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Structure and Functional Morphology of the Ovipositor of *Homolobus truncator* (Hymenoptera: Ichneumonoidea: Braconidae)

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Abstract.—The following morphological structures of the ovipositor of *Homolobus truncator* (Say) are described and hypotheses of their functions are proposed. A pre-apical notch on the exterior surface of the dorsal valve locks the ovipositor into the host cuticle. A series of sharp ridges on the distal surface of the notch helps maintain a grip on the inner surface of the host cuticle. An internal longitudinal ridge, the sperone, on the dorsal valve directs eggs away from inner surface of the ventral valves. A flap-like structure near the apex of each ventral valve covers the portal through which eggs pass and reduces evaporation of fluids in the egg canal between oviposition events. An internal hollow reservoir near the base of each ventral valve acts as a conduit to facilitate passage of fluids (venom) to more distal areas of the egg canal, where they provide hydrostatic pressure to help force eggs out of the ovipositor. An internal valve-like structure on each ventral valve, the valvillus, plays a role in maintaining egg position within the ovipositor and acts like the stopper of a hypodermic needle to push fluid against the egg and force it out of the ovipositor. Ctenidia on the inner surface of the ventral valves are instrumental in moving eggs along the basal half of the egg canal, but their role in egg movement apical to the valvillus is questionable. Ctenidia may also play a role in preventing the valvilli from scraping all fluid from the walls of the egg canal. Recurved barbs at the apex of each ventral valve hook into the inner surface of the host cuticle to maintain purchase while the thick dorsal valve is inserted. Most of these structures are widespread throughout Ichneumonoidea and our discussions are likely to pertain in whole or in part to these taxa.

Parasitoid Hymenoptera utilize a diverse range of hosts that occupy a wide array of microhabitats. This diversity is reflected in a variety of adaptations in ovipositor morphology. Thus, ovipositor morphology can provide insights into host utilization and life history. For example, Quicke (1991) noted that the dorsal and ventral valves of *Zaglyptogastra* and *Pristomerus* varied in thickness along their length, giving the ovipositor a sinuous appearance. This characteristic allows the ovipositor to bend with differential relative positions of the dorsal and ventral valves. Quicke (1991) reasoned that this bending allows the ovipositor to navigate through preexisting openings in the host substrate in order to locate a host. Subsequent field observations confirmed this hypothesis (Quicke and Laurenne 2005).

An understanding of functional morphology allows for inferences of biology when only morphology is known. For example, Belshaw et al. (2003) examined ovipositor characteristics of numerous Ichneumonoidea with known biologies in order to discover features that correlate with endo- or ectoparasitism. Based on these correlations they predicted the mode of parasitism of taxa using morphological data. In some instances, behavior can also be inferred from ovipositor morphology.

Lenteren et al. (1998) described the "ovipositor clip" on the dorsal valve of *Leptopilina heterotoma* (Thompson) (Hymenoptera: Figitidae) and explained how it functions to restrain the host during oviposition.

Here we describe the morphology of the ovipositor of *Homolobus truncator* (Say) and speculate on the function of numerous structures, viz., the pre-apical notch on the exterior surface of the dorsal valve; the series of sharp ridges on the distal surface of the notch; the internal longitudinal ridge, the sperone, on the dorsal valve; the flap-like structure near the apex of each ventral valve; the internal hollow reservoir near the base of each ventral valve; the ctenidia on the inner surfaces of the ventral valves; the internal valve-like structure on each ventral valve, the valvillus; and the recurved barbs at the apex of each ventral valve.

Homolobus truncator is a nocturnal, koinobiont, endoparasitoid of numerous species of exposed, lepidopterous larvae, primarily in the families Geometridae and Noctuidae. Among its recorded hosts are a number of economically important agricultural pests such as *Agrotis ipsilon* (Hufnagel), *Helicoverpa zea* (Boddie), *Spodoptera exigua* (Hübner), and *Spodoptera frugiperda* (Smith) (Yu et al. 2004). *H. truncator* is found in all major biotic realms except Australia (van Achterberg 1979).

MATERIAL AND METHODS

All specimens of *H. truncator* used in this study were collected with Malaise traps in Hardy County, Virginia, USA. Species were identified using the key in van Achterberg (1979) and later confirmed by comparison with specimens of *H. truncator* determined by van Achterberg.

Specimens were stored in 95% ETOH, and dissected in the same solution. For SEM preparation, specimens were chemically dried using hexamethyldisilazane (HMDS), following the protocol of Heraty and Hawks (1998), and then coated with gold palladium. SEM images were taken with a HitachiS-800 scanning electron microscope.

The terminology used here follows Quicke et al. (1999). Comprehensive studies of the hymenopteran ovipositor include Snodgrass (1933), Oeser (1961), Scudder (1961), Smith (1970), and Quicke et al. (1992). The muscular mechanics of hymenopteran oviposition were described by Vilhelmsen (2000).

The ovipositor is composed of a dorsal valve and paired ventral valves. The dorsal and ventral valves interlock by a 'tongue and groove' system in which each ventral valve has a longitudinal groove that interlocks with a pair of longitudinal rails (tongues) that protrude from the ventral surface of the dorsal valve. The two ventral valves are capable of sliding independently along the length of the rails of the dorsal valve (Fig. 1C). The 'tongue and groove' system is properly termed the olistheter mechanism. Together, the internal concave surfaces of the dorsal and ventral valves form the egg canal (Fig. 1C, E). These same features are ubiquitous throughout Hymenoptera with only rare exceptions.

RESULTS AND DISCUSSION

Ovipositor morphology of Homolobus truncator (Figs 1A, B, D, E, 2A–E, 3A–F, 4A–F, 5A–D)

The ovipositor of *Homolobus truncator* is short (Fig. 1A) and relatively thick and rigid except near the apices of the ventral valves (Fig. 2D, E). The dorsal valve is blunt (Fig. 1B) and contains a pre-apical notch. Immediately basal to the notch, the dorsal valve thickens to approximately twice the diameter of any point more distal (Fig. 1B, E). There are many sensory structures in this area (Figs 1B, 3E, F), which appear to be campaniform tactile sensillae (Fig. 22, 'SC' in Quicke et al. 1999). The remainder of the dorsal valve gradually increases in diameter basally.

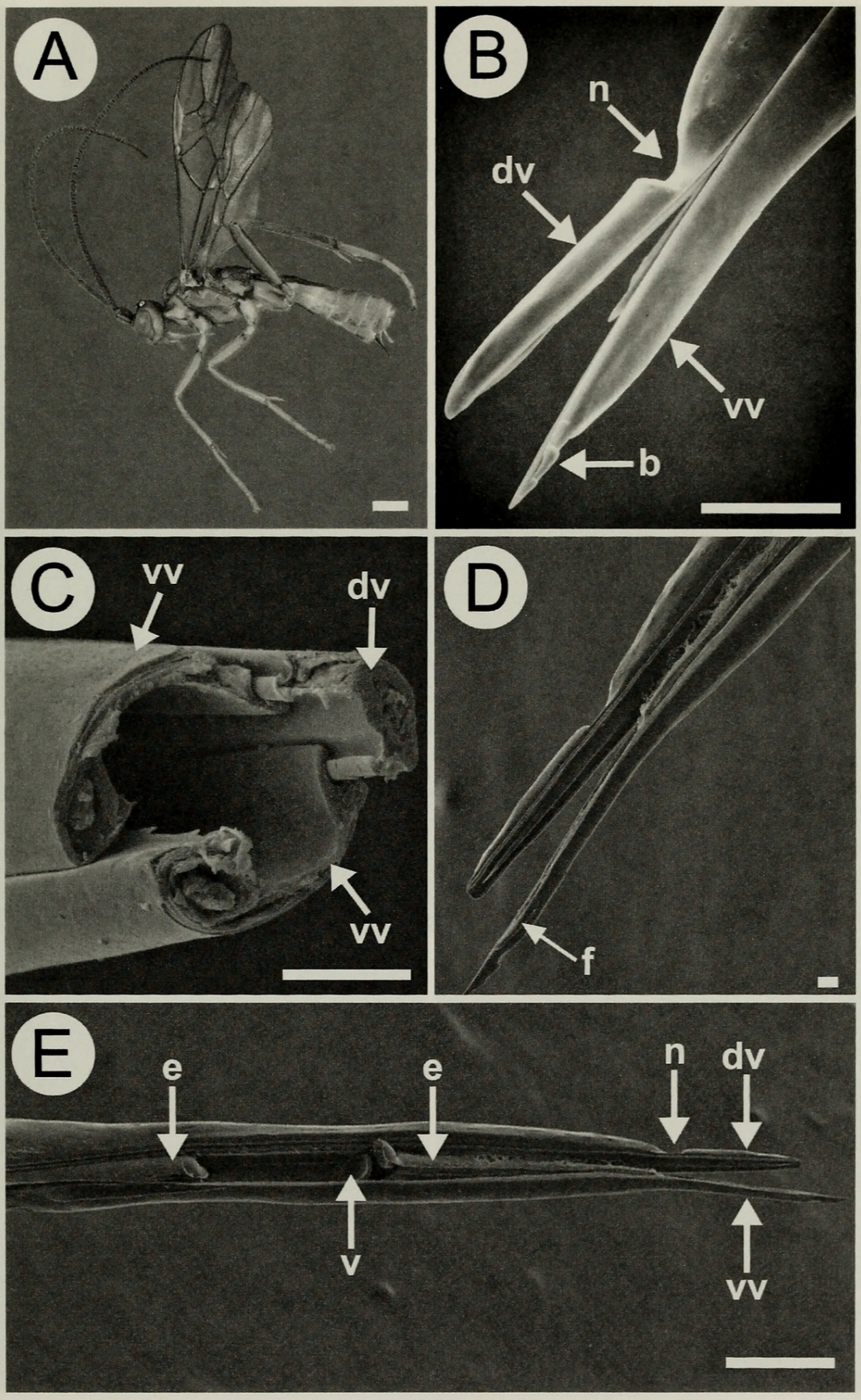

Fig. 1. A. *Homolobus truncator*: lateral habitus, scale bar = 1 mm. – B. *H. truncator*: lateral view of the distal region of the ovipositor, scale bar = 75 µm. – C. *Meteorus sp.*: the distal region of the ovipositor is broken, scale bar = 10 µm. – D. *H. truncator*: ovipositor apex with one ventral valve removed, arrow indicates a flap on the ventral valve, scale bar = 10 µm. – E. *H. truncator*: lateral view of entire ovipositor with one ventral valve removed, scale bar = 100 µm. (Abbreviations: b = barbs, dv = dorsal valve, e = egg, f = flaps, n = notch, v = valvillus, vv = ventral valve).

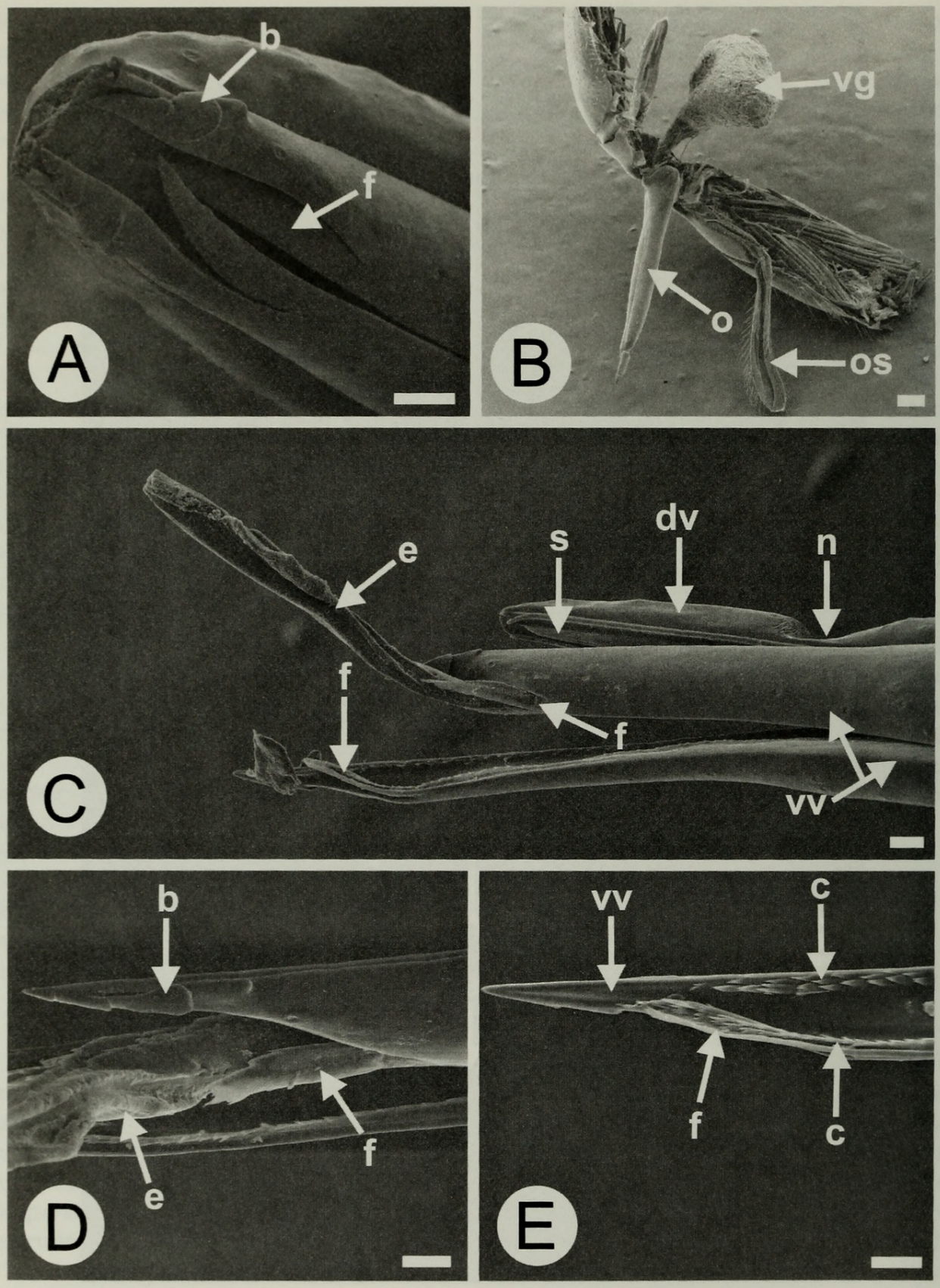

Fig. 2. A–E. *Homolobus truncator*. – A. Ventral view of the ovipositor showing a flap on each ventral valve, scale bar = 10 µm. – B. View of the entire ovipositor and venom gland, scale bar = 100 µm. – C. Latero-ventral view of the ovipositor with an egg exiting from the flap on the ventral valve, scale bar = 10 µm. – D. Lateral view of the exterior ventral valve with an egg exiting from the flap on the ventral valve, scale bar = 10 µm. – E Lateral view of the interior ventral valve, scale bar = 10 µm. (Abbreviations: see Fig. 1, c = ctenidia, o = ovipositor, os = ovipositor sheath, s = sperone, vg = venom gland).

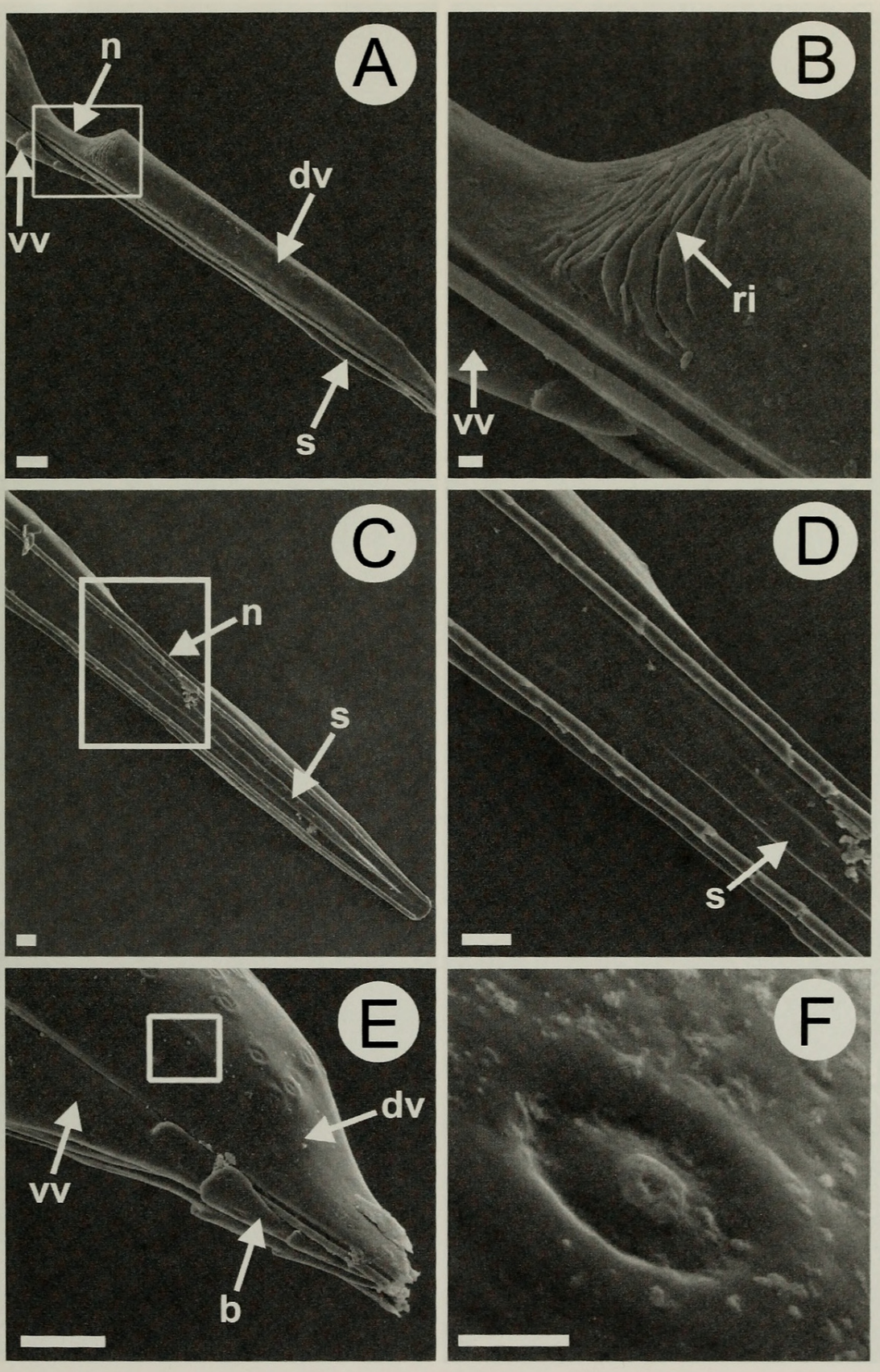

Fig. 3. A–F. *Homolobus truncator*. – A. Lateral view of the ovipositor with a rectangle outlining the location of Fig. 3B, scale bar = 10 µm. – B. High magnification of the outlined region in Fig. 3A, scale bar = 1 µm. – C. Ventral view of the dorsal valve with a rectangle outlining the location of Fig. 3D, scale bar = 10 µm. – D High magnification of the outlined region in Fig. 3C, scale bar = 10 µm. – E Lateral view of the ovipositor with the tip broken at the notch. The rectangle outlines the sensory structure in Fig. 3F, scale bar = 1 mm. – F. High magnification of the outlined region in Fig. 3E, scale bar = 1 µm. (Abbreviations: see Fig. 1 and Fig. 2, ri = ridges).

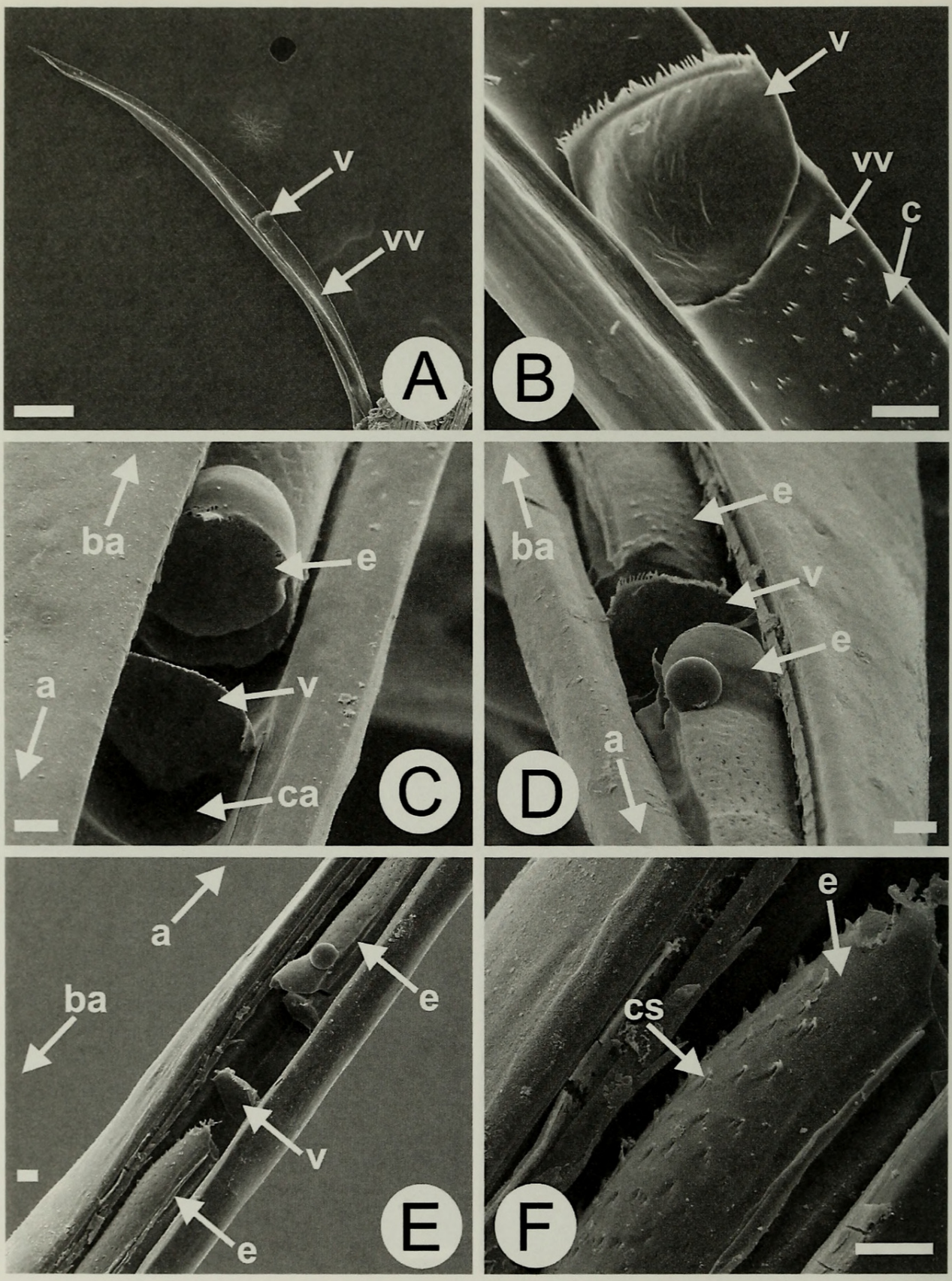

Fig. 4. A–F. *Homolobus truncator*. – A. Lateral view of the ventral valve interior, scale bar = 100 µm. –B. The valvillus, scale bar = 10 µm. – C. Lateral view of the ovipositor with one ventral valve removed, scale bar = 10 µm. – D. Lateral view of the ovipositor with one ventral valve removed, scale bar = 10 µm. – E. Lateral view of the ovipositor with one ventral valve removed, scale bar = 10 µm. – F. Lateral view of the ovipositor with one ventral valve removed showing high magnification of an egg in the egg canal, scale bar = 10 µm. (Abbreviations: see Fig. 1 and Fig. 2, a = apical direction of ovipositor, ba = basal direction of ovipositor, ca = cavity for valvillus, cs = ctendial scars).

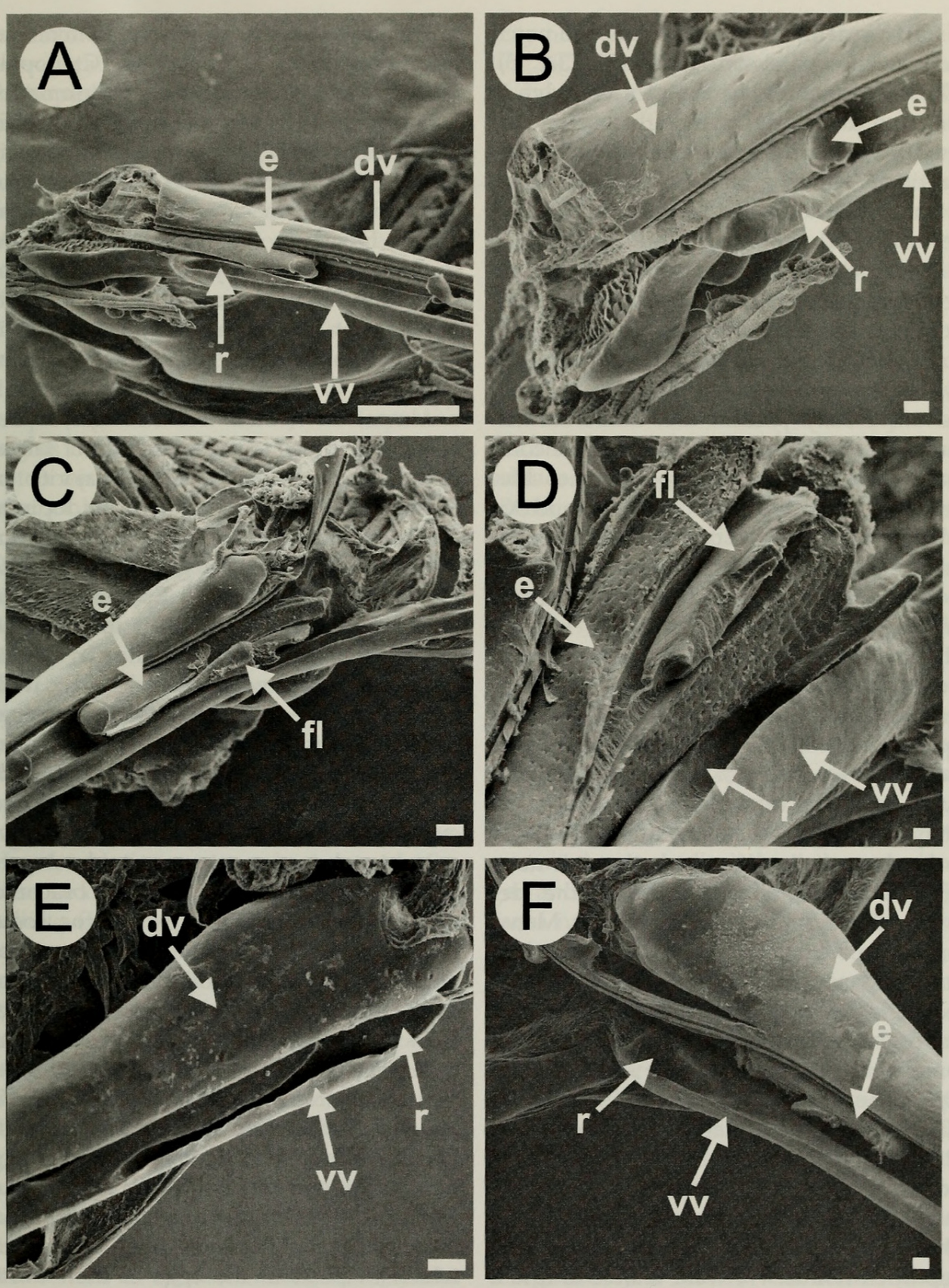

Fig. 5. A–D. – A. *Homolobus truncator*: Latero-ventral view of the ovipositor with one ventral valve removed, scale bar = 100 µm. – B. *Homolobus truncator:* Lateral view of the ovipositor with one ventral valve removed, scale bar = 10 µm. – C. *Homolobus truncator:* Latero-ventral view of the ovipositor with one ventral valve removed, scale bar = 10 µm. – D. *Homolobus truncator:* Latero-ventral view of the ovipositor with one ventral valve removed, scale bar = 10 µm. – E. Blacinae: lateral view of the ovipositor base with one ventral valve removed, scale bar = 10 µm. – F. *Austrozele sp.* (Macrocentrinae): Latero-ventral view of the ovipositor with one ventral valve removed, scale bar = 10 µm. (Abbreviations: see Fig. 1, fl = congealed fluid, r = reservoir).

The distal surface of the pre-apical notch has a series of sharp ridges (Fig. 3A, B). The scarp (acute) surface of each ridge is directed anteriorly. Although we did not quantify the ridges, the peak-to-peak separation of the ridges is approximately 1 μm, and the peak-to-valley height is a few hundred nanometers.

The pre-apical notch is widespread in Ichneumonoidea. It is clear that at least some occurrences of the ovipositor notch are convergent. Braconid subfamilies where the pre-apical notch is commonly or universally present are: Amicrocentrinae, Charmontinae, Euphorinae, Helconinae, Homolobinae, Macrocentrinae, Meteorinae, Microtypinae, Orgilinae, and Xiphozelinae. Presence of a pre-apical notch is rare in the braconid subfamilies Cardiochilinae and Cenocoeliinae. When present in the Cenocoeliinae, the notch is very shallow. The frequency of the pre-apical notch in Aphidiinae and Blacinae is unknown; in both subfamilies there are species with and without the pre-apical notch but we have not surveyed sufficiently to provide reasonable estimates. The shape of the pre-apical notch in Aphidiinae is fundamentally different in that it is not a simple indentation but rather the depressed area is relatively quite long. Many, or perhaps most, Alysiinae have a structure that appears much like a pre-apical notch which may even function in certain aspects like those of the aforementioned braconids. In members of the Alysiinae, the tip of the dorsal valve is swollen and the diameter decreases rapidly; however this decrease in diameter remains relatively constant toward the base, though it gradually thickens. The structure at the apex of the dorsal valve in Alysiinae may be a modified nodus. We were unable to find a pre-apical notch in any ichneutine genera including *Ichneutes*, although Rahman et al. (1998, char. N) coded the pre-apical notch present for Ichneutinae (*Ichneutes* sp.). Ichneumonid subfamilies where the pre-apical notch is commonly or universally present are: Anomaloninae, Banchinae, Campopleginae, Cremastinae, Ctenopelmatinae, Neorhacodinae, Ophioninae, Oxytorinae, Tatogastrinae, and Tersilochinae (David Wahl, *pers. comm.*). Approximately half of the genera in Metopiinae and Orthocentrinae possess a pre-apical notch. However, the notch tends to be shallow to moderately shallow when present. Although most members of Stilbopinae do not possess a pre-apical notch, it can be found in *Notostilbops fulvipes* Townes.

Almost all ichneumonoids with a pre-apical notch are endoparasitoids of larval holometabolous insects. The majority of these ichneumonoids attack Lepidoptera, but some attack larval Diptera or Coleoptera. Exceptions to these generalities can be found in many genera of Euphorinae that are endoparasitoids of adult insects. The presence of a pre-apical notch is not constrained by ovipositor length; it is found in species with long ovipositors that probe deep into substrates such as wood and leaf-rolls, as well as in species with short ovipositors that oviposit directly into exposed hosts. We did not observe a pre-apical notch in any ectoparasitoids. A pre-apical notch was absent in all braconid cyclostome subfamilies, except for some Aphidiinae. We did not detect a pre-apical notch in any of the following non-cyclostome subfamilies: Adeliinae, Agathidinae, Cheloninae, Ichneutinae (however see Rahman et al. 1998, char. N), and Sigalphinae. The endoparasitoid subfamilies Agathidinae and Sigalphinae are peculiar amongst the Braconidae in that they deposit the egg in a ganglion of the host (Shaw and Quicke 2000) and therefore very precise deposition is necessary. Members of Cheloninae, which oviposit in the eggs of their hosts and emerge from the larvae, do not have a pre-apical notch, and this may be true for all egg-larval ichneumonoid parasitoids, though we have not conducted a detailed survey. Within the Ichneumonidae, some Stilbopinae (*Stilbops* spp.) are egg-larval

parasitoids, and these species also lack a pre-apical notch. Only one species of Stilbopinae, *Notostilbops fulvipes*, has a pre-apical notch and the biology of this rare species is unknown. It is unreasonable to assume that *N. fulvipes* is an egg-larval parasitoid since some other Stilbopinae (*Panteles schuetzeanus* (Roman)) are endoparasitoids of Lepidoptera larvae (Quicke 2005).

There is a well-developed sperone, an internal median longitudinal ridge, near the apex of the ventral surface of the dorsal valve (Figs 1D, 3A, C, D). The sperone begins immediately basal to the pre-apical notch and is most pronounced near the apex of the dorsal valve where it projects into the egg canal. Rahman et al. (1998, characters O, P) surveyed the distribution of the sperone and pre-apical notch in the Braconidae, as did Quicke and Belshaw (1999, chars. 52, 53). These studies reported that a sperone is present in many non-cyclostome Braconidae, as well as in the ichneumonid *Xorides*. Both studies demonstrated an association between the pre-apical notch and the sperone in that all taxa with a pre-apical notch also had a sperone. However, a sperone may be present in the absence of a pre-apical notch, e.g. *Trioxys pallidus* (Haliday).

The two ventral valves of *H. truncator* narrow toward the apices and are sharply pointed. Each valve has a small series of recurved barbs near the apex (Fig. 2D), which are ubiquitous across Hymenoptera. On the outer (ventral surface), there is a flap-like structure on each valve, immediately basal to the apex (Fig. 2A) and mesal to the barbs. Rahman et al. (1998, character N) examined the distribution of the flap-like seal within the Braconidae and found it absent in all cyclostome taxa and present in the majority of non-cyclostome braconids. We examined those non-cyclostome braconids coded by Rahman et al. (1998, character N) as absent and found this feature present in *Eubazus* (Fig. 6A), Macrocentrinae (Fig. 6C), and Euphorinae (Fig. 6B). The subfamilies Microgastrinae (Fig. 6D) and Meteorinae (Fig. 6E) appear to be the only non-cyclostome subfamilies without flap-like structures on the apex of the ventral valves, however denser taxon sampling is needed to make a firm conclusion. Other microgastroid subfamilies and Euphorinae genera have the flap-like structure present. We suggest these flap-like structures are a putative synapomorphy of the non-cyclostome Braconidae.

The ventral valves quickly increase in diameter and then remain relatively constant in diameter toward their bases. The surface of the egg canal of most hymenopterans and many other insects is covered with scattered ctenidia or scales that are set almost flat against the surface with the basal end attached and the distal end free (Smith 1968; Austin and Browning 1981; Rahman et al. 1998). In specimens of *H. truncator* the ctenidia are absent from the dorsal valve except for a small number present on the sperone. The ctenidia on the ventral valves are scattered over most of the inner surface, except near the apex where they are absent medially and concentrated marginally, where they are longer and less rigid (Figs 2E, 4A). The egg canal narrows considerably near the apex of the ovipositor.

Internally, near mid-length, each ventral valve has one chitinous valvillus (Fig. 4A–E). The valvilli rotate over a 90° arc; from perpendicular to the egg canal axis to parallel with the axis and directed apically. The ovipositor valvilli have no intrinsic musculature, therefore the movement of the valvilli is controlled by the relative motion of the valves and perhaps by fluid pressure in the egg canal. Each valvillus is margined by a narrow but dense fringe (Fig. 4B). The valvillus is deeply imbedded within the wall of the egg canal, and immediately apical to the valvillus the egg canal is excavated to allow the valvillus to lay flat (Fig. 4C). When a valvillus is perpendicular and blocking the egg canal,

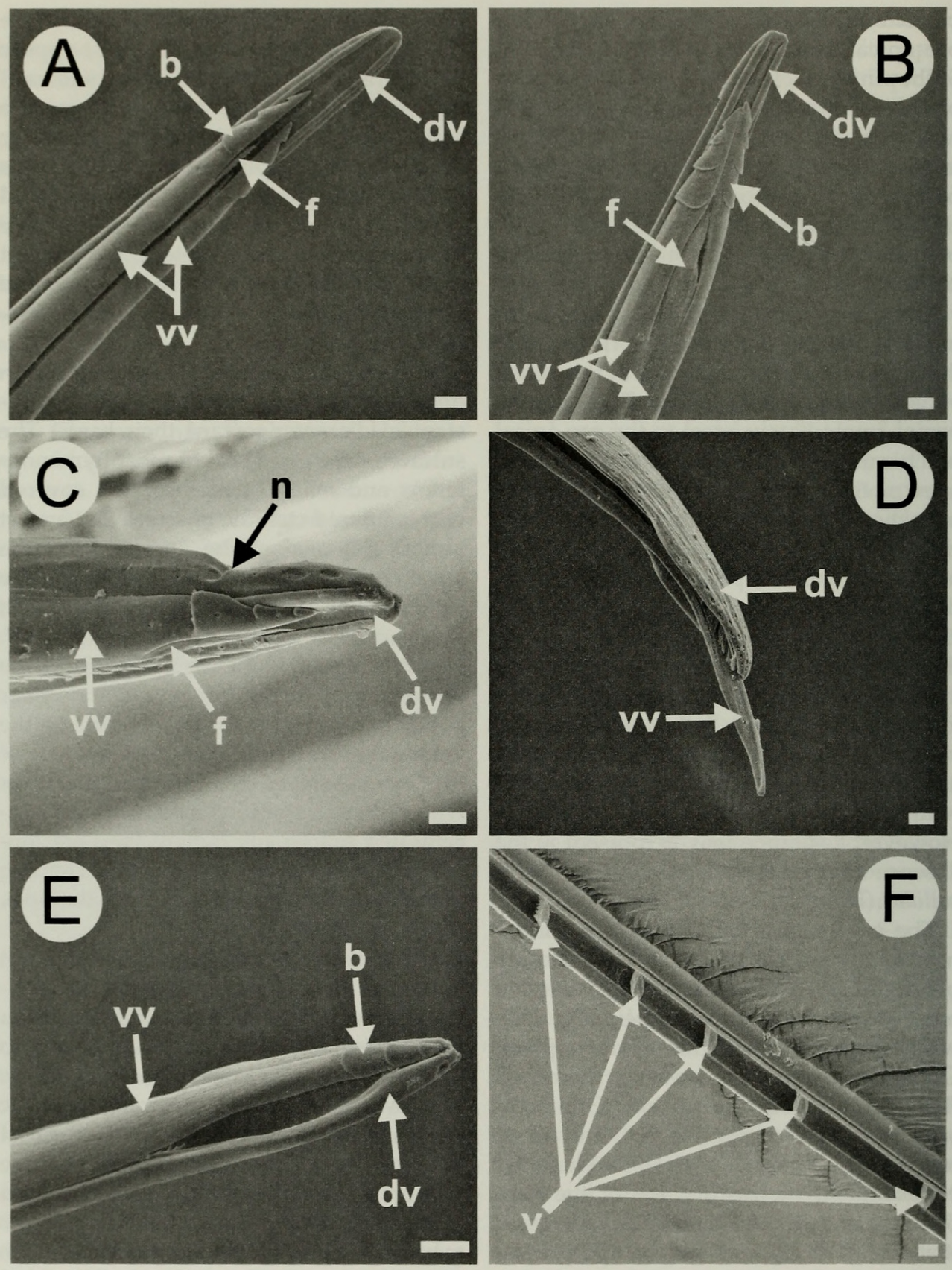

Fig. 6. A. *Eubazus* (Helconinae): ventral view of the ovipositor apex, scale bar = 10 µm. – B. *Streblocera* (Euphorinae): ventral view of the ovipositor apex, scale bar = 10 µm. – C. Macrocentrinae: latero-ventral view of the ovipositor apex, scale bar = 10 µm. – D. Microgastrinae: lateral view of the ovipositor apex with one ventral valve removed, scale bar = 10 µm. – E. *Meteorus* (Meteorinae): ventral view of the ovipositor apex with one ventral valve removed, scale bar = 10 µm. – F. *Wrougtonia sp.* (Helconinae): lateral view of the ovipositor with one ventral valve removed, scale bar = 10 µm. (Abbreviations: see Fig. 1).

it appears to be of a shape and size capable of sealing the egg canal (Fig. 4C, D)

An internal, excavated reservoir near the base of each ventral valve (Fig. 5A–D) is described here for the first time. It is approximately 170 μm long, 20 μm wide at the base, and it tapers to a point apically. It appears to be about as deep as it is wide (Fig. 5A, B). A preliminary survey of braconid subfamilies found this feature to be present in Blacinae (Fig. 5E), Helconinae (*Wroughtonia* sp.), Homolobinae, Meteorinae (*Zele* sp.), and Macrocentrinae (*Austrozele* sp.) (Fig. 5F). The reservoir is well developed in *Homolobus* and least developed in *Wroughtonia* and *Zele*. We did not find a reservoir present in Agathidinae, Braconinae, Doryctinae, Rogadinae, or *Campoletis sonorensis* (Cameron) (Ichneumonidae). The reservoir may represent a synapomorphy for a subset of non-cyclostome braconids, but obviously more taxon sampling is needed to test this idea.

Functional morphology

Our hypotheses on the functions of various ovipositor structures are based on careful examination of morphological structures. Direct observations are difficult because most of the events that we postulate take place inside the host or inside the ovipositor. The strengths, and weaknesses, of the hypotheses are based primarily on their explanatory power, i.e., their power to explain the morphology of the observed structures in a parsimonious and logical manner. In some cases arguments may be convincing, and in other cases we are offering what amounts to little more than speculation.

In the following paragraphs hypotheses on the oviposition process in *H. truncator* are broken down into four phases: penetration of the host cuticle, locking mechanism, egg movement, and egg-laying and ovipositor withdrawal. A concise overview is presented in the next paragraph and illustrated in Fig. 8. In Fig. 8, the host cuticle is represented by a horizontal black line. The dorsal valve is light grey and on the right. On the left there are two ventral valves, one is light grey and in the foreground, the other is dark grey and in the background. The arrows on the left indicate the movement of the ventral valves; the color of the arrow corresponds to the movement of the valve with the same color. An arrow on the right indicates movement of the dorsal valve. In Fig. 8F, the dark grey ventral valve moves upward as indicated by the dark grey arrow; this movement is visually obstructed by the light grey ventral valve in the foreground because the dark grey ventral valve is in the background. This visual obstruction also occurs in Fig. 8M, N, Q, and R. The valvilli are represented by horizontal ovals, where the dark grey valvillus is attached to the dark grey ventral valve and the light grey valvillus is attached to the light grey ventral valve. When the valvillus is represented by a thin oval, the valvillus is perpendicular and blocks the egg canal. When the valvillus is represented by a large oval, the valvillus is parallel to, and allows movement through the egg canal. In Fig. 8H, venom enters the egg canal, which is represented by the color yellow. The egg is represented by the white, vertical oval. In Fig. 8O, the egg begins to exit the ovipositor from a flap on the ventral valve. In Fig. 8R the egg is visually obstructing the flap.

Before a host is encountered, the egg is positioned near the tip of the ovipositor (see later). It is held in place basally by the valvilli and apically by the narrow apex of the egg canal and the sperone, which blocks the aperture of the egg canal. The tip of the ovipositor comes into contact with the host cuticle with all three valves aligned apically and flush with the surface of the host cuticle (Fig. 8A). The sharp ventral valves penetrate the host cuticle (Fig. 8B, C) to a depth sufficient to create a relatively large wound (Fig. 8D, E). The ventral valves are then partially withdrawn to the point where their sharp barbs

engage the internal surface of the host cuticle (Fig. 8F). At this time the blunt dorsal valve is pushed into the newly formed wound. The dorsal valve enters the host until the pre-apical notch slips onto the host integument (Fig. 8G, H). The ventral valves are then reinserted and egg-laying is effected with a series of alternating thrusts of the ventral valves (Fig. 8I–T). Although the egg is positioned at the apex of the ovipositor by movement of the ventral valves and the gripping force of the ctenidia, we speculate that once the egg is past the valvilli, further movement of the egg is facilitated by the valvilli pushing venom against the egg. On its way to the ovipositor apex, friction of the egg against the sperone forces the egg through the flaps in the ventral valves (Fig. 8O–Q). As the egg emerges from the ovipositor, elastic energy stored in the chorion provides additional force to help move the egg out of the ovipositor (Fig. 8R, S).

Phase 1: Ovipositor penetration of the host cuticle.—We postulate that the dorsal and ventral valves contact the host cuticle in unison with an egg positioned near the tip of the ovipositor (Fig. 8A). Contact with either one of the valves independently would cause unnecessary risk of fracturing one of the valves as both appear to be relatively fragile by virtue of their small cross-sectional area and barbs which act as stress raisers. The sharp ventral valves then penetrate the host (Fig. 8B, C) and are pushed deeply into the host to create a wound sufficiently large to facilitate entry of the relatively blunt dorsal valve (Fig. 8D–H). This explains the sharp points of the ventral valves and the rapid increase in the diameter of the ventral valves near the apex. It is uncertain if the ventral valves thrust in unison or in opposition; video of wood-boring ectoparasitic Ichneumonidae shows the ventral valves moving in opposition (Skinner and Thompson 1960). For this reason, Fig. 8 shows the ventral valves thrusting in opposition, however it is not beyond reason that the ventral valves move in unison. One exception is shown in Fig. 8F where the ventral valves are withdrawn in unison. This is necessary to prevent damaging the egg (see below). After penetration, we posit that the ventral valves are withdrawn to the point where the recurved barbs hook onto the inner surface of the host cuticle (Fig. 8F). At this point the wound is much larger than the diameter of the apical portion of the ventral valves that occupy it, thereby leaving room for the thick dorsal valve to enter. If the ventral valves were deeply inserted while the blunt dorsal valve was entering the host, the resulting wound would be excessively large and this would impede the effectiveness of the locking mechanism (see below).

Phase 2: Locking mechanism.—Belshaw et al. (2003) stated that the "pre-apical notch in the upper valve is tentatively assumed to be associated with moderating penetration of the host cuticle..." (p. 217) and van Veen (1982) observed that the ovipositor of *Banchus femoralis* Thomson is inserted into the host cuticle no further than the notch. Little else has been mentioned in the literature concerning this ubiquitous modification of the ovipositor. We suggest that the function of the notch is two-fold; it is part of a temporary locking mechanism that ensures continuous engagement with the host during oviposition, and in agreement with van Veen (1982), we suggest that it facilitates the correct depth of ovipositor penetration.

After initial penetration, the dorsal valve is pushed into the host until the host cuticle comes in contact with the base of the notch (Fig. 8G, H). This region is covered in campaniform sensillae (Fig. 3E, F) which we presume signal the wasp to stop thrusting the dorsal valve. After the cuticle of the host slips onto the notch on the dorsal valve, we posit that the ventral valves are pushed further into the host to a point where the thick section of the ventral valves align across from the dorsal notch (Fig. 8I–L). At this point, the notch,

and all other surfaces of the ovipositor, would be pressed firmly against the host cuticle, effectively locking the ovipositor into the host. During oviposition the ventral valves move in opposition to one another to effect movement of the egg (see below). During this activity the pressure between the exoskeleton of the host and the ovipositor remains constant because the diameter of the parts of the ventral valves that come into contact with the host cuticle is uniform (Fig. 8K–N). During the process of locking into the host cuticle, Fig. 8E, F, we suggest that the ventral valves must be withdrawn in unison, either directly or indirectly through abdominal movement. If the ventral valves withdraw in opposition to each other in Fig. 8E, F, their ctenidia would resist proximal movement of the egg toward the valvilli. If the ventral valves move in unison, the egg would remain supported by the ventral valves. Since the dorsal valve lacks ctenidia proximal to the notch, movement of the ventral valves in unison would not damage or cause distal movement of the egg.

Sharp transverse ridges are located over the distal surface of the pre-apical notch (Fig. 3A, B). This is the area of the dorsal valve in contact with the inner surface of the host cuticle. The sharp surfaces of the ridges face anteriorly and appear to be able to efficiently grip the inner surface of the host cuticle by creating numerous shallow penetrations. The sharp ridges and the resulting reduction in contact area would result in greater traction to be applied to the inner surface of the host's cuticle, much like the jaws of a pipe wrench. We have not conducted a systematic survey across the Ichneumonoidea for this feature.

Quicke et al. (1999) speculated that the pre-apical notch might be a point of articulation, "as if the tip might be able to hinge upwards, perhaps to assist exit of the egg." (p. 204). There are several reasons why we think that this is not likely to be the case in *H. truncator*. First, this scenario necessitates that the olistheter mechanism be derailed and it is hard to imagine a method to re-couple the interlocked dorsal and ventral valves (Fig. 1C), especially if they are not parallel to each other. Secondly, the idea lacks explanatory power in that if the dorsal and ventral valves were not in contact during oviposition it fails to explain the function of the sperone and the flaps near the apices of the ventral valves (see below)

Phase 3: Mechanism of egg movement along the egg canal.—Ctenidia on the surface of the egg canal in Hymenoptera and other insects are thought to act like stiff brushes to grip and push the egg down the canal and to prevent backward movement of the egg. In his study of the common black field cricket, *Gryllus assimilis* (Fabricius), Severin (1935) observed a direct correlation between alternating valve thrusts and movement of the egg through the ovipositor. Austin and Browning (1981) confirmed that the alternating action of the valves is responsible for egg movement in the gryllid *Teleogryllus commodus* (Walker) by directly manipulating the valves of anaesthetized specimens with fine forceps. It has also been shown that the egg is moved down the canal with alternating thrusts of the two ventral valves in some Hymenoptera. Cole (1981) observed specimens of the ichneumonid parasitoid *Itoplectus maculator* (Fabricius) ovipositing into the lepidopterous hosts *Galleria mellonella* (Linnaeus) and *Ephestia kuehniella* (Zeller). He noted that the ventral valves moved rhythmically back and forth after the ovipositor was inserted into the host. He also demonstrated that the egg must move down the egg canal after the ovipositor was inserted into the host because the parasitoids were capable of selecting the sex of their offspring, an action that logically follows contact and assessment of the host with the ovipositor.

The ctenidia of *H. truncator* are involved in egg movement in the basal half of the egg canal. Fig. 4E shows an egg of a specimen of *H. truncator* positioned near

the base of the egg canal. The surface of the egg is marked with indentations and small scars caused by contact with ctenidia. To create these scars, ctenidia of the ventral valves must have been firmly imbedded into the surface of the egg and any apical movement of the valves would necessarily result in a corresponding movement of the egg.

The fact that many aculeates (e.g. Fig. 7C, D) have ctenidia on some parts of the inner surface of the sting suggests that, at least for these species, the ctenidia have functions other than gripping and pushing eggs. The diverse morphology of ctenidia across Hymenoptera also implies multiple functions. We suggest that, besides moving eggs along the egg canal, the ctenidia may decrease friction by aiding lubrication. Ctenidia may also help to maintain a minimal amount of liquid in the egg canal. When one ventral valve moves apically relative to the other ventral valve, the valvillus of the former rubs against the inner surface of the latter. If the walls lacked ctenidia, all liquids, some of which may have a lubricating function (Bender 1943; Robertson 1968) would be scraped away. It stands to reason that the small separation of the ctenidia from the egg canal wall could acts as a miniscule fluid reservoir whereby wetting of the fluid into the gap would result in some fluid retention surrounding the ctenidia, thus improving lubricity. Conceivably, this would make it easier for eggs to pass by decreasing frictional forces against the egg canal wall via the action of lubricating fluid. In the "venom canal" of *Vespa crabro* Linnaeus, thick ctenidia similar to those found in the egg canals of parasitoids are found only on the dorsal valve (Fig. 7C, D). Further research is necessary to test these conjectures.

Although there is convincing evidence showing that ctenidia, in conjunction with alternating thrusts of the ventral valves, move the egg along the basal portion of the egg canal, we suggest that the valvilli may assist movement of the egg in the distal half of the egg canal. Ichneumonoids and aculeate Hymenoptera are unique among Hymenoptera in that many members possess valvilli (Figs 4A–F, 7A, B). These are valve-like structures in the ventral ovipositor valves that are able to block the egg canal; in the ichneumonoidea there are typically one pair per ventral valve but there may be as many as 5, as for example in *Wroughtonia sp.* (Fig. 6F). Because the aculeate sting does not function as an egg-laying device, the valvilli of aculeates are almost certainly employed as valves to pump venom into their hosts and/or potential predators (Janet 1898; Snodgrass 1925, 1956; Marle and Piek 1986). Rogers (1972), in his study of the ichneumonid endoparasitoid *Venturia canescens* (Gravenhorst), suggested that the valvillus functions to maintain the egg in place near the apex of the ovipositor. Quicke et al. (1992), noting the different uses of the ovipositor in aculeates and parasitoids, suggested that valvilli may have different functions in the two groups; presumably they meant egg positioning in parasitoids and venom injection in aculeates.

Figure 1E shows the ovipositor of *H. truncator* with the right ventral valve removed. Two eggs are visible in the egg canal. One is situated basally and the other is positioned near the apex with its basal end abutting the valvillus and its distal end aligning with the notch on the dorsal valve and the point where the ventral valve narrows. We suggest that this is the typical position of an egg ready for oviposition. We dissected numerous ovipositors of *H. truncator* and, with few exceptions we found an egg in this apical position. One exception is illustrated in Figures 2C and 2D, where the apical end of an egg may be seen extruding from the flap-like structures near the apex of the ventral valves. The "loaded" egg position is undoubtedly obtained with alternating thrusts of the lower valves in conjunction with friction provided by the apically directed ctenidia.

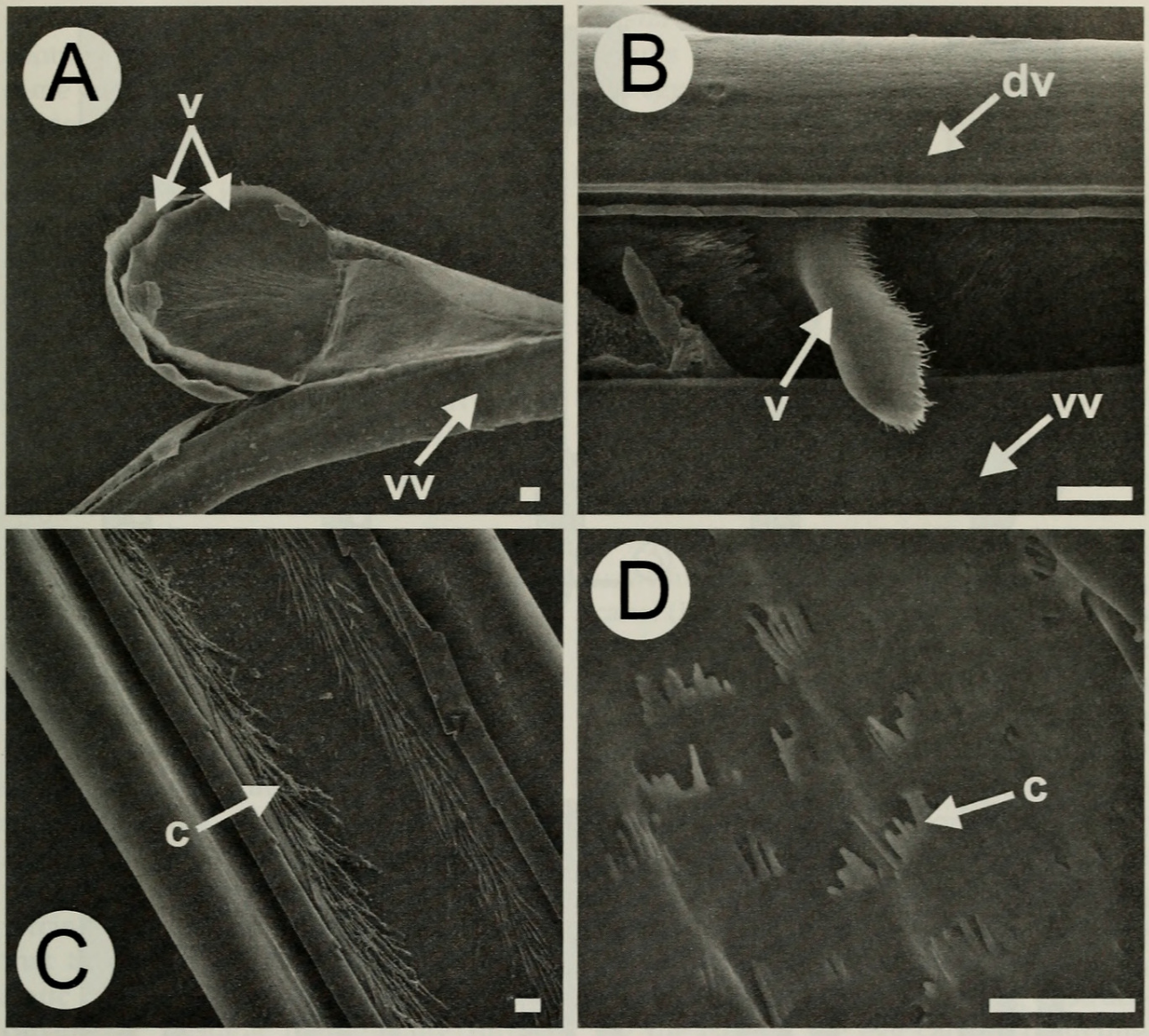

Fig. 7. A. *Sphex nudus* (Sphecidae): lateral view of the valvilli (2 valvilli lay side-by-side in all aculeates with valvilli), scale bar = 10 µm. – B. *Campoletis sonorensis* (Ichneumonidae): lateral view of the valvillus, scale bar = 10 µm. – C. *Vespa crabro* (Vespidae): ventral view of dorsal valve showing ctenidia near ovipositor apex, scale bar = 10 µm. – D. *Vespa crabro* (Vespidae): ventral view of dorsal valve showing ctenidia near ovipositor base, scale bar = 10 µm. (Abbreviations: see Fig. 1 and Fig. 2).

In agreement with Rogers (1992), it appears that one function of the valvillus in *H. truncator* appears to be to lock the egg into this loaded position. This is clearly not the case in all ichneumonoids, because, as mentioned previously, Cole (1981) showed that the egg of *Itoplectus maculator* must move down the entire length of the egg canal after the ovipositor is inserted into the host. If Rogers (1992) and we are correct, then unlike that of *I. maculator*, the sex of the eggs of *H. truncator* is determined before contact with the host.

The phylogenetic positions of Aculeata and Ichneumonoidea among the apocritan Hymenoptera are controversial; however they are usually thought to be sister-groups (Rasnitsyn 1988; Dowton et al. 1997; Ronquist et al. 1999). The putative morphological synapomorphies supporting this relationship are the shape of the metasomal-propodeal articulation and the presence of valvilli (Mason 1983; Rasnitsyn 1988). The presence of valvilli is clearly ground-plan for both taxa. We know that the function of the valvilli of aculeates is to

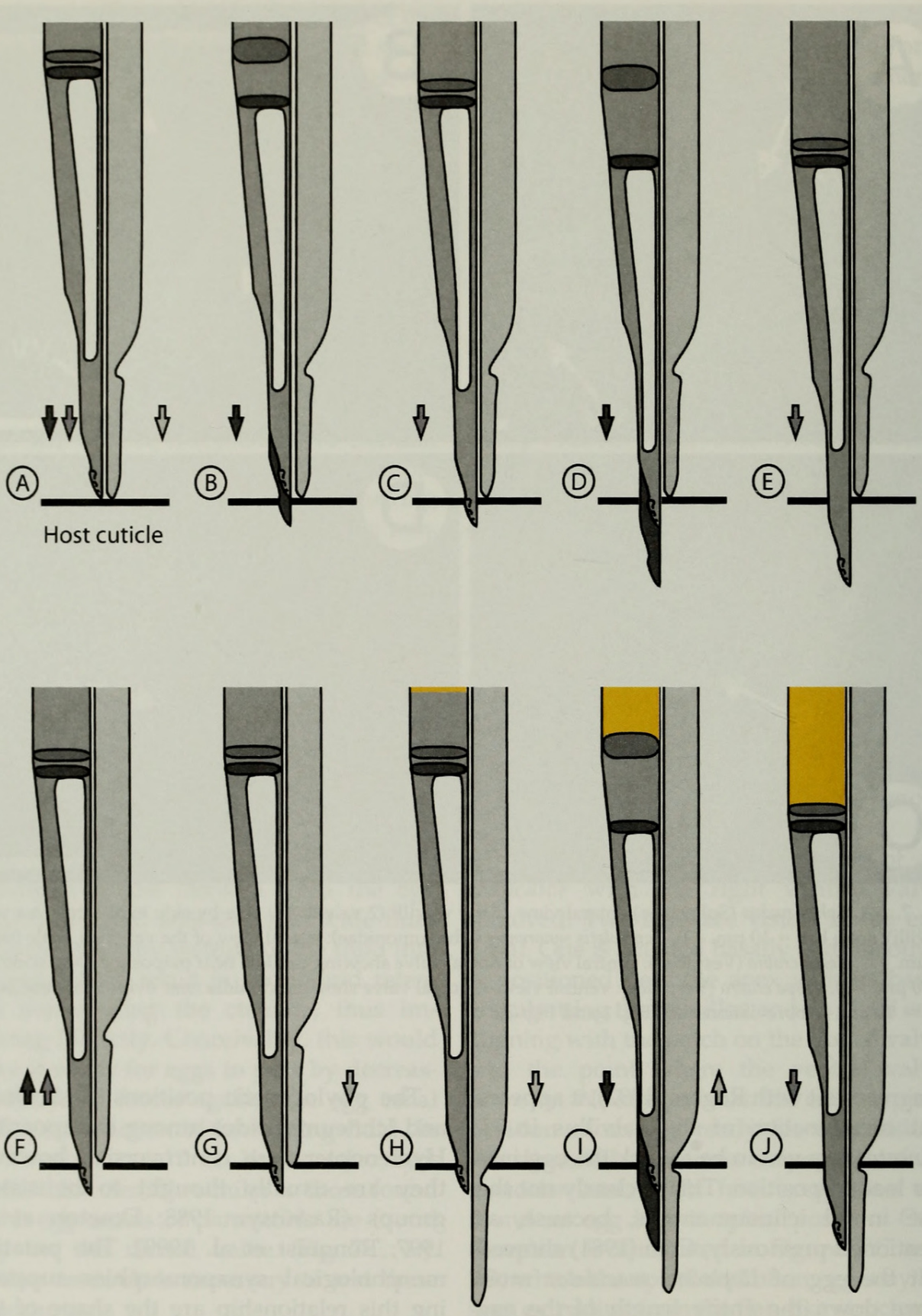

Fig. 8. Illustration of the proposed oviposition sequence in lateral view. A horizontal black line represents the host cuticle. There are two ventral valves, one is light grey and in the foreground, the other is dark grey and in the background. The arrows on the left indicate the movement of the ventral valves; the color of the arrow indicates the movement of the dark grey ventral valve, the light grey ventral valve, or both. An arrow on right indicates movement of the dorsal valve. The valvilli are represented by horizontal ovals, where the dark grey valvillus is attached to the dark grey ventral valve and the light grey valvillus is attached to the light grey ventral

push fluids, and without evidence to the contrary, it is parsimonious to assume the same function for members of Ichneumonoidea. The fluids injected by *H. truncator* are unknown to us, but typically braconid endoparasitoids inject substances that control the immune response of their hosts (Vinson and Iwantsch 1980). Braconid ectoparasitoids usually inject paralyzing venom and have highly muscled venom glands, whereas braconid endoparasitoids only rarely paralyze prey and have relatively weakly muscled, thin walled, venom glands (Edson and Vinson 1979). Though it may be possible that ectoparasitoid braconids pump venom with muscular contractions of the venom gland, the weak musculature of the venom glands of endoparasitoids implies that other mechanisms are employed to deploy venom.

Once the egg is in the loaded position (Fig. 1E), more force would be needed to move the egg due to the bottle-neck formed by the relatively narrow apical section of the ovipositor. We suggest that the valvillus plays the central role in forcing the egg out of the ovipositor and that this is accomplished through hydrostatic pressure. The valvillus by itself is not capable of pushing the egg any further than the position shown in figure 1E. To force the egg completely out of the ovipositor, the lower valve would have to be pushed to a point where the valvillus is aligned with the tip of the dorsal valve. We have never seen an ovipositor in this position and believe it to be impossible in an intact system. We propose that liquid from the venom gland is moved into the egg canal; the valvilli then prevent proximal fluid flow (acting like check valves which allow flow in only one direction). Distal movement of either ventral valve results in hydrostatic pressure that forces the egg distally. The convex-apical shape of the valvilli also suggests that they act as one-way valves. Any pressure on the apical side of a valvillus will flatten it and create a larger radius of curvature and hence transmit more sealing pressure against the wall of the canal (much like a water dam on a river), whereas any pressure on the proximal side of a valvillus will deform the shape to a smaller radius of curvature and hence result in the loss of seal between the valvillus and the canal wall.

When a ventral valve is pulled back, its valvillus is flush with the wall of the egg canal (Figs 4B, 8G). When a ventral valve is pushed apically, the valvillus blocks the egg canal and pushes against any liquids apical to it (Figs 4D, 8K–L). The hydrostatic force is applied to eggs in the apical or loaded position. This action would create negative pressure in the portion of the egg canal basal to the valvillus, which would cause more fluid, and perhaps the next egg to be drawn into it. A problem with this simple scenario is illustrated in figure 1E, where there appears to be an egg obstructing the base of the egg canal. To circumvent this blockage, which we observed in most specimens, there is a reservoir at the base of each ventral valve (Fig. 5A–D). We propose that when a ventral valve is pulled back and while the opposing valve is being pushed forward and creating negative pressure in the egg canal, the reservoir fills with venom and forms a conduit through which fluids flow into more apical parts of the egg canal (Fig. 8H–T). A preliminary survey found basal reservoirs, similar to those found in *H. truncator*, present in the

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valve. When the valvillus is represented by a thin oval, the valvillus is perpendicular and blocks the egg canal. When the valvillus is represented by a large oval, the valvillus is parallel to, and allows movement through the egg canal. In Fig. 8H, venom enters the egg canal, which is represented by the color yellow. The egg is represented by the white, vertical oval. In Fig. 8O the egg begins to exit the ovipositor from a flap on the ventral valve.

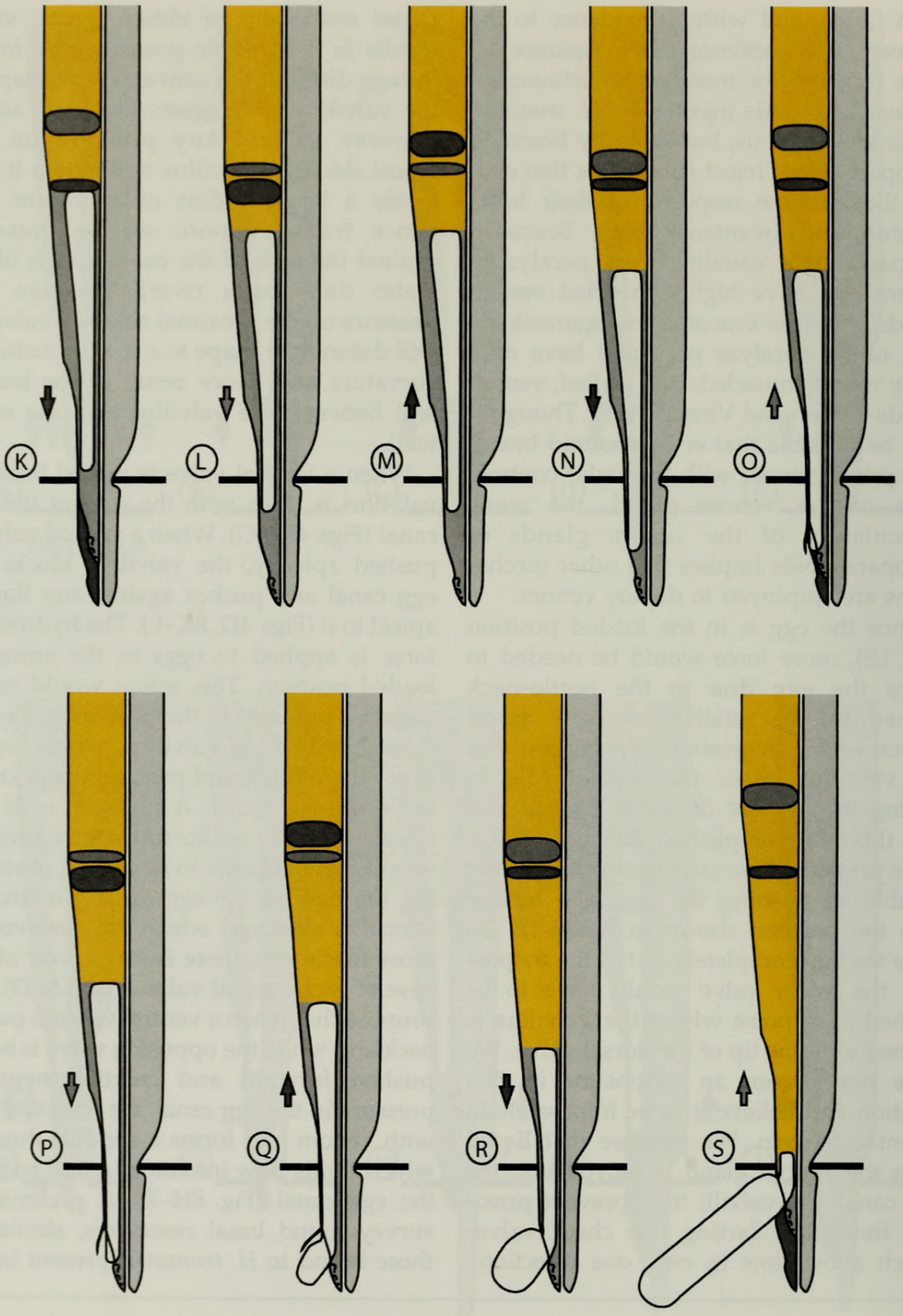

Fig. 8. Continued.

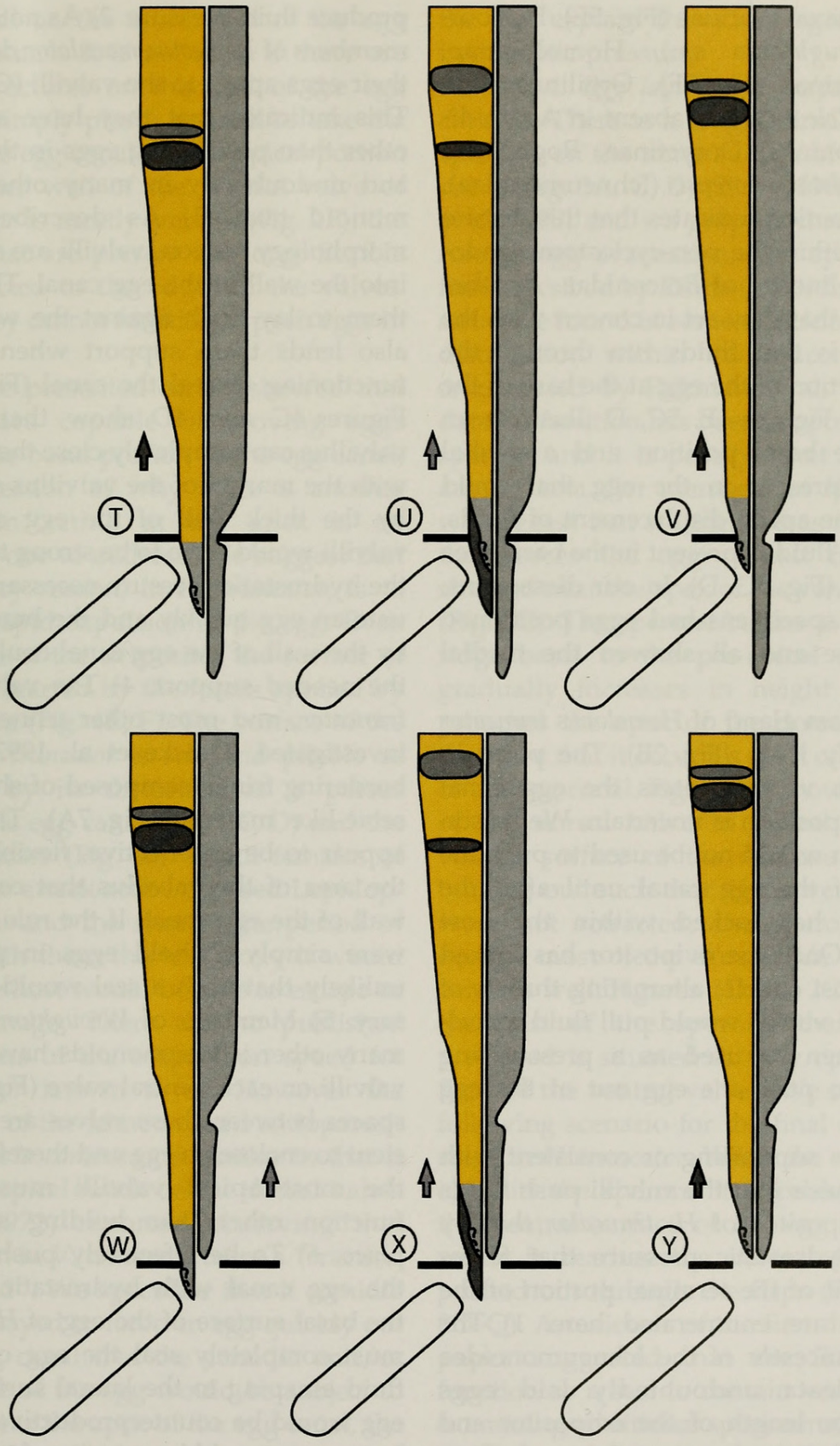

Fig. 8. Continued.

following taxa: Blacinae (Fig. 5E), Helconinae (*Wroughtonia sp.*), Homolobinae, Macrocentrinae (Fig. 5F), Orgilinae, Meteorinae (*Zele sp.*), and absent in Agathidinae, Braconinae, Doryctinae, Rogadinae, and *Campoletis sonorensis* (Ichneumonidae). This distribution indicates that this feature evolved within the non-cyclostome endoparasitoid lineage of Braconidae. Another possibility that may act in concert with the reservoirs is that fluids run through the medial portion of the egg at the base of the egg canal. Figures 4E, 5C, D illustrate an egg in the basal position and a medial divide is present in the egg that could facilitate the apical displacement of fluids. Congealed fluid is present in the basal area of the egg (Fig. 5C, D). In our dissections, numerous specimens had eggs positioned at the base and all showed the medial division.

The venom gland of *Homolobus truncator* is relatively large (Fig. 2B). The point in time when venom enters the egg canal during oviposition is uncertain. We reason that venom would not be used to push the egg out of the egg canal until after the ovipositor has locked within the host (Fig. 8H). Once the ovipositor has locked into the host cuticle, alternating thrusts of the ventral valves would pull fluid apically, and then be used as a pressurizing medium to push the egg out of the egg canal.

Evidence supporting or consistent with the hypothesis that the valvilli push fluids in the ovipositor of *H. truncator* thereby creating hydrostatic pressure that forces the egg out of the terminal portion of the egg canal are enumerated here. 1) The common ancestor of the Ichneumonoidea and Aculeata undoubtedly laid eggs through the length of the ovipositor and it is clear that the function of the valvilli in the Aculeata is to inject fluids. That the valvilli had the same function in the common ancestor of these two taxa implies that at least in the ground plan of the Ichneumonoidea the valvilli function to produce fluid pressure. 2) As noted earlier, members of *Itoplectus maculator* do not load their eggs apical to the valvilli (Cole 1981). This indicates that they have a function other than positioning eggs in this species and undoubtedly in many other ichneumonoid taxa. 3) As described in the morphology section, valvilli are set deeply into the wall of the egg canal. This allows them to lay flush against the wall, but it also lends them support when they are functioning to seal the canal (Fig. 2C, D). Figures 4C and 4D show that a single valvillus can completely close the egg canal with the margin of the valvillus supported by the thick wall of the egg canal. The valvilli would have to be strong to produce the hydrostatic pressure necessary to evacuate an egg quickly and the brace formed by the wall of the egg canal could provide the needed support. 4) The valvilli of *H. truncator*, and most other ichneumonoids investigated (Quicke et al. 1992), have a bordering fringe composed of short, thick, setae-like material (Fig. 7A). This would appear to be an effective, flexible seal for the area of the valvillus that contacts the wall of the egg canal. If the role of valvilli were simply to hold eggs in place it is unlikely that such a seal would be necessary. 5) Members of *Wroughtonia sp.* and many other ichneumonoids have multiple valvilli on each ventral valve (Fig. 6F). The spaces between these valves are not sufficient to enclose an egg and therefore all but the most apical valvilli must have a function other than holding an egg in place. 6) To be effectively pushed out of the egg canal with hydrostatic pressure, the basal surface of the egg of *H. truncator* must completely seal the egg canal. Any fluid escaping to the lateral surface of the egg would be counterproductive, not only because it would be a waste of venom, but also because it would press the lateral surface of the egg against the wall of the egg canal thereby increasing frictional forces and making it more difficult to move the egg. Figures 5B and 5C show

such a seal on the apical end of the egg of *H. truncator*. It is not clear if there are special structures on this end of the egg or if it is simply plastic enough to take the form of the egg canal. 7) The apical portion of the inner wall of the ventral valve of *H. truncator* is mostly smooth (Fig. 2E); the ctenidia that are present are long, flexible and restricted to the edges of the valves. Clearly they cannot function to push eggs in this area.

Evidence presented earlier showed that ctenidia are capable of moving eggs through the basal portion of the egg canal, so the question of why there is another mechanism acting at the apex is an important one to address. We suggest that the primary reason, in *H. truncator*, is to facilitate rapid expulsion of the egg. Even at the base of the ovipositor the surface of the egg is scarred by the forces applied by the ctenidia (Fig. 4E, F). The surface of the egg of *H. truncator* is soft and pliable as indicated by its distortion as it passes through the egg canal (Fig. 5C, D) and the ctenidial scars (Fig. 4F). Adult females of *H. truncator* attack active exposed Lepidoptera larvae and the shorter the period of contact with them the less likely it would be that the host would be able to escape or inflict damage. There are no published observations of the oviposition speed for *H. truncator* known to us. However the ovipositor of the ichneumonid endoparasitoid *Venturia canescens* is similar in that it has a pre-apical notch on the dorsal valve. Rogers (1972) reported observing that oviposition in *V. canescens* takes a "fraction of a second". We speculate that if ctenidia were employed to force an egg quickly out of the egg canal that the ctenidia and/or the surface of the egg would be subject to tearing. Near the apex of the egg canal, the egg is tightly packed into a small space that becomes increasingly narrow. The force needed to move an egg is greater here than it would be at the base of the egg canal. The need for a quick delivery of the egg and the greater frictional forces in the apical part of the egg explain the advantages of using hydrostatic pressure.

Phase 4: Egg laying and ovipositor withdrawal.—There is a longitudinal ridge, the sperone, on the inner surface of the dorsal valve (Fig. 3A, C, D) (Zinna 1960; Rahman et al. 1998) and we propose that it plays a role in egg evacuation. The sperone was first described by Zinna (1960) for a similar structure found in some chalcidoids and its distribution within the Braconidae was enumerated by Rahman et al. (1998). To date no function has been proposed for the sperone and it is possible that this varies among taxa. For members of *H. truncator* we suggest that it functions as a substrate that forces the egg to exit from flaps situated near the apex of the ventral valves (Fig. 2C). The sperone begins as a shallow ridge basal to the pre-apical notch and gradually increases in height as it approaches the apex of the dorsal valve to the point that it occupies most of the lumen of the egg canal. Figure 3A shows the apex of the dorsal valve in lateral view; the ventral surface of the sperone is visibly bulging out such that, if the ventral valve were not retracted, the sperone would occupy most of the dorsal side of the egg canal as well. Figures 1D and 2C show that the part of the sperone that is most produced is situated directly opposite the flaps of the ventral valves. We suggest the following scenario for the final egg laying stage in *H. truncator*. When the apex of the egg hits the sperone it is pushed toward the ventral surface of the egg canal and when it reaches the ventral flaps it is pushed out through the flaps (Figs 2C, D, 8O–Q). A reviewer of the first draft of this paper suggested that the flaps could function as a seal to contain venom. After examining additional specimens, we found one instance of the egg partially exiting the ovipositor from the ventral valve flaps (Fig. 2C, D). Furthermore, if the sole function of the flaps were to seal venom, one would expect them to be present in aculeate taxa. We examined specimens of

Scoliidae, Chrysididae, Rhopalosomatidae, Vespidae, Sphecidae, Halictidae, and Megachilidae under a scanning electron microscope and none possesses flaps at, or near, the apex of the ventral valves. Furthermore, since the eggs of *H. truncator* are loaded at the tip of the ovipositor they effectively block any fluids from escaping. Finally, the flaps are relatively thin and flexible and they lack muscle; it seems that little pressure need be exerted on them to cause them to open. In the closed position the flaps would provide a weak seal that would prevent evaporation of the little fluid remaining on the surface of the egg canal while the next egg moves into the loaded position.

The eggs are elongated when they are compressed in the egg canal (drawn to scale in Fig. 8A–P) and it would undoubtedly require multiple alternating thrusts of the ventral valves to effectively evacuate the entire egg. Since fluids fill the contents of the egg canal between the valvilli and the base of the egg, these too would flow out of the flaps following oviposition.

As the egg emerges from the ovipositor, elastic strain energy stored in the chorion provides additional force to assist egg evacuation from the ovipositor (Fig. 8Q–T). Assuming that the chorion does not undergo any molecular restructuring during oviposition and that it has not undergone appreciable plastic deformation throughout its volume, we can assume that it is purely elastic. As such, the initial energy required to deform the egg, to allow passage through the egg canal, will be returned in full. The relaxed shape of the eggs has a larger degree of sphericty (lower surface area) than does the deformed shape in the canal, thus the amount of elastic strain energy stored in the chorion is proportional to the change in the sphericty (i.e., change in surface area). In the egg canal the egg is constrained in an exaggerated elongated shape (metastable state) and upon emergence from the canal the constraint is removed and the relaxed shape of the egg is attained, which is more stable and more spherical. The stored elastic strain energy is returned when the egg exits the canal to attain its stress free shape; the elastic strain energy is a strong driving force for egg extraction. As the egg first emerges from the flaps in the lower valves the egg expands in the radial direction, and contracts in the axial direction (Fig. 8Q–T). The shape change, in particular the axial contraction, assists egg extraction. Upon emergence the egg's diameter increases, so the contractile force of the chorion returning to its original stress-free state pulls the remainder of the egg out of the canal, much like a siphon. Undoubtedly a suction force occurs within the egg canal which could draw other eggs or fluid apically in the canal. A video demonstration of this phenomenon can be seen in the oviposition of *Rhyssella curvipes* (Gravenhorst) and *Pseudorhyssa alpestris* (Holmgren) (Skinner and Thompson 1960). Fig. 8Q–Y depicts an approximation of the size and shape of an egg exiting the egg canal, as the exact dimensions of the egg upon exiting the ovipositor is unknown. The apical narrowing of the egg canal also reveals that a larger back pressure will exist just before the apical end of the egg reaches the flaps, preventing the egg from slipping out unnecessarily; more energy will be required to deform the egg through the smaller opening, hence the egg would tend to relax by moving distally. The valvilli play an important role here to prevent back flow, as does the higher number, density, and distribution of ctenidia at the apical end of the ventral valves and flaps (Fig. 2E).

Withdrawal of the ovipositor from the host would necessarily begin with the ventral valves (Fig. 8S). Once the apices of the ventral valves are recessed to a point where they are near the notch of the dorsal valve (Fig. 8S–V), the surface of the ovipositor would no longer be pressed against the host cuticle and the entire ovipositor could be withdrawn without resistance

(Fig. 8W–Y). The shallow angle of the preapical notch facilitates easy extraction; it permits only a small axial force in opposition to withdrawal and actually helps to disengage the dorsal valve from the host cuticle as compared to a recurved barb which would hold fast (Fig. 1B).

Summary

We wish to reiterate the point that we are proposing hypotheses not facts. It is our hope that the ideas presented here will stimulate future research that will hopefully result in corroboration, but perhaps refutation, of our hypotheses. Of greatest interest to us is the function of the valvilli. They show great variability in form, location, and number throughout the Ichneumonoidea, and an understanding of their functional morphology could provide many insights into life history traits. Furthermore, the taxonomic distribution of the reservoirs at the bases of the ventral valves, the presence of apical flaps, and undoubtedly many other ovipositor characters could provide useful information for phylogenetic studies of the ichneumonoidea.

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