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Arthropod Structure & Development 31 (2003) 329–337

ARTHROPOD STRUCTURE & DEVELOPMENT

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Female reproductive system of the decapitating fly *Pseudacteon wasmanni* Schmitz (Diptera: Phoridae)

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Received 2 June 2002; accepted 16 September 2002

Abstract

Pseudacteon wasmanni is a South American decapitating fly that parasitizes workers of *Solenopsis* fire ants. We used light microscopy (historesin serial-sectioning stained with Haematoxylin/Eosin) and scanning electron microscopy to show and analyze internal and whole external views of the female reproductive system. All specimens analyzed ($n = 9$) by light microscopy showed post-vitellogenic oocytes inside the ovaries. The lack of typical follicles (oocyte-nurse cell complexes) in all specimens suggests that oogenesis occurs during the pupal stage. The total number of eggs found ranged from 31 to 280 ($X = 142 \pm 73$, SD). The egg has a slugform or torpedo shape (about 130 by 20 μm) with a pointed apex at the posterior pole as defined by the fly; the micropyle appears to be in a depression or invagination at the anterior pole. An acute hypodermic-like ovipositor is evaginated from the hard sclerotized external genitalia during egg laying. The existence of a muscular bulb associated with the end of the common oviduct suggests that the egg is injected into the ant's body by a strong contraction of the bulb which probably is stimulated by bending of several ventral sensilla. During contraction, the abdomen extends out along a large fold between the sixth and seventh tergites in such a way that the sclerotized genitalia is rotated ventrally into a slightly anterior orientation in preparation for oviposition.

Keywords: Ovary; Fire ant; *Solenopsis*; Biocontrol; Ovipositor; Brazil

1. Introduction

The decapitating fly *Pseudacteon wasmanni* Schmitz parasitizes *Solenopsis* fire ants in South America like almost 20 other species of flies in this genus (Porter and Pesquero, 2001). These flies have the potential to be used as fire ant biocontrol agents because they are host specific (Porter et al., 1995b; Gilbert and Morrison, 1997; Porter 1998b; Porter and Alonso, 1999; Porter, 2000), broadly distributed across habitat and season (Borgmeier and Prado, 1975; Fowler et al., 1995), and they have had sufficient impact on fire ant populations to have caused evolution at a suite of defensive behaviors (Feener and Brown, 1992; Orr et al., 1995; Porter et al., 1995a).

The insect female reproductive system is generally formed by a pair of ovaries, oviducts, spermatheca, accessory glands and vagina. Each ovary is formed by ovarioles whose number and physiological states are closely related to egg production. The female reproductive system of phorid flies is best described for the saprophytic fly *Megaselia scalaris* (Benner, 1985; Benner and Curtis, 1988). However, relatively little is known about internal morphology and histology of female reproductive system in parasitic phorids including the genus *Pseudacteon* (Wasmann, 1918; Borgmeier, 1930).

Diptera have typical meroistic polytrophic ovarioles (Telfer, 1975; King and Büning, 1985; Büning, 1994). Several parasitic phorid flies have been looked at (Wasmann, 1918; Borgmeier, 1930), but it is not clear whether pre-vitelogenesis or new oocyte-nurse cell complexes occur in adult flies. Basic information about the number, development, and production of eggs in *Pseudacteon* flies is important for biocontrol efforts because it provides answers to important questions about their potential fecundity and how they should be reared. The primary objective of this work is to describe by histological analyses and scanning electron microscopy (SEM) the morphology of the adult female reproductive system of *P. wasmanni*, the

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PII: S1467-8039(02)00049-X

number of eggs, their developmental stage and morphology, and the general structure and function of the ovipositor. We will also discuss how reproductive structure and physiology of phorid flies is related to parasitic or saprophytic life habits.

2. Materials and methods

Females of *P. wasmanni* were caught in the state of São Paulo (state road SP-191 between Rio Claro and Araras, São Paulo, Brazil) during February 1994. For histological preparations, nine specimens had their abdomens severed to facilitate fixing with a modified paraformaldehyde solution for 4–8 h at room temperature (4 g of paraformaldehyde in 90 ml of distilled water; after dissolving, add 0.75 g NaCl, 0.23 g Na_2HPO_4 and 0.27 g KH_2PO_4; finally, complete to 100 ml with 0.1 M sodium phosphate buffer, pH 7.4).

After fixation, the abdomens were transferred to a sodium phosphate buffer (0.1 M, pH 7.4), dehydrated in an ethanol grade (70 to 95%), and then infiltrated and embedded in JB-4/Polysciences resin. The specimens were serially sectioned (4–5 μm thickness) with glass knives in a Sorvall/DuPont microtome and the sections were stained with Mayer's haematoxylin and aqueous eosin (HE). The sections were examined with a Zeiss photo microscope.

The total number of eggs was determined by using the egg nucleus as an identifying parameter. During analysis of the consecutive sections, each egg showed the same nucleus as a dark- or pale-basophilic dot; to avoid over counting, only the egg section having the darkest stained dot was counted.

Two additional abdomens were processed for SEM. These abdomens were fixed as described earlier and some of their tergites and sternites were removed to expose internal structures. They were dehydrated in ethanol (from 70% to absolute ethanol), transferred to absolute ethanol and oxide propylene solutions (2:1, 1:1 and 1:2), then to absolute oxide propylene. The samples for SEM analysis were critical point dried (Balzers/CPD 030), mounted on stubs and sputter coated with gold (Balzers/SCD 050). The preparations were observed with a Jeol P15 tabletop SEM.

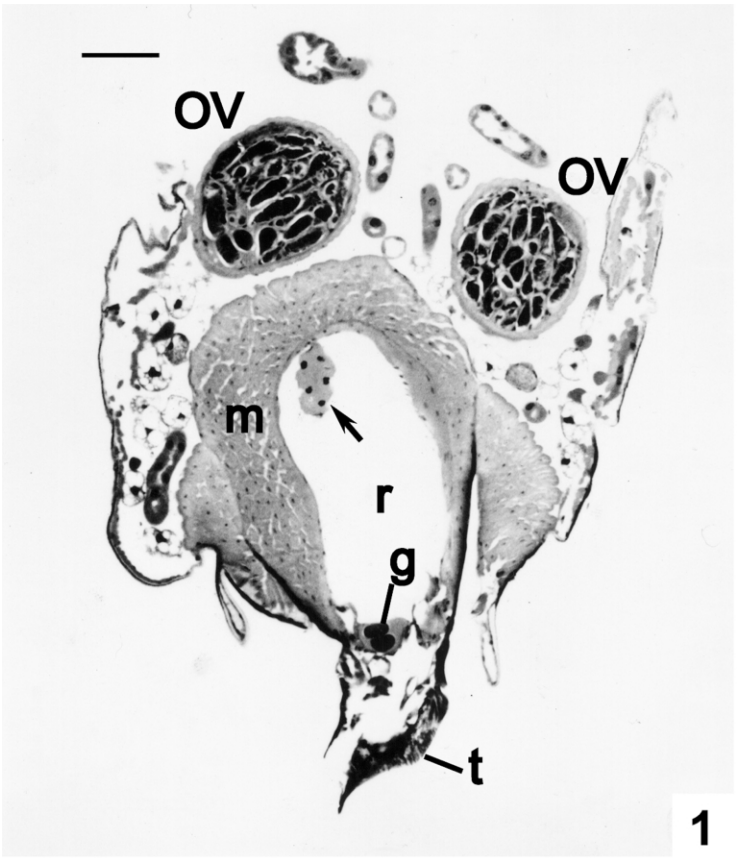
3. Results

The histology and SEM analyses showed that the ovaries of *P. wasmanni* are spherical (Figs. 1, 2 and 4). Each ovary was enclosed by a thick muscular sheath (Fig. 2) in which muscle fibers appear to be perpendicular to the longitudinal axis of the ovary. The peritoneal sheath is seen beneath this muscular sheath as a thin epithelium (Fig. 2). The muscular sheath that covered each ovary is contiguous with the lateral and common oviducts. In the anterior portion of the common oviduct, the lumen is enlarged defining a reservoir of eggs (Figs. 3 and 4). From this region the width of the common oviduct decreased as it approached the vagina. The eggs in the oviduct seemed to be oriented with the pointed end down from the ovary toward the vagina (Fig. 3). However, in the beginning of the common oviduct (reservoir) the eggs are normally found folded or curved near each other. Inside the ovarioles, we did not observe nurse cells or a follicular epithelium that would nourish and cover the oocyte. It was not possible to identify follicles from which we could determine the basic follicle structure of the meroistic polytrophic ovarioles found in other dipterans; instead, we only observed clefts filled with what appeared to be post-vitellogenic eggs (Figs. 1 and 2). Several whole mount squash preparations of ovaries of late-stage pupae also showed only mature eggs indicating that oogenesis is completed earlier in the pupal stage.

Inside the ovarioles, the eggs were extremely basophilic and surrounding them we observed some small nuclei (Fig. 2). These nuclei are the only evidence of the possible presence of follicular epithelium covering the eggs. The eggs within the ovarioles, oviducts and vagina were embedded in an acidophilic-gelatinous material (Figs. 2, 3 and 7). Inside the eggs, the nuclei were easily distinguished in the ooplasm as a dark, elliptical, and basophilic dot (Figs. 2, 8 and 9). In all analyzed specimens no cytological features were observed which could determine the occurrence of oösorption.

The analysis of serial-sectioned eggs observed in the oviduct lumen (Figs. 6–10) showed a pointed posterior pole with a cap formed by a thicker chorion layer. In this region, the ooplasm also had an acid nature that stained darker than the rest of the ooplasm by haematoxylin (Figs. 7 and 10). In the anterior pole, the egg had a rounded surface with a common depression which could be interpreted as being the

Figs. 1–5. Histology and SEM features of the female reproductive system and partial digestive system of the scuttle fly *P. wasmanni*. Fig. 1: Whole view of a dorsal section through the abdomen showing sectioned ovaries (OV) and muscular bulb (m), rectal lumen (r), sclerotized genitalia (t) and oval structure (arrow); in the posterior portion of the common oviduct, eggs (g) are seen separated by a thin epithelium beneath the lumen of the intestine. Fig. 2: Detail of a longitudinal section of an ovary showing eggs (g) and their dark nuclei (arrows); note the peritoneal sheath (p) and the muscular sheath that covers each ovary (s) which is contiguous with the lateral oviduct (L). Fig. 3: Lateral view of the internal structure of the abdomen showing the tergites (roman numerals from II to VII), the acidophilic material (a), the reservoir of eggs (rv), the common oviduct (CO), the genitalia compartment (ch) and spermatheca (z). Figs. 4 and 5: Whole view SEM preparation showing the ovaries (ov), lateral oviduct (L), reservoir of eggs (rv), muscular bulb (m), and colon (c). Further details can be found in the text. Scales: 100 μm (Figs. 1 and 3), 50 μm (Figs. 2 and 4) and 15 μm (Fig. 5).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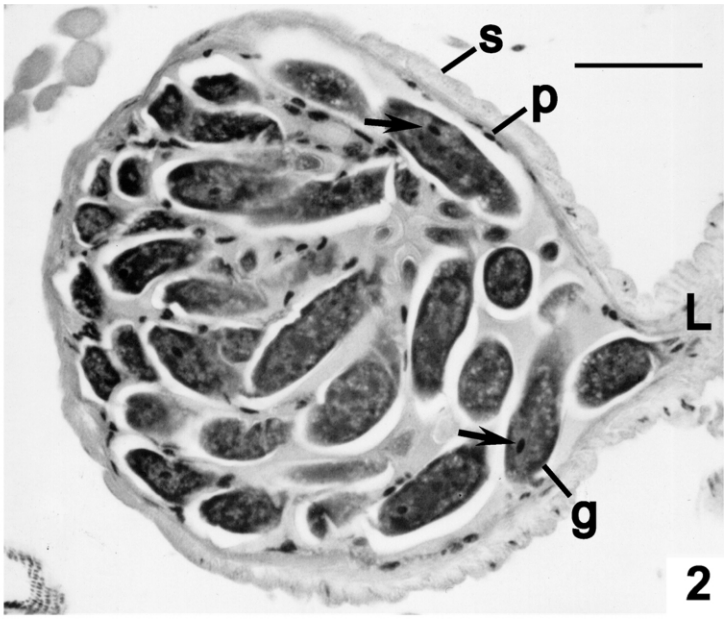
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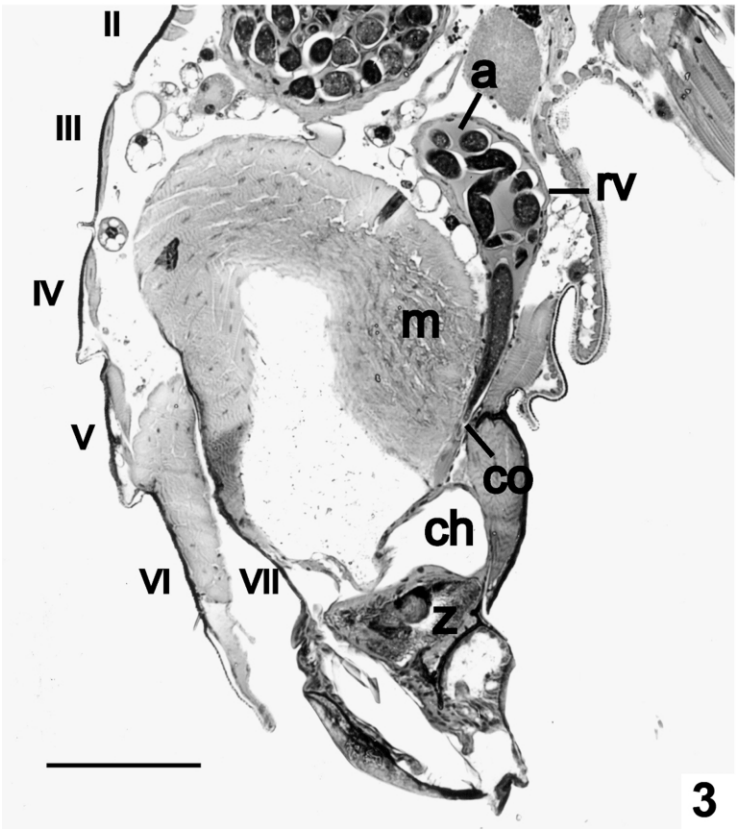
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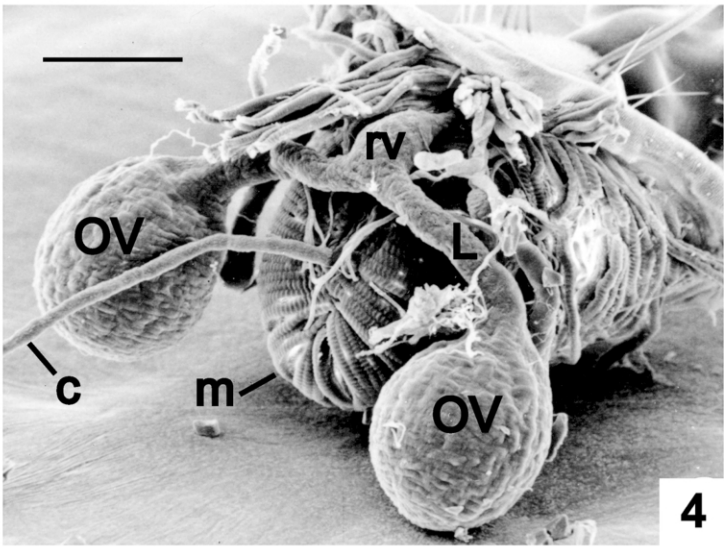
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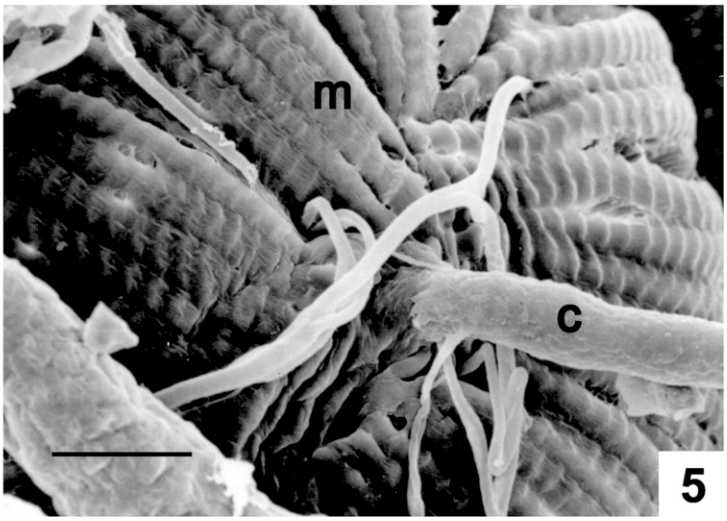
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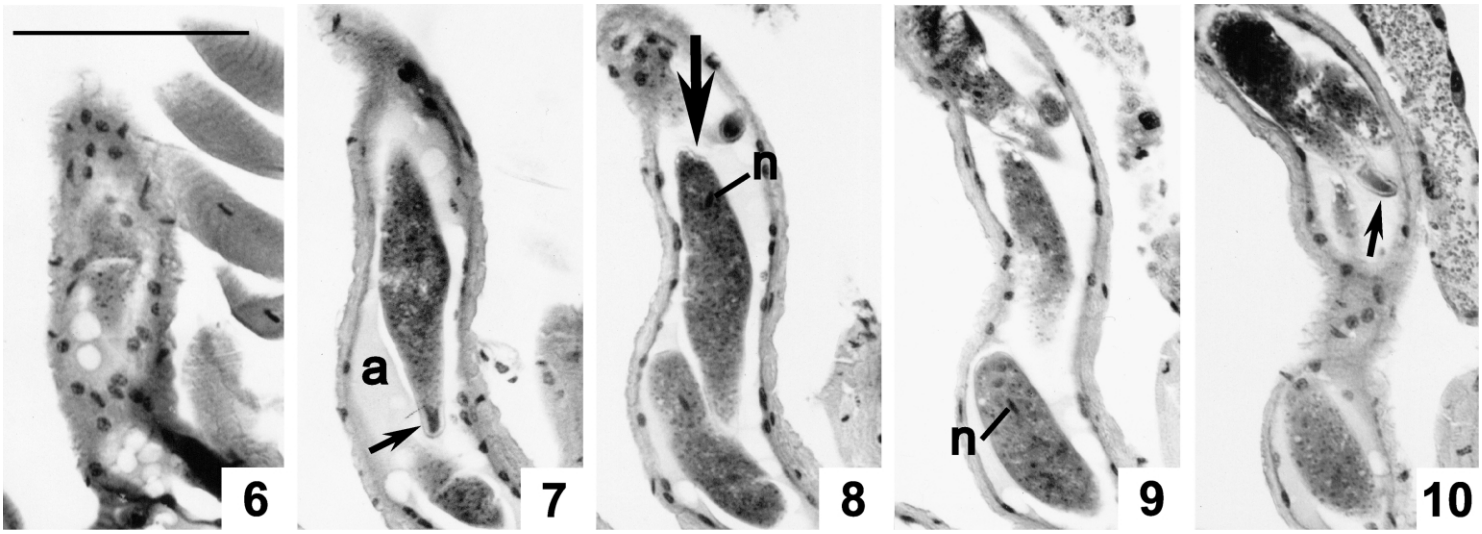


Figs. 6–10. Serial sections of the lateral oviduct of the scuttle fly *P. wasmanni*. Note that in Figs. 7 and 10 that each egg has a differentiated posterior pole (small arrows). Fig. 8: In the anterior portion of one of the eggs shown in this section it is possible to observe the micropyle depression (large arrow). Scale: 100 μm; a, acidophilic material; n, egg nucleus.

micropyle (Fig. 8). In some eggs observed in the ovaries, a basophilic hair-like structure was distinguished inside this depression. There was no evident ornamentation on the surface of the chorion of the eggs.

The number of eggs found inside both ovaries of the nine specimens examined ranged from 23 to 277 ($X = 130.2 \pm 73.8$, SD). The number of eggs in the oviducts ranged from 3 to 25 ($X = 11.4 \pm 6.3$, SD). The total number of eggs found per specimen varied from 31 to 280 ($X = 141.6 \pm 73.1$, SD). During histology analyses, a few eggs were in an appropriate orientation for measurement (not folded or sectioned transversely) in the genital tract. The eggs of *P. wasmanni* have a slugform or torpedo shape about 130 μm in length and 20 μm maximum width. Exploratory dissections (fresh squashes of whole ovaries) indicate that the eggs of several other *Pseudacteon* species (i.e. *P. tricuspis*, *P. litoralis*, *P. curvatus*, and *P. obtusus*) are of similar size and shape.

As shown in Figs. 1 and 4, a large bulbous muscular structure is centrally placed between both ovaries. This structure also was observed in other *Pseudacteon* species (*P. tricuspis*, *P. curvatus*, *P. litoralis*, *P. obtusus*, and *P. solenopsidis*; unpublished data and *P. formicarum*; Wasmann, 1918). In *P. wasmanni*, this structure was formed by two oblique muscular layers which resembled a bulb covering the final portion of the digestive epithelium or rectum (Figs. 1, 3–5); the former structure showed its lumen enlarged, where it was possible to observe sparse-granular material interpreted as feces and a oval structure which was formed by large cells having big nuclei; the epithelium that formed the rectum was thin with only its small basophilic nuclei visible (Figs. 1 and 3). In the posterior portion of the common oviduct, several eggs were visible separated by a thin epithelium beneath the lumen of the intestine (Fig. 1). In sagittal sections, it was possible to identify at the end of the abdomen a ventral compartment or chamber closely related to the posterior end of the common oviduct and the rectal lumen (Fig. 3); this chamber seems to be continuous to the rectal lumen and its posterior end is also separated by a thin epithelium. It was observed that the spermatheca was filled with spermatozoa in all specimens analyzed and in some sections the tubular projections of the spermatheca could be located near the bulb.

As shown in Fig. 3, the seventh sclerite is almost completely covered by the fifth and sixth sclerites. When the muscular bulb contracts, the fluid inside the bulb is apparently forced posteriorly, causing a hydraulic extension of a dorsal and lateral membrane out from under the fifth and sixth segment. Occasionally, this extension and rotation occurs for several seconds during grooming behavior (SDP, pers. obs.); however, the primary purpose is almost certainly to orient the ovipositor during oviposition.

The SEM and histology pictures show the position and structure of the muscular bulb (Figs. 1, 3, 4, 11 and 13). The external muscular layer of the bulb consisted of ventral sutured muscle fibers forming a depression, with the common oviduct running out of this suture. A narrow and long colon is clearly linked to the bulb (Figs. 4 and 5). The initial portion of the common oviduct (reservoir) was clearly enlarged due to accumulation of eggs (Fig. 4). In addition, other structures were observed near the bulb which appear to be related to the genital tract, i.e. accessory glands. During histological analyses no rectal pads were distinguished in the rectal epithelia indicating that feces, if any, may be not dehydrated.

The external morphology analysis of the severed abdomens showed two sets of three sensilla on the sternite just before the external genitalia (Fig. 11). A close inspection revealed lateral–posterior incomplete pegs (Fig. 12) around the base of each sensilla, probably defining

the bending direction for effective nerve stimuli. It seems likely that these sensilla may function as 'trigger hairs' for extension of the ovipositor and/or injection of an egg.

An acute hypodermic-like ovipositor was found by removing the cuticle of the terminal abdominal segments which form the hard sclerotized external genitalia (Fig. 13). An invaginated membrane (Fig. 14) was located between these segments. Although histological sectioning was done, the best views of the ovipositor were produced by SEM (Figs. 15 and 16). The ovipositor was sharp pointed and furrowed resembling an hypodermic needle. It was about 30 μm long or only about 1/4 the length of an egg. The ovipositor was like a tube folded longitudinally with the dorsal face sclerotized and the ventral face formed by a thin collapsed membrane. At the ventral ending of the ovipositor, the collapsed membrane had a circular aperture marked by a ring out of which the eggs apparently emerge during oviposition (Fig. 16).

4. Discussion

No pre-vitellogenesis or vitellogenesis features were seen in the histological sections or whole mount squashes. This was surprising because it indicated that oogenesis is completed before emergence, probably during the pupal stage. The ovaries of *P. formicarum* are also filled with vitellogenic eggs (Wasmann, 1918). In contrast, several saprophytic phorid flies apparently do have follicles (oocyte-nurse cell complex) and developing oocytes (Borgmeier 1930; Benner, 1985).

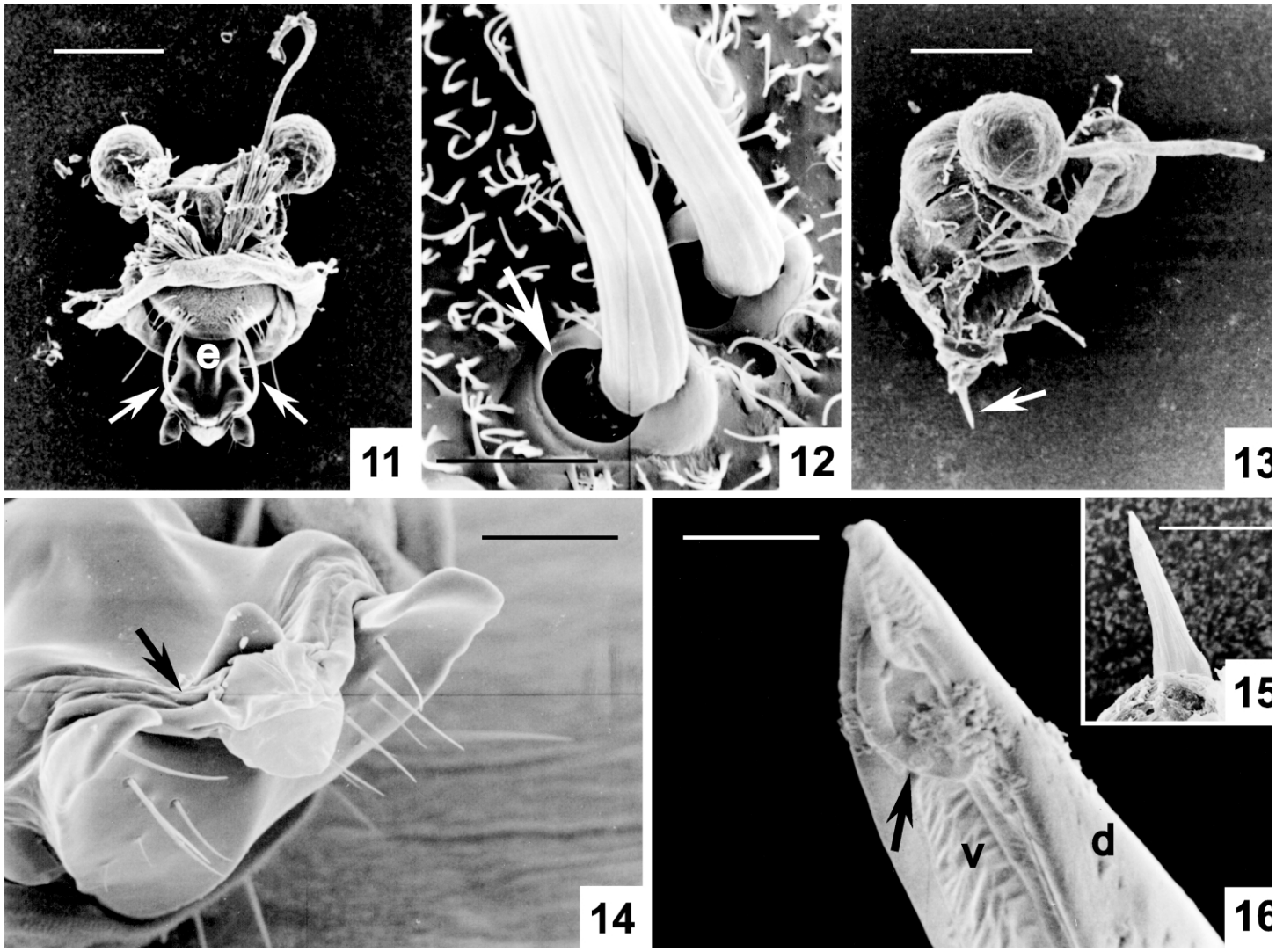
The occurrence of only mature or nearly mature eggs inside both ovaries of all *P. wasmanni* flies analyzed characterizes a strict pro-ovigeny condition (Quicke, 1997) and this is probably related to the short life span of the fly and the need to lay large numbers of eggs rapidly after mating. Most *Pseudacteon* females only live several days and do not feed much during this time (Porter, 1998a). Also, embryos and larvae have an abundant supply of protein in the host and the pupal stage provides a long period for egg development (Cônsoli et al., 2001). At least two species of *Pseudacteon* flies (*P. tricuspis*, *P. curvatus*) are ready to mate and lay eggs within 3–4 h of emerging from the pupa (SDP, unpub. data). The fact that all specimens of *P. wasmanni* that we inspected had mature or nearly mature eggs in their ovaries indicates that *P. wasmanni* also has the potential for laying large numbers of eggs shortly after emergence. As discussed earlier and like hymenopteran parasitoids (Quicke, 1997), the completion of oogenesis before the emergence of the adult stage, or strict pro-ovigeny, is probably an adaptation for a parasitoid life history. According to Büning (1994) shifting egg growth into pre-imaginal stages is related to a high evolutionary pressure to shorten oocyte growth phase, which is time and energy consuming.

Transmission electron microscopy TEM of newly emerged specimens of *P. solenopsidis* Schmitz (Zacaro and Porter, 1997) showed that each egg had an unornamented chorion and was enclosed by a thin layer of flattened post-vitellogenic follicular cells. Each ovary, in this species, was enclosed by a double muscular layer and the ovarioles were separated from each other only by a thin tunica propria. Among the ovarioles of *P. solenopsidis*, it was possible to observe a few muscle cells and phagocytes. A strong characteristic of the follicular cells was the presence of large amounts of rough endoplasmic cisternae with a dilated lumen which led us to think that all ovarioles were in chorionogenesis. The comparison between the data obtained by transmission electron microscopy TEM of the ovary of *P. solenopsidis* (Zacaro and Porter, 1997) and the results obtained for *P. wasmanni* described in this work showed, however, that similarities can be found between fine structure of the chorion of *P. solenopsidis* and the acidophilic-gelatinous material found in *P. wasmanni*; this gelatinous material may be related to the exochorion, which changes its composition from a sparse/fibrillar configuration to a condensed one, depending on the stage of the chorionogenic process.

The number of eggs found in the specimens of *P. wasmanni* varied greatly ($X = 142 \pm 73$, SD) indicating either that the flies have different reproduction capacities for egg production or that some flies had already laid most of their eggs. It is tempting to assume the latter possibility since flies that had the most eggs in the ovaries had fewer in the oviducts. At least, 50 eggs are visible in the ovaries of *P. formicarum* (Wasmann, 1918).

In addition to muscle movements produced in oviducts, the presence of a muscular sheath covering each ovary of *P. wasmanni* may represent the power supply from which ovarioles are compressed, moving eggs to the enlargement of the anterior portion of the common oviduct. In *P. wasmanni*, SEM photos of the surface of the ovary clearly demonstrate that the muscular sheath forms an almost continuous layer probably ensuring ovariole compression rather than facilitating entry of resources from the hemolymph as in the stable fly *Stomoxys calcitrans* (Cook and Peterson, 1989).

With few exceptions, such as for the eggs of the genus *Megaselia* and *P. tricuspis* (Furukawa and Kaneko, 1981; Disney, 1994; Cônsoli et al., 2001), detailed SEMs are not available. Descriptions of egg sizes are available for phorids in several genera: *Megaselia* species (0.30–0.55 mm length), several *Puliciphora* species (0.4–0.7 mm length), *Diplonevra mortimeri* (0.7 by 0.3 mm) and *Apocephalus attophilus* (0.33 by 0.12 mm) (Kaneko and Furukawa, 1983; Disney, 1986a,b, 1988, 1991, 1993, 1994; Feener and Moss, 1990). The smallest recorded egg sizes measured before oviposition are from *P. formicarum* (0.065 by 0.017 mm; Wasmann, 1918) and *P. wasmanni* (0.130 by 0.020 mm, this paper). Cônsoli et al. (2001) reported that eggs of *P. tricuspis* are oval shortly after oviposition and 0.0325 mm long × 0.0135 mm wide.



Figs. 11–16. SEM of the external genitalia and ventral sensilla of the scuttle fly *P. wasmanni*. Fig. 11: Ventral view of the abdomen in which is possible to observe the external and sclerotized genitalia (e) and the two sets of major sensilla (arrows). Fig. 12: Detail of the insertions of the major sensilla in which are noted incomplete cuticle pegs (arrow). Fig. 13: Lateral view of an abdomen from which pieces of the external genitalia were removed exposing the ovipositor (arrow). Fig. 14: Frontal view of the posterior ending of the abdomen shown in Fig. 11 in which portions of a smooth membrane protrude (arrow). Fig. 15: Ventral view of the entire ovipositor. Fig. 16: Detail of the ventral posterior ending of the ovipositor showing a circular aperture marked by a ring (arrow); the ovipositor resembles a tube longitudinally folded in which the dorsal face (d) is sclerotized and the ventral face (v) is formed by a thin and collapsed membrane. Scales: 100 μm (Figs. 11 and 13), 5 μm (Fig. 12), 25 μm (Fig. 14), 20 μm (Fig. 15) and 3 μm (Fig. 16).

Apparently, they loose their pointed apex and increase in size rather quickly after oviposition. Eventually, post-oviposited eggs of *P. tricuspis* increase approximately 10 times in size before hatching the first instar larvae (Cônsoli et al., 2001). This growth is the characteristic of hydropic eggs also found in hymenopteran parasitoids (Cônsoli et al., 2001).

No similarities are found between the morphology of the eggs of *Pseudacteon* and phorid flies in other genera like a pointed posterior pole. Some other species of the genus *Megaselia* such as *M. halterata*, *M. stenoterga*, and *M. oxybelorum* have a smooth chorion, but their eggs are spherical or oval in shape.

Cônsoli et al. (2001) confirmed that *P. tricuspis* eggs are injected into fire ant workers. The elongated shape, the smooth surface, the pointed posterior pole, and the small size of *Pseudacteon* eggs are probably adaptations for injection as is the muscular bulb associated with genital chamber and the hypodermic design of the ovipositor. The solitary observation that *P. obtusus* lays its eggs externally (Williams and Banks, 1987) is probably not normal in view of several exploratory dissections which showed that *P. obtusus* also has an hypodermic shaped ovipositor and eggs similar to those of *P. wasmanni*. Acute hypodermic-like ovipositors have also been reported in other *Pseudacteon* species (Wasmann, 1918; Borgmeier, 1925, 1931) and other

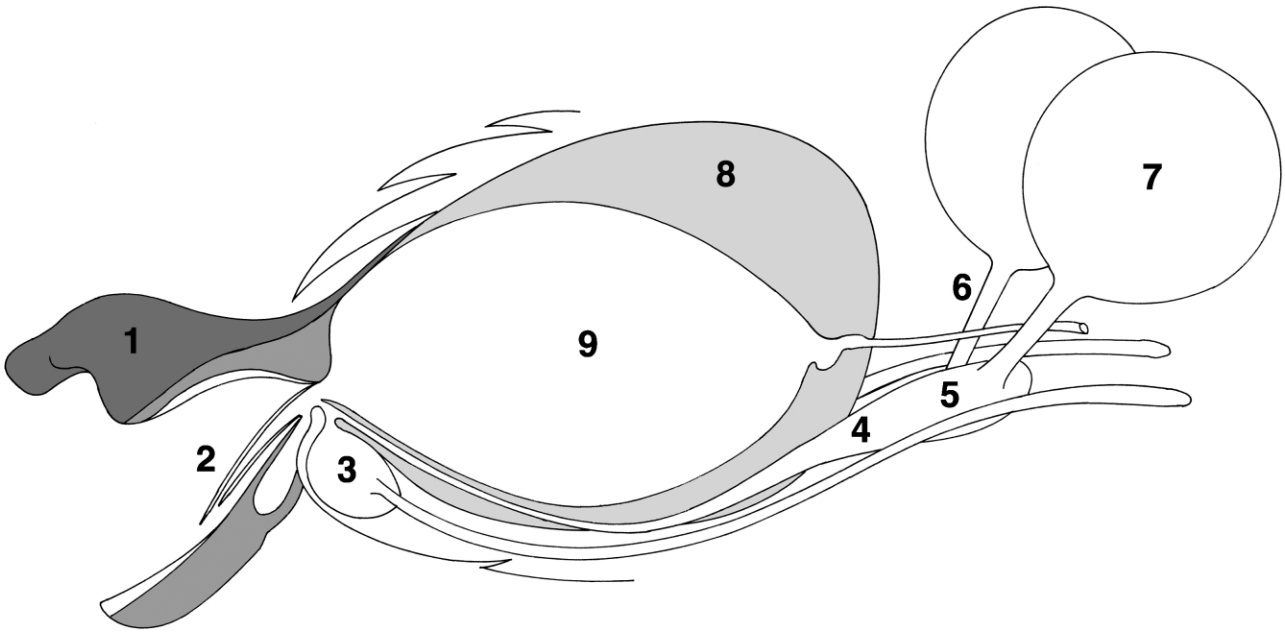


Fig. 17. Schematic drawing of the female reproductive system showing likely spatial organization in relation to the posterior digestive system and muscular bulb; (1) external genitalia, (2) ovipositor, (3) spermatheca, (4) common oviduct, (5) reservoir of eggs, (6) lateral oviduct, (7) ovary, (8) muscular bulb, and (9) hind gut.

parasitic phorids (Disney, 1986b; Disney and Schroth, 1989). However, saprophytic phorids can also have acute sclerotized ovipositors (Disney and Schroth, 1989).

Like parasitoid wasps (Quicke, 1997), parasitoid phorids may be subdivided into two major groups depending on how oogenesis takes place: (a) those that are synovigenic, which can be characterized by the saprophitic phorids and (b) those that are pro-ovigenic, which can be characterized by truly endoparasitoid phorids. Also, the term koinobiont, which is commonly used to designate hymenopteran endoparasitoids, may be applied to the truly endoparasitoid phorids, since they seem to share some features like endoparasitism, specialists, small eggs, pro-ovigeny, no oösorption, and short adult life span.

According to Le Ralec (1995), hydropic eggs correlate positively with pro-ovigenic species that do not feed on the host or on a protein diet. Hydropic eggs have electron dense ooplasm due the presence of numerous ribosomes and mitochondria, and the yolk having few lipoid globules lacks protein bodies. In contrast, species with anhydropic eggs have yolk rich in lipoid and protein bodies. *P. wasmanni* produces basophilic eggs and this stain feature can be related to protein or ribosome rich egg content. In the ooplasm of *P. solenopsidis* eggs, Zacaro and Porter (1997) observed electron dense inclusions probably related to protein bodies.

Egg production may be related to saprophytic (synovigeny) versus endoparasitic (pro-ovigeny) life strategy. Oogenesis occurs mainly during the adult stage in saprophytic phorids; in contrast, parasitic phorids appear to complete oogenesis before emergence. Saprophytic phorids have the potential to lay eggs in batches while parasitic phorids probably inject one egg into each host. However, scuttle flies that parasitize termites carry few mature eggs (Disney, 1988).

Although histological and morphological studies were done in this report, no evident fusion of the final portions of the oviduct and rectum in a common chamber was observed. Considering the stunned reaction of the ant during *P. wasmanni* attack and oviposition; the oviposition apparently occurs with relative violence probably in the coxal region of the host thorax (Cônsoli et al., 2001). These further arguments added to the presence of the muscular bulb around the rectum and mechanoreceptors give clues about how egg laying may occur. To accept this scenario we have to assume that the anus and vagina open into a common chamber (Fig. 17). According to Feener and Brown (1997), dipteran parasitoids do not inject venom into the host during oviposition; thus, developing larvae may have other ways of countering the host's immune system. Muscle contraction providing injection of additional fluids is found in the sting apparatus of some Aculeata (i.e. Vespidae and Pompilidae; von Marle and Piek, 1986). Analogies to these systems can be found in the muscle bulb-like structure formed by large cells in the anterior portion of the lumen of the bulb which would work to limit the reflux of liquid feces and the muscle sheath itself providing the power supply for injection. In addition, liquid feces may also be injected during egg oviposition and might trigger the immobilized ant's reaction as observed in the field (Porter et al., 1995a).

Pseudacteon flies hover 3–5 mm above prospective hosts while attacking fire ant workers (Porter, 1998a). Details of the oviposition process in *P. wasmanni* and other species in this genus are largely unknown because the

process is rapid (~0.1 s; Porter, 1998a) and these flies are very small (1.0–1.5 mm). During oviposition, *P. wasmanni* appears to briefly latch onto the side of the host thorax oriented with its head up and the abdomen down. At this point, the six large ventral sensilla are probably depressed triggering a nerve impulse that may cause a contraction of the large muscular bulb. The contraction of the muscular bulb forces out fluid that appears to cause a hydraulic extension of the abdomen between the sixth and seventh dorsal segments. This extension causes the external ovipositor to rotate downward about 90° on a ventral hinge placing the actual ovipositor at an angle to inject an egg slightly forward compared to the fly. The assumption is that the external genitalia of *P. wasmanni* and other *Pseudacteon* flies are used in a lock-and-key fashion to position the short hypodermic-shaped ovipositor at a specific location on the thorax of the ant where an egg can be successfully injected (Porter, 1998a). The coxal region seems likely based on observations with *P. tricuspis* (Cônsoli et al., 2001), but the exact location is unknown for *P. wasmanni*.

Acknowledgements

Thanks are extended to Marcos Pesquero (UNESP, Rio Claro, Brazil) for helping to collect the flies, Colleen Kay Porter (University of Florida, Gainesville, USA) for helping to improve the English of early versions of this manuscript. Carlos Frankl Sperber (Universidade Federal de Viçosa—UFV, Brazil) helped with translation of several German papers. We also thank Fernando L. Cônsoli (Texas A&M University, USA) and Carminda da Cruz Landim (UNESP, Rio Claro, Brazil) for reading the manuscript and providing valuable suggestions. We also thank Pedro Arimatéia Ribeiro (UFV—DBG) for the assistance in doing the final art work shown in the scheme. We are grateful to Department of Biology, Laboratories of Histology and Electron Microscopy (UNESP, Rio Claro, Brazil) for permission to execute this work.

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