



## Oviposition mechanisms in the whitefly parasitoids *Encarsia transvena* and *Eretmocerus mundus*

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**Abstract.** The ovipositors of two whitefly parasitoids, *Encarsia transvena* and *Eretmocerus mundus* were examined using scanning and transmission electron microscopy. That of *Encarsia* is straight, has an apparently hard and sharply pointed upper valve, and appears well-suited to penetrating a hard substrate. That of *Eretmocerus* is curved, thick-walled, but has a blunt and apparently flexible tip. These features correlate well with what is known of the mode of oviposition and host feeding in the two taxa, with *Encarsia* and *Eretmocerus* ovipositing internally and externally respectively.

**Key words:** Aphelinidae, *Bemisia tabaci*, Chalcidoidea, cuticle, host feeding, host penetration, ovipositor, parasitic wasp

### Introduction

Whiteflies are hosts to several genera of parasitoid wasps, the most common of which are members of the aphelinid genera *Encarsia* and *Eretmocerus* (Gerling et al., 1980). Although both develop as solitary endoparasitoids of whitefly nymphs, their developmental biology differs markedly (Gerling et al., 1991). *Encarsia* females deposit their eggs into the haemolymph of the whitefly nymph by standing on it and penetrating the dorsal cuticle with their ovipositor (van Lenteren et al., 1976). Consequently, it is to be expected that their ovipositor will have a sharp, pointed, tip, and that it will be sturdy enough to drive through the host's dorsal cuticle. *Eretmocerus* females, on the other hand, oviposit while standing beside their host and driving their ovipositor between the venter of the host nymph and the leaf surface. The egg is deposited under the whitefly and hatches there. Penetration into the host is a complex procedure that apparently involves the 1st instar parasitoid

larva puncturing the host with its mandibles and the host actively engulfing the young larva (Gerling et al., 1990, 1991).

The fact that females of *Eretmocerus* do not penetrate the host's cuticle while egg laying and have weaker ovipositors than *Encarsia* females was inferred by Gerling (1966) as a possible explanation for the host-feeding habit of *E. californicus* Howard *sic*. The latter was accomplished by penetration of the host's vasiform orifice rather than penetration of the tough dorsal cuticle as in *Encarsia pergandiella* Howard. These differences in host-feeding and oviposition behaviour have subsequently been observed in several other species of the two genera, but no detailed examinations of the associated ovipositor features have ever been made. Consequently, we set out to examine the comparative structure of the ovipositors of *Encarsia transvena* (Timberlake) and *Eretmocerus mundus* Mercet (Hymenoptera: Aphelinidae) both of which attack *Bemisia tabaci* (Gennadius), an important pest of several crops.

## Materials and methods

Both parasitoid species were reared on *B. tabaci* nymphs in temperature-controlled cabinets at 25 °C. *Encarsia transvena* originated from a culture received from Dr L. Osborne (University of Florida) several years ago. *Eretmocerus mundus* was originally collected in the field in Israel.

Microscopic examination included the use of scanning and transmission electron microscopy (SEM and TEM, respectively). For SEM, the material was fixed over night with 3% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4; washed with the same buffer, dehydrated in increasing concentrations of ethanol, critical point dried and then coated with gold. We used a JEOL 840 SEM operating at 25 kV.

For transmission electron microscopy the material was fixed in the same way as for SEM for 24 hours at 4 °C, and then rinsed in sodium cacodylate buffer. Post fixation was performed for 1 h with 1% osmium tetroxide in the same buffer. The material was then rinsed as before and stained in saturated uranyl acetate for 20 min, dehydrated through increasing concentrations of ethanol and embedded in Epon 812. Sections were cut using an LKB Ultramicrotome, double stained in uranyl acetate, followed by lead citrate and then examined with a JEOL 1200 CX transmission electron microscope.

### *Basic morphology and terminology*

Full descriptions of hymenopteran ovipositor morphology have been provided by Smith (1968, 1969, 1970). Ovipositor structures have had a

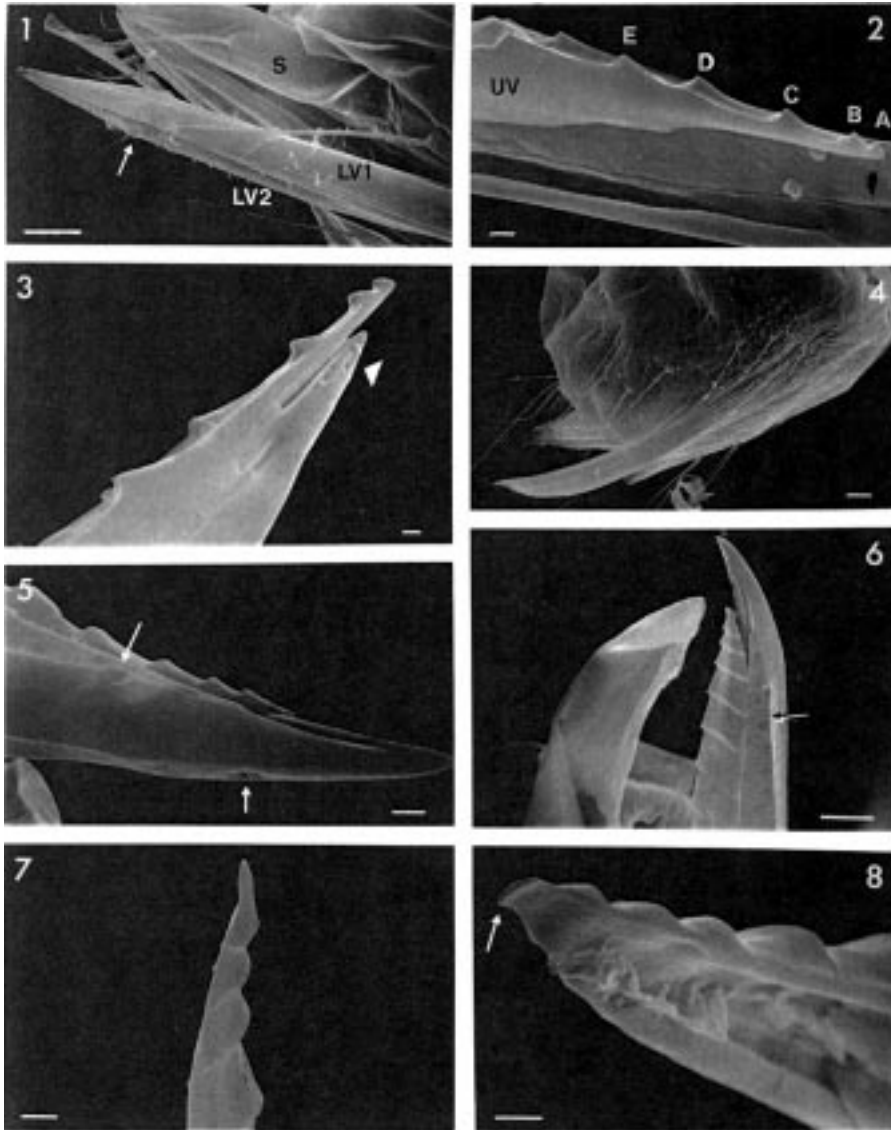
complicated history of terminology, and so for simplicity, we use the following system (Quicke et al., 1994). The ovipositor proper comprises 3 parts, the fused gonapophyses of the 9th abdominal (8th metasomal) segment are referred to as the upper ovipositor valve (Figure 2: UV), and the pair of gonapophyses of the 8th abdominal segment (7th metasomal), referred to as the lower ovipositor valves (Figure 1: LV). The upper valve is interlocked with the lower valve on each side by a 'T'-section, tongue and groove joint called the olistheter, which allows the valves to slide to-and-from relative to one another as required for substrate penetration and oviposition. The upper and lower valves each have a lumen filled with haemolymph and living tissues such as nerves and tracheae. Collectively, the three valves surround the egg canal, the walls of which are furnished with various forms of microsculpture (see Figures 9–11) which is believed to assist with moving the egg (Austin and Browning, 1981; Raliman et al., in press). In most if not all chalcidoids the ovipositor is asymmetric with the two halves of the upper valve fully fused at the apex but joined medio-dorsally by membranous cuticle for the rest of its length, and the two halves typically overlap one another (Figure 13). The lower valves are similarly asymmetric in many species with the ventral margin of one overlapping the other (Figure 12).

## Results

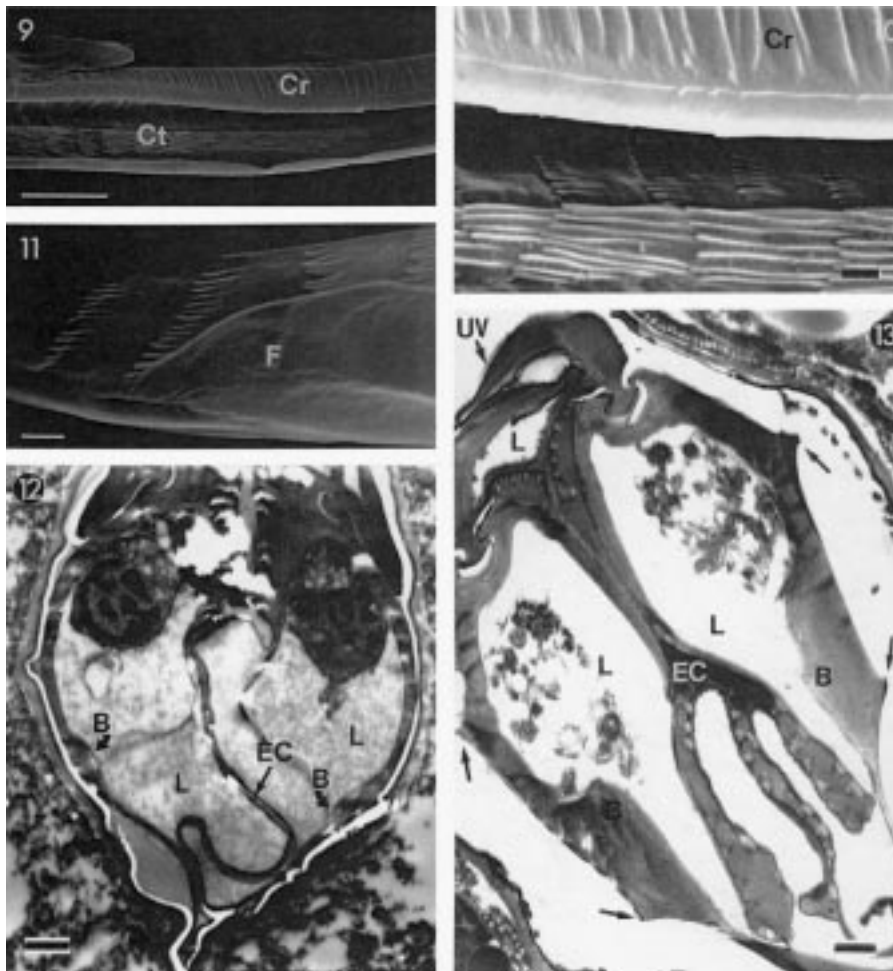
### *External ovipositor morphology*

Our findings regarding the ovipositor of *Encarsia* are in agreement with those of Le Ralec (1991). The whole structure is relatively narrow and more or less straight (Figure 1). The upper valve is furnished at the apex with a number of serrations (teeth). The three distal ones are symmetric (Figure 2: A–C), i.e. they are centred medially and extend more or less across the dorsal width of the valve. The five more posterior teeth are asymmetrically distributed, alternating on left and right sides like the off-set teeth of a saw (Figure 2, teeth D, E). No conspicuous sensilla were found on the upper valve, but 5 distinct sensilla comprising two distinct morphotypes were present on the apical 3  $\mu\text{m}$  of each lower ovipositor valve (Figure 3, arrow). In addition to this sensilla cluster, 3 other putative sensilla occur between 15 and 20  $\mu\text{m}$  from the lower valve tip (Figure 1, arrow). These three all appear different morphologically, the middle one being rather like a transverse nick.

The ovipositor of *E. mundus* is far more robust than that of *Encarsia* (Figure 4 cf. 1), it is wider, the lower valves are relatively deeper, and the whole is distinctly upcurved. The tip of the upper valve is unique as far as is known, in that it is distinctly truncate, even slightly expanded (Figures 6 and



Figures 1–8. Scanning electron micrographs of external ovipositor morphology. 1–3, *Encarsia transvena*: 1, ventral view showing left and right lower ovipositor valves (LV1 and LV2 respectively), ovipositor sheath (S) and pre-apical lower valve sensilla (arrow); 2, apex of upper valve (UV) showing serrations (A–E), note that the distal most pair are positioned medially while more posterior ones alternate on right (C,E) and left sides (D); 3, cluster of sensillae (arrow head) at apex of lower valve. 4–8, *Eretmocerus mundus*: 4, general view showing depth and upwards curvature of ovipositor; 5, detail of apex of lower valve showing two sensilla (arrows) located some distance from tip; 6, showing angular crease (arrow) of lower valve and blunt apex of upper valve; 7 and 8, two views of apex of upper valve. Scale bars: 1, 4 = 10  $\mu\text{m}$ ; 2, 3, 5, 7, 8 = 1  $\mu\text{m}$ ; 6 = 5  $\mu\text{m}$ .



Figures 9–13. 9–11, Scanning electron micrographs of lower valve egg canal wall of *Eretmocerus mundus*, anterior to right: 9, general view showing ctenidial sculpture (Ct) of the egg canal and crenular sculpture (Cr) of upper part of outer wall; 10, detail of 9; 11, detail of sculpture near apex with the origin of one of the folds (F) of the egg canal wall. 12, 13, Transmission electronmicrographs of transverse sections through medial part of ovipositor: 12, *Encarsia transvena* showing the evenly thin walls of lower valve with weakly developed internal longitudinal boss (B) protruding into lumen (L) and the intricate meeting of the egg canal walls leaving only a narrow egg canal when ovipositor is not in use; 13, *Eretmocerus mundus* showing upper valve (UV), relatively thicker cuticle of lower valves with more strongly developed internal longitudinal bosses (B) protruding into the lumen, complex foldings of egg canal (EC) walls, and pair of longitudinal lateral ridges (arrows). Scale bars: 9 = 10  $\mu\text{m}$ ; 10–13 = 1  $\mu\text{m}$ .

8). In lateral aspect, the upper valve tip appears more like a tongue (Figure 7), and in different specimens was variously orientated, suggesting that it may be rather flexible. The 5 distal serrations are symmetric whereas the more anterior ones, although medially divided by the notal membrane that joins the two more sclerotised lateral parts, are not markedly off-set (Figure 6). Moreover, the whole set of serrations appears much less pointed and sharp than those of *Encarsia transvena* (Figures 6 and 7 cf. 2). The lower valves, although smoothly rounded laterally at the apex, become distinctly angular some 15  $\mu\text{m}$  further towards the base. Both faces have fine transverse crenulate micro sculpture (Figures 9 and 10). However, as with *E. transvena*, there are no conspicuous sensilla on the upper valve. Further, all sensilla on the lower valves are located further from the apex than in *E. transvena*. A sensillum occurs some 13  $\mu\text{m}$  from the tip in a more or less ventral position (Figure 5, lower arrow), and additional morphologically similar ones occur dorso-laterally at approximately 17  $\mu\text{m}$  and 30  $\mu\text{m}$  from the tip.

#### *Transverse sections and egg canal microsculpture*

Transverse sections through the ovipositor at about its midlength show the different profiles of the lower valves: smoothly rounded in *E. transvena* (Figure 12) and strongly angular with two longitudinal ridges on each side in *E. mundus* (Figure 13). In addition, the sections revealed a complex arrangement of membranous cuticle lining the egg canal (EC). The egg canal walls of the two lower valves are closely apposed and the egg canal is not open in these sections, that were taken from non-ovipositing females. The outer wall of the lower valve in *E. transvena* ranges approximately from 300 to 500 nm thick. In *E. mundus*, the cuticle of the outer walls is about 200 nm thick ventrally, but reaches a maximum of approximately 1.5  $\mu\text{m}$  opposite the more ventral of the two longitudinal ridges, i.e. at the level of the longitudinal internal thickening (Figure 13, B).

The complex folding of the membranous walls of the lower valve canal make it very difficult to assess where the different microsculptures occur. In section the membranous cuticle appears dentiform (Figures 9–11), and is very similar in both taxa. These comb-like micro sculpture arrangements are referred to as ctenidia (Rabman et al., in press) and are similar in general form to those found in many other parasitic wasps. Each ctenidium is aligned more or less transversely, but the details differ on different regions of the egg canal membrane. The ctenidia on the more ventral part of the egg canal wall comprise parallel sets of long ridges with those of one ctenidium starting just after the tips of those of the preceding ctenidium (Figures 9, 10). The ctenidia of the more dorsal part of the egg canal wall comprise more discrete combs of teeth.

## Discussion

The differences observed in external ovipositor morphology, especially that of the upper valve, appear to reflect the different oviposition and host feeding behaviours in the species *E. transvena* and *E. mundus* as representative of the two genera to which they belong. The slender, rather sharply pointed and apparently firm upper ovipositor valve of *Encarsia* is clearly well-suited as a penetrative organ. Penetration through the dorsal cuticle of the host for oviposition will be further facilitated by the generally slender form. A cutting role is also suggested by the off-setting of the majority of the upper valve serrations, which suggests a saw-like function which will minimise vibration (Quicke, 1997). In contrast, the apex of the upper ovipositor valve of *Eretmocerus* is blunt and apparently unsuited for 'drilling'. Indeed, its features seem well suited for an organ that is designed for penetrating the space between the leaf and the delicate lower epidermis of the whitefly nymph, while avoiding the penetration or wounding of the latter, an act that would render the host unsuitable for the development of *Eretmocerus* progeny. The difference in function between the ovipositors of these two aphelinid genera is also indicated by their general form, slender and straight in *Encarsia* versus robust and upcurved in *Eretmocerus*. In *Eretmocerus*, the shape of the ovipositor is apparently adapted for sliding it along the leaf surface and levering. The latter assumption is supported further by the presence of ridges on the lower valves. These ridges are associated with cuticular thickenings which presumably give it more strength. In comparison, the outer cuticle of the lower valves of *Encarsia* is of more or less even thickness. The serrations in the two genera also differ in their sharpness and general form. The fact that the internally-ovipositing, *Encarsia*, and the externally-ovipositing, *Eretmocerus*, both possess serrations on the ovipositor's tip warrants consideration. The utility of serrations as a cuticle penetrating tool in *Encarsia* is almost self-evident, that is not the case for *Eretmocerus*, where nothing has to be penetrated and the ovipositor has only to be slid between two surfaces in order to lay an egg. Our observations do not furnish an unequivocal explanation for the presence of serrations in *Eretmocerus*, they may act as retro-stoppers or anti-sliding devices with the upward-pointing, rounded, serrations enabling the parasitoid to insert the ovipositor while holding on to the ventral cuticle of the host and advancing toward the desired oviposition spot without either wounding the host or sliding back during the act. Once oviposition is completed, we presume that the parasitoid can release the ovipositor valve by turning it about its axis and thus disengaging it from the host's venter.

The distribution and range of morphotypes of the ovipositor sensilla is also potentially informative with regard to function (LeRalec et al., 1996).

The endoparasitic *Encarsia* has several distinct types of sensilla, including a group at the tip of the lower valve. In *Eretmocerus*, the sensilla appear to comprise a single morphotype and are widely spaced with no apical cluster. An apical cluster would seem well-suited for an endoparasitoid given that it may need to obtain sensory feedback about environment surrounding the tip of the ovipositor so that the ovipositing female can release the egg into the proper chemical and physical media. Such feedback has been demonstrated in allowing the parasitoid to detect host suitability in numerous publications, e.g. Klomp et al. (1980), van Alphen and Thunnissen (1983), Gerling et al. (1987), and van Alphen and Visser (1990). In addition, in some *Encarsia* species, like *E. formosa* also display ovicidal behaviour in that if a female locates an host that has recently been attacked by another, she will locate the previous female's egg and pierce it with her ovipositor (Arakawa, 1987). Such discriminatory behaviour and multifunctionality of the ovipositor in this genus compared with *Eretmocerus*, is consistent with the considerably greater diversity and more heterogeneous arrangement of sensilla in *Encarsia*.

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