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Morphology of the male reproductive tract in two species of phytophagous bugs (Pentatomidae: Heteroptera)

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Abstract
Heteroptera is a diverse group with wide variety of life stories. Most Pentatomidae species are phytophagous and can be configure as important agricultural pests. In this work, the anatomy and histology of the male reproductive tract of *Thyanta perditor* (Fabricius, 1794) and *Oebalus ypsilongriseus* (De Geer, 1773) was described using light microscopy. In these species the male reproductive system consisted of a pair of testes, with four follicles each, a pair of deferent ducts, accessory glands, ejaculatory bulb and an ejaculatory duct. The deferent ducts were long and thin and flow into the ejaculatory bulb, where secretions from the accessory glands was also released. The ejaculatory bulb was continuous with the ejaculatory duct, which is connected to the aedeagus. In this work, the morphology of the reproductive system in two species of bedbugs expanding knowledge about the reproductive biology of Pentatomidae.

Keywords: Hemiptera, reproductive biology, testicles, deferent ducts

1. Introduction

Hemiptera is among the five most diverse types of insects, with a wide variety of body plans and eating habits, such as hematophagia, zoophagy and phytophagia [1-3]. Adaptations of mouthparts for suction and drilling located on a straight crest parallel to or near the gular region are synapomorphies of the order [1]. Currently, around 90,000 species has been described [4, 5] and it is estimated that more than 103,500 [6-8] are distributed worldwide in the suborders: Auchenorrhyncha, Coleorryncha, Heteroptera and Sternorrhyncha [4].

Considering the ecological and economic importance of Heteroptera, the intensive reviews address the biology and taxonomy of the group [9-14]. The Pentatomomorpha infraorder has around 15,000 essentially terrestrial species and the majority are phytophagous [1, 15, 16]. Several hypotheses seek to elucidate the evolutionary history of the clade [1, 17-22], however there are still doubts about its phylogenetic relationships. The use of new characters, such as those derived from the internal structures of the reproductive tract, indicated as potential for insect systematics [23-28].

For Pentatomidae, the morphology of the male reproductive system has already been described in some species [25, 26, 29-43], generating information about the functionality of the histological regions and the potential to apply them in the control population of some species of pests in agriculture.

Several species of Pentatomidae are identified as major agricultural pests in South America. In Brazil, the species *Thyanta perditor* (Fabricius, 1794) and *Oebalus ypsilongriseus* (De Geer, 1773) are widely distributed and are considered polyphagous pests that can cause significant economic damage affecting the quantity and quality of production [44]. In this work, we describe the anatomy and histology of the reproductive system in two species of bedbugs, *T. perditor* and *O. ypsilongriseus*, with the aim of expanding knowledge about the reproductive biology of Pentatomidae.

2. Materials and Methods

2.1. Insects

Ten adult males of *Thyanta perditor* (Fabricius, 1794) and *Oebalus ypsilongriseus* (De Geer, 1773) were collected in the municipality of Rio Paranaíba (19° 12'S 46° 07' W), Minas Gerais state, Brazil. The collections were carried out with the aid of entomological nets.

2.2. Light Microscopy

The insects were cryo-anesthetized at -5 ^{0}C, and their reproductive tracts were dissected in 0.1 M NaCl and transferred to 2.5% glutaraldehyde in sodium cacodylate buffer, 0.1 M pH 7.2 for 2 hours at room temperature. Then, the samples were washed in 0.1 M sodium cacodylate buffer at pH 7.2, dehydrated in a graded ethanol series (50, 70 90 and 95%) and embedded in historesin (Leica Historesin). Semithin sections (2 µm thick) were obtained with a glass knife in a rotary microtome (Leica RM 2255), stained with 1% toluidine blue sodium borate and analyzed and photographed with light microscopy Olympus BX-60 (Japan) and then drawn with Indian ink and graphite.

3. Results

The reproductive tract in *T. perditor* and *O. ypsilongriseus* consisted of a pair of testes with four follicles each, a pair of deferent ducts, an ectodermal sac, paired accessory glands, a complex ejaculatory bulb and an ejaculatory duct (Figs. 1A-D). The testes were covered by a peritoneal sheath which in *T. perditor* had orange pigments and testes elongated in shape (Fig. 1A) and in *O. ypsilongriseus* the pigments was red and testes ovoid (Fig. 1B).

Efferent ducts departed from each testicular follicle, which fused to form the deferent ducts. The deferent ducts were long, cylindrical and covered by a peritoneal sheath of the same color as the testes (Figs. 1A-B).

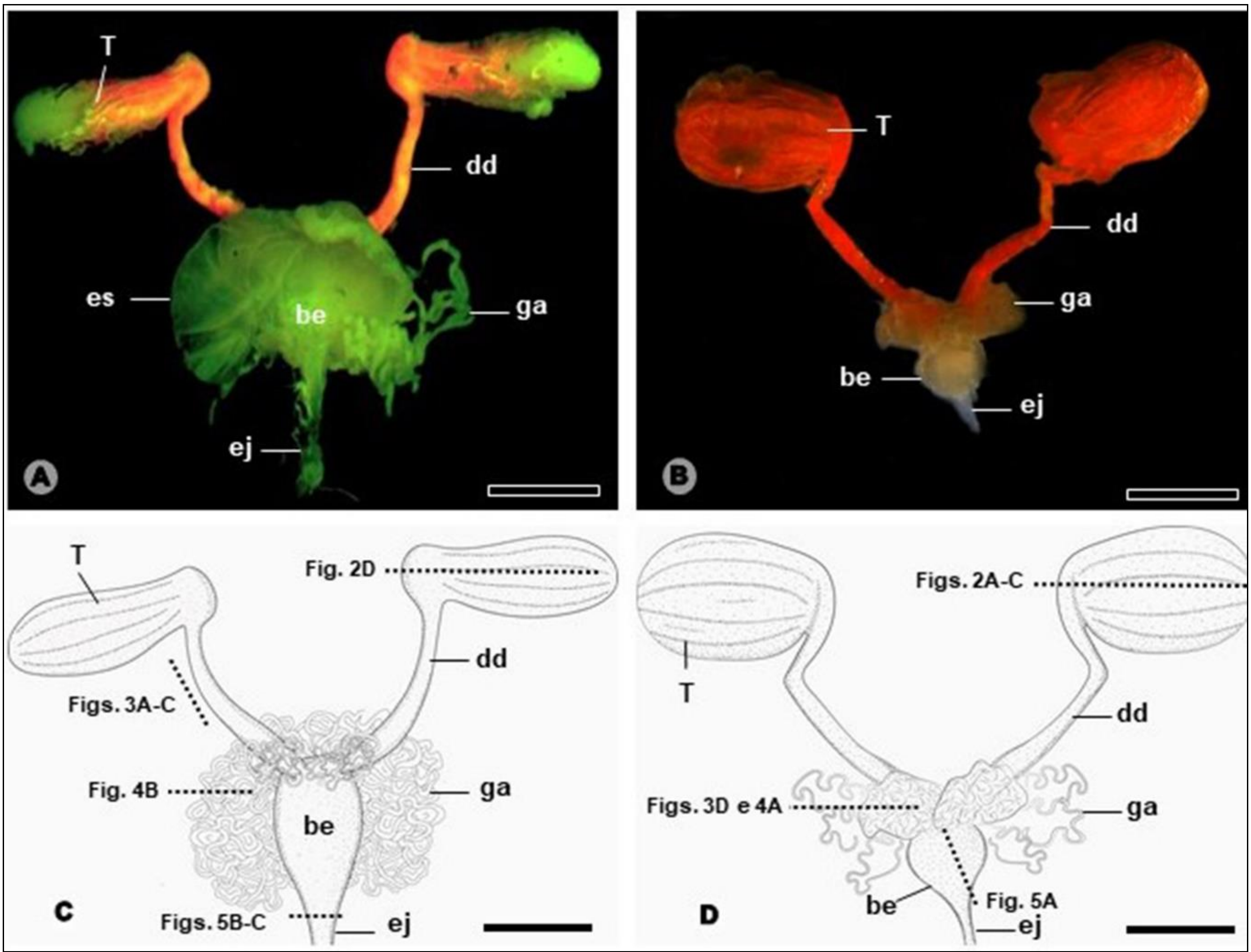

Fig 1: Photography and schematic drawing of the anatomy of the reproductive tract of *Thyanta perditor* (A-C) and *Oebalus ypsilongriseus* (B-D), showing the testis (T), deferent ducts (dd), accessory gland (ga), ejaculatory bulb (be), ectodermal sac (es) and ejaculatory duct (ej). (B) Corresponding transverse sections. Bars: 0.15 cm.

In both species, the testicular follicles had cells in different stages of spermatogenesis (Figs. 2A-D), which can be classified into three development zones: (a) in the growth zone, the spermatogonias separate from the germ cell and are surrounded by the cystic cell. Spermatogonia undergo mitosis and differentiate into spermatocytes; (b) maturation zone where there are two meiotic divisions and differentiation in spermatids and (c) in the differentiation zone, spermatids undergo elongation and condensation of chromatin and differentiate into spermatozoa. The sperm was grouped into cysts, forming bundles, which leave the testicles and was broken as soon as they reach the deferent ducts (Figs. 2B-D).

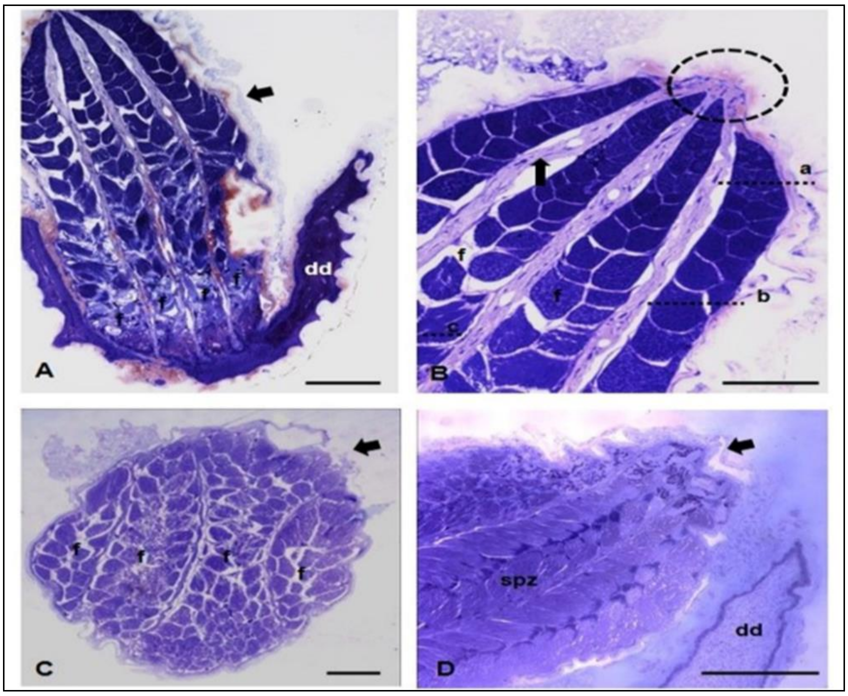

Fig 2: Histology of the male reproductive tract of *Thyanta perditor* and *Oebalus ypsilongriseus*. A-C Longitudinal section of the testes of *O. ypsilongriseus* showing the four testicular follicles (f) and connection with the deferent ducts (dd). The arrow indicates the pigmented peritoneal sheath that covers the testes and deferent ducts. The path circle indicates the junction region of the efferent ducts forming the deferent duct. Note the zone of growth (a), maturation (b) and differentiation (C). D- Longitudinal section of the *T. perditor* testes showing the four testicular follicles (f), the conjunctive capsule (arrow) and the different ducts. Bars = 150 µm.

There were not observed morphological changes in the histology of the deferent ducts to differentiate as a seminal vesicle region and spermatozoa were observed throughout the length of the ducts (Figs. 3A-B). The epithelium of the vas deferens was formed by cubic cells with spherical nuclei, central and covered by a muscular layer (Figs. 3A-B).

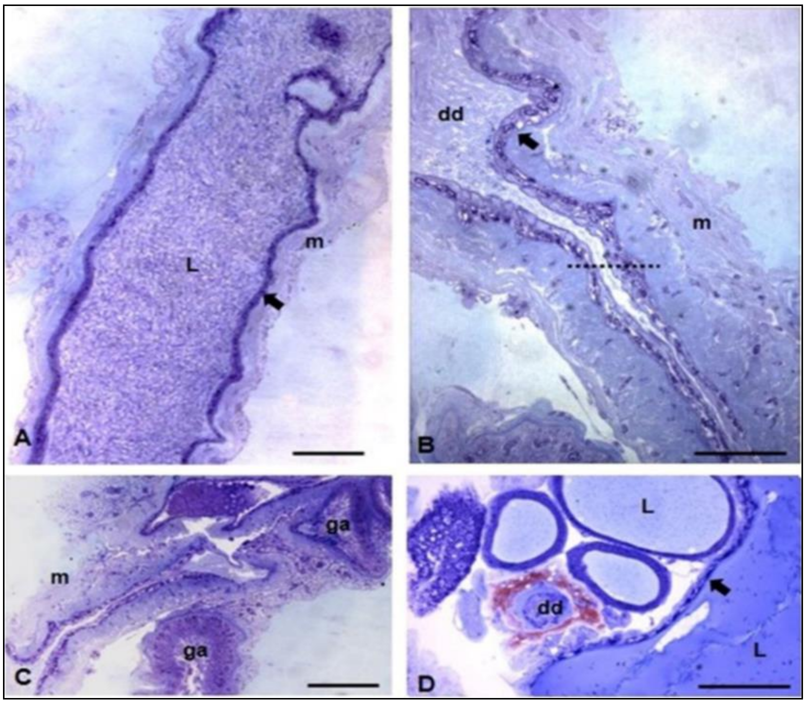

Fig 3: Histology of the male reproductive tract of *Thyanta perditor* and *Oebalus ypsilongriseus*. A-C- Longitudinal section of the *T. perditor* deferent duct showing the lumen (L) filled spermatozoa, the epithelium (arrow) and the muscular layer (m). In (B-C) the narrowing of the duct in the region of arrival in the ejaculatory bulb is observed, where accessory glands (ga) also flow. D- Cross section of the accessory glands (ga) of *O. ypsilongriseus*, showing the lumen full of secretion and the outlet of the deferent ducts (dd). Bars = 120 µm.

The accessory glands in *T. perditor* and *O. ypsilongriseus* were paired, located in the posteroventral region of the abdomen and emptied into the ejaculatory bulb (Figs 4A-B and 5A). They consisted of three different regions, a thin muscular layer, the prismatic glandular epithelium with spherical nuclei of varying heights and the lumen (Fig. 4A-B). Different types of secretion were observed in the lumen, with a homogeneous and granular appearance (Figs. 4A-B).

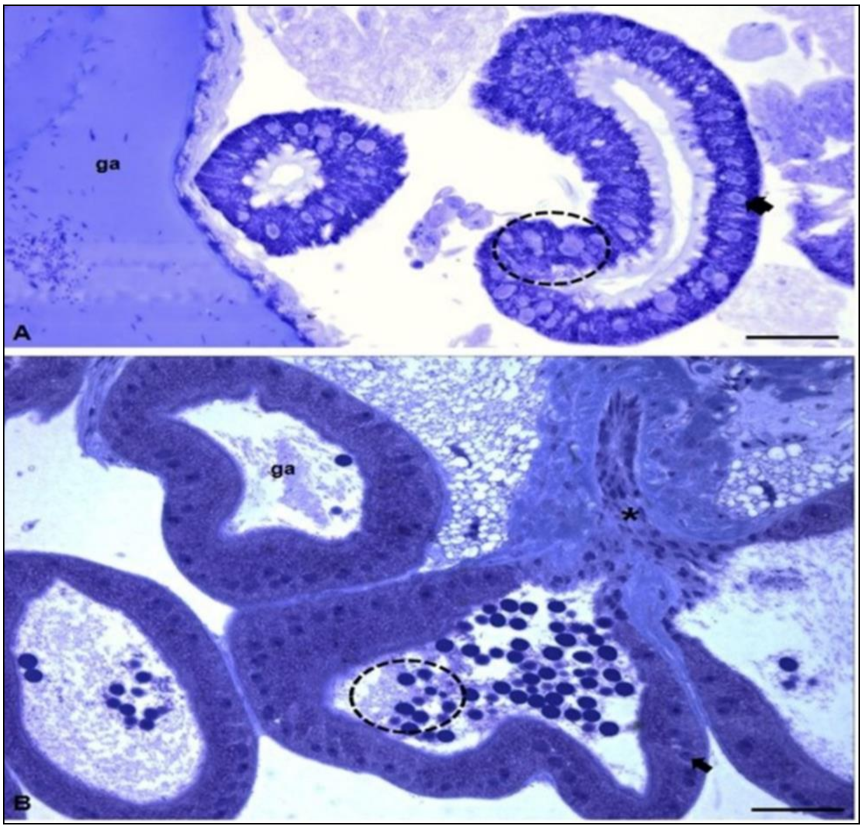

Fig 4: Histology of the male reproductive tract of *Thyanta perditor* and *Oebalus ypsilongriseus*. A- Cross section of the accessory gland (ga) of *O. ypsilongriseus*, the arrow indicates the spherical and median nucleus in the cubic epithelium and the dashed circle indicates the secretions in the cell's cytoplasm. B- Cross section of the accessory gland (ga) of *T. perditor*, the arrow indicates the spherical and median nucleus in the cubic epithelium, the dashed circle indicates the granular secretions in the lumen and the asterisk the junction region for the excretion canal of the gland (dd). Bars = 100 µm.

The final portion of the deferent ducts opened into the ejaculatory bulb, which was long, fusiform and with a complex formation (Figs. 5A-C). The ejaculatory duct was internally covered by an ectodermal cuticle (Figs. 5A-C).

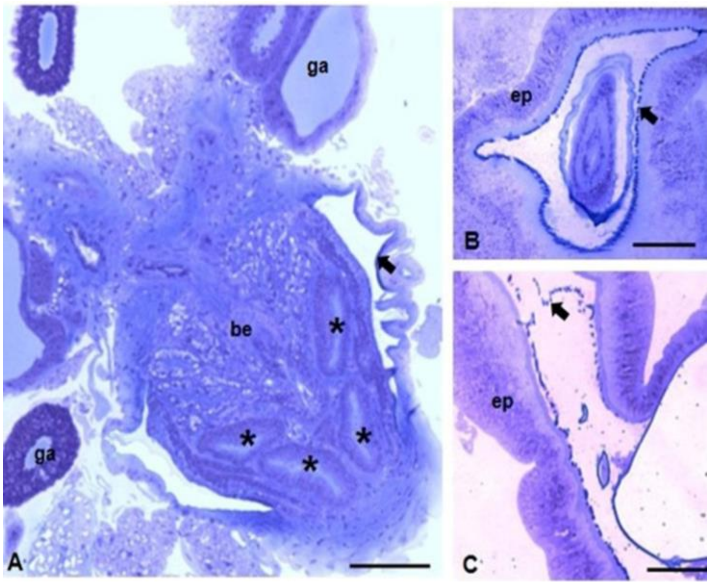

Fig 5: Histology of the male reproductive tract of *Thyanta perditor* and *Oebalus ypsilongriseus*. A- Longitudinal section of the *O. ypsilongriseus* ejaculatory bulb illustrating the active groups of secretory cells, the accessory glands (ga), the mouth opening in the bulb (asterisk) and the portion of ectodermal origin shown by the cuticle (arrow). B-C- Cross section of the *T. perditor* ejaculatory duct. The arrow indicates the ectodermal cuticle that covers the epithelium (ep). Bars = 120 µm.

4. Discussion

The anatomy of the reproductive tract with a pair of testes, deferent ducts long, paired accessory glands that opened into a complex ejaculatory bulb, an ejaculatory duct and the absence of a different region in the seminal vesicle, follows a pattern in the studied Pentatomidae species [26, 29-43], apparently it's a plesiomorphy of the group.

The color of the peritoneal sheath that covers testes and deferent ducts varies between the Heteroptera taxa. For Pentatomidae, this sheath is usually red [38, 41, 45, 46] as observed in *O. ypsilongriseus*. In *T. perditor* an orange pigmentation pattern was observed, as well as in pentatomid *Oebalus poecilus* [32] and common in Coreidae species [32, 38, 47]. For Pentatomidae was also observed a sheath with an internal yellow layer and an external red layer [48]. These studies demonstrated that there is no pattern of relationship between the color of the peritoneal sheath and taxa of Hemiptera.

The elongated shape of the testes observed in *T. perditor* is characteristic in many species of Pentatomidae [45,48-51], ranging from elongated-ovoid [26, 38, 41], ovoid in *O. ypsilongriseus* and other species [39, 41] and cylindrical [40].

Among the Heteroptera there is a large variation in the number of testicular follicles, including intraspecific variation [29, 33, 34, 52, 53], not being a character identified as a potential for systematic analysis of Hemiptera. In Pentatomidae, the number of testicular follicles varies between four and seven for most of the studied species. In *O. ypsilongriseus* and *T. perditor*, four follicles were observed in each testis; ranging from four to six in *Dolycoris baccarum* [26], five in *Carbula scutellata* and *Graphosoma lineatum* [38, 39], six in *Halys dentatus* [42] and seven in *Apodiphus amydglai* and *Eurydema ventrali* [40, 41].

The spermatogenesis process, including the release of sperm in bundles of the testes, is similar among Pentatomidae [52, 53, 54, 55]. Testes with cells at different stages of spermatogenesis indicate adult sperm production and the potential for multiple copulations [56, 57].

The deferent ducts in insects commonly have a differentiated and dilated region, called the seminal vesicle, specialized in the storage of sperm [26, 39-41, 58]. However, in the species studied here, no morphological differences were observed that differentiate a region of the seminal vesicle and the different ducts assume the function of storing sperm, as also seen in other Hemiptera [33, 58, 59]. The presence of a muscular layer along the ducts of the reproductive tract of insects is commonly found and is believed to be the main mechanism used for regulation and control of ejaculation [57]. The epithelium found in the spermatic ducts along the tract is similar to that described for other insects [23, 24, 25, 57, 60, 61, 62].

The secretions of the accessory glands make up the fluid in the semen and its functions are believed to act on sperm transfer, insemination and alter the female's reproductive behavior [50, 63, 64, 65]. In *O. ypsilongriseus* and in *T. perditor*, the accessory glands showed different secretions that can also keep viable sperm in the female's tract for a longer time, increasing reproductive success. The accessory glands discharge into the ejaculatory bulb, a complex structure commonly found in Pentatomidae species [31, 36, 37, 40, 42, 43]. The single ejaculatory duct, medium and with a cuticle, shows its ectodermal origin, as in most insects [57, 62].

5. Conclusion

Anatomical differences were observed between the two species studied, associated with the shape of the testes and the color of the peritoneal sheath. The number of testicular follicles and histological characteristics were similar between *O. ypsilongriseus* and in *T. perditor*. In this work, we describe the reproductive tract morphology in two species of polyphagous agricultural pests, generating characters that can expand knowledge about the reproductive biology and evolutionary history of Hemiptera.

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