

# Further notes on the biology of *Pseudavga flavicoxa* Tobias, 1964 (Hymenoptera, Braconidae, Rhyssipolinae)

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## Abstract

*Pseudavga flavicoxa*, a solitary koinobiont ectoparasitoid of *Bucculatrix thoracella*, attacks host larvae early rather than late in their final instar, subsequent development of the parasitoid then occurring within the host cocoon. This paper supplements an earlier contribution outlining other aspects of its biology and taxonomy. The host is stung repeatedly, both into the thoracic region and elsewhere on its body, causing temporary paralysis and also enabling host-feeding, which preceded oviposition in all observed cases. Stung hosts then intercepted without oviposition occurring recovered and (like parasitized hosts) resumed feeding and then constructed a cocoon, but suffered developmental arrest as a prepupa. The egg is invariably laid onto the arthrodial membrane between the first and second thoracic segments, to which it is glued. This site is first prepared by the female by a to-and-fro motion involving contact by the lower valves of the ovipositor, which are somewhat blade-like and sharp-edged. It is unclear whether dried traces of a 'glue', presumably originally liquid, subsequently seen at this site were of host origin resulting from a small wound or arose as a secretion from the female wasp, but the former is suggested. The egg was seen to issue from the extreme base of the ovipositor, at most guided onto the prepared patch by the parted lower valves. Although clearly partly plurivoltine, *P. flavicoxa* is remarkably long-lived as an adult, both sexes being easily kept alive under semi-natural conditions (Edinburgh, U.K.) during the late summer and autumn, males for 8 weeks and females for up to 20, despite their small size (ca 2.2–2.4 mm long). Dissection of gravid females showed that each of the 4 ovarioles carried just one mature egg at a time, with submature eggs remaining only poorly developed until the mature egg was expended.

## Keywords

Host-feeding, venom effect, developmental arrest, egg placement, ovipositor structure, longevity

## Introduction

Some aspects of the biology of the small rhytipoline braconid *Pseudavga flavicoxa* Tobias as a koinobiont ectoparasitoid of the bucculatricid moth *Bucculatrix thoracella* (Thunberg) were outlined by Shaw and Sims (2015). However, in that study attempts to witness oviposition in captivity were unsuccessful, and various aspects and consequences surrounding this intimate interaction therefore could not be elucidated. The present account fills in behavioural and mechanistic details of the oviposition process and the effect of venom on the host.

## Materials and methods

Larvae of *B. thoracella*, some parasitized, were collected by Dr Ian Sims at Jealott's Hill, Berkshire, England as they descended from leaves of *Tilia* × *vulgaris* for cocoon formation in the period 9.ix–4.x.2014. The resulting host cocoons were sent to Edinburgh and received on 10.x.2014, where they were immediately placed in a fully shaded and unheated outdoor wooden shed (cf. Shaw 1997). Some adult *P. flavicoxa* emerged in the autumn of 2014 (Shaw and Sims 2015) but the great majority during July 2015, and this paper concerns experiments performed with these 2015 adults. Until being used for experiments, inexperienced adults were kept in groups of up to six females with several males in upright 7.5 × 2.5 cm corked glass tubes with continuous access to honey:water supplied initially as droplets of a 1:3 dilution suspended on the wall of the tube, replenished or re-diluted as necessary. Copulation was frequently observed, and at least the majority of the females used in experiments were mated. After being introduced to hosts, females were regarded as experienced and kept separately from other females, under similar conditions but each with its individual log.

After a brief period as a leaf miner, the larva of *B. thoracella* feeds on the parenchyma of *Tilia* leaves from the leaf underside, leaving a windowed pattern of damage visible from the upperside. To accomplish its moults, the larva constructs a temporary small and dense silken shelter, in which ecdysis occurs. When fully fed, the larva descends from the leaf on a silken thread, and spins its characteristic ribbed cocoon upon landfall. Following recent range expansion (outlined by Shaw and Sims 2015) *B. thoracella* has become abundant in Edinburgh, U. K., where *Tilia* × *vulgaris* is a frequently planted amenity tree. Final and penultimate instar larvae of *B. thoracella* to be used in experiments were collected in suburban Edinburgh from *Tilia* × *vulgaris* during the late summer and autumn of 2015 and kept with *Tilia* leaves in closed plastic boxes bottom-lined with absorbent tissue (lavatory roll) until needed. Rearing a sample of 150 *B. thoracella* larvae collected in the same area during late summer and autumn of 2014 indicated that this Edinburgh population was free from parasitoids.

After being fed for at least 25 days on dilute honey, when dissections of others indicated that eggs would certainly be mature, females of *P. flavicoxa* were introduced



**Figure 1.** Experimental arena.

singly to an undisturbed final instar *B. thoracella* larva beside its feeding damage on a cut piece of *Tilia* leaf trapped by the cork of a  $7.5 \times 2.5$  cm upright glass tube. Because the parasitoids were generally very slow to successfully parasitize the hosts, several tubes were set up to be observed simultaneously (Fig. 1). Periods of continuous observation (generally not more than 3 hours at a time, after which even half-hearted interactions tended to cease) were made at various time of day, with no consistent difference of outcome, intermittently over the period 8.viii–9.ix.2016 when the last successful attack was seen (subsequent trials did not result in ovipositions). When a wasp accepted the host, by grasping and repeatedly stinging it, it was sufficiently preoccupied for some time for the host+parasitoid to be removed from the tube, whether still on the leaf or by tipping them out of the tube, without unduly disturbing the interaction. Observations were then made under a Wild M5A stereomicroscope, down one arm of which single shot photographs were taken using a hand held Canon Powershot S110. Some larvae that received eggs were given fresh (uneaten) portions of *Tilia* leaf in order to assess the extent of their subsequent feeding prior to cocoon construction. Transverse thin sections (8 microns) of the ovipositor of specimens initially stored in 70% ethanol then dehydrated and wax-embedded were prepared using a Leica RM2245 microtome and stained with haematoxylin and eosin before photography using an Olympus BX 51 microscope with an Olympus UC 30 camera attachment. Dissections of the ovipositor followed maceration in aqueous potassium hydroxide (c. 10%), and these structures were photographed using a Leica M16 microscope and phototube with an Olympus C-5060 camera, in some cases with multiple images stacked using ZereneStacker®.

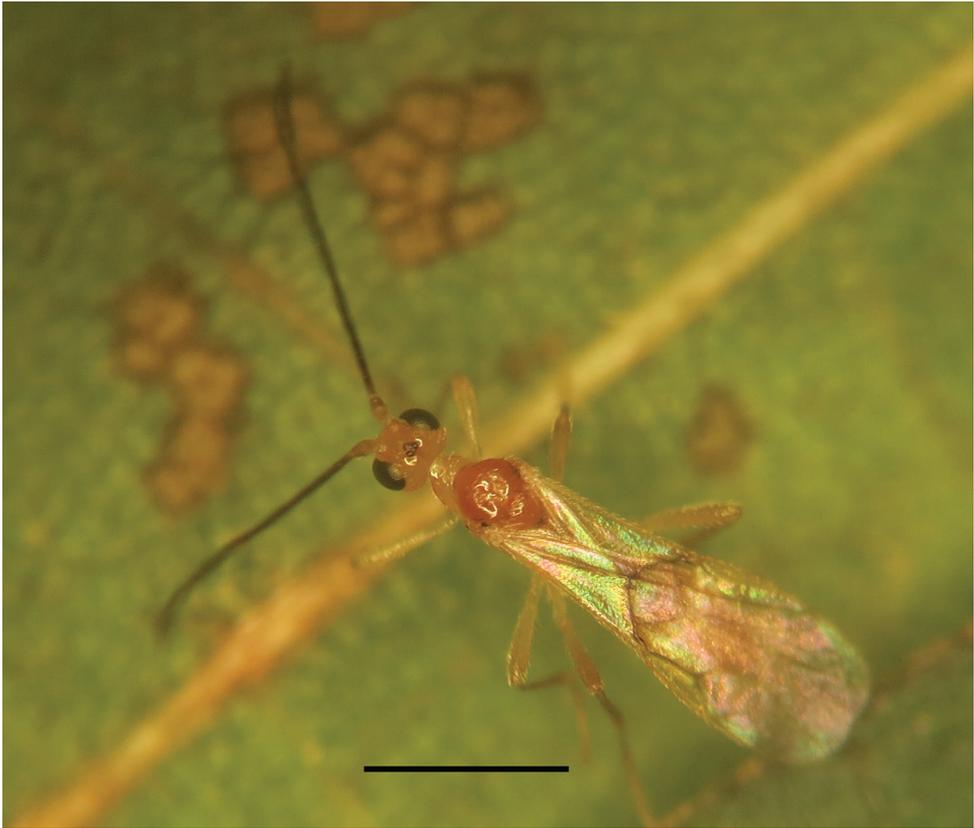
## Results and discussion

### Emergence and longevity

Emergence of *P. flavicoxa* adults from the overwintered cocoons was during the period 6–20.vii.2015. Males (N=42) emerged on average 5.25 days before females (N=32). When provided with dilute honey, both sexes lived for an extraordinary length of time given their small size (ca 2.3 mm long); males routinely for at least eight weeks, and some females for as long as 20 weeks (the last two females were killed by the first frost recorded in the outdoor rearing shed on 1.xii.2015). The adult progeny from the 2015 captive ovipositions emerged in July 2016. This suggests that *P. flavicoxa* is essentially univoltine, although Shaw and Sims (2015) recorded a substantial emergence in late autumn, soon after cocoon formation, when cocoons were kept indoors.

### Host acceptance

No interactions were seen between the parasitoid and penultimate instar hosts, nor hosts in their moulting cocoons. Henceforth ‘host’ refers to the final instar. The female wasps showed only very slight interest in the feeding damage caused by hosts (Fig. 2), or host faeces (which are ejected to end up far from the host), though they occasionally tested the leaf surface, with their outstretched antennae then about 30° apart (usually about 90°) but scarcely curled. The wasps either completely ignored the hosts for the duration of the ca 3 hr observation period (at least half the exposures), or very gradually approached the hosts over a long period (regularly up to 45 minutes), withdrawing repeatedly whenever the host responded before re-approaching later. This usually started with the parasitoid standing motionless about 1–1.5 cm away from the host for tens of minutes. The antennae were not used to touch the host directly as the wasp gradually moved closer, sometimes almost circling the host, which occasionally responded by flicking its caudal end (as though ejecting faeces) or walking away. Sometimes the host would be sufficiently alarmed by the approach of the wasp to throw itself from the leaf and remain suspended on a short thread (usually only 1–2 cm, but sometimes secondarily extending a further 1–2 cm if disturbance continued), for a time varying from a few minutes to much more than an hour. In these cases the wasp did not follow it, but sometimes waited (usually fruitlessly) for up to a few tens of minutes at the leaf edge from which the host was suspended, though normally by the time the host re-climbed (and consumed) its silken escape line the wasp had left. On other occasions the wasp gradually lost interest in the host it was stalking. Otherwise, in a minority of interactions, the stalking of the host was successful to the point when the wasp made a sudden pounce and grasped the host, stinging it repeatedly in various positions to cause temporary paralysis, usually of a few tens of minutes duration. Often, but not always, the briefly



**Figure 2.** Adult parasitoid on underside of *Tilia* leaf with feeding damage by host. Scale bar 1mm.

writhing pair fell from the leaf, then usually were suspended in mid air by the host's escape line. In a very few cases the host was successfully pounced upon within just a few minutes of the arena being set up, without prolonged stalking. In a few other cases, when the containers were left unattended for much longer than the 3 hour observation period, subsequently it was seen that an egg had been laid (even after as much as 24 hrs later), but what prompted the parasitoid's interest after its prolonged indifference was unclear: normally during the observation period it was evident that a wasp that had been unsuccessful in more than two or three attempts lost interest. However, it might be that a new dawn prompted fresh exploration (night-time activity appeared to be absent).

It gradually became clear that hosts late into their final instars were unattractive, and perhaps genuinely unsuitable, which might have accounted for a proportion of the cases in which hosts were completely ignored, and also the failure to obtain ovipositions in the autumn of 2014 (Shaw and Sims 2015). However, several of the hosts rejected in 2015 were offered as early- and mid-final instars.

## Host-feeding and oviposition

No host-feeding interactions were seen apart from the concurrent host-feeding that preceded each oviposition. Accepted hosts were stung repeatedly as temporary paralysis ensued, and in all observed cases pierced in both thoracic and abdominal regions, though apparently at random. Although it is possible that venom was administered preferentially into one or other of these sites, no difference in behaviour was noted. During and following the stinging process the wasp made repeated traverses and turns along the long axis of the host (Fig. 3), apparently feeding at puncture sites as it went (although no seepage of host haemolymph was seen), but also clearly orientating itself eventually to the head end of the host. After about 30 seconds to two minutes (exceptionally five minutes) of this, the wasp settled transversely across the dorsum of the host just behind the head (Fig. 4), and pressed its ovipositor across it with the lower valves in contact, with the short ovipositor sheaths still supporting the ovipositor. The extended ovipositor was then moved to-and-fro along the arthrodial membrane connecting the host's first and second thoracic segments for a few seconds, before the short and stout egg issued from the genital opening at the extreme base of the ovipositor and was apparently at most guided by the separated lower valves of the ovipositor transversely onto the membrane, a process that took about 30 seconds (exceptionally 2 minutes) to accomplish (Fig. 5). During actual oviposition the ovipositor sheaths were cocked upwards away from the ovipositor itself. Subsequently, the female parasitoid always walked from the host and then flew off, a displacement activity that would tend to avoid renewed contact with that particular host individual.

While many parasitoid Hymenoptera ovipositing in (or on) exposed hosts are quick to do the deed, instances of extreme patience or slow stalking have been recorded in several other studies (e.g. Shaw 1981, 2011, Kloss et al. 2016).

## Egg attachment

The egg, measuring  $0.28 \times 0.13$  mm, was positioned with its long axis transversely along the membrane between the first and second thoracic segments on all 14 hosts on which an egg was laid, as had been the case for all seven hosts with an egg observed in nature in 2014 (Shaw and Sims 2015). The egg was generally positioned more or less dorsally or dorsolaterally (Fig. 6), though in one case it was fully lateral (Fig. 7). At first the egg is translucent and not easy to see, but within half an hour it becomes opaque enough for its outline to be clear. The moment of oviposition was clearly seen but unfortunately could not be photographed, and the disturbance of trying to manipulate the pair to achieve a good view for the camera led to two hosts being abandoned after site preparation but before an egg was laid. Thus the prepared site showing the effect of the to-and-fro motion could later be photographed (although after the seepage or secretion had dried; Fig. 8). The substance by which the egg is glued to the host could often be seen at the edges of the egg (Figs 6, 7). Rather surprisingly, in view of the



**Figure 3.** Host-feeding on envenomed host. Scale bar 1mm.



**Figures 4, 5.** Parasitoid in position for oviposition at the head end of the host.

restricted area available for oviposition, one host was parasitized twice by the same female after the standard 3 hr period of observation had elapsed (Fig. 9), but neither parasitoid developed successfully.



**Figures 6, 7.** Eggs positioned on the intersegmental membrane between the host's prothoracic and mesothoracic segments.



**Figure 8.** Site prepared for oviposition (photographed several hours later).



**Figure 9.** Two eggs, both on the usual intersegmental membrane.

### **Egg maturation**

Dissections of females in the first weeks after their emergence suggested that eggs were rather slow to mature (with access to dilute honey only), and it was not until they were about 3 weeks old that they were judged all to have fully mature eggs, one in each of the two paired ovarioles making four in all, with subsequent eggs in each ovariole remaining greatly underdeveloped until the mature egg had been laid (Fig. 10).

### **Effect of venom**

The temporary paralysis caused to facilitate oviposition wears off after a few tens of minutes, and the host gradually resumes apparently undiminished feeding activity within an hour (exceptionally only after 3 hours). The approximate minimum and maximum extent of this before cocoon construction (after at least 1 and more often 2



**Figure 10.** Dissection to show the full maturity of only one egg per ovariole. Scale bar 0.2mm.

or even 3 days) is shown in Figs 11 and 12, respectively. The two hosts that had been temporarily paralysed but abandoned before an egg was laid (see above) duly recovered, resumed feeding and then prepared their cocoons in the usual way. They were removed as prepupae and found to be clearly in developmental arrest (Figs 13, 14), pupal features of the host eventually becoming evident (Fig. 14) in much the way seen in hosts envenomed by the species of the related *Rhysipolis* (Shaw 1983) and indeed the choreutid moth *Anthophila fabriciana* (Linnaeus) envenomed as larvae by the endoparasitoid rogadini braconid *Clinocentrus cunctator* (Haliday) when no egg had been laid (figured by Shaw 1981, as *C. gracilipes*).

This suggests a reason why hosts too late in their final instar are rejected; if the arrest suffered by the host results from a disturbance to the endocrine system, as has been deduced to be caused by *Rhysipolis* species (Shaw 1983), the causative agent must presumably be administered early enough in the instar for it to be effective. There is a clear adaptive advantage for this, as arrested hosts cannot moult and slough off the parasitoid egg.



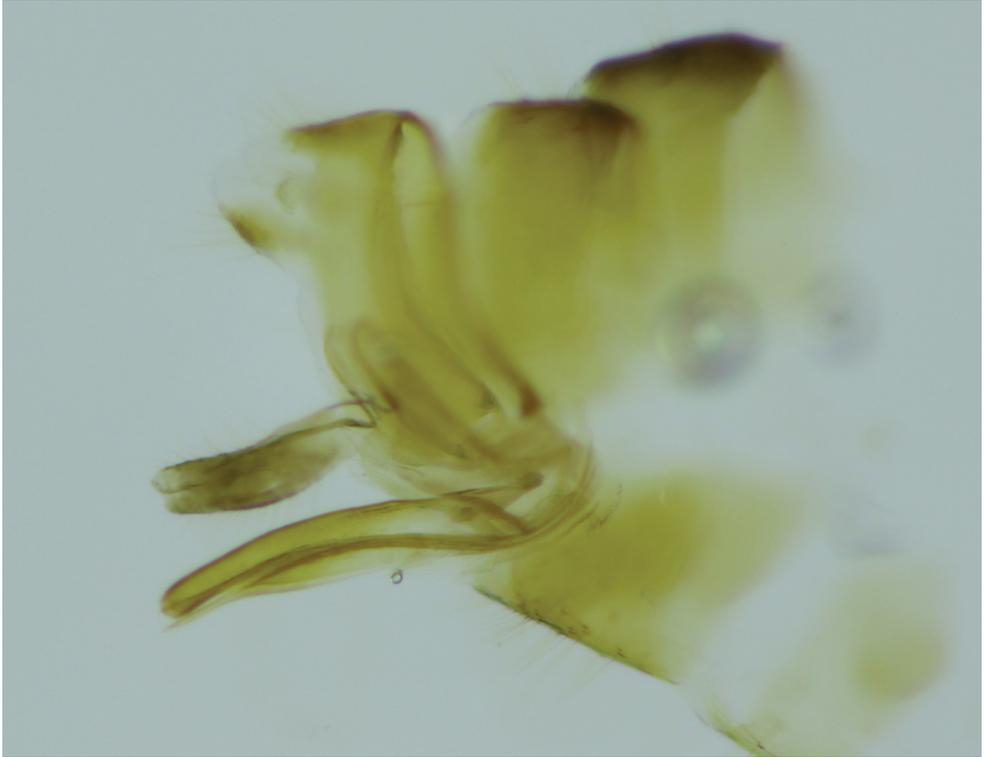
**Figures 11, 12.** Amount of feeding by host following oviposition until cocoon formation 1 and 3 days later respectively (not to same scale).



**Figures 13, 14.** Host stung but not oviposited on then removed from cocoon, after respectively 11 and 20 days.

### Structure of the ovipositor

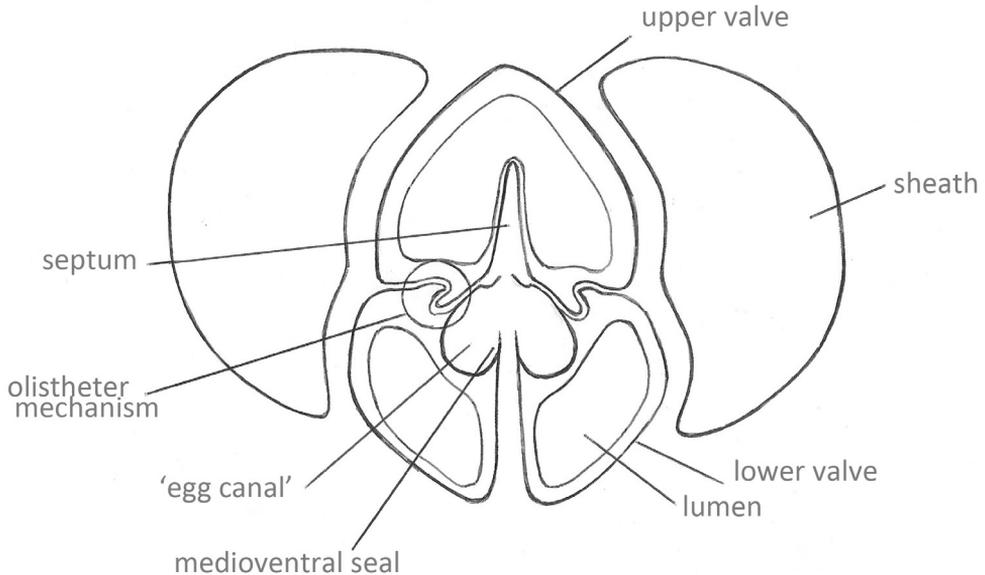
The ovipositor overall has an unusual structure, with a strong but blunt upper valve and the acute lower valves appearing blade-like except in their apical fifth (Figs 15, 16). Thin sections showed that the lower valves help to define a canal, with a medioventral seal evident (especially clearly seen in Fig. 18). This canal, that in other systems might be termed the egg canal, was discernable in all thin sections but to a variable extent. As the egg had been directly observed to issue at the very base of the ovipositor, essentially direct from the genital opening, it is evident that the canal in this case serves only to conduct venom. However, the lower valves extend ventrally considerably beyond the medioventral seal (Figs 18–21), and they readily separated during dissection



**Figure 15.** Apex of metasoma of parasitoid, to show ovipositor and sheaths.



**Figure 16.** Cleared dissection of ovipositor apparatus.

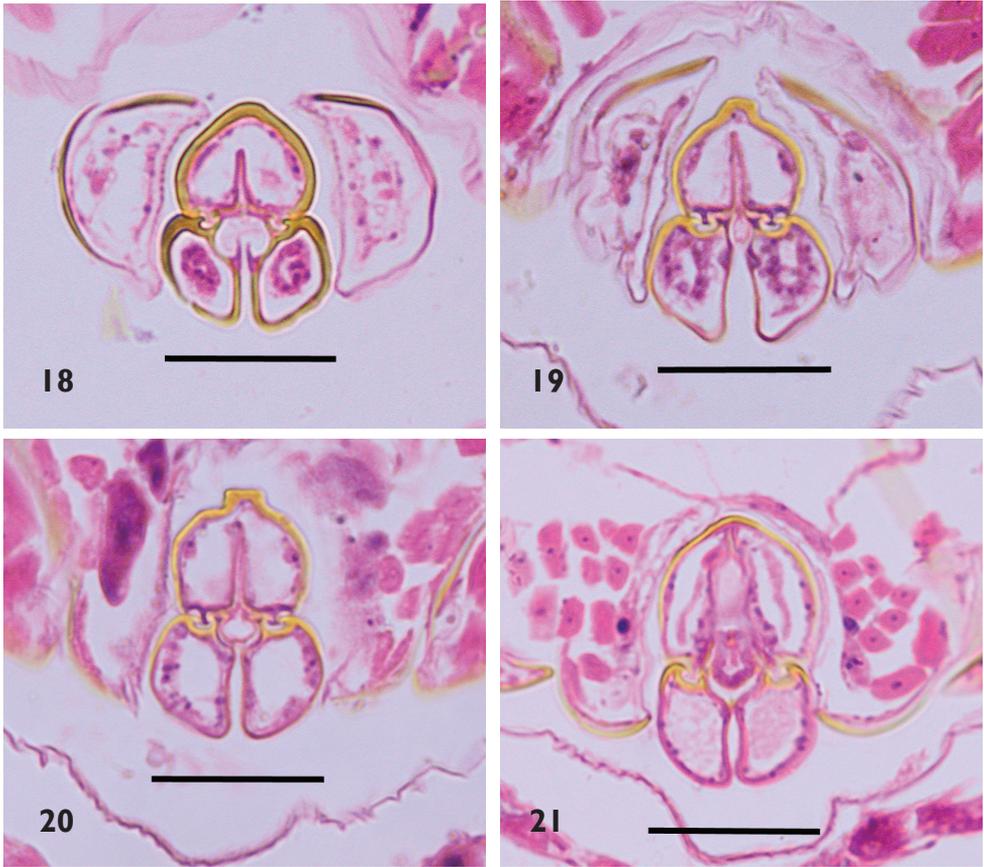


**Figure 17.** Diagram illustrating the parts of the ovipositor in TS.

(Figs 16, 22). This is consistent with the supposition that the parted lower valves steer the egg onto the prepared intersegmental membrane of the host. The large septum, at its widest basally (Fig. 21), and the robust olistheter mechanism evident in all thin sections (Figs 18–21), suggest that the lower valves are capable of wide separation for that purpose, as indeed would be necessary bearing in mind the comparatively enormous ovarian egg (Fig. 23), in diameter roughly 3 times the width of the entire ovipositor.

A serrated tip (presumably serving to hold the ovipositor in the host while venom is administered) is present on the upper edge of the lower valves (Fig. 22) for about a fifth of their length, but otherwise the valves are without teeth or nodes (the kink seen in the lower valve in Fig. 16 is evidently an artefact, not seen in another preparation (Fig. 22)). The contrast in sharpness between the rounded upper valve and the acute lower valves suggest that the latter have the primary role in puncturing the host's integument. The stubborn persistence of an air bubble in different dissections (partly in liquid) of the lower valve (Fig. 22) could conceivably indicate the presence of a pore, perhaps sensory, but this has not been further investigated.

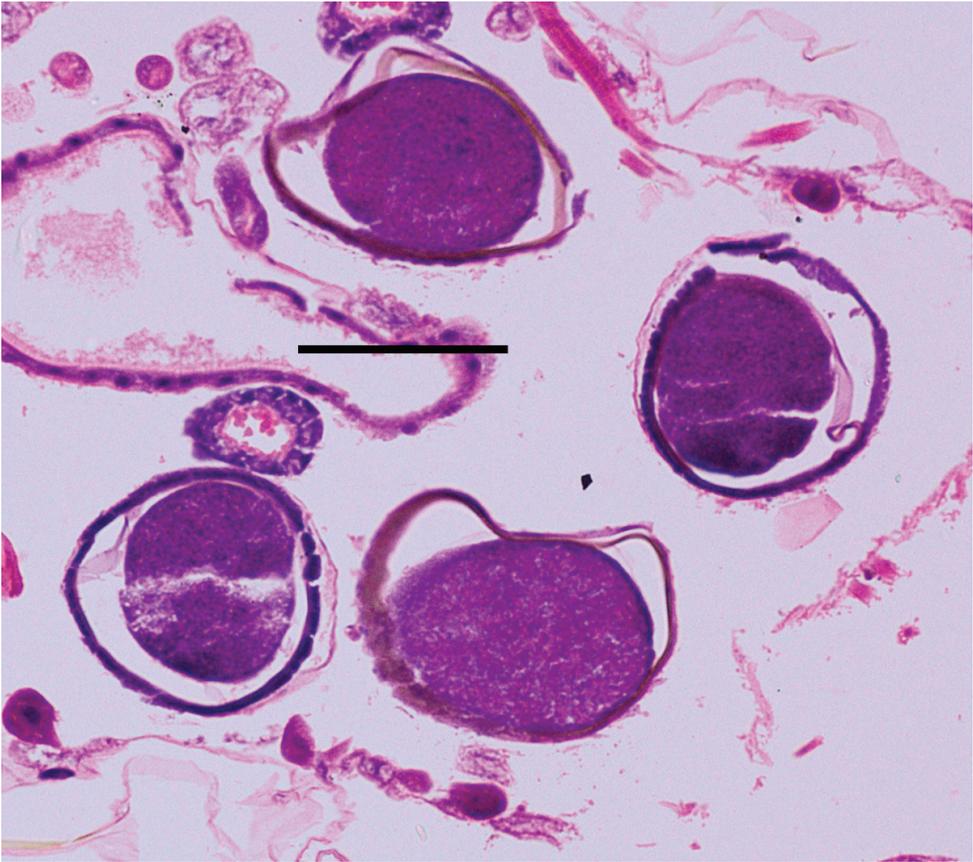
In principle the to-and-fro motion of the ovipositor immediately preceding oviposition could have been associated either with host-wounding or with spreading a secretion produced by the wasp. The lower valves of the ovipositor are rather blade-like for most of their length and, although not (on their lower edge) serrated (Fig. 22), they do have sharp lower angles in the crucial place (Fig. 19), especially bearing in mind their very small size. This might suggest that the substance that glues the egg to the host is host haemolymph resulting from a wound. That is also suggested by the variation in the amount of the 'glue', which can sometimes be seen to surround the egg to a greater



**Figures 18–21.** Thin sections across ovipositor. **18** just apical of the broad part of lower valve as it starts to narrow **19** about half way along broad part **20** near origin of sheaths **21** near extreme base. Scale bars 0.04 mm.



**Figure 22.** Lower valve of ovipositor (dissection in water).



**Figure 23.** Cross section of metasoma, showing 4 mature eggs. Scale bar 0.1 mm.

extent than appears necessary (Figs 6, 7), and indeed the apparently excessive extent of the supposed seepage seen in Fig. 8. However, although the ovipositor looks far less suitable for spreading a secretion produced by the parasitoid, that possibility cannot be entirely ruled out.

### Acknowledgements

I am grateful to Ian Sims, who first reared *P. flavicoxa* in Britain and subsequently collected and sent host cocoons containing the parasitoid to enable this study; to Viv Allison (Department of Comparative Physiology, University of Edinburgh) who prepared the ovipositor sections; and to Richard Lyszkowski for taking and processing the stacked photographs of ovipositor dissections. In review Istvan Miko made valuable remarks on the ovipositor.

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