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RESEARCH ARTICLE

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Morphology of the male and female reproductive tracts of virgin and mated *Chrysoperla externa* (Hagen, 1861) (Neuroptera: Chrysopidae)

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Abstract

Predatory insects have reproductive organs rich in complex changes that may be responsible for the success of their population growth. The species *Chrysoperla externa* is a predator used in biological control programs in Latin America. However, there is no morphological data about the morphology of the reproductive tract in this insect. This study describes the morphology of the reproductive organs of virgin and mated *C. externa* male and female. The male has a pair of testes yellow in color and five pairs of accessory glands closely associated with the seminal vesicles. The testis follicles are twisted filled with cysts in different developmental stages. The pair of ovaries in the females shows asymmetry with 9–11 ovarioles per ovary with oocytes in different developmental stages and a spherical spermatheca. Virgin and mated males have no differences in the size of the testes, seminal vesicle, and accessory glands. *C. externa* females show morphological changes in the reproductive tract according to sexual maturation, which is triggered by mating. The ovary activation occurs after female mating. The ovaries are of merotistic polytrophic type. The spermathecal reservoir is lined by a flattened epithelium with a thin cuticular intima and associated with well-developed muscles. It is concluded that the reproductive tract of *C. externa* is similar in virgin and mated males and females. Egg production is activated only after mating and the development of reproductive tract structures is faster in mated females.

KEYWORDS
green lacewing, morphology, ovary, testis, ultrastructure

1 | INTRODUCTION

The genus *Chrysoperla* Steinmann, 1964 is formed by green insects known as chrysopids and which belong to the family Chrysopidae. Their larvae are predatory of small caterpillars, aphids, mealybugs, mites and white flies. Some species of this genus have been used in biological pest control programs for crops of economic importance in various parts of the world (Albuquerque, Tauber, & Tauber 2001; Pappas, Broufas, & Koveos 2011; Ismoilov et al., 2020). For this reason, research aiming to search for information about processes related to the reproduction of chrysopids can contribute to a better behavioral and biological understanding of the species and consequently allow improvements in their breeding and use in biological pest control programs.

The reproductive organs of chrysopids are responsible for giving rise to new generations and their structures may vary according to the species. In the embryonic phase they are similar in males and females, differing, however, during post-embryonic development. The female reproductive tract of insects has a pair of ovaries, two lateral oviducts connected to a single common oviduct opening in the vagina, which may be associated with a storage spermatozoa organ, the spermathecae (Snodgrass, 1935).

The ovaries have a set follicle, the ovarioles, which change in number according to insect species and are classified in panoistic (without nurse cells associated with oocyte) or meroistic (with nurse cells) (Büning, 1994). In Chrysopidae, the ovaries have from eight to 24 ovarioles per ovary (Bitsch, 1984; Garbiec & Kubrakiewicz, 2012; Kubrakiewicz, Jedrzejowska, & Biliński 1997; Principi, 1949). Likely in other insects, the ovaries and lateral oviducts have mesodermic origin, whereas the common oviduct and the vagina are ectodermical with a thin cuticular intima covering the epithelium (Hwang & Bickley, 1961; Serrão, Naves, & Zanuncio, 2011). The spermathecae have a spherical reservoir with a sclerotized cuticle opening in a folded spermathecal duct (Barnard & Brooks, 1984).

The male reproductive tract of insects has a pair of testis, *vas deferens*, a single ejaculatory duct opening in the aedeagus. In some insects the spermatozoa are temporally stored in seminal vesicles (Snodgrass, 1935). In Chrysopidae the testes are long and twisted lined by a peritoneal sheath (Bitsch, 1984). The testes open in the *vasa deferentia* that are elongated and narrowed, except in the proximal region where they enlarge to form the seminal vesicles, which are associated with some accessory glands, opening in the ejaculatory duct (Hwang & Bickley, 1961; Principi, 1949).

In some insects, mating stimulates behavioral and physiological changes in the females (Ávila, Sirot, LaFlamme, Rubinstein, & Wolfner, 2011). The ovary activation in insects, control of ovarian development is determined by the quantity and quality of food, whereas in others, the nutrients required for development are acquired from male seminal fluids (Poiani, 2006; Wheeler, 1996). Studies on the maturation of the reproductive tract in *Chrysoperla externa* (Hagen, 1861) females and the importance of copulation in oocyte development are rare.

In Brazil, the species *C. externa* has potential to be used in integrated pest management programs due to its voracity and easy mass rearing (Albuquerque, Tauber, & Tauber, 1994; Freitas & Penny, 2001).

To date, the knowledge on *C. externa* is restricted to its life cycle (Albuquerque, Tauber, & Tauber, 1994; Fonseca, Carvalho, Cruz, Souza, & Ecole, 2015), predatory capacity (Gamboa, Souza, & Morales, 2016; Salamanca, Devia, & Amaya, 2010), and laboratory mass rearing (Bezerra, Amaral, & Souza, 2017). Surprisingly, there are poor data about the reproductive tract of Chrysopidae, which was described in the pioneering study by Stitz (1909) for *Chrysoperla carnea* (Stephens, 1835) and *Chrysopa septempunctata* Wesmael, 1841 (Principi, 1949), *Chrysopa oculata* Say, 1839 (Hwang & Bickley, 1961), and *Chrysopa perla* (Linnaeus, 1758) (Philippe, 1972). However, information on the morphology of the reproductive system and reproductive strategies in *C. externa* males and females is scarce.

This study describes the reproductive organs of virgin and mated *C. externa* males and females contributing to fill the gap to understand reproductive biology this natural enemy, which is fundamental for mass rearing strategies to produce high number of insects to be used in biological control programs.

2 | MATERIALS AND METHODS

2.1 | Insects

C. externa adults were obtained from a mass rearing in the Laboratory of Insect Biology of the Department of Entomology of the Federal University of Lavras. These insects were maintained at 25 ± 2°C and 60 ± 10% relative humidity, and 12:12 h (light: dark) photoperiod. The larvae were fed on eggs of *Ephestia kuehniella* Zeller, 1879 (Lepidoptera: Pyralidae) a*d libitum*, and adults were fed on 1:1 yeast and honey (Carvalho & Souza, 2009; Freitas & Penny, 2001).

Newly-emerged adults were sexed and individualized in glass tubes (2.5 cm diameter × 8.0 cm height) to avoid mating, and they are referred to as virgins. Another set of newly-emerged pairs (1♀:1♂) were kept in PVC cages (10 cm high × 10 cm diameter) until mating.

2.2 | Anatomy

The reproductive tracts of virgin insects were dissected daily for 30 days after adult emergence and mated ones daily for 30 days after mating. The following male and female reproductive tract parameters were evaluated: number of ovarioles, number of oocytes, ovary length, testis length, and length of male accessory glands and seminal vesicles. The length of the young and mature oocytes, the number of ovarioles/ovary, oocytes/ovary (right and left), and testis area were obtained with the computer program Leica Application Suite (version 4.0) in the stereomicroscope Leica MZ 10F (Jena, Germany).

2.3 | Histology

Ten males and 10 females were caged together for 10 days after adult emergence. Then, the reproductive tracts of the insects were dissected in saline solution for insects (0.1 M NaCl, 0.2 M KH_2PO_4, 0.2 M Na_2HPO_4) and transferred to Zamboni fixative solution (Stefanini, De Martino, & Zamboni, 1967). Then, samples were dehydrated in a graded acetone series (30, 50, 70, 90, and 100%) and embedded in historesin (Leica, Jena, Germany). Sections (3-μm thick) were stained with hematoxylin and eosin and analyzed under an Olympus BX-60 light microscope 142 (Olympus Corporation, Tokyo, Japan).

2.4 | Statistics

The normality of the morphometric data of the reproductive tracts was verified using Shapiro–Wilk tests. Data that did not meet the normality assumption were submitted to Kruskal–Wallis tests using R software (R Development Core Team, 2011).

3 | RESULTS

3.1 | Male

The development of the reproductive organs of *C. externa* was evaluated with daily dissections, and no anatomical differences were found in both virgin and mated insects from newly-emerged to 30-day-old ones. Thus the following description refers to general aspects in both virgin and mated males.

The male reproductive tract of *C. externa* was in the dorsal region between the sixth and seventh abdominal segments with a pair of testes, *vas deferens*, seminal vesicles, accessory glands, and an ejaculatory duct. The testes were bright yellow with elongated and twisted-like shapes (Figure 1).

Each testis was completely enveloped by a peritoneal sheath (Figure 1a) and the testis follicles lined by flattened epithelium. All testis follicles were filled with cysts containing germ cells in different developmental stages. In the proximal portion, each testis opened into a long and narrowed *vas deferens*, which enlarged in the proximal (basal) region to form the seminal vesicles that open in the ejaculatory duct (Figure 1a,b,f).

The aedeagus exhibited a brown sclerotized feature, the "gonarcus" (Figure 1c,d), which was between the anal segment and the ninth sternite and was below the rectum. The gonarcus was formed by a pair of arc-shape lateral projections folded down (entoprocessus) (Figure 1e).

There were no morphometric differences between the reproductive organs of the virgin and mated males. The mean length of the

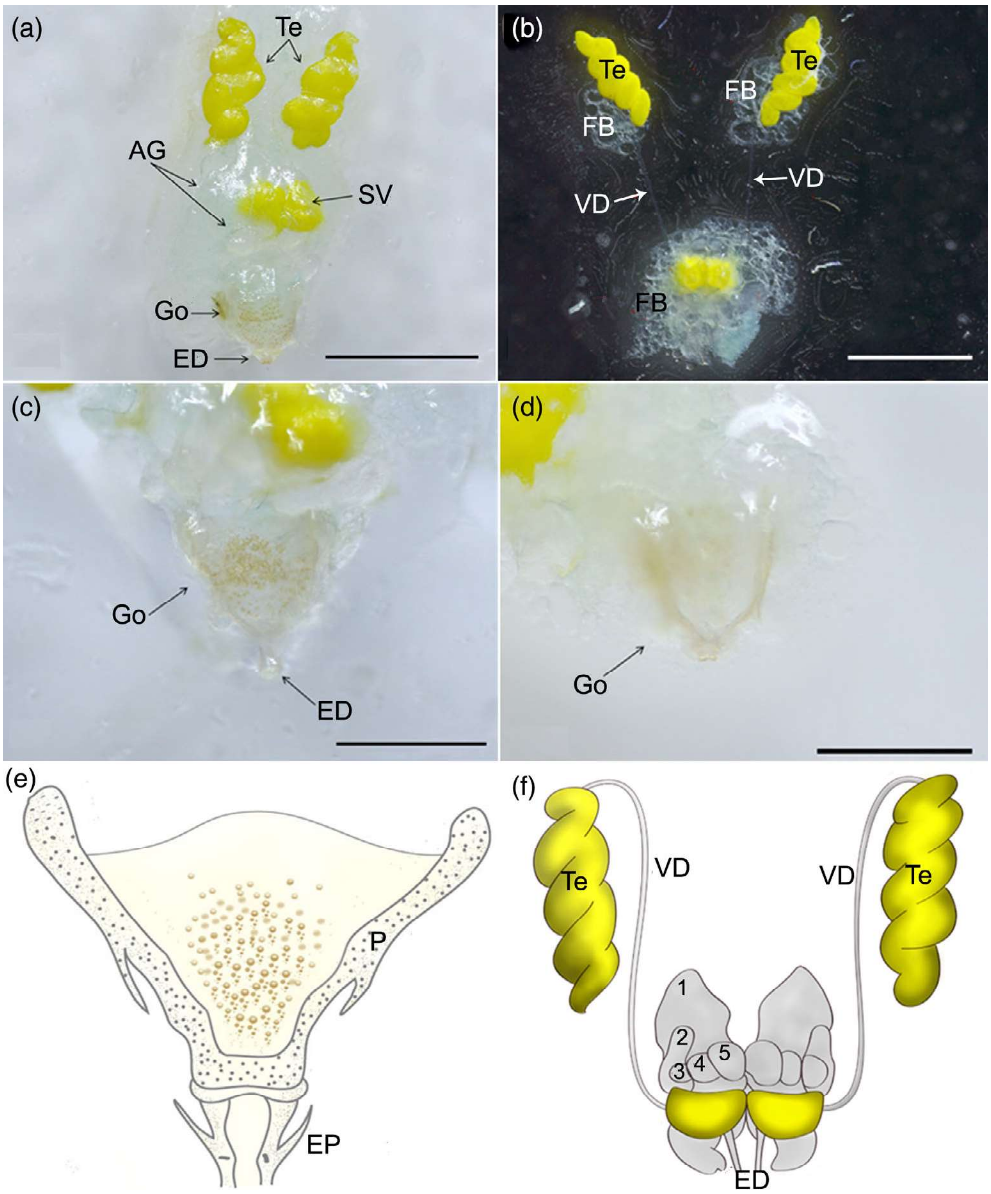

FIGURE 1 Male reproductive tract of *Chrysoperla externa*. (a) General aspect showing testes (Te), seminal vesicle (SV), accessory glands (AG), ejaculatory duct (ED) and gonarcus (Go). (b) Detail showing the *vas deferens* (VD) and fat body (FB) associated with organs. (c) Ventral view of the gonarcus (Go). (d) Dorsal view of the gonarcus (Go). (e) Schematic drawing of side plates (P) and the entoprocessus (EP). (f) Schematic drawing of the reproductive tract showing testes (Te), *vas deferens* (VD), ejaculatory duct (ED) and the five pairs of accessory glands (1–5). Scale bars = 1 mm (a and b); 0.5 mm (c and d). (e) and (f): not drawn with scale [Color figure can be viewed at wileyonlinelibrary.com]

right testis was 0.775 mm (χ^2 = 1.55, p = 0.21), left testis was 0.806 mm (χ^2 = 2.08, p = 0.14), the total size was 0.161 (χ^2 = 1.55, p = 0.21) and 0.167 mm^2 (χ^2 = 0.077, p = 0.78) for virgin and mated insects, respectively. The accessory glands were 0.276 and 0.336 mm^2 (χ^2 = 2.08, p = 0.14), seminal vesicles 0.098 and 0.218 mm^2 (χ^2 = 2.77, p = 0.09) in virgin and mated males, respectively.

The testes had spermatogenesis in different developmental stages with spermatozoa (Figure 2a) and spermatids (Figure 2b,c). The *vasa deferentia* had a single layer of narrow epithelium with some muscles. The seminal vesicles were ovoid with a simple cubic epithelium associated with muscle and the lumen filled with spermatozoa and secretion (Figure 2d).

There were found five pairs of accessory glands with different sizes and shapes, including spherical, ovoid, and lobed (Figure 1f). The accessory glands were lined by a single layer of epithelial cells that ranged from cubic to columnar, and the lumen was filled with acidophilic content (Figure 2d).

3.2 | Female

The female reproductive tract of *C. externa* was located between the abdominal segments 4–7 and had a pair of ovaries connected by a pair of lateral oviducts that opened in a common oviduct associated with a spermatheca and a collateral gland (Figure 3a–c). The ovaries of *C. externa* were asymmetrical, presenting a varied number of ovarioles with oocytes in different developmental stages (Figure 3a,b). There were between 9 and 11 ovarioles per ovary, totaling an average of 20 ovarioles, and the same number of ovarioles was rarely found in the right and left ovaries.

Differently from found in males, in females, there were morphological differences in the reproductive tract between virgin and mated females.

In virgin females 24 h after adult emergence, the reproductive tract was not activated with small and narrowed ovaries. In five-day-old females, ovary activation began with developing oocytes. Eleven-day-old virgin females had few developing oocytes. The complete

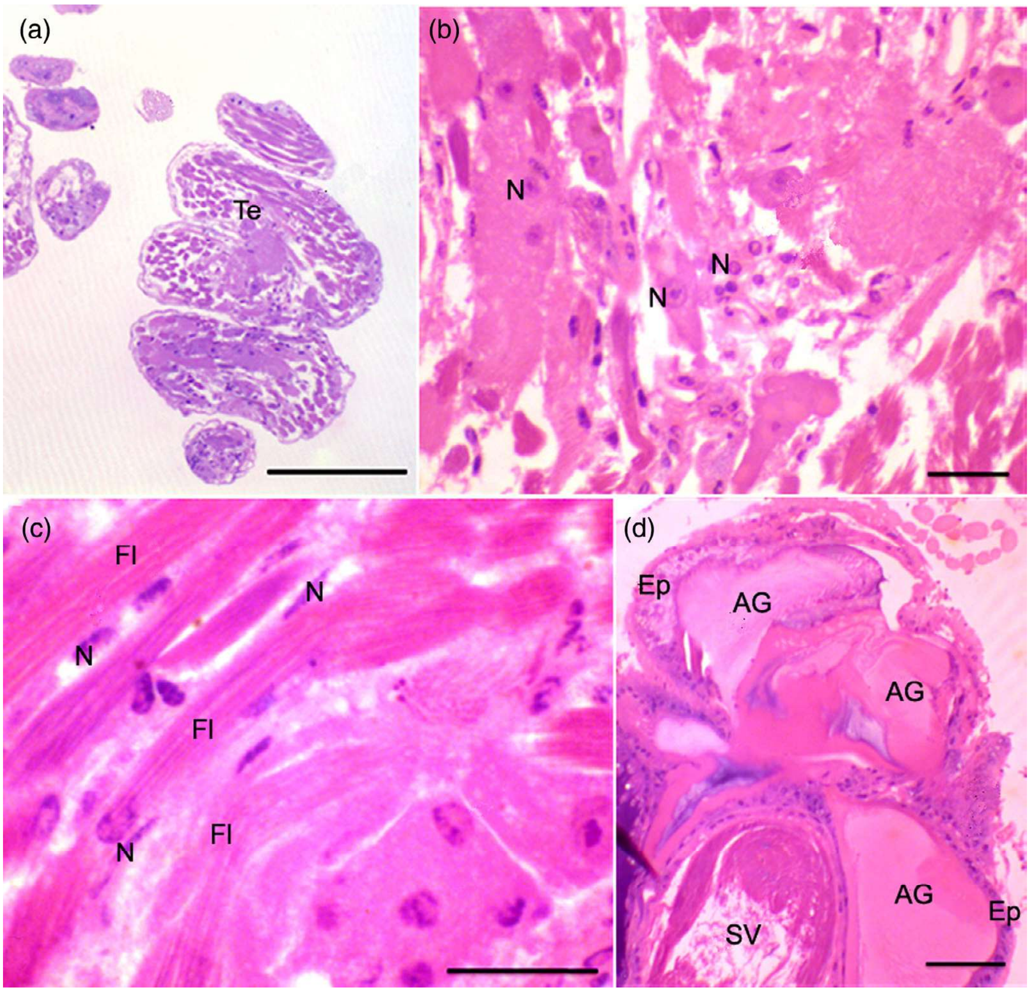

FIGURE 2 Light micrographs of the male reproductive trac of *Chrysoperla externa*. (a) Longitudinal section showing twisted testis (Te) with different spermatogenesis stages. (b) Detail of young spermatids characterized by spherical nucleus (N). (c) Spermatids in advanced stage of differentiation with elongated nucleus (N) and flagella (Fl). (d) Seminal vesicle (SV) filled with spermatozoa closely associated with accessory glands (AG). Ep = Epithelium. Scale bars = 30 μm [Color figure can be viewed at wileyonlinelibrary.com]

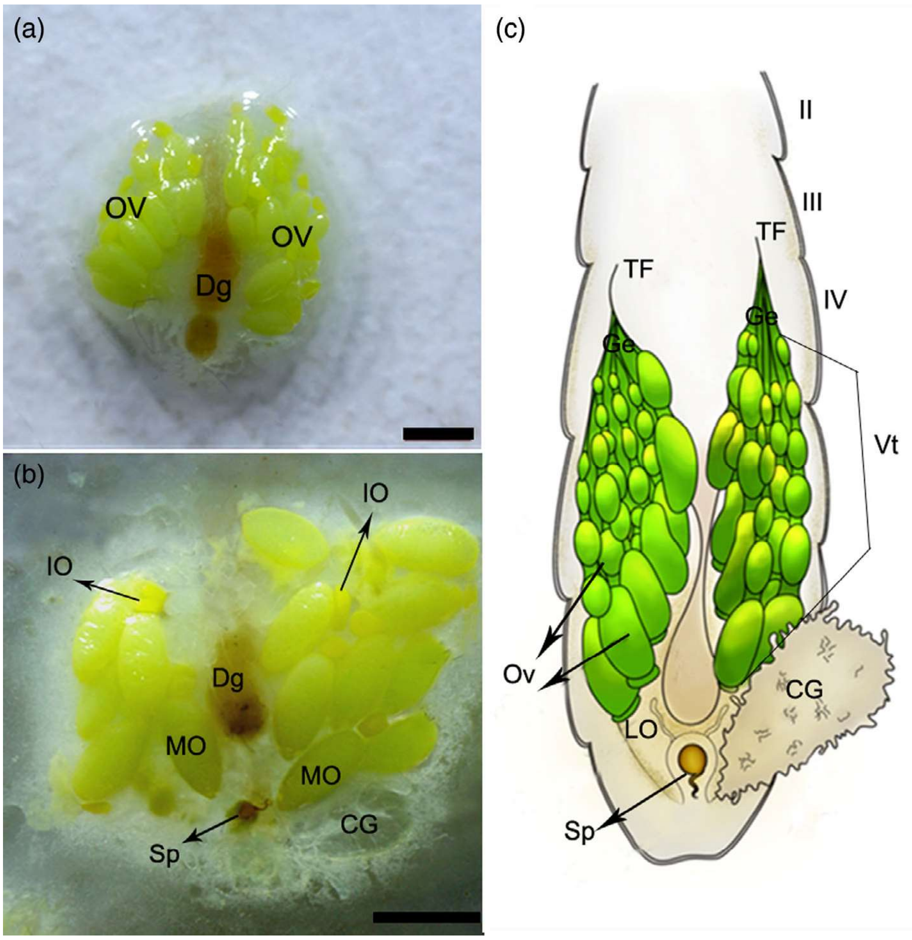

FIGURE 3 The female reproductive tract of *Chrysoperla externa*. (a) General aspect of the ovaries (OV). (b) Ovaries showing ovarioles with immature (IO) and mature oocytes (MO), spermatheca (Sp) and collateral gland (CG). (c) Schematically drawing of the ovaries with ovarioles (Ov) showing the terminal filament (TF), germarium (Ge), vitellarium (Vt), lateral oviduct (LO), spermatheca (Sp) and the collateral gland (CG). Not drawing with scale. Dg = digestive tract, II–IV = abdominal segments. Scale bars = 1 mm [Color figure can be viewed at wileyonlinelibrary.com]

activation occurs in the 15th day after adult emergence with many oocytes, including mature ones in the basal region of the ovarioles closely to the lateral oviducts.

In virgin females, the spermathecal reservoir had a sclerotized structure with dark color and uniform diameter.

Whereas virgin females maintained ovaries with an immature appearance, mated ones showed rapid development and maturation of their ovaries.

The number of more basal oocytes in the ovaries of mated females was 30.46 ± 0.70 with 0.63 ± 0.02 mm in length, whereas in virgin ones was 17.33 ± 0.70 with 0.25 ± 0.09 mm in length.

The ovaries were of meroistic polytrophic types, and each ovariole was enveloped by a peritoneal sheath (Figure 4a). Oocytes were surrounded by a single layer of follicular cells with well-developed nuclei (Figure 4a). In the apical part of the ovariole, the germarium had prefollicular cells and germ cells in different differentiation stages (Figure 4a).

The young oocytes, near the germanium region, were in the early developmental stage lined by cubic follicular cells (Figure 4a). During oocyte maturation, the follicular cells became cubic with acidophilic cytoplasm (Figure 4b). Mature oocytes were characterized by the chorion and storage of yolk granules (Figure 4c).

The spermathecal has a well-developed reservoir and a duct (Figure 4d). The wall of the spermathecal reservoir had a narrow epithelium lined by a cuticle (Figure 4e), and there was an external muscular layer at the junction of the reservoir with the spermathecal duct to form a spermathecal pump.

4 | DISCUSSION

The twisted and elongated shape *C. externa* testis is similar to those described the Chrysopidae *C. septempunctata*, *C. perla* (Principi, 1949), and *Anisochrysa ventralis* (Curtis, 1834) (Philippe, 1972). The testes are lined with a follicular epithelium and have several internal extensions that are divided into compartments in which germ cell maturation occurs (Canard, Séméria, & New, 1984). Although there are a few number of species evaluated, those findings suggest that male reproductive tract has a possible bauplan within Neuroptera.

The gonarcus occurs in all Neuroptera (Tjeder, 1936, 1954, 1956) however, during development, there are morphological variations in some species, such as in *C. perla*, *Chrysopa ciliata* Wesmael, 1841, *Chrysopa albolineata* Killingston, 1935, *C. carnea*, *Chrysopa ventralis* Curtis, 1834, *Chrysopa phyllochroma* Wesmael, 1841, *C. septempunctata*, and *C. oculata* (Killington, 1936, 1937; Principi, 1949). The gonarcus is the main feature of male genitalia for taxonomy (Aspöck & Aspöck, 2008). The male genitalia of *C. externa* is composed by the complex gonarcus-mediuncus-entoprocessus with enlarged lateral

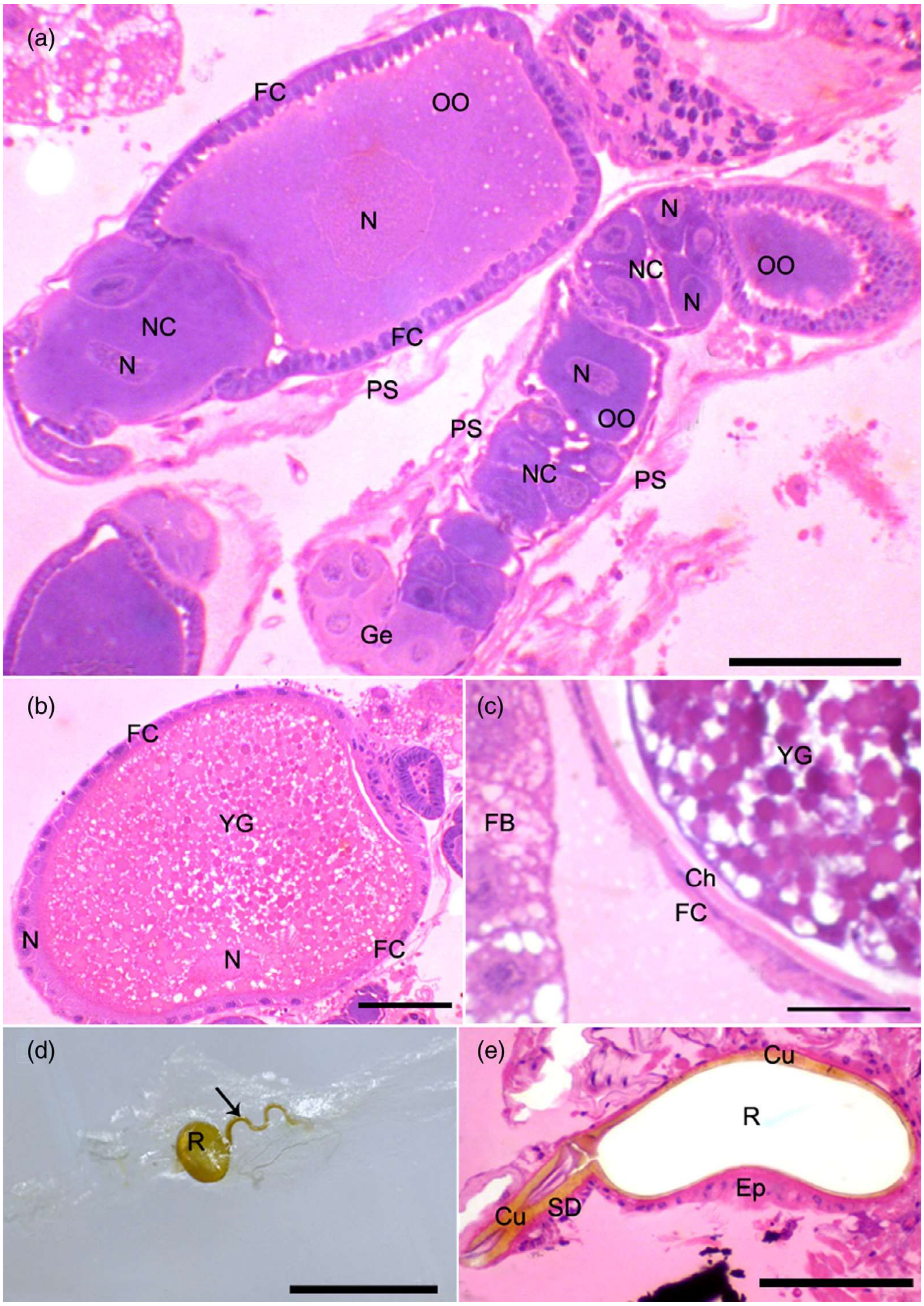

FIGURE 4 Light micrographs of the female reproductive tract of *Chrysoperla externa*. (a) Ovarioles showing the germarium (Ge), nurse cells (NC), oocytes (OO) in different developmental stages, follicular cells (FC), nucleus (N), and peritoneal sheath (PS). (b) Vitellogenic oocyte showing cytoplasm with yolk granules (YG). Note cubic follicular cells (FC). (c) Detail o mature oocyte with yolk granules (YG), chorion (Ch), and flattened follicular cells (FC). (d) The spermatheca showing the reservoir (R) and spermathecal duct (arrow). (e) Detail of the spermatheca showing the reservoir lumen (R) and spermathecal duct (SD) lined by cuticle (Cu) and epithelium (Ep). FB = fat body. Scale bars = 30 μm [Color figure can be viewed at wileyonlinelibrary.com]

plates with narrowed prolongations and lateral arm robust different from those found in *Ceraeochrysa paraguaria* (Navás, 1920) with narrow gonarcus and *Ceraeochrysa claveri* (Navás, 1911) with a small finger-like projection in the front of the gonarcus (Aspöck & Aspöck, 2008). Although the taxonomy of Chrysopidae is discussed according to morphological features including wing venation, head, abdomen, and genitalia (Aspöck & Aspöck, 2008; Brooks & Barnard, 1990; Winterton, Hardy, & Wiegmann, 2010) our findings suggest that the gonarcus may be a useful character for future phylogenetic analyses.

The seminal vesicle of *C. externa* is filled with spermatozoa and some secretion. The seminal vesicles are closely associated with five pairs of accessory glands, which have morphological diversity and may play a role in the production of compounds to support the spermatozoa storage, moving through female reproductive tract, including the spermathecal duct, and stimulate ovarian activity after mating (Chen et al., 1988; Flanders, 1939; Happ, 1992; Liu & Kubli, 2003). *C. externa* males have well-developed accessory glands, which have morphological diversity and may play a role in the production of compounds in the seminal vesicles peptides that stimulate ovarian activity after mating. Male accessory gland secretions have substances with major compounds that are glycoproteins, which may change female and male reproductive behavior after mating as well as have antibiotic effects (Chen et al., 1988; Heifetz, Tram, & Wolfner, 2001; Herndon et al., 1997). Thus is it is plausible to suggests that the sperm may have additional functions other than germ cell transference to the female.

The morphology of the reproductive tract of *C. externa* females, including the polytrophic meroistic ovary is similar to what has been described for the lacewings *C. carnea* (Garbiec & Kubrakiewicz, 2012), *Ceraeochrysa cubana* (Hagene, 1861) (Vacacela et al., 2017) however, the ovaries of *C. oculata* are classified as panoistic (without the presence of nurse cells) (Hwang & Bickley, 1961). The type of ovary is always of the same in the insect orders and evaluating figure 7 from Hwang and Bickley's paper, it shows an anatomical draw of elongated ovarioles with many developing oocytes, all them with a small chamber associated, which is an evidence that the ovary is of the meroistic polytrophic types a feature that can be evaluated only in histological analyses.

C. externa females are synovigenic because their oocytes grow after adult emergence independently of the mating status, although with some delay in virgin females. A common source of nutrients used by synovigenic females is the male seminal fluid transferred to females during mating (Friedel & Gillott, 1977; Hayashi & Kamimura, 2002). We found that mated females have two-folds the number of oocytes and bigger oocytes than virgin ones. The presence of male during the reproductive period of species belonging Chrysopidae do not stimulate oviposition behavior (Carvalho & Souza, 2009) but the oogenesis activation seems to be triggered by proteins found in the seminal fluid transferred to females (Chen et al., 1988; Coleman, Drähn, Petersen, Stolorov, & Kraus 1995; Engelmann, 1970; Wheeler, 1996; Wolfner, 1997), which may occur in the lacewing here studied.

Unlike other insects that exploit nutrients acquired in the immature stages for reproductive tract development and reproduction (Alcock & Gwynne, 1991; Huffaker & Gutierrez, 1999), *C. externa* females require nutrient acquisition in adult stage to support reproduction. Females using this reproductive strategy may benefit from nutritional and/or regulatory molecules from seminal fluid (Jervis & Ferns 2005; Liu & Kubli, 2003).

Ovary activation in *C. externa* occurs more rapidly after successive mating, and the absence of copula affects the formation of oocytes, delaying activation resulting in few oocytes of small sizes. Therefore, in addition to the role of hormonal stimulation, the transfer of fluid from male seminal vesicles and/or accessory glands (Gillott, 1995, 2003; Wheeler, 1996) may represent an essential source of compounds for the activation of the female reproductive tract in this predator.

The number of ovaries varies according to the lacewings species. In *C. perla* and *C. carnea*, there are 8 to 12 ovarioles (Canard et al., 1984), and in *C. septempunctata* 20 ovarioles (Principi, 1949), which is similar to *C. externa* found in this study. In *C. oculata*, mature oocytes have between 0.125 and 0.145 mm in length (Hwang & Bickley, 1961), whereas, in *C. externa*, they are 0.634 mm. Together, these findings suggest that number of ovarioles and oocyte size change according to the lacewing species.

In *C. externa*, the spermatheca is a dark, sclerotized, spherical structure with a concavity in its ventral surface, which has been termed as a ventral impression (Tjeder, 1956). This organ is responsible for sperm storage until the oocyte fertilization (Snodgrass, 1956).

Despite the contributions made by morphological, behavioral, and phylogenetic studies in the literature for *C. externa*, it is important to include new characteristics, such as those related to the reproductive tract. Identifying differences in morphological patterns in the organs and cells of the reproductive tract of *C. externa* reveal important data of the species and contribute to understanding the reproductive biology.

We conclude that the reproductive tract of *C. externa* is similar in virgin and mated males and females. Egg production is only activated after mating, and the development of reproductive tract structures is faster in mated females. This information is very important for the successful creation and multiplication of the predator *C. externa* for use in biological pest control programs.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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