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CELLULAR AND MOLECULAR BIOLOGY

Morphology of the Female *Receptaculum Seminis* of *Euschistus heros* (FABRICIUS, 1798) (INSECTA: HEMIPTERA: PENTATOMIDAE)

EDMILSON A. SOUZA, RAFAELA M.M. DO VAL, LUCIANE C.O. LISBOA, FLÁVIO L. FERNANDES & JOSÉ E. SERRÃO

Abstract: *Euschistus heros* (Fabricius, 1798) (Heteroptera: Pentatomidae) is a soybean pest in Brazil. It has a reproductive success that may be associated with the female *receptaculum seminis* or spermatheca, a reproductive organ for spermatozoa storage until the oocyte fertilization. This study describes the anatomy and histology of the female *receptaculum seminis* in *E. heros*. The female *receptaculum seminis* was dissected and analyzed with a stereomicroscope, following standard procedures for histological and histochemical analyses. The female *receptaculum seminis* of *E. heros* has a spherical *caspula seminalis* and a duct. The epithelial cells of the *capsula seminalis* have two cell layers with basal one presenting columnar cells with a collecting canaliculus opening in the lumen. The apical layer has cuboidal cells. The cytoplasm in both cell layers was rich in protein. These features suggest a secretory function of these cells. The duct has four regions characterized by muscular pars *intermedialis* with anterior and posterior cuticular flanges, an elongated and narrow distal *ductus receptaculi*, a well-developed enlarged vesicular area, and a proximal *ductus receptaculi*, which indicate several functions, such as control the release and transport of spermatozoa and secretory. These results contribute to the comprehension of the reproductive biology of this agricultural pest.

Key words: Spermatheca, brown stink bug, secretion, spermatozoa.

INTRODUCTION

Heteroptera is a monophyletic group of insects with *ca*. 40000 species that are majority phytophagous but there are also hematophagous or predators (Schuh & Slater 1995, Weirauch & Schuh 2011).

Among Heteroptera, the Neotropical brown stink bug, *Euschistus heros* (Fabricius,1798) (Pentatomidae), is an important soybean pest in Brazil, but can also attack other plants (Panizzi & Slansky Jr 1985, Panizzi et al. 2012). This pest produces a lot of offspring synchronously with the plant reproductive stage, which difficult its population control (Panizzi et al. 2012). A possible factor that contributes to its reproductive success is the presence of a female *receptaculum seminis* or spermatheca, an organ storing sperm after copula until the fertilization of the eggs (Chapman 2013, Pascini & Martins 2017, Barros et al. 2021).

The female *receptaculum seminis* of insects has a *capsula seminalis* or spermathecal reservoir, a *pars intermedialis* or muscular duct, and, in some species, accessory glands (Martins & Serrão 2002, Cardoso et al. 2008, Farder-Gomes et al. 2019a, b, Barros et al. 2021). After mating, the spermatozoa migrate from the proximal part of the female reproductive tract to the *capsula seminalis* (Tombes & Roppel 1972, Bailey &

Nuhardiyati 2005, Oppelt & Heinze 2007, Pascini & Martins 2017), where they are nourished and protected against substances present on female hemolymph and mechanical damages (Collins et al. 2004, Al-Lawati et al. 2009, King et al. 2011, Pascini & Martins 2017).

The morphology of the *capsula seminalis* varies according to species, including spherical, tubular, and kidney-shaped (Souza et al. 2008, 2016, Candan et al. 2012, 2014, 2015, Viscuso et al. 2015, Pascini & Martins 2017, Monteiro et al. 2019). It usually has a monolayer of cells lined by a cuticular intima in the luminal surface (Martins & Serrão 2002, Martins et al. 2005, Souza et al. 2008, Pascini & Martins 2017, Monteiro et al. 2019).

The *capsula seminalis* is connected to muscular *pars intermedia* and is responsible for the transport and control of the spermatozoa release to the common oviduct or vagina during ovulation for egg fertilization (Chapman 2013, Pascini & Martins 2017). The female *receptaculum seminis* duct has morphological variations among species, which can be short, long, narrow, or dilated (Martins et al. 2005, Souza et al. 2008, 2016, Stacconi & Romani 2011, Pascini & Martins 2017).

The accessory gland occurs in some species and has been claimed to be responsible for the production of substances that contribute to the maintenance of spermatozoa viability (Martins & Serrão 2002, Cardoso et al. 2008, Farder-Gomes et al. 2019a, b). However, in insects without these glands, the *caspsula seminalis* and duct cells seem to have a secretory function (Schoeters & Billen 2000, Souza et al. 2016, 2019, Pascini & Martins 2017).

Despite the number of studies on the anatomy of the female *receptaculum seminis* in Pentatomidae (Hemiptera), the histology of this organ is poorly understood (Candan et al. 2014, 2015, Stacconi & Romani 2011). Therefore, this study describes the anatomy, histology, and histochemistry of *E. heros* female *receptaculum seminis*, contributing new data to the reproductive biology of this crop pest.

MATERIALS AND METHODS

Insects

Ten *E. heros* females were collected in soybean crops in Rio Paranaíba, Minas Gerais, Brazil (19.217415° S, 46.224279° W). The specimens were then transferred to the Laboratório de Biologia Celular e Estrutural at Universidade Federal de Viçosa - Campus Rio Paranaíba.

Histology and histochemistry

The insects were cryo-anesthetized at -4 ºC for 3 minutes, dissected in 125mM NaCl, and the female *receptaculum seminis* transferred to Zamboni's fixative solution (Stefanini et al. 1967) at 5oC for 12 hours. Then, the female *receptaculum seminis* were dehydrated in a graded ethanol series (70%, 80%, 90%, and 95%) for 10 min each and embedded in historesin (Leica) following the manufacturer's instructions. Tissue slices 2 µm thin were obtained with glass knives in a rotatory microtome and stained with toluidine blue sodium borate buffer. Some sections of the female *receptaculum seminis* were submitted to the histochemical tests mercury-bromophenol for detection of total protein and periodic-acid Schiff (P.A.S) for detection of neutral polysaccharides and glycoconjugates according to Bancroft & Gamble (2008).

RESULTS

The female *receptaculum seminis* of *E. heros* has a spherical *capsula seminalis* opening in duct with different anatomical regions: i) a muscular *pars intermedialis* with anterior and posterior cuticular flanges, ii) an elongated and narrow

distal *ductus receptaculi*, iii) a well-developed enlarged vesicular area, and iv) a proximal *ductus receptaculi* (Fig. 1).

The epithelium of *capsula seminalis* is formed by two layers of cells and a lumen lined by a 10µm-thick cuticular intima (Fig. 2a). In the basal layer, there are columnar cells with a well-developed nucleus rich in decondensed chromatin, the cytoplasm with vesicles of different sizes, and a terminal apparatus including a collecting canaliculus (Fig. 2a-c). The canaliculus of each cell is long and opens individually in the reservoir lumen (Fig. 2b). Contents of the cytoplasm vesicles and the reservoir lumen are positive for total proteins, polysaccharides, and glycoconjugates (Fig. 2b-c). The apical layer of cells in the reservoir epithelium is formed by cuboidal cells with homogeneous and basophilic cytoplasm, and a nucleus with the predominance of decondensed chromatin (Fig. 2a). The cytoplasm of these cells is positive for proteins but negative for polysaccharides and glycoconjugates (Fig. 2b-c).

The *pars intermedialis* opening in the *capsula seminalis* is formed by single-layered columnar epithelium that changes to flattened to the distal portion (Fig. 2d-g).

The cuticular intima lining the lumen is folded resulting in proximal and distal cuticular flanges (Fig. 2d). The cuticular flanges seem to be associated with muscle fibers (Fig. 2d-e). The epithelial cells have the cytoplasm positive for proteins (Fig. 2f), whereas polysaccharides and glycoconjugates are mainly in the basal cell region (Fig. 2g).

The distal *ductus receptaculi* is elongated and narrow, with a single layer of cuboidal cells lined by a cuticular intima, but without muscles (Fig. 3a).

The vesicular area is swelled lined by a simple columnar epithelium with a cuticular intima (Fig. 3b). These columnar cells have

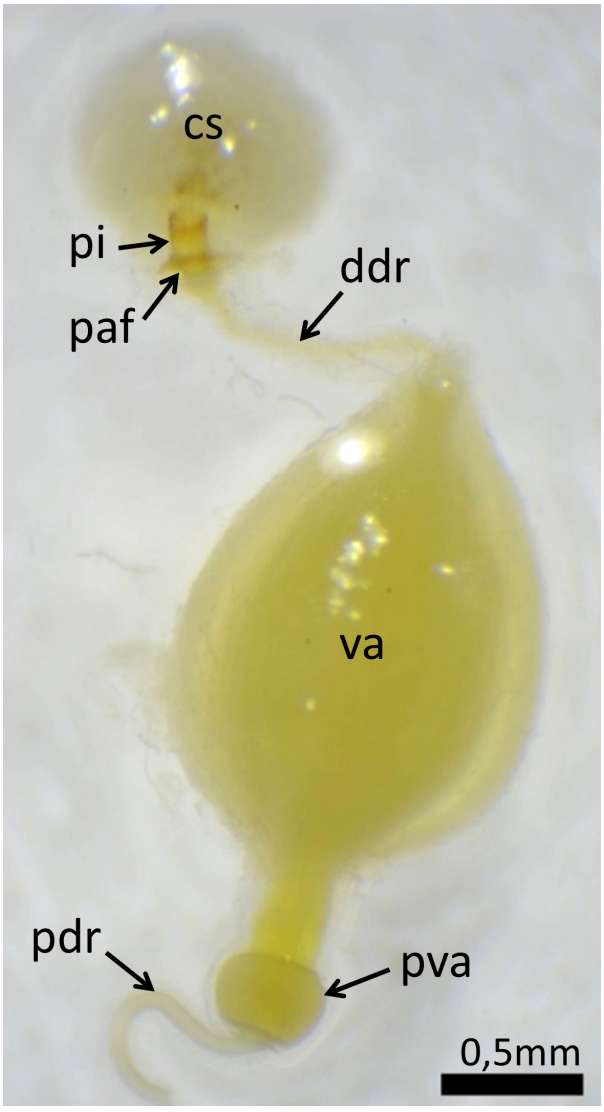

Figure 1. ***Euschistus heros*** **female** ***receptaculum seminis*** **anatomy:** ***Capsula seminalis*** **(cs),** ***pars intermedialis*** **(pi), posterior annular flange (pag), distal** ***ductus receptaculi*** **(ddr), vesicular area (va), proximal vesicular area (pva), proximal** ***ductus receptaculi*** **(pdr).**

the cytoplasm positive for proteins (Fig. 3b), and polysaccharides and glycoconjugates concentrated in the basal region (Fig. 3c).

The proximal vesicular area is a small spherical dilation with a single layer of columnar cells with a well-developed nucleus rich in decondensed chromatin, and a cuticular intima lining the lumen (Fig. 3d). These cells are positive for total proteins (Fig. 3e). This portion

ends in a proximal *ductus receptaculi*, a short tubular region with cuboidal cells positive for proteins (Fig. 3f), without polysaccharides and glycoconjugates deposits. This duct opens in the common oviduct.

DISCUSSION

The female *receptaculum seminis* of *E. heros* with a spherical *capsula seminalis* is similar to those reported in other Heteroptera representatives of Dinidoridae (Kocorek & Danielczok-Demska

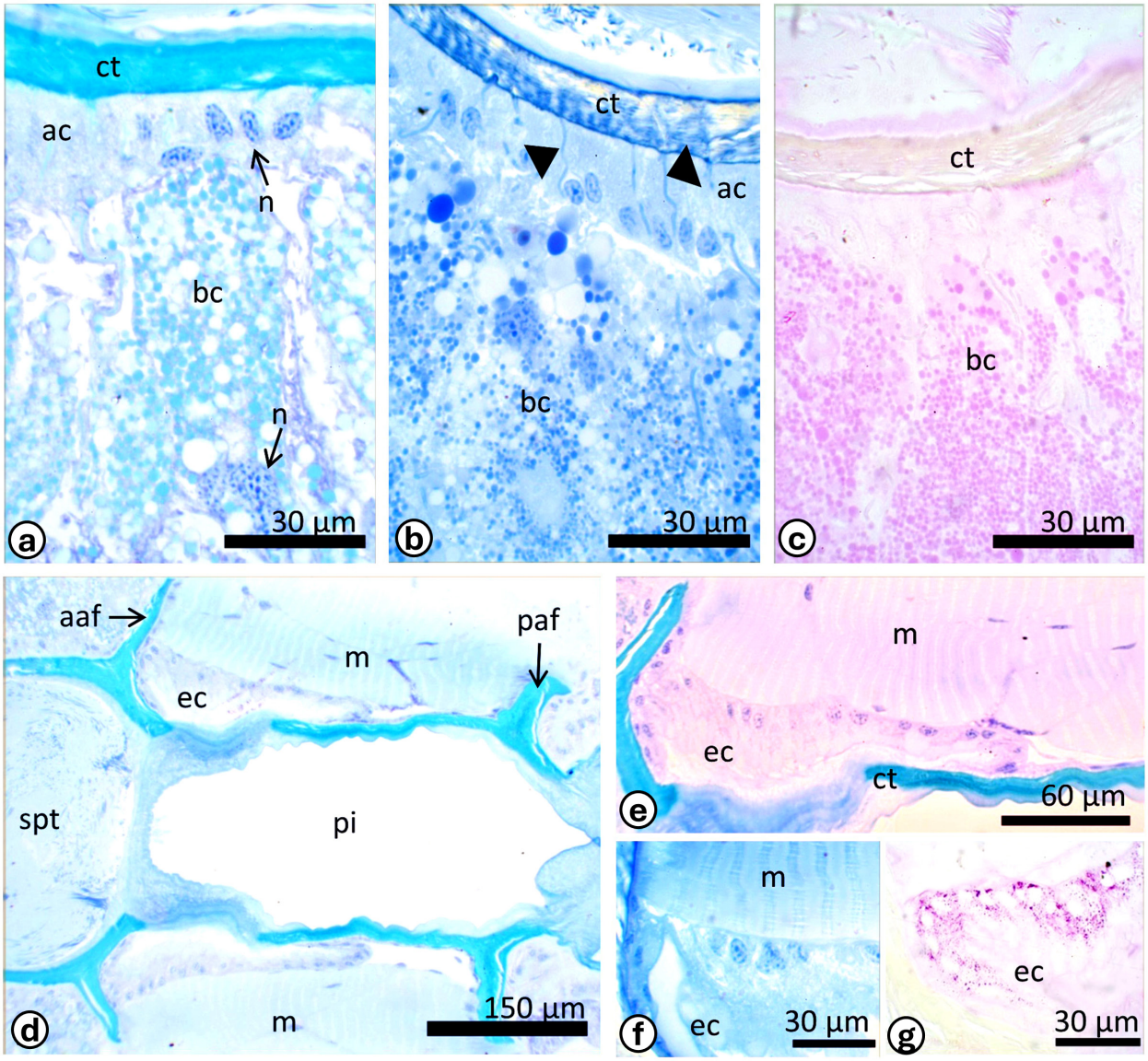

Figure 2. Histology of *Euschistus heros capsula seminalis* and *pars intermedialis*: a: Apical cells (ac) associated with the cuticle and basal cells (bc) of the *capsula seminalis* stained with toluidine blue. b: *Capsula seminalis* with apical cells (ac) and basal cells (bc) positive for proteins. c: Basal cells (bc) positive for glycoproteins and glycoconjugates. d: *Pars intermedialis* (pi) with anterior (aaf) and posterior cuticular flanges (paf). e: *Pars intermedialis* (pi) showing epithelium with columnar and flattened cells. e: *Pars intermedialis* (pi) with columnar epithelial cells (ec) positive for proteins. g: *Pars intermedialis* (pi) with epithelial cells (ec) positive for glycoproteins and glycoconjugates in the basal cell region. Ct: cuticle, ac: apical cells, ec: epithelial cells, n: nucleus, arrowhead: collector canaliculi, spt: spermatozoa, m: muscle, aaf: anterior annular flange, pag: posterior annular flange.

2002), Pentatomidae (Candan et al. 2015), and Coreidae (Souza et al. 2016), but in some families it is oval, semispherical, or kidney-shaped (Stacconi & Romani 2011, Candan et al. 2014, 2015, Pluot-Sigwalt & Moulet 2017), suggesting a possible morphological variation that may be related with the phylogeny of these insects, which needs should be further evaluated since the phylogenetical relationships in the Heteroptera representatives are well understood.

The *capscula seminalis* of *E. heros* has a two-layered epithelium, with basal columnar cells showing a terminal apparatus, including conducting canaliculus, characterizing a class III secretory cells according classification of Noirot & Quennedey (1991). Each conducting canaliculus opens in the lumen of the *capsula seminalis* indicating the release of the cell products in this region. A *capsula seminalis* with two layers of cells, with the basal ones being secretory, has been reported in Heteroptera,

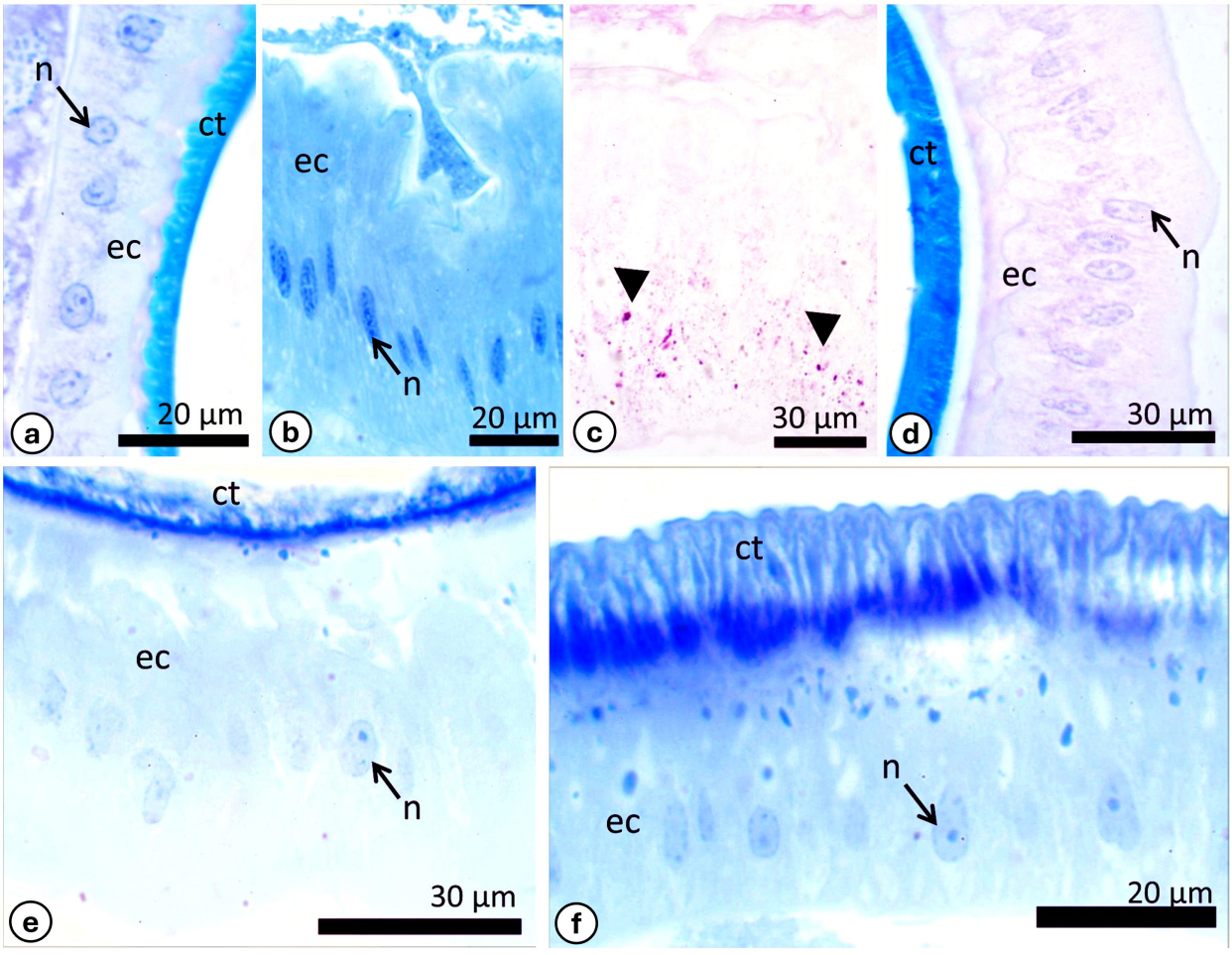

Figure 3. Histology of *Euschistus heros ducts receptaculi* and vesicular area. a: Distal *ductus receptaculi* (ddr) with cuboidal epithelium (ec) lined by cuticle (ct). b: Vesicular area (va) with columnar epithelium (ec) positive for proteins. c: Vesicular area (va) with columnar epithelium with polysaccharides and glycoconjugates (arrowheads) in the basal region. d: Proximal vescicular area (va) with columnar epithelium (ec) and cuticle (ct). e: Columnar epithelium of the proximal vescicular area (va) positive for proteins. f: Proximal *ductus receptaculi* (pdr) composed of a layer of cuboidal cells positive for proteins. Ct: cuticle, ec: epithelial cells, n: nucleus, arrowhead: positive for glycoproteins and glycoconjugates.

including representatives of Lygaeidae (Gschwentner & Tadler 2000), Coreidae (Souza et al. 2016), and Pentatomidae (Rodrigues et al. 2008, Stacconi & Romani 2011).

The histochemical tests here evaluated shown that class III secretory cells of the basal columnar epithelium in the *capsula seminalis* are rich in proteins, carbohydrates, and glycoconjugates, which are probably stored in the reservoir lumen for the maintenance of spermatozoa viability. The proteins and carbohydrates produced by the accessory glands are released to the reservoir lumen and are claimed to be responsible for keeping functional spermatozoa in the female *receptaculum seminis* of Hymenoptera (Cruz-Landim & Serrão 2002, Martins et al. 2005, Cardoso et al. 2008, Gotoh et al. 2009, Souza et al. 2008, Farder-Gomes et al. 2019a) and Diptera (Pascini & Martins 2017, Farder-Gomes et al. 2019b). However, in the female *receptaculum seminis* of *E. heros* there is not an associated spermathecal gland, like in other Heteroptera (Kocorek & Danielczok-Demska 2002, Stacconi & Romani 2011, Candan et al. 2014, Souza et al. 2016). Therefore, it seems plausible to suggest that class III secretory cells in the *capsula seminalis* may produce the components necessary for spermatozoa maintenance in the absence of an accessory gland.

In addition to basal epithelium with class III columnar cells, the epithelium of *capsula seminalis* of *E. heros* has an apical layer of cuboidal cells covered by a cuticular intima. These cells might be responsible for the production of the cuticle, without a significative role in the production of components for spermatozoa maintenance, such as reported in *Lygaeus simulans* (Gschwentner & Tadler 2000).

The female *receptaculum seminis* duct of *E. heros* has four anatomical portions. That opening in the *capsula seminalis* has a *pars intermedialis*, a well-developed muscle layer such as reported in other insects as responsible for the control of the spermatozoa for egg fertilization (Gschwentner & Tadler 2000, Candan et al. 2012, Souza et al. 2016). In Heteroptera, this region commonly has cuticular flanges at both ends (Kocorek & Danielczok-Demska 2002, Stacconi & Romani 2011, Candan et al. 2014, Souza et al. 2016), which also occurs in *E. heros*.

The second and elongated portion duct (distal *ductus receptaculi*) of *E. heros* with flattened epithelium and narrow lumen diameter, suggests that this portion has the function transport of spermatozoa. A similar narrow duct portion has been reported in the Heteroptera Dinidoridae *Thalma secunda*, *Byrsodepsus sundanus*, *Colpoproctus pullus*, *Eumenotes obscura* and *Sagriva vittate* (Kocorek & Danielczok-Demska 2002).

In *E.* heros, after the distal ductus receptaculi there is a well-dilated region (vesicular area) such as in other Heteroptera (Kocorek & Danielczok-Demsk 2002, Rodrigues et al. 2008, Stacconi & Romani 2011, Candan et al. 2014, Souza et al. 2016).

The proximal region of the vesicular area of *E. heros* is spherical, opening in the proximal *ductus receptaculi*, an anatomical feature described for the first time in Heteroptera since it is not reported in previous studies with Pentatomidade representatives (Rodrigues et al. 2008, Stacconi & Romani 2011, Candan et al. 2015).

The epithelia in the *pars intermedialis*, vesicular area, and spherical proximal vesicular area portions of *E. hero* duct have cells storing neutral polysaccharides in the basal cell region, which are the main fuel for energy conversion that may be used for active transport of molecules ions from the hemolymph. In addition to polysaccharides, these columnar

cells have proteins and glycoconjugates, which may be released to the female *receptaculum seminnis* duct lumen, such as in *L. zonatus* (Souza et al. 2016). Considering the secretory features of the epithelial cells in the female *receptaculum seminnis* duct, it seems plausible to suggest that they release substances that contribute to the maintenance and movement of spermatozoa for egg fertilization, likely in the bumble bee *Bombus terrestris* (Schoeters & Billen 2000) and the earwing *Doru luteipes* (Souza et al. 2019).

That is the first histological and histochemical description of the female *receptaculum seminis* of *E. heros*. The results indicate that both the *capsula seminalis* and duct have secretory epithelia, probably producing components that contribute to the maintenance viability and transport of the spermatozoa stored in this female organ until egg fertilization. Altogether, these results add new morphological characters of the female reproductive tract of *E. heros*, expanding the knowledge about the reproductive biology of this important agricultural pest.

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EDMILSON A. SOUZA[1]
https://orcid.org/0000-0003-1164-0591

RAFAELA M.M. DO VAL[1]
https://orcid.org/0000-0003-0855-4166

LUCIANE C.O. LISBOA[1]
https://orcid.org/0000-0002-7365-6540

FLÁVIO L. FERNANDES[2]
https://orcid.org/0000-0003-0532-8593

JOSÉ E. SERRÃO[3]
https://orcid.org/0000-0002-0477-4252

[1]Universidade Federal de Viçosa, Instituto de Ciências Biológicas e da Saúde, Rodovia MG 230, Km 8, 38810-000 Rio Paranaíba, MG, Brazil

[2]Universidade Federal de Viçosa, Instituto de Ciências Agrárias, Rodovia MG 230, Km 8, 38810-000 Rio Paranaíba, MG, Brazil

[3]Universidade Federal de Viçosa, Departamento de Biologia Geral, Avenida Peter Henry Rolfs, s/n, 36570-000 Viçosa, MG, Brazil

Correspondence to: **Edmilson Amaral de Souza**
E-mail: edmilson.souza@ufv.br

Author contributions

EDMILSON A. DE SOUZA and RAFAELA M.M. DO VAL: conception, design, data collection, analysis and writing of the article. FLÁVIO L. FERNANDES: data collection and writing of the article. LUCIANE CRISTINA O. LISBOA and JOSÉ EDUARDO SERRÃO: analysis and writing of the article.