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Evaluation of the presence of glandular structures in preserved crickets (Orthoptera, Grylloidea, Phalangopsidae) using a comparative scanning electron microscopy technique

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Abstract

We studied the occurrence of glands in preserved specimens of *Vanzoliniella* spp., using a new comparative scanning electron microscopy technique. The method proposed here is an alternative to histological studies that cannot proceed due to the unavailability of well-preserved or recently collected specimens. It can also be used to study other subsurface structures. Some hypotheses to explain the function of the meso and metanotal glands in male crickets are presented.

Key words

Vanzoliniella, courtship feeding, metanotal glands, precopulatory courtship

Introduction

During copulation, the ancestors of the Ensifera (Orthoptera) probably assumed the female-above position (Alexander & Otte 1967). This position is still maintained in the recent Phalangopsidae. In this position, the female's head is motionless behind the male's wings, which are held almost vertical, and the female feeds at the secretions on the surface of the male's metanotum (Alexander & Brown 1963, Mello & Reis 1994). The metanotal prominences, where the secretions are found, are called metanotal glands. In this paper we describe findings made by scanning electron microscope (SEM) observations of the metanotal prominences in preserved specimens of *Vanzoliniella* spp. This technique, using secondary and backscattered electron detection, suggests that there are secretory glands, possibly producing nonvolatile products, below the metanotal sclerites.

Materials and Methods

Collection of the Specimens.— Specimens of *Vanzoliniella* spp. were collected with pitfall traps, in Atlantic Forest remnants, Viçosa, Minas Gerais State, Brazil, in 1996. They were preserved in 70% alcohol until 2001, when this study was conducted.

Scanning Electron Microscopy.— To analyze the metanotal surface we used Secondary Electron detection (SE). For this analysis, one specimen was dried for 1h in a chamber with silica gel and covered with gold, through sputtering (Balzers MED 010). To analyze the layer below the cuticular surface, we used Backscattered Electron detection (BSE). For this second analysis, another specimen was dried 15h, and covered with carbon through evaporation (Balzers MED 010). This covering allows the detection of backscattered electrons, which come from deeper layers of the material. We photographed both specimens in a Scanning Electron Microscope (Zeiss DSM 940 A). The second specimen was also photographed in a variable pressure SEM (LEO 435 VP). To compare the surface of each pore with the layer below it, we photographed exactly the same region of the metanotum with SE and BSE. Afterwards, we made a drawing of each surface pore on transparent paper, overlaid the paper upon the BSE image and evaluated the juxtaposition of the pores and the electron-dense regions.

Hypotheses

In this study we tested if the metanotal sclerites cover secretory glands, forming the observed prominences, and if there are nonvolatile substances secreted by these glands. If there are secretory glands below the prominences, we would expect that on the metanotal surface there would be pores, evidenced by SE. If these glands secrete nonvolatile substances, we would expect that there would be electron-dense regions, exactly below the surface pores, and with a greater diameter than the pores, evidenced by BSE. Nonvolatile substances present elements of high atomic number (Vilela & Della Lucia 1987), resulting in brighter regions in the BSE image in comparison to the cuticular surface. Another evidence for the secretion of nonvolatile substances would be the presence of some kind of residue over the pores.

Results

Vanzoliniella spp. present well-developed metanotal prominences (Fig. 1A,B), with pores throughout their surface (Fig. 1A). There were also pores in the mesonotum (Fig. 2F,H), but occurring more sparsely than in the metanotum (Fig. 2E,G). There were electron-dense regions on the layer below several of the metanotal pores (Fig. 2B), with a greater diameter than the pores themselves (Fig. 2A). Pores with little secretion on the surface presented small electron-dense points, not greater than the pore diameter (Fig. 2A,B).

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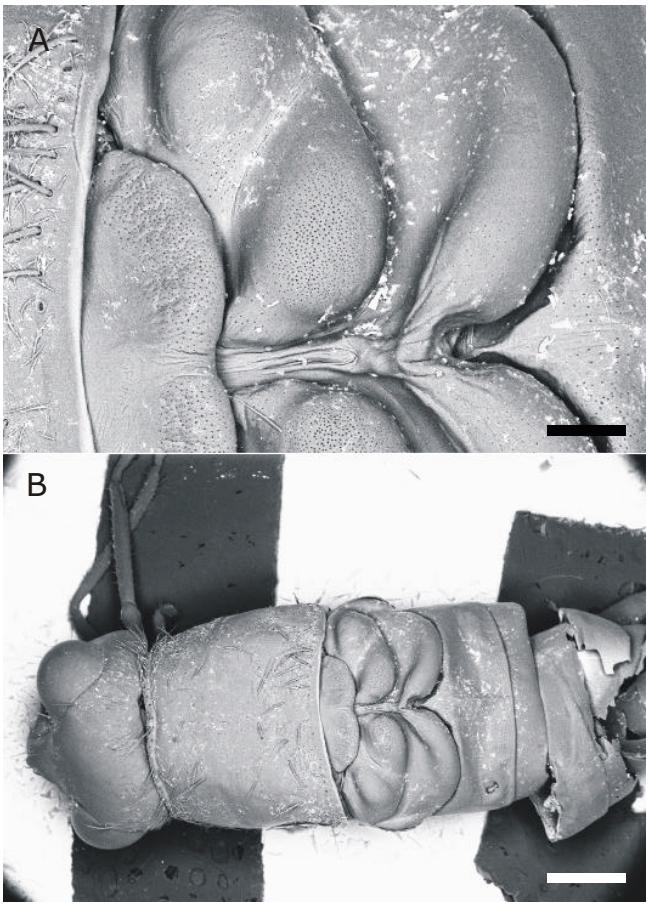


Fig. 1. *Vanzoliniella* sp., male, SEM-VP photomicrographs. **A**. meso and metanotum with glandular pores; **B**. dorsal view of male head and thorax, showing conspicuous metanotum prominences. Wings, part of the abdomen and legs removed. Specimen sputter-covered with carbon. Scale bars: 200µ (A), 800µ (B).

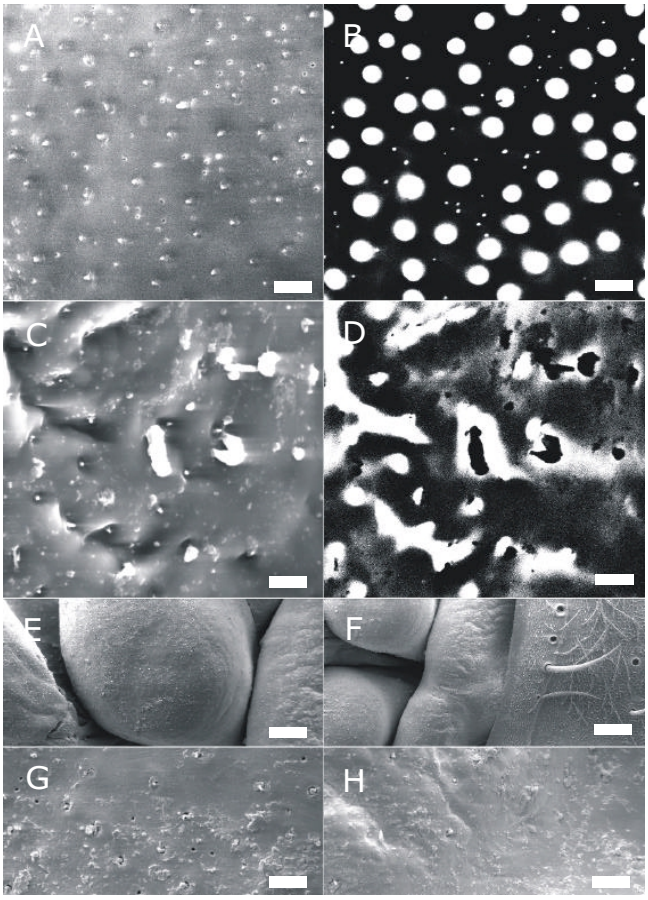


Fig. 2. *Vanzoliniella* sp., male, SEM photomicrographs. **A**. secondary electron image (SE) of metanotum. Secretion is easily seen in the greater part of the pores; **B**. backscattered electron image (BSE) of metanotum. Compare the position of the white circles (electron-dense regions) with the pores in A; **C**. mesonotum (SE). Compare the white amorphous body in the center of the picture with the same region in D; **D**. mesonotum (BSE); **E**, **F**. part of meta and mesonotal prominences respectively; **G**. detail of metanotal prominences showing pores; **H**. same on mesonotal prominences. Note that the pores of the mesonotum are more sparse. Scale bars: 10µ (A-D,F), 645µ (E), 8µ (G), 125µ (H).

There were also pores without any electron-dense region detected by BSE.

We observed several bright corpuscular bodies (Fig. 2A, C, G, H), apparently being secreted by the pores. These corpuscular bodies included both secretion and 'dirt'; the secretion could be distinguished by comparing SE and BSE images. We considered as 'dirt' any amorphous body in the SE image which did not correspond to an electron-dense region in the BSE image (Fig. 2A-D).

Discussion

The presence of pores on the metanotal prominences confirms that the metanotal sclerites cover secretory glands, and these glands secrete a nonvolatile substance. Even though the analyzed specimens were preserved in alcohol for over 5 y, the secretion was not washed out. This suggests that the secretion is sticky, possibly an adaptive character to prevent it from running off the male's dorsum.

The metanotal prominences appear to be a consequence of the accumulation of glandular tissue below the cuticle. Our work shows that below the surface of most pores there is an accumulation of secretion, with elements of higher atomic number than in the exoskeletal material. This secretion could be stored in two possible ways: superficial glandular structures, or along ducts of deeper glands. As the cuticle of the metanotum forms prominences, we judge the latter hypothesis the most plausible. Examination of cross sections of these regions should elucidate this point. The pores with small or no electron-dense points in the BSE image have probably secreted their content, as evidenced by the absence of secretion in the SE image.

As *Vanzoliniella* spp. possess neither a stridulatory vein nor auditory tympana (personal observation), the mechanism by which males attract females may involve, for instance, the production of sexual pheromone. Our results do not discount the possibility that such volatile substances are secreted in these or other glands, to attract the females. Olfactometer experiments could evaluate whether these or

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other male structures are involved in precopulatory courtship.

Entomologists frequently face difficulties in carrying out histological studies, due to the absence of well-preserved or recently collected specimens. The comparative method we present here overcomes these limitations. It can be widely used to evaluate the presence of subsurface structures, such as glands that secrete nonvolatile substances.

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