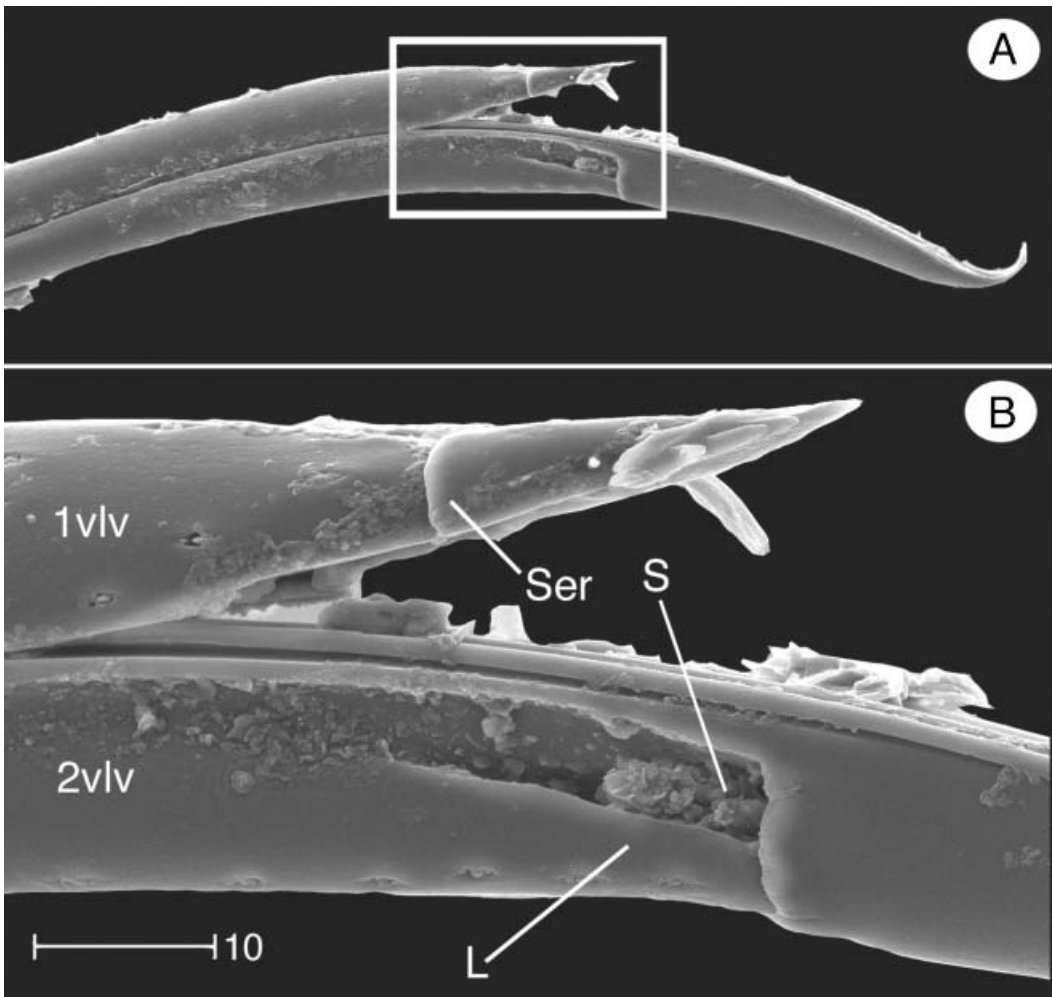


Figure 1. Electron micrographs of the ovipositor clip of *Neralsia* (Figitinae). (A) Overview of ovipositor tip, lateral side; (B) enlargement of boxed area in (A). 1vlv, first valvulae; 2vlv, second valvulae; L, lobe; S, slot; Ser, apical serration. Scale bar: 10 μ m.

examined are deposited in the Entomology Research Museum, UC Riverside (voucher nos 56878–56906).

Host records

Table I summarizes published and unpublished host records of semi-concealed hosts known for Eucoilinae and Figitinae. Only records in which a qualified systematist participated in the identification of the reared parasitoids, or co-authored the record, are shown to eliminate the possibility of erroneous host records. The list would be expanded considerably if secondary records were included.

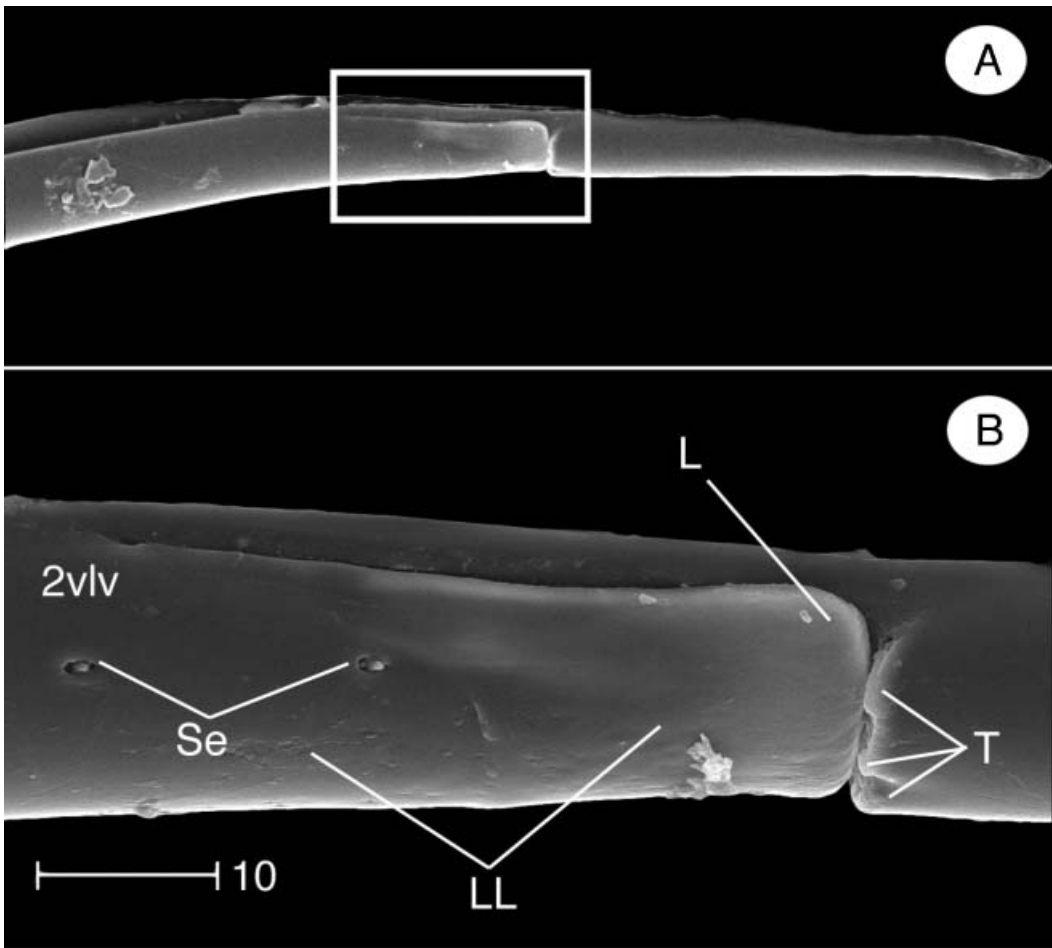


Figure 2. Electron micrographs of the ovipositor clip of *Trybliographa* (Eucoilinae). (A) Overview, ventral side; (B) enlargement of boxed area in (A). 2vlv, second valvulae; L, lobe; LL, lateral lip; Se, sensillae; T, teeth. Scale bar: 10 μ m.

Specimen preparation—light microscopy

Dried specimens identified as candidates for dissection were hydrated with water or ethanol and removed from their insect pin. The metasoma of each specimen was separated from the mesosoma + head, and dissected in 80% ethanol in a watch glass using insect pins fitted to pin vices. Once exposed, the entire ovipositor was transferred to a watch glass containing 80% ethanol, then to a second watch glass containing 95% ethanol. The ovipositor was then transferred to either euparal (specimens prepared at TAMU) or Hoyer's mounting medium (specimens prepared at UCR; voucher nos 56907–56933). The latter mounting medium was ultimately preferred due to its superior optical index. Coverslips of Hoyer's-mounted specimens were sealed with Glyptol[®].

Images were captured through a Zeiss Axioskop-2 using DIC lighting. Images were captured by a JVC KY-70 3-CCD digital camera fitted to the Axioskop-2 via a 2/3 \times reduction c-mount. The resolution of the JVC camera is 1360 \times 1024 pixels. Image capture control was achieved using Auto-Montage v. 5.0 (Synoptics Ltd, UK). Images were

Table I. Summary of Figitidae possessing ovipositor clips that attack semi-concealed dipteran hosts.

Figitid taxon	Host	Host niche	Reference
Figitinae			
<i>Neralsia hyalinipennis</i> Ashmead	Muscidae	Dung	Combs and Hoelscher (1969) , Thomas and Morgan (1972), Blume (1986)
<i>Figites anthomyiarum</i> (Bouché)	Anthomyiidae	Carrion	James (1928)
<i>Figites</i> spp.	Anthomyiidae, Calliphoridae, Muscidae, Sarcophagidae	Dung/carrion	Thompson (1955), Wharton (1979)
<i>Xyalophora</i> spp.	Muscidae	Dung/carrion	Blickle (1961), Turner et al. (1968), Wylie (1973), Wharton (1979)
Eucoilinae			
<i>Aganaspis</i> spp.	Tephritidae	Fruit (on ground)	Wharton et al. (1981), Jiron and Mexzon (1989), Ovruski (1995)
<i>Cothonaspis</i> sp.	Sepsidae	Dung/carrion	Figg et al. (1982), Nordlander (1982)
<i>Dettemeria euxestae</i>	Otitidae	Fruit (on ground)	Valicente (1986)
Borgmeier			
<i>Dicerataspis</i> sp.	Drosophilidae	Rotting vegetation	Wharton et al. (1998)
<i>Eucoila keilimi</i> Kieffer	Anthomyiidae	Rotting vegetation	Keilin and Baume Pluvinel (1913)
“ <i>Eucoila</i> ” spp.	Anthomyiidae, Muscidae, Sarcophagidae, Sepsidae, Calliphoridae	Dung/carrion	Turner et al. (1968), Figg et al. (1982), Blume (1986), Nordlander (1982)
<i>Eutrias tritoma</i> Thomson	Sepsidae, Sarcophagidae	Dung/carrion	Blume (1986)
<i>Ganaspis</i> spp.	Muscidae, Drosophilidae	Rotting vegetation	Shivpuje (1977), Diaz and Gallardo (1996)
<i>Hexacola</i> spp.	Ephydriidae, Chloropidae	Algae beds	Beardsley (1989), Simmonds (1952)
<i>Kleidotoma japonica</i> Huzimatu	Ephydriidae	Algae beds	Huzimatsu (1940)
<i>Kleidotoma</i> spp.	Anthomyiidae, Sepsidae, Muscidae, Sarcophagidae, Ephydriidae, Canacidae	Algae beds, dung carrion	Depner (1968), Figg et al. (1982), Blume (1986), Beardsley (1993)
Eucoilinae			
<i>Leptopilina heterotoma</i> (Thompson)	Drosophilidae	Rotting vegetation	Nordlander (1980) and references therein, van Alphen et al. 1991
<i>Leptopilina fimbriata</i> (Kieffer)	Drosophilidae	Rotting vegetation	Boness (1975)
<i>Lopheucoila anastrephae</i> (Rohwer)	Lonchaeidae	Fruit (on ground)	Wharton et al. (1998)
<i>Nordlandiella semirufa</i> (Kieffer)	Agromyzidae	Asteraceous flower heads	Buffington (2004a)
<i>Odontosema anastrephae</i> Borgmeier	Tephritidae	Fruit (on ground)	Wharton et al. (1981, 1998)
<i>Rhoptromeris strobigena</i> Nordlander and Grijpma	Chloropidae	Conifers	Nordlander and Grijpma (1991)
<i>Rhoptromeris heptoma</i> (Hartig)	Chloropidae	Cereal grasses, plant material	Meyer (1923), Nordlander (1978) , Jonsell et al. (1999)
<i>Trichoplasta</i> sp	Muscidae, Drosophilidae, Lonchaeidae	Rotting vegetation, fungi	Nordlander (1982)
<i>Triplasta</i> sp.	Muscidae	Rotting vegetation	Diaz and Gallardo (1996)

Table I. Continued.

Figitid taxon	Host	Host niche	Reference
<i>Trybliographa trichopsila</i> (Hartig) ^a	Calliphoridae, Muscidae, Sarcophagidae	Dung/carrion	Sychevskaya (1974)
<i>Trybliographa</i> spp.	Anthomyiidae	Vegetation, fungi	Nordlander (1982)

^aThis species is probably not *T. trichopsila* but a species of *Eucoila* (M. Forshage, personal communication).

captured as a series of focal planes and “montaged” to produce a composite focused image. All images are deposited on MorphBank (www.morphbank.com).

Specimen preparation—scanning electron microscopy

Two species, *Neralsia* sp. (Figitinae) and *Trybliographa rapae* Westwood (Eucoilinae), were examined using SEM. Specimens were dissected as described above. Following the second ethanol bath (95%), samples were transferred to hexamethyldisilazane (HMDS; Heraty and Hawks 1998) for final dehydration. Samples were then mounted to SEM stubs covered by double-sided mounting adhesive and sputter-coated with gold–palladium for three 30 s intervals, changing the angle of the stubs relative to the ion cloud at each interval. A Phillips XL-30 SEM was used for imaging using a 10 kV electron beam. All images were deposited on MorphBank. SEM stubs are deposited at the Entomology Research Museum, UC Riverside (voucher no. 56877).

Character mapping

Two phylogenetic hypotheses (one parsimony based, one Bayesian based) of figitid subfamily and sub-group relationships from Buffington et al. (2007) were used for mapping the presence/absence of the ovipositor clip. The phylogeny of Buffington et al. (2007) is based on total-evidence analyses of 28S D2+D3, COI, 18S, and morphological data partitions (1855 total characters). The difference in tree topology between the two competing hypotheses was minor but both are included here for examining alternative evolutionary scenarios (Buffington et al. 2007). The presence/absence of the ovipositor clip was included as a morphological character (character 163), and it should be noted that removal of this character from the analysis did not affect tree topology in either of the parsimony or Bayesian analyses.

Results

Presence of the ovipositor clip

The ovipositor clip (*sensu* van Lenteren et al. 1998) was found to occur in all Figitinae (Figure 4) with the exception of *Lonchidia*, and one (parsimony) or two (Bayesian) clades of Eucoilinae (Figure 7). The morphology of the clip between the figitine (Figure 4) and eucoiline (Figure 5) clades was entirely similar, and matched the description the clip for *L. heterotoma* (van Lenteren et al. 1998). Within the Figitinae + Aspicerinae clade, an additional feature of the paired valve (1 vlv), the apical serration (Ser, Figure 1B), was observed. Some but not all eucoilines were also observed to possess this feature.

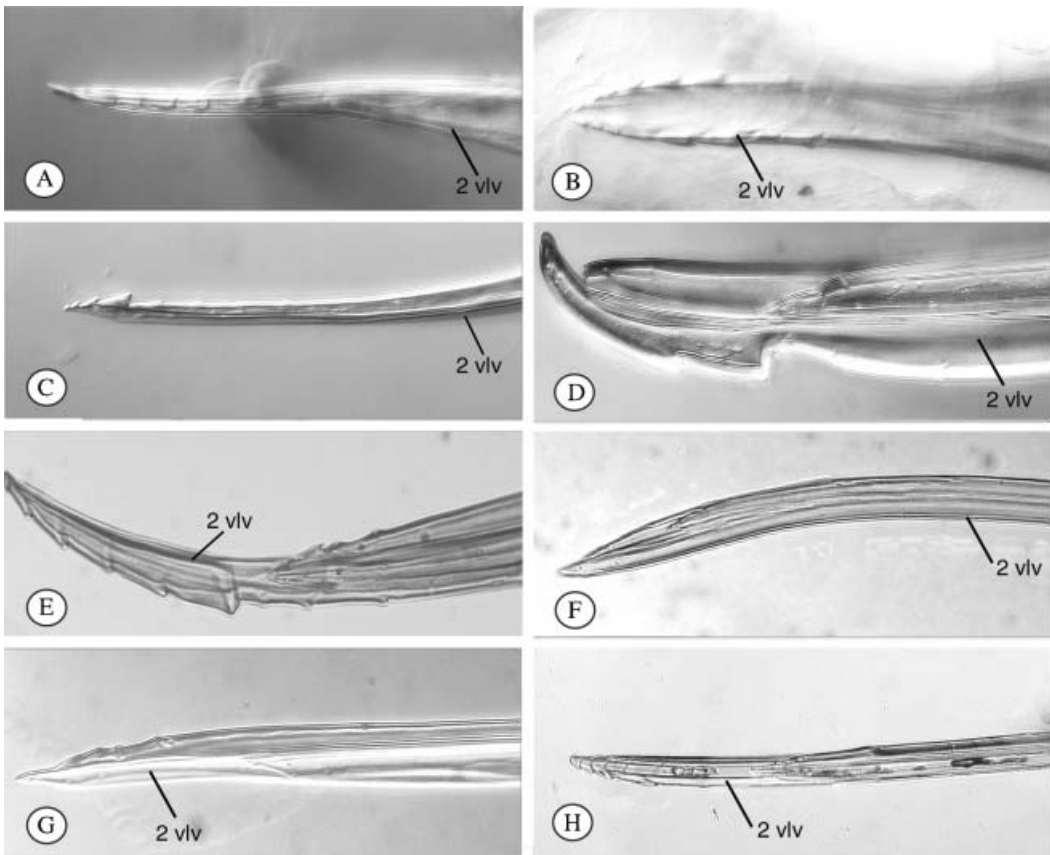


Figure 3. Examples of Figitidae that lack the ovipositor clip. (A–C) Anacharitinae: (A) *Aegilips*, (B) *Xyalaspis*, (C) *Anacharis*; (D, E) Aspicerinae: (D) *Melanips*; (E) *Callaspidea*; (F) Charipinae (*Alloxysta*); (G) Emargininae (*Thoreauella*); (H) Thrasorinae (*Euceroptres*). 2 vlv, fused ovipositor valve.

Members of Figitidae that lack an ovipositor clip include Anacharitinae (Figure 3A–C), Aspicerinae (Figure 3D, E), Charipinae (Figure 3F), Emargininae (Figure 3G), Thrasorinae (Figure 3H), and *Lonchidia* (currently classified as Figitinae; not shown). Among the Eucoilinae, the *Gronotoma* group (Figure 6A–C) and *Zaeucoila* group (Figure 6D–H) clades lacked the ovipositor clip. Buffington et al. (2007) recovered these groups as sister-groups (Figure 7A, B). A common feature among figitid groups that lack the ovipositor clip is the presence of serrations at the tip of 2 vlv (fused valve, Figure 3A–E, H), including the Anacharitinae, Aspicerinae, and Thrasorinae. Within Aspicerinae, the depth of these serrations (Figure 3D, E) are near the depth of the cavity portion of the ovipositor clip in Figitinae (Figure 4A, C–D).

Character mapping

Figure 7 summarizes the result of mapping the presence (black bars) of the ovipositor clip on the best parsimony (Figure 7A) and Bayesian (Figure 7B) total evidence trees. In either topology, the clades whose taxa possess the ovipositor clip are separated by numerous clades that lack the clip. For Figitinae, ACCTAN or DELTRAN optimization (Swofford

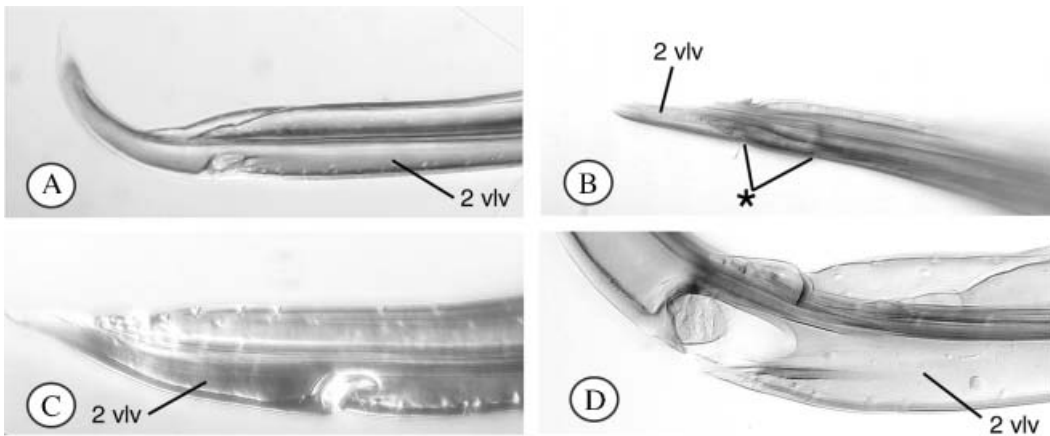


Figure 4. Examples of the ovipositor clip present within Figitinae. (A) *Neralsia*; (B) *Amphithectus*; (C) *Trischiza*; (D) *Xyalophora*. The viewing angle in (B) is dorsal and not lateral; the asterisk and lines are meant to delineate the extent of the clip. 2 vlv, fused ovipositor valve.

and Maddison 1987) does not affect how the presence of the clip maps using parsimony or Bayesian analyses (single gain, Figitinae clade). Within Eucoilinae in the parsimony tree, the ovipositor clip maps as a single gain at the base of (core Eucoilinae (*Kleidotoma* group + *Zamischus* group) clade (ACCTRAN or DELTRAN). The ovipositor clip maps on to the eucoiline Bayesian tree differently, showing two steps in both ACCTRAN (single gain at Eucoilinae node with secondary loss in *Gronotoma* + *Zaeucoila* groups) or DELTRAN (parallel gain in core Eucoilinae and *Kleidotoma* + *Zamischus* groups).

Buffington et al. (2007) favoured the parsimony-based results of the total evidence analysis for several reasons, including recovering the Parnipinae as sister-group to all figitid subfamilies and recovering the eucoiline (*Gronotoma* + *Zaeucoila* group) clade as sister-group to the (core Eucoilinae (*Kleidotoma* + *Zamischus* group) clade. Based on the number of steps required to map the ovipositor clip on the two competing phylogenies presented here, the parsimony tree is again preferred over the Bayesian tree (two steps parsimony, three steps Bayesian).

Discussion

The following discussion refers to a *concealed host* as a host whose feeding niche is within a form of encasement that prevents the host from freely moving away from the niche space. Examples of this type of feeding niche include galls, leaf and stem mines, previously parasitized insects, and seeds. A *semi-concealed* host is one whose feeding behaviour within a substrate prevents obvious detection by a parasitoid (e.g. by olfaction), but if the parasitoid enters the host substrate, the host could be detected by the parasitoid. As a caveat, the host larva can readily escape from the parasitoid by fleeing into the substrate. Examples of this type of host-feeding niche include carrion, dung, roots, algae, and rotting fruit. An *exposed* host is one that feeds externally (either phytophagous or predaceous) and is either less likely or not able to escape parasitization by seeking refuge within its niche. Examples of this type of host-feeding niche include external foliovores and predators of external foliovores.

The occurrence of the ovipositor clip within Figitidae is strongly correlated with a preference for attacking semi-concealed (as defined above) dipterous larvae. Evidence

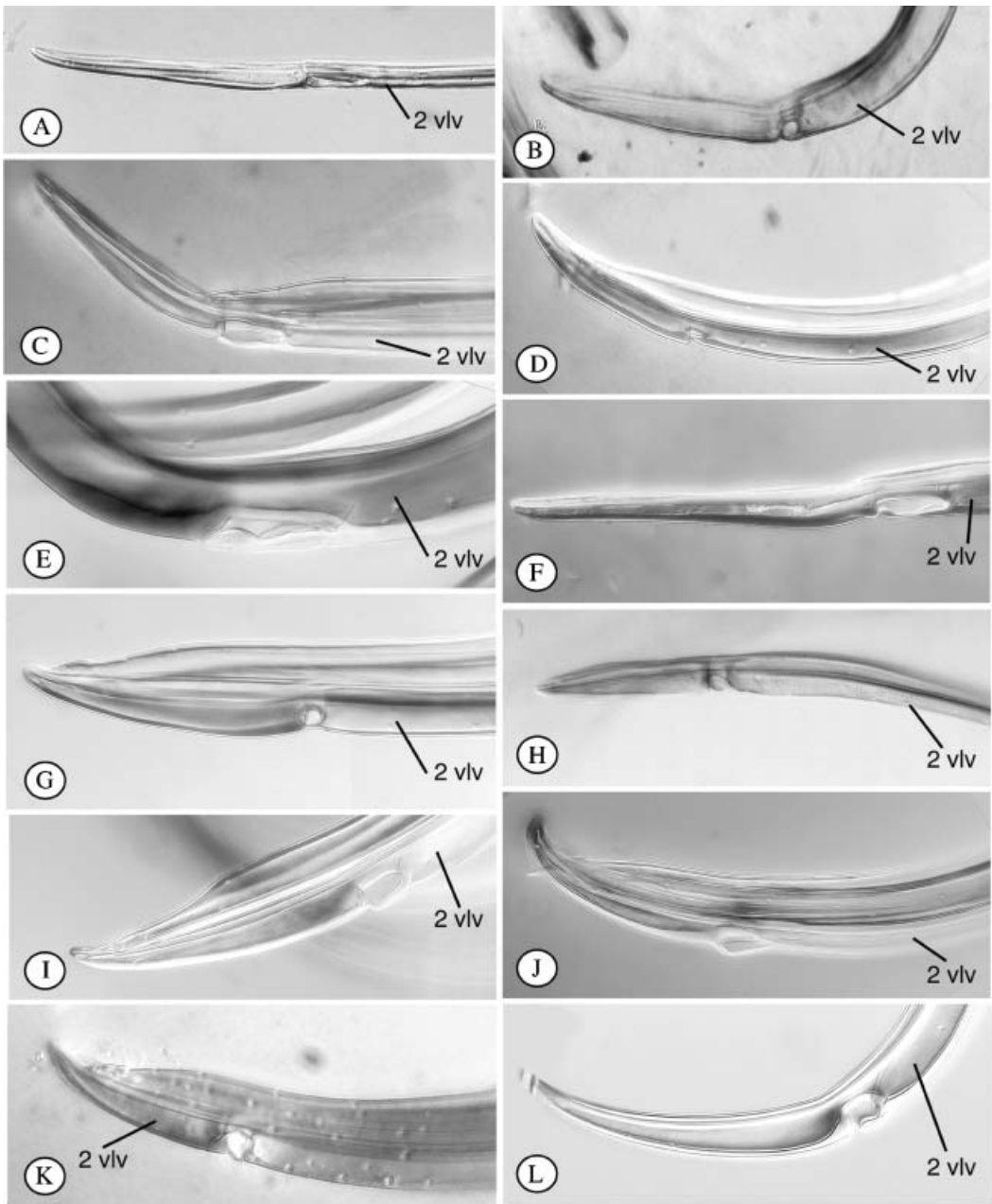


Figure 5. Examples of the ovipositor clip within Eucoilinae. (A) *Aganaspis daci* (Weld); (B) *Leptopilina*; (C) *Dieucoila*; (D) *Nordlandiella*; (E) “*Eucoila*” *impatiens* Say; (F) *Odontosema anastrephae* Kieffer; (G) *Ganaspis mundata* Foerster; (H) *Triplasta*; (I) *Glauraspidia*; (J) *Trybliographa*; (K) *Kleidotoma*; (L) *Aporeucoela*. 2 vlv, fused ovipositor valve.

supporting these host preferences is summarized in Table I. Figitids attacking concealed hosts (as defined above) universally lack the ovipositor clip. These figitids include Parnipinae, which attack cynipid gall inducers on *Papaver* (Papaveraceae) (Ronquist and Nieves-Aldrey 2001) and Thrasorinae which attack hymenopteran gall inducers on *Quercus*

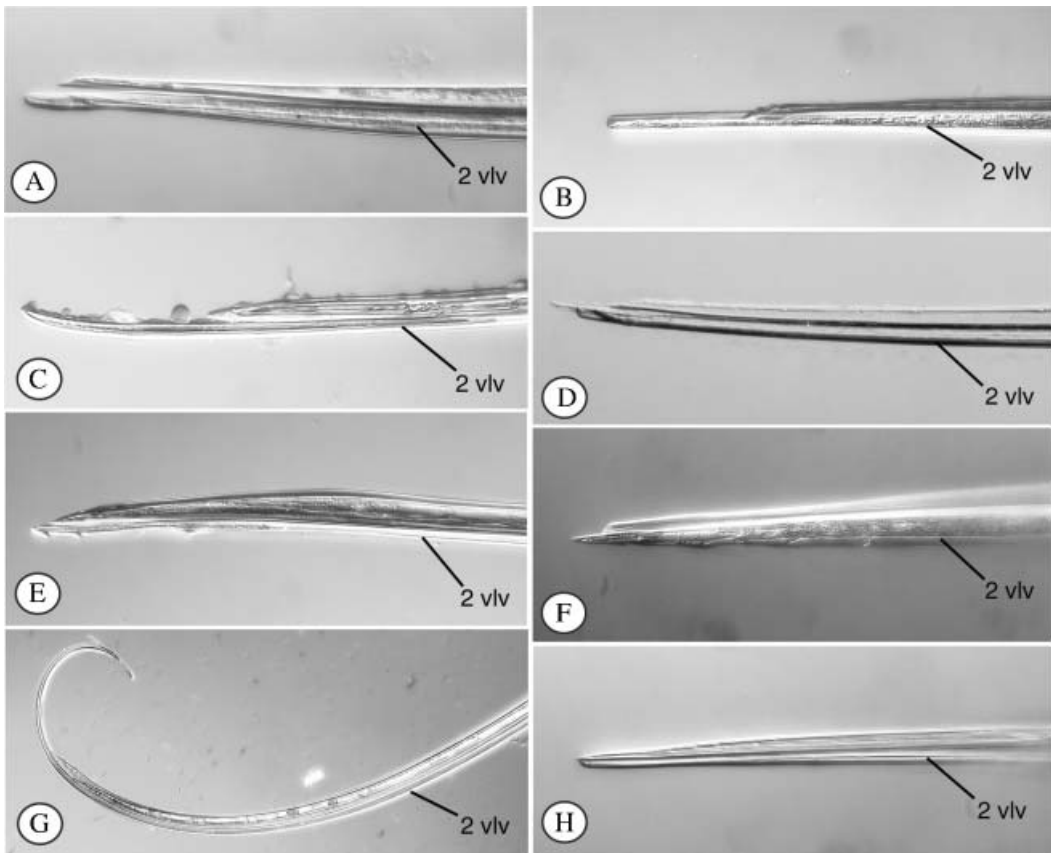


Figure 6. Examples of Eucoilinae that lack the ovipositor clip. (A) *Ganaspidium nigrimanus* (Kieffer); (B) *Gronotoma*; (C) *Ealata*; (D) *Tropideucoila*; (E) *Rhabdeucoela*; (F) *Lopheucoila anastrephae* Rohwer; (G) *Dettmeria*; (H) *Dicerataspis*. 2 vlv, fused ovipositor valve.

spp. (Fagaceae), *Mimosa* spp. (Mimosaceae), and *Nothofagus* spp. (Nothofagaceae) (Ronquist 1999). Within Eucoilinae, the *Gronotoma* and *Zaeucoila* genus groups, which attack leaf-mining Agromyzidae on various plants (Buffington 2002, 2004a, 2004b; Fontal-Cazalla et al. 2002), also lack the ovipositor clip.

Figitids that attack exposed hosts also lack the ovipositor clip. These include the Aspicerinae that attack exposed Syrphidae larvae (Thompson 1955; Burks 1979) and Anacharitinae that attack exposed Neuroptera larvae (Burks 1979; Cave and Miller 1987).

The ovipositor clip is associated with host restraint during the early stages of oviposition prior to envenomation (van Lenteren et al. 1998). Concealed hosts cannot readily escape parasitism, and it follows that figitids specializing on concealed hosts do not require the ovipositor clip for host restraint. Furthermore, when attacking a concealed host, the ovipositor must first penetrate the substrate encasing the host. Quicke et al. (1999) reviewed the various morphological traits associated with ovipositor tips of parasitoids that attack concealed hosts. In most cases, these modifications came in the form of denticles and serrations that were postulated to be associated with drilling and sawing through a substrate to reach the host. Similar modifications were found in some Chalcidoidea (Heraty and Quicke 2003) and Cynipoidea (Fergusson 1988) that oviposit in plant tissues. Quicke et al.

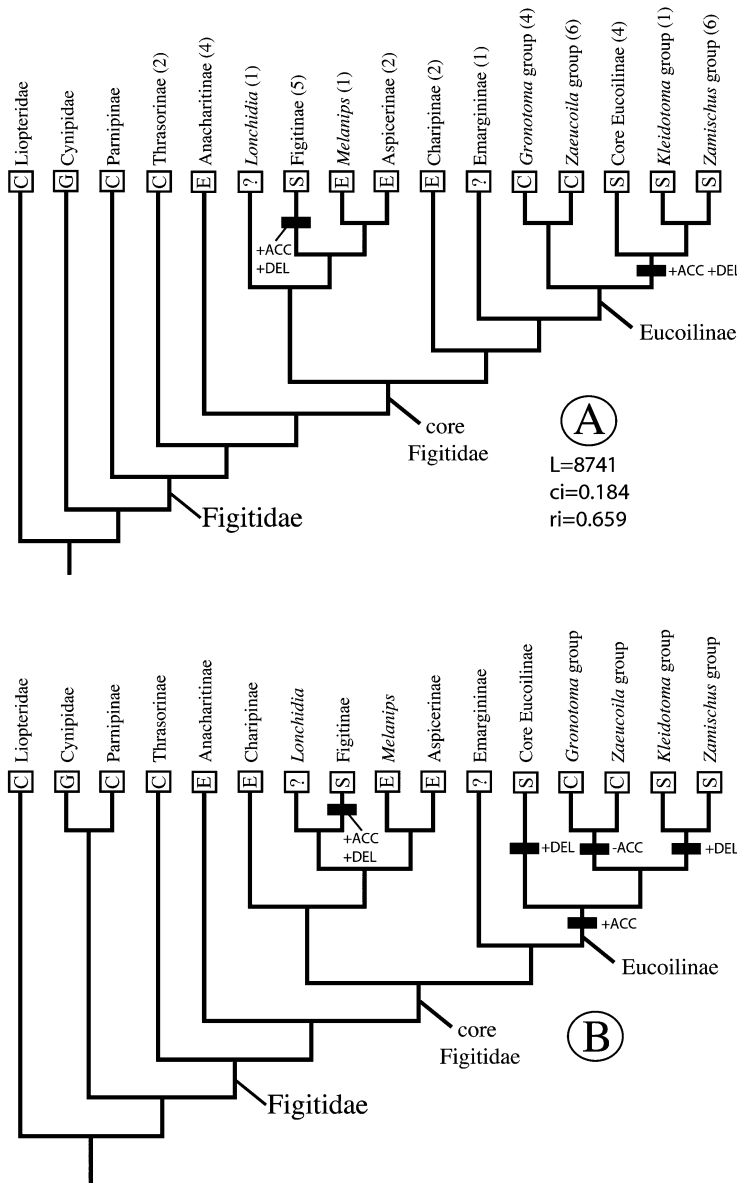


Figure 7. Presence of the ovipositor clip mapped on to two possible cladograms for relationships of Figitidae (from Buffington et al. forthcoming). (A) Parsimony analysis; (B) Bayesian analysis. Numbers next to terminal names indicate the number of taxa sampled. Letters in boxes at tree tips refer to niche type in which the host is attacked: C, concealed; S, semi-concealed; E, exposed; G, gall inducer/inquilline; ?, unknown. Black bars indicate clades for which all members possess the ovipositor clip. “+ACC” indicates presence of character based on ACCTRAN optimization; “+ DEL” indicates presence of character based on DELTRAN optimization (Swofford and Maddison 1987).

(1999) also noted sub-apical notches were common in several lineages of koinobiont endoparasitic Ichneumonoidea that attacked both concealed and exposed hosts.

Charipinae specialize on hyperparasitism of aphidophagous Braconidae and Chalcidoidea in aphid mummies (Clausen 1940; Menke and Evenhuis 1991) as well as

Chalcidoidea parasitic on immobile Psylloidea (Menke and Evenhuis 1991; Ronquist 1999). An ovipositor clip is probably not needed in this system since these hosts are both immobile and concealed.

There are other reasons to explain why Figitidae that attack exposed hosts lack the ovipositor clip. Anacharitinae are quick-strike parasitoids of chrysopid and hemerobiid second and third instar larvae (Miller and Lambdin 1985; Cave and Miller 1987; G. Miller, personal communication). The later instar larvae of these neuropterans are both precocious and aggressive, and both traits are probably responsible for the anacharantine mode of oviposition.

Aspicerinae (including *Melanips* as suggested in Buffington et al., 2007) attack exposed Chamaemyiidae and Syrphidae larvae (Ronquist 1999; M. L. Buffington, personal observation). The main difference between these exposed hosts, and the semi-concealed hosts of Figitinae and Eucoilinae, can be found in the host's niche. Chamaemyiids and syrphids are typically predators of phytophagous insects on vegetation (Clausen 1940), whereas the cycloraphan hosts of Figitinae and Eucoilinae are typically detritous feeders and scavengers on or in the soil (Table I). Though they may lack the ovipositor clip for the same reason anacharitines do (quick-strike oviposition), aspicerines do possess well-developed serrations on 1 vlv (as in Figitinae) and 2 vlv (Figure 3D, E). Unfortunately oviposition observational studies are currently lacking for Aspicerinae. Though hosts are unknown for species of *Lonchidia* (Figitinae), Emargininae, and Pycnostigminae, ovipositor morphology (Fergusson 1988, Figure 3G) and phylogenetic position suggest that hosts are likely to be concealed Diptera (i.e. ovipositor clip lacking; no host restraint needed).

Members of Eucoilinae that lack the ovipositor clip belong to the *Gronotoma* and *Zaeucoila* groups of genera. Buffington et al. (2007) recovered these clades as sister-groups in both parsimony and Bayesian analyses (Figure 7). The majority of members of these clades parasitize leaf-mining Agromyzidae (Buffington 2000, 2002, 2004a, 2004b; Buffington and Ronquist 2006), though some members of the *Zaeucoila* group (*Detmeria*, *Lopheucoila*, *Dicerataspis*, Figure 6F–H) are known from Tephritoidea (Wharton et al. 1998). As in the other figitids that attack concealed hosts, the ovipositors of members of the *Gronotoma* and *Zaeucoila* groups must first penetrate the leaf surface in order to reach the host. *Ganaspidium nigrimanus* (Kieffer) (*Gronotoma* group) was observed ovipositing on *Liriomyza* sp. (Agromyzidae) (M. Johnson, personal communication). It was noted that *G. nigrimanus* first located the leaf mine itself through “drumming” the leaf surface with the antennae. Once the mine was located, the female began probing it with her ovipositor along the entire length of the mine until a host larva was detected. The same behaviour was observed for an unidentified species of *Agrostocynips* (*Zaeucoila* group) attacking an unidentified agromyzid on *Celtis* sp. (Ulmaceae) (M. L. Buffington, personal observation).

The precise oviposition behaviour of *Detmeria*, *Lopheucoila*, and *Dicerataspis* is unknown. Interestingly, these taxa lack the ovipositor clip (Figure 6F–H), though they have been reared from hosts that other eucoilines possessing the ovipositor clip also attack (e.g. *Aganaspis* spp., Table I; Wharton et al. 1998). This may be explained by phylogenetic constraint since lack of the ovipositor clip is the plesiomorphic condition within the *Gronotoma* + *Zaeucoila* group clade (these taxa belong to the *Zaeucoila* group of genera).

Hosts for Figitidae possessing the ovipositor clip (Figures 4, 5) are summarized in Table I. Though the microhabitats for these hosts vary from algae beds (Ephydriidae) and roots of plants (Anthomyiidae) to dung (Muscidae, Sepsidae) and carrion (Sarcophagidae), all of these hosts are semi-concealed in that they do not specifically conceal themselves in

specialized structures that could restrict escaping parasitization. Arguably some species of Muscidae “conceal” themselves within a cow pat, however, the ovipositing wasp is able to enter the centre of the cow pat through holes and crevices to oviposit successfully (R. A. Wharton, personal communication; M. L. Buffington, personal observation). If the host larva detects the presence of the wasp, it can escape deeper into the cow pat; a host in a gall or leaf-mine is unlikely to be able to do this (Godfray 1994).

Mapping of the presence of the ovipositor clip (Figure 7) results in two different interpretations of the evolution of the feature. On the preferred tree (Figure 7A, parsimony) the ovipositor clip maps on to the Figitinae clade (excluding *Lonchidia*; one step) as well as the (core Eucoilinae (*Kleidotoma* group + *Zamischus* group)) eucoilinae clade (one step). In the alternate scenario (Bayesian tree, Figure 7B), the ovipositor clip maps in the same way as the parsimony tree for Figitinae (one step), but maps as a parallel gain in Eucoilinae (two steps) or as a single gain with subsequent loss (two steps). A third scenario, requiring the highest degree of loss, would require the ancestor of all core Figitidae to possess the ovipositor clip. Forcing the homology of the clip between Figitinae and Eucoilinae would require a sister-group relationship between Figitinae and Eucoilinae (single origin of clip, one step), with subsequent loss of the clip in the *Gronotoma* and *Zaeucoila* groups (one step). On the parsimony tree, this requires an additional 39 steps.

Conclusion

A classification scheme for ovipositing behaviour into various host types has not been proposed (van Lenteren et al. 1998). The system described here would suggest that one category should exist in which the host is physically restrained during the early stages of oviposition. This type of oviposition is correlated with parasitoids that attack semi-concealed hosts that could escape parasitization if not quickly subdued with some other means (e.g. venom). In contrast, fully exposed hosts may be more readily parasitized without host restraint. In these cases, the parasitoid attacks as quickly as possible to prevent possible injury inflicted by the host defending itself. If hosts are fully concealed within a specific substrate (e.g. a gall or leaf-mine), a method of restraint is not necessary since the host cannot readily escape. In these cases, the ovipositor is unmodified to allow maximum ovipositional efficiency.

Most Eucoilinae and all Figitinae that attack semi-concealed dipterous hosts universally possess the ovipositor clip. The best parsimony and Bayesian trees, however, suggest the clip is not homologous between these lineages unless loss of the clip is accepted on five separate lineages.

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Appendix 1. List of Figitidae examined

An asterisk after a taxon name indicates the voucher specimen is located at the Department of Entomology, Texas A&M University; otherwise, the voucher is housed at the Entomology Research Museum, UC Riverside.

Thrasorinae: *Plectocynips* sp.; *Euceroptres* sp.

Anacharitiniae: *Aegilips clarimontis* Kieffer; *Anacharis* sp.; *Hexacharis* sp.; *Xyalaspis* sp.

Charipinae: *Alloxysta brassicae* Ashmead; *Phaenoglyphis ambrosiae* (Ashmead).

Emargininae: *Thoreauella* sp.

Aspicerinae: *Aspicera* sp.; *Callaspidea* sp.; *Melanips* sp.

Figitinae: *Amphithectus* sp.; *Figites* sp.; *Lonchidia* sp.; *Neralsia* sp.; *Trischiza* sp.; *Xyalophora* sp.

Eucoilinae: *Aganaspis daci* (Weld)*; *Agrostocynips* sp.*; *Aporeucoela* sp.; *Caleucoela* sp.; *Dettmeria* sp.*; *Dicerataspis* sp.*; *Dieucoila* sp.; *Disorygma pacifica* (Yoshimoto); *Ealata* sp.*; “*Eucoila*” *impatiens* (Say); *Ganaspis mundata* Förster*; *Ganaspidium nigrimanus* (Kieffer); *Glauraspida* sp.; *Gronotoma* sp.*; *Kleidotoma* sp.; *Leptopilina* sp.; *Lopheucoila anastrephae* Rohwer*; *Nordlandiella semirufa* (Kieffer); *Odontosema anastrephae* Borgmeier*; *Rhabdeucoela* sp.*; *Tropideucoila* sp.*; *Trybliographa rapae* Westwood.