MORPHOLOGY OF THE DIGESTIVE AND REPRODUCTIVE SYSTEMS OF *PEREGRINUS MAIDIS* (HOMOPTERA: DELPHACIDAE)

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ABSTRACT

The corn delphacid, *Peregrinus maidis* (Ashmead) (Homoptera: Delphacidae) is the only known vector of maize stripe tenuivirus and maize mosaic rhabdovirus in tropical and subtropical areas. The morphology of its digestive and reproductive systems was studied by light microscopy and illustrations were made to aid in dissections and injections of pathogenic innoculum for vector studies. Two salivary glands, located one on each side of the head, extend into the mesothorax. Each gland is divided into two types of lobes which form the principle and accessory glands. The esophagus, a narrow muscular tube, extends from the cibarial diaphragm to the abdomen and empties into the midgut. Parallel to the esophagus, extending from the midgut into the head, is the anterior diverticulum. This closed sac is often filled with air bubbles. The midgut, a tube of uniform diameter, winds in a consistent pattern from the esophagus to the rectum. No filter chamber was observed. The two pairs of Malpighian tubules originate at the posterior end of the midgut with each pair being fused for the proximal two-thirds of their length. The hindgut consists of a rectal sac and rectum. *P. maidis* females have two large ovaries containing an average of 16 ovarioles. They open successively into the lateral oviduct, the median oviduct, the common oviduct, and the vagina. The spermatheca and the bursa copulatrix open into the common oviduct. A spermathecal gland is also present. The male has two lateral testes with three to four follicles each. A pair of accessory glands fill the abdomen, often extending into the thorax.

Key Words: Internal morphology, anatomy, planthopper, maize, rhabdovirus, tenuivirus.

RESUMEN

El delphacido del maiz *Peregrinus maidis* (Ashmead) (Homoptera: Delphacidae) es el único vector conocido del tenuivirus de la hoja rayada del maíz y del rabdovirus del mosaico del maíz en areas tropicales y subtropicales. Se ha estudiado la morfología de su sistema digestivo y reproductivo bajo microscopio y se han hecho ilustraciones para ayudar en las disecciones e inyección del patógeno en los estudios de vectores. Dos glandulas salivares, localizadas a cada lado de la cabeza, se extienden hasta el mesotorax. Cada glandula se divide en dos tipos de lobulos llamados las glandulas principales y accesorias. El esófago, un tubo estrecho musculoso, se extiende desde el diafragma hasta el abdomen y se vacía en el estomago medio. El divertículo anterior se encuentra paralelo al esófago, extendiendose desde el estomago medio hasta la cabeza. Este saco cerrado a menudo se llena de burbujas de aire. El estomago medio, un tubo con diametro uniforme, mantiene una forma consistente desde el esofago hasta el recto. No se observó camara de filtramiento. Los dos tubos de Malpighi se originan en el final posterior del estomago medio, y cada par está ligado aproximadamente a los dos tercios de su parte final. El estomago inferior, el saco rectal y el recto. Las hembras de *P. maidis* tienen dos ovarios grandes los cuales contienen un promedio de 16 ovariolas. Estas ovariolas se abren sucesivamente en el oviducto lateral, el oviducto medio, el oviducto común y la vagina. La espermateca y la bolsa copulatrix se abren en el oviducto común. La glandula de la espermateca también está presente. El macho tiene dos testiculos laterales, y cada uno tiene de tres a cuatro foliculos. Un par de las glandulas accesorias llena el abdomen, extendiendose a veces hasta el torax.

In tropical and subtropical areas, maize (*Zea mays* L.) can become infected with destructive viruses including maize stripe tenuivirus (MStV) and maize mosaic rhabdovirus (MMV) (Tsai & Falk 1988). These two viruses are transmitted by the planthopper *Peregrinus maidis* (Ashmead), their only known vector (Nault & Knoke 1981, Tsai & Falk 1988). MMV and MStV were first reported by Kunkel (1921) and Storey (1936), respectively. However, according to Brewbaker (1979), the presence of these viruses has been evident for many centuries and they may have played a role in the disappearance of the classic Mayan civilization. This point is disputed by Nault (1983) who argues that *P. maidis*, MStV and MMV originated in Africa and were not introduced to the Americas until post-Columbus times.

Because of the economic importance of MStV and MMV in tropical and subtropical countries, both viruses and their vector have been the subject of many studies. These studies have included the biology of *P. maidis* (Nault & Knoke 1981, Tsai & Wilson 1986), transmission of MMV and MStV (Ammar et al. 1987, Gingery et al. 1979, 1981, Nault & Gordon 1988, Tsai 1975, Tsai & Zitter 1982, Tsai & Falk 1988), characterization of the viruses (Falk & Tsai 1983, 1984, Gingery et al. 1981, Lastra & Carballo 1983, Tsai & Falk 1988), and the discovery of the presence of MMV and MStV in various organs and tissues of *P. maidis* by electron microscopy and serological technology (Ammar 1985, 1987, Ammar & Nault 1985, Falk et al. 1988, Herold & Munz 1965, Falk & Tsai 1984, 1985, Nault & Gordon 1988). These latter studies involved dissections of the insects to assay for the presence of the virus in different organs. Because it is difficult to perform these dissections with accuracy, a guide to the internal morphology is needed, since information on internal structures of *P. maidis* is limited to reports by Ammar (1985, 1986, 1987), who described some major organs of *P. maidis* on an ultrastructural level, and Backus (1985), who studied the structure of auchenorrhynchan mouth parts and the mechanisms of feeding behavior. Accordingly, we initiated this detailed study of the internal morphology of *P. maidis* for the purpose of facilitating the study of the fate MMV and MStV in *P. maidis* after its acquisition or injection.

Materials and Methods

Peregrinus maidis was reared in the laboratory on *Z. mays* L. var. Saccharata "Guardian" in a growth room at 24° C and 12 h light. Approximately 500 adults of both sexes were dissected. Insects that had been placed in a freezer at 0° C for periods of 15 min to 24 h were dissected in distilled water on paraffin using fine dissecting needles and forceps. Initially, the insects were dissected in a stain solution of one part safranin red to 60 parts deionized water. The organs removed were left in the solution for a period of 5-30 minutes, rinsed, and observed with a dissecting microscope and a light microscope at magnifications from 10X to 400X. When stains were not used, the organs were placed on a black background in order to observe the delicate structures. For observation of other fine structures, such as the salivary glands and the spermathecal gland, cold-anesthetized insects were kept at 7.5° C and dissected in Clarke's solution (Sogawa 1965) using neutral red (Sogawa 1965) or methylene blue chloride to stain the living tissue. For structural study of the salivary glands, a 1:1 solution of the above stains was used.

Results and Discussion

Digestive System

Salivary glands. The salivary glands of *P. maidis*, which are present on each side of the thorax and head (Sg in Fig. 1A), consist of principal and accessory glands. The principal gland contains six to eight acini, or follicles (P sg in Fig. 1B). Eight have been observed in *P. maidis* by Ammar (1985, 1986) and in Delphacidae in general by Sogawa (1965). Approximately 80% of the 500 insects dissected for this study contained seven acini, which varied in size and shape. The variation in numbers and morphology of acini could be due to the age of the insect or to physiological factors. The principal gland is found mainly in the prothorax whereas the accessory gland is found in the head (A sg in Fig. 1B). The ducts from the acini in the principal gland join to form the principal duct (P sd in Fig. 1B). At this juncture the accessory duct (A sd in Fig. 1B) also connects to the principal duct. The two principal ducts unite to form the common salivary duct

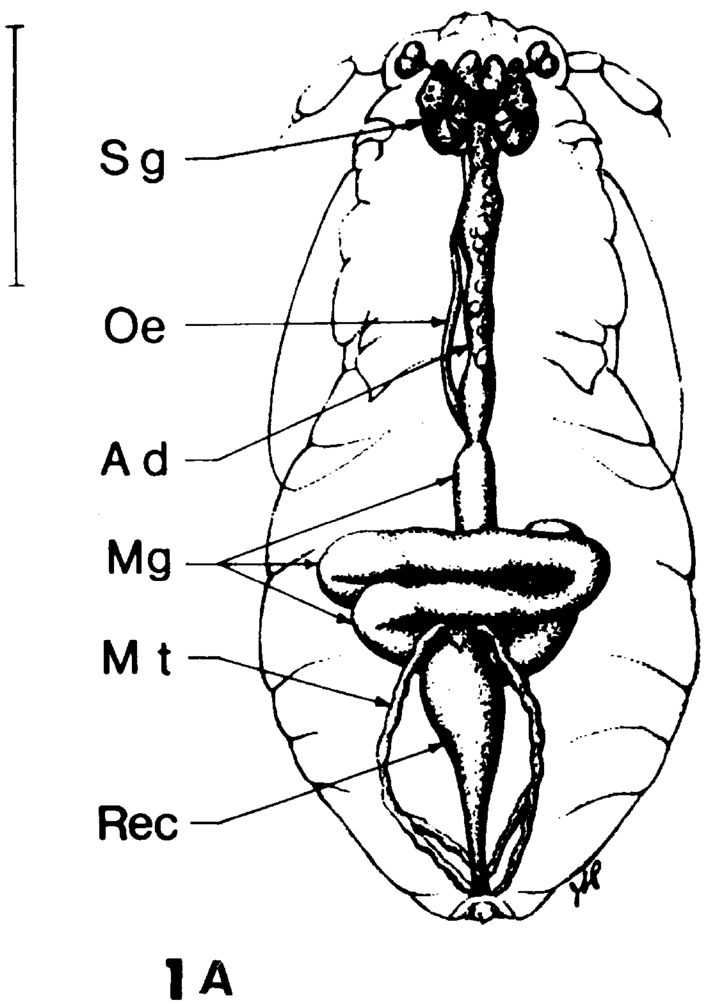

Fig. 1A. Dorsal view of digestive system of adult female. S g, salivary gland; Oe esophagus; A d, anterior diverticulum; Mg midgut; M t, Malpighian tubules; Rec, rectum. Vertical bar = 1.0 mm.

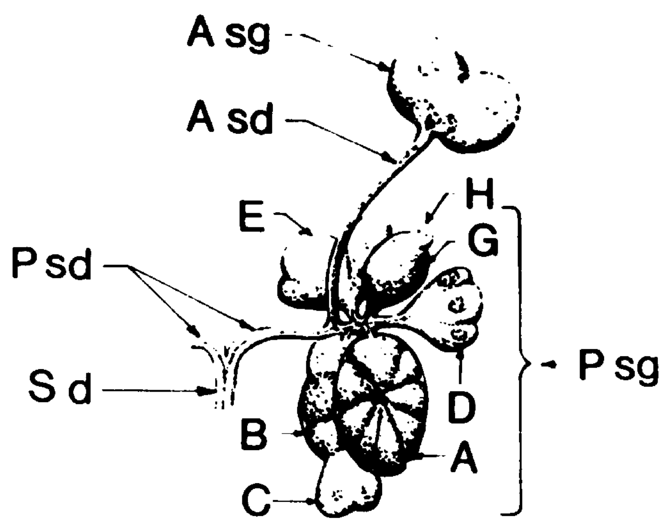

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Fig. 1B. Detail of salivary glands. P sg, principal salivary gland; A sg, accessory salivary gland; p sd, principal salivary duct; A sd, accessory salivary duct; S d, salivary duct; A-H, acini types. Vertical bar = 0.5 mm.

(S d in Fig. 1B) which extends into the head to the salivary syringe which in turn opens into the salivary canal in the stylets (Ammar 1985, Backus 1985, Sogawa 1965). Because numerous fat bodies fill the head and surround the salivary glands, it is sometimes difficult to separate the smaller and more translucent acini from the fat bodies.

Foregut. Backus (1985) described the ciberial and pharyngeal regions of the mouthparts of several leafhoppers. The foregut begins at the bases of the mandibular and maxillary stylets with the precibarium. Fluid passes from the stylets to the precibarium then into the cibarium (or sucking pump) and then to the esophagus. However, the structure of the cibarium and pharynx in planthoppers appears to be different from that in leafhoppers (Ammar 1985). In *P. maidis*, the esophagus is a narrow tube that originates posterior to the cibarium and extends to the anterior of the abdomen where it empties into the midgut (Oe in Fig. 1). At this point, there appears to be a slight constriction which may indicate the esophageal valve (Ammar 1985). Because of its muscular sheath (Snodgrass 1935), the tissue of the esophagus appears stronger and more elastic than that of the midgut.

Midgut. Parallel to the esophagus, extending from the midgut into the head, is a closed tube or sac (A d in Fig. 1, 3). This structure, described by Ammar (1985) as the anterior diverticulum, is often filled with air bubbles. Compared to the esophagus, the tissue in this region is more fragile and resembles that of the midgut. Again there seems to be a slight constriction between the anterior diverticulum and the midgut.

The midgut of *P. maidis* is a long tube of uniform diameter, which winds from left to right toward the rectum (Mg in Fig. 1, 2). No sheath or membrane enclosing the midgut has been observed, which would suggest there is no filter chamber (Ammar 1985). The pattern of winding of the midgut, similar to the patterns observed by Mishra (1980) and Goodchild (1966), suggest the possibility of a different type of filtering device. Figures 4 and 5 illustrate the central loop of the midgut pulled away to reveal the contact of the anterior and posterior regions of the midgut.

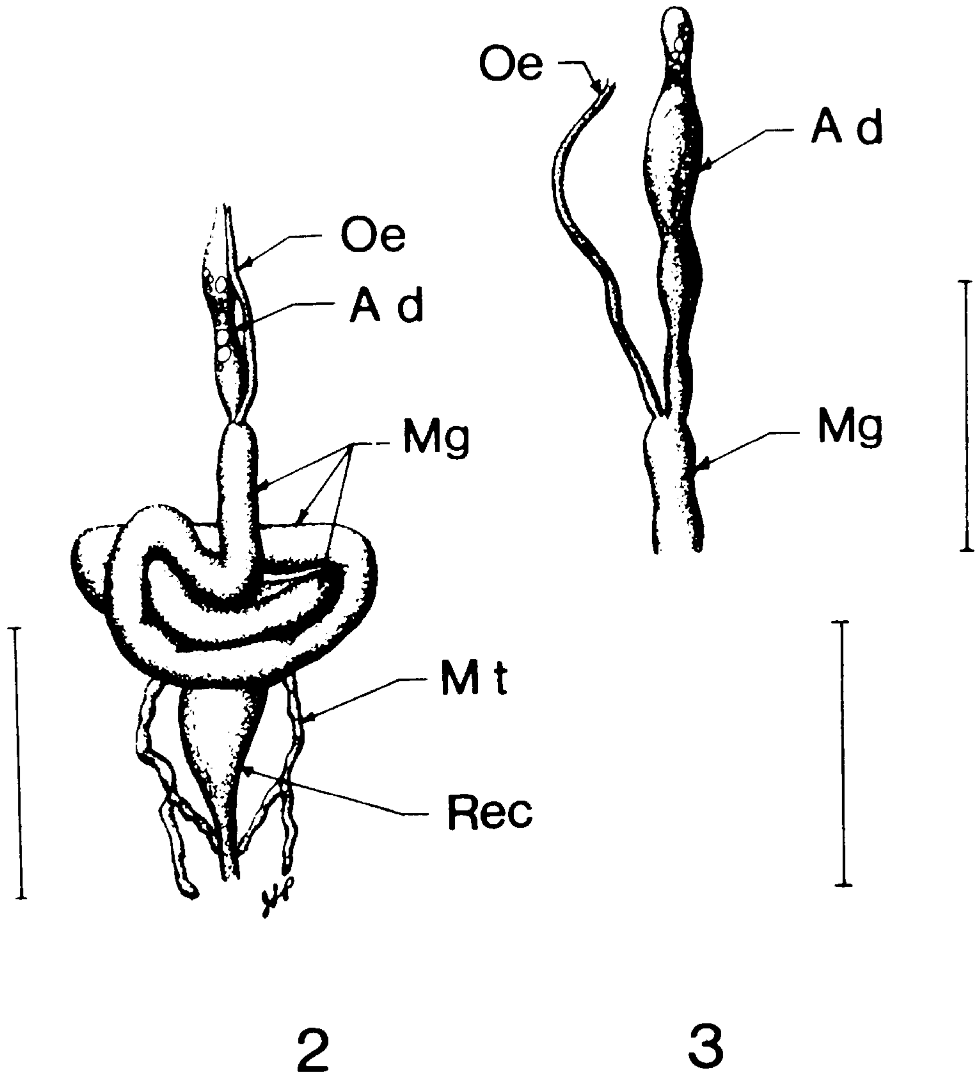


Fig. 2. Ventral view of alimentary canal. Oe, esophagus; A d, anterior diverticulum; Mg, midgut; M t, Malpighian tubules; Rec, rectum. Vertical bar = 1.0 mm.

Fig. 3. Foregut and midgut. Oe, esophagus; A d, anterior diverticulum; Mg, midgut. Vertical bar = 1.0 mm.

Malpighian Tubules. At the posterior end of the midgut there is a slight swelling from which the two pairs of Malpighian tubules arise (Mt in Fig. 1, 4). Each pair is fused (Ammar 1985) for the proximal two-thirds of its length, at which point the distal ends form a fork extending toward the anus.

Hindgut. Posterior to the origins of the Malpighian tubules, the midgut opens into the hindgut (Hg in Fig. 4). At this junction, a pyloric value (Ammar 1985) is visible. The hindgut consists of a rectal sac and a rectum (Rec in Fig. 4). These tissues are translucent but elastic and strong.

Reproductive System

Female. The ovaries of *P. maidis* consist of an average (N = 250) of 16 ovarioles (16 ± 2). The ovariole is comprised of developing oocytes and eggs connected to the lateral oviduct by a pedicel (Ovl, L od, Ped in Fig. 6). Each ovariole ends anteriorly with a terminal filament which unites with those of the other ovarioles to form a suspensory ligament (S lg in Fig. 6) (Snodgrass 1935). These in turn appear to attach with the ligaments of the other ovary to form a median ligament (M lg in Fig. 6) which attaches to a fat body (Ammar 1985) in the thorax.

The ovaries each form a calyx before connecting to the lateral oviducts (Cal, L od in Fig. 6) which join to form a short median oviduct (Snodgrass 1935). This in turn opens into a common oviduct (C od in Fig. 6). At this juncture a curved tubular spermatheca (Ammar 1985) opens into the common oviduct (Spt, C od in Fig. 6). The contents of this structure are often orange in color. At its distal end a long fragile tube extends to the spermathecal gland (Ammar 1985) which seems to partially surround the neck of the bursa copulatrix (Spt gl, B cp in Fig. 6). The bursa copulatrix is a globular, narrow-necked structure that opens into the common oviduct at its junction with the vagina (Vag in Fig. 6), which then connects with the ovipositor. In our study, we have not observed the presence of oviduct glands (Asche 1985).

Male. Peregrinus maidis males have two lateral testes which are light red in color. These are found ventrally in the posterior of the abdomen (Tes in Fig. 7). Sixty-five percent of the 250 insects observed appeared to have three follicles per testes (Ammar 1985). Upon more careful dissection of 20 insects, a fourth follicle was observed on ten of these. The absence of the fourth follicle could be due to their fragile nature. The follicles join together and then open into a short, narrow vas deferens. This organ enlarges to form the seminal vesicle (Sem v in Fig. 7) which opens into a common ejaculatory duct (Ej d in Fig. 7).

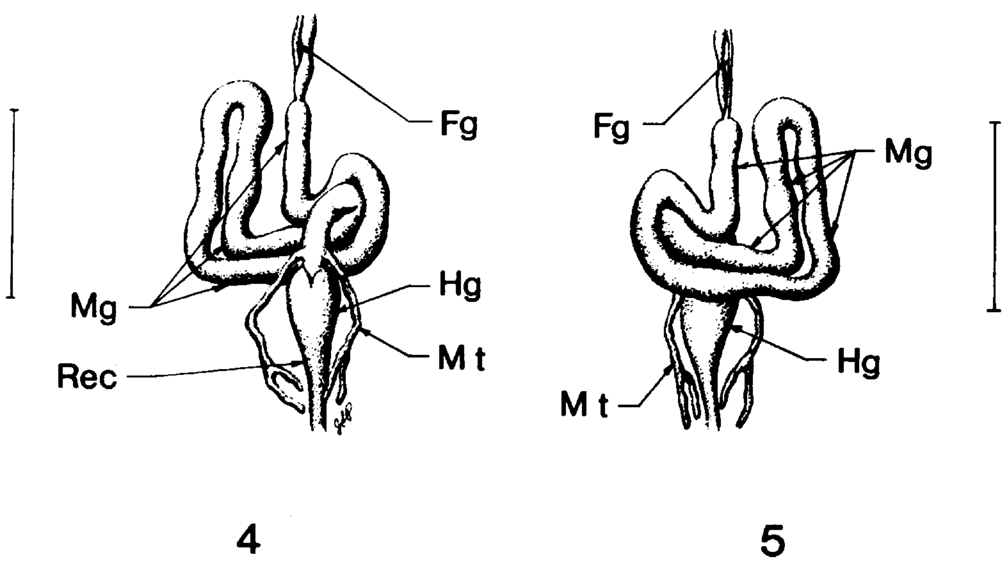


Fig. 4-5. Alimentary canal with midgut partially straightened: 4., dorsal view; 5., ventral view. Fg, foregut; Mg, midgut; Hg, hindgut; M t, Malpighian tubules; Rec, rectum. Vertical bar = 1.0 mm.

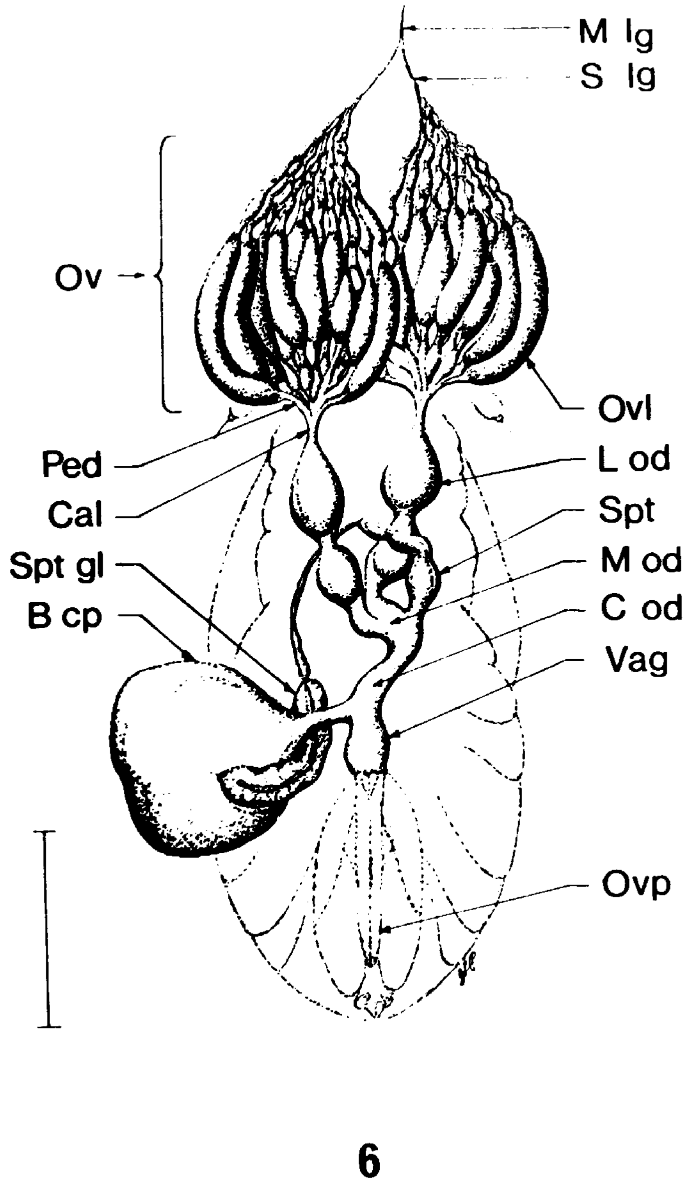
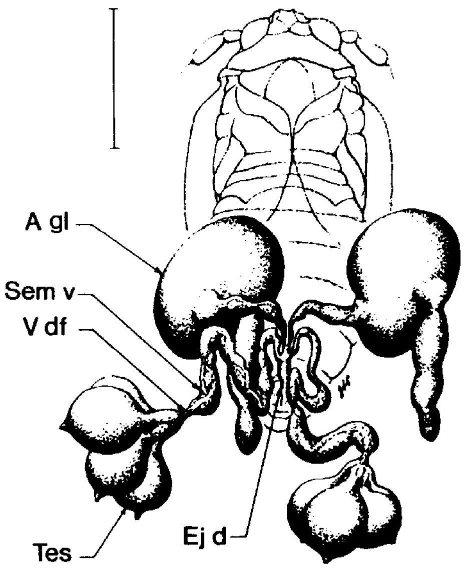


Fig. 6. Female reproductive system. Ventral view. Ov, ovary; Ovl, ovariole; S lg, suspensory ligament; M lg, median ligament; Ped, pedicle; Cal, calyx; L od, lateral oviduct; M od, median oviduct; C od, common oviduct; Spt, spermatheca; Spt gl, Spermatheca gland; B cp, bursa copulatrix; Vag, vagina; Ovp, ovipositor. Vertical bar = 1.0 mm.

The two lateral accessory glands also open into the ejaculatory duct (A gl, Ej d in Fig. 7). They are basically tube-shaped (see Fig. 8 which illustrates the accessory gland of an immature male). In mature males, these glands enlarge to fill the abdomen and the posterior of the thorax. The membrane surrounding the milky substance of these glands is delicate and easily ruptured, which presents difficulties when making dissections and injections.



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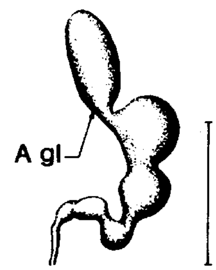

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Fig. 7. Male reproductive system. Dorsal view. Tes, testes; V df, vas deferens; Sem v, seminal vesicle; A gl, accessory gland; Ej d, ejaculatory duct. Vertical bar = 1.0 mm.

Fig. 8. Young adult male accessory gland showing basic tubular structure. A gl, accessory gland. Vertical bar = 1.0 mm.

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