



## Research paper

## A novel epidermal abdominal gland in the cricket *Ectecous segregatus* Gorochov, 1996 (Orthoptera: Grylloidea: Phalangopsidae)



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## ABSTRACT

*Ectecous segregatus* (Grylloidea: Phalangopsidae) presents a modification in some abdominal tergites hypothesized to be openings for epidermal glands. This feature however has been described in the literature only on the basis of its external morphology. This study describes the morphology of *E. segregatus* abdominal glands associated with cuticular modifications. Histological and ultrastructural data show the presence of abdominal glands in males of *E. segregatus*, represented by an epidermis with columnar cells classified as type I glands. This is the first study that shows the presence of an abdominal gland associated with external cuticular modifications in crickets.

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### 1. Introduction

Insects present great diversity of exocrine glands distributed throughout the body, with secretions having functions in defense, feeding, behavior and reproduction (for review see Billen and Sobotnik, 2015). Gland presence in orthopteroids is almost always associated with the positioning of the female on the male during copulation, which is presumably a behavior ancestral to Pterygota (Alexander and Brown, 1963).

The presence of glandular structures evidenced by cuticular morphology in Grylloidea has been reported since Hancock (1905), who describes a metanotal gland, present only in the male, as “a moderately deep fossa, situated in the center of the metanotum”. Three other types of cuticular structures, in Grylloidea, are reported to be associated with glands by Otte (1992): (i) tibial glands present on the spurs of the posterior tibia of some Nemobiinae, (ii) tegminal glands located on the outer surface of the wings of *Adenopterus* sp. and *Archenopterus* sp. (Otte et al., 1987) and (iii) those with tegminal glands on the entire outer

surface. However, there are six other types of external structures with possible glandular function reported in Grylloidea: (i) tergal gland on the supra-anal plate (Bolfarini and De Mello, 2012), (ii) apex of the tegmina with glandular underside (Chopard, 1956), (iii) head with deep glandular depressions associated with bristles (Gorochov, 1996), (iv) abdominal tergites morphologically differentiated in glands (Gorochov, 1996), (v) anal gland (Walker and Masaki, 1989) and (vi) epiphallallic gland (De Mello and Camargo e Mello, 1996).

The presence of male-specific glands in some crickets and its use during copulation involving behaviors associated with females has been well documented (Hancock, 1905; Fulton, 1915; Alexander and Otte, 1967; Walker and Gurney, 1967; Walker, 1978; Bell, 1980; De Mello and Reis, 1994; Brown, 1997; Gwynne, 1997; Ono et al., 2004; Souza-Dias et al., 2015). The main assumptions about the behavioral effect of metanotal glands in crickets involve attraction and female maintenance during mating (Hancock, 1905) and the female maintenance after fixing the spermatophore on the copulatory papillae, avoiding that the female eats the spermatophore before the sperma reaches the spermatheca (Boldyrev, 1913; Engelhardt, 1914; Fulton, 1915). Both hypotheses converge on female maintenance function for this gland during copulation increasing the sperm transfer. In addition, the supply of any substance present in the secretion of the gland could act as a nuptial

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gift, to ensure an effective mating or as a form of parental investment.

The pioneering studies above assume that external modifications of body cuticle in Grylloidea may represent internal glands, but they have been described from inferences based on behavioral aspects and without the support of histological or histochemical studies. Only Engelhardt (1914) and Fulton (1915) presented a brief histological description of the metanotal gland in *Oecanthus* species (tree crickets). Within the diversity of exocrine glands in Grylloidea, *Ectecous segregatus* Gorochov, 1996 is the only species of this genus with a hypothesized male-specific glandular structure in the abdomen. However this is based only on external morphology of abdominal tergites. As such, the objective of this work was to evaluate the histology and ultrastructure of the modified abdominal tergites in males of *E. segregatus* to evaluate the presence of dorsal abdominal glands, and to contribute to the understanding of the reproductive function of these glandular structures.

## 2. Materials and methods

### 2.1. Insects

Individuals of *E. segregatus* were collected from the “Reserva Biológica Augusto Ruschi” (19°45’S; 40°27’W) and the “Estação Biológica de Santa Lúcia—EBSL” (19°57’S; 40°32’W), municipality of Santa Teresa, Espírito Santo State, Brazil (ICMBio authorization number 37717).

The crickets were collected manually with a plastic bottle and transferred to the laboratory where they were kept at  $25 \pm 2^\circ\text{C}$  with  $80 \pm 5\%$  relative humidity.

### 2.2. Scanning electron microscopy

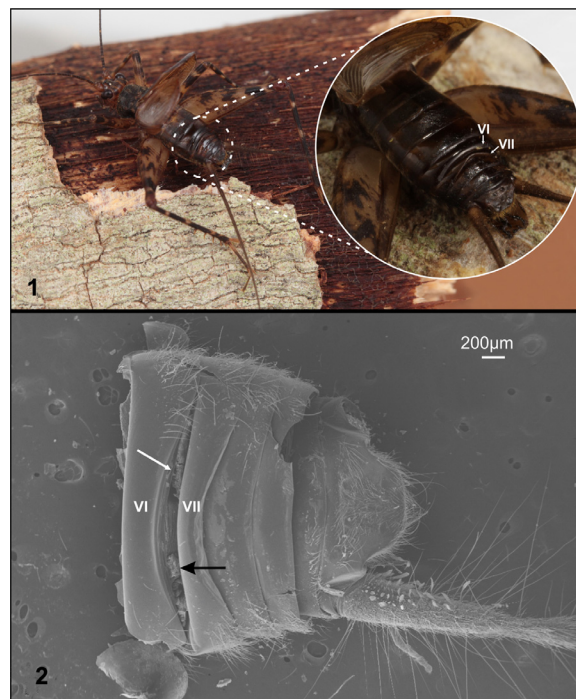
Males of *E. segregatus* kept in 80% ethanol had the final portion of the abdomen removed from the fifth tergite. This portion of the abdomen was dehydrated in a graded ethanol series (80, 90, 95 and 100%), transferred to hexamethyldisilazane, air dried, gold covered (20 nm) and analyzed with a scanning electron microscope LEO VP1430 in the Nucleus of Microscopy and Microanalyses at the Universidade Federal de Viçosa (NMM).

### 2.3. Histology and histochemistry

Five males were cryoanesthetized at  $-4^\circ\text{C}$  and dissected in physiological solution for insects (0.1 M NaCl, 0.1 M  $\text{KH}_2\text{PO}_4$ , 0.1 M  $\text{Na}_2\text{HPO}_4$ ) to remove the final portion of the abdomen from the fifth tergite, which was transferred to Zamboni’s fixative solution for 24 h, dehydrated in a graded ethanol series (70, 80, 90, 95 and 100%) and embedded in historesin JB-4. Three  $\mu\text{m}$  thick slices were stained with hematoxyline and eosin. Some sections were subjected to histochemical tests of PAS+ Alcian blue to characterize polysaccharides and glycoconjugates, mercury bromophenol blue to determine total proteins and 1% osmium tetroxide for lipid characterization. The sections were analyzed using light microscopy.

### 2.4. Transmission electron microscopy

The VI, VII and VIII abdominal tergites from three males of *E. segregatus* were dissected and transferred to 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.2, and 0.3 M sucrose, for 24 h. Samples were washed in sodium cacodylate buffer and postfixed in 1% osmium tetroxide in the same buffer for two hours. After being washed twice in the buffer, the pieces were dehydrated in a graded ethanol series (70, 80, 90, 95 and 100%) and embedded in LR White resin. Ultrathin sections were stained with 1% uranyl acetate



**Figs. 1 and 2.** 1. Photograph of a living male of *Ectecous segregatus* showing the VI and VII tergites with pronounced curvature. 2. Scanning electron micrograph of the final abdomen region in *E. segregatus* male, showing that the VI and VII tergites have material accumulated between them (arrow).

and lead citrate and analyzed with a Zeiss EM 109 transmission electron microscope at the NMM.

## 3. Results

The VI and VII tergites of the males presented a curvature absent in the other abdomen segments (Fig. 1). High resolution analyses with scanning electron microscope showed that in the VI and VII tergites the curvature was so pronounced that the membranous region of the cuticle was exposed, allowing substances to accumulate between these tergites (Fig. 2). High resolution analyses showed that in this region of the abdomen the surface of the cuticle is smooth, without the presence of glandular openings or cuticular pores.

Histological analyses showed that the V and VIII tergites presented an epidermis formed by flattened cells (Fig. 3), while those in modified tergites (VI and VII) showed columnar cells with vacuolated cytoplasm and basal nuclei with predominance of decondensed chromatin (Fig. 4). Onto the apical surface of columnar epidermal cells are some small nuclei with heterochromatin (Fig. 4).

The columnar epidermis in the VI and VII tergites had cells with weak reaction to PAS (Fig. 5) and some granules reactive to the osmium tetroxide in the apical cytoplasm (Fig. 6).

Amorphous material accumulated externally onto the cuticle between the VI and VII tergites, showed strong reaction to PAS test (Fig. 7), with some granules stained by osmium tetroxide (Fig. 8). Both the columnar cells and the accumulated material on the cuticle presented weak reaction to the mercury bromophenol blue test for proteins.

The ultrastructure showed that the apical surface of the columnar epidermal cells had short microvilli (1–2  $\mu\text{m}$ ) with cytoplasm rich in mitochondria and electron-dense granules (Fig. 9). The nuclei of these cells presented predominance of decondensed chromatin (Figs. 9, 10) and the perinuclear and basal cytoplasm showed

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