Boletín de la Sociedad Entomológica Aragonesa (S.E.A.), nº **55** (31/12/2014): 289–293.

STRUCTURE OF THE STING APPARATUS AND ASSOCIATED EXOCRINE GLANDS IN *DINOPONERA QUADRICEPS* (SANTSCHI, 1921) (HYMENOPTERA: FORMICIDAE)

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Abstract: *Dinoponera quadriceps* (Santschi, 1921) (Formicidae: Ponerinae) has an aggressive and predatory behavior, using its functional sting for defense and predation. This study describes the sting apparatus and the associated exocrine glands in *D. quadriceps* from three different populations in the Brazilian semiarid regions: Caetité, Feira de Santana and Manoel Vitorino. Like other species, the Ponerinae studied in these populations have their sting apparatus associated to the Dufour and venom glands. In the sting the lancets have at the distal end a serrated surface, which may be important to inject venom into the prey. The venom gland has secretory cells with excretory canaliculi opening into the lumen of the gland, and, specifically, the convoluted gland shows that the cells are in direct contact with the venom. This is the first structural description of the sting apparatus, the venon and Dufour glands in *D. quadriceps*.
Key words: Hymenoptera, Formicidae, Ponerinae, *Dinoponera quadriceps*, sting apparatus, Dufour gland, Brazil.

Estructura del aguijón y glándulas exocrinas asociadas en *Dinoponera quadriceps* (Santschi, 1921) (Hymenoptera, Formicidae)
Resumen: *Dinoponera quadriceps* (Santschi, 1921) (Formicidae: Ponerinae) presenta un comportamiento agresivo y predatorio, usando su aguijón funcional para la defensa y depredación. Este estudio describe el aparato del aguijón y las glándulas exocrinas asociadas en *D. quadriceps* de diferentes poblaciones de tres regiones del semiárido brasileño: Caetité, Feira de Santana y Manoel Vitorino. Como otras especies, los Ponerinae estudiados en estas poblaciones tienen el aparato del aguijón asociado a la glándula de Dufour y las glándulas de veneno. En el aguijón, las lancetas tienen una superficie serrada en su extremo distal, lo que puede ser importante para inyectar la ponzoña en la presa. La glándula de veneno tiene células secretoras con canalículos excretores que desembocan en el lumen de la glándula, y, específicamente, el complejo glandular muestra que las células están en contacto directo con la ponzoña. Ésta es la primera descripción estructural del aguijón y de las glándulas de Dufour y de veneno en *D. quadriceps*.
Palabras clave: Hymenoptera, Formicidae, Ponerinae, *Dinoponera quadriceps*, aguijón, glándula de Dufour, Brasil.

Introduction

Ponerinae ants have a functional sting apparatus that is used to inject venom, which may play important behavioral roles as defense, alarm pheromones and predation (Hermann & Blum, 1981; Caetano *et al*., 2002; Hölldobler & Wilson, 1990; Monnin & Peeters, 1998). Associated with the sting apparatus, in aculeate Hymenoptera there are two exocrine glands related to the production of pheromones and venom: the Dufour gland (colaterial gland) and the venom gland (Abdalla, 2002).

Dufour gland is located at the base of the sting apparatus, ventrally to the venom gland. In ants, the Dufour gland opens in the sting apparatus, without reproductive functions, but its secretion play important roles as trail marker, alarm and aggregation pheromones (Abdalla, 2002).

On the contrary to what occurs with some Hymenoptera, the sting is not lost when ponerine ants introducing it into the prey, and thus the venom gland becomes an important tool for ant survival (Caetano *et al*., 2002).

The venom gland, also known as acid gland or poison gland, consists of a long, thin, convoluted tubule located in the posterior abdomen, generally with the bifurcated distal end, linking up, to its proximal portion to a bag-shaped reservoir. The reservoir opens in the sting by an excretory duct (Nocelli *et al*., 2002). Associated with the reservoir there is a set of paired filamentous glands that converge into a single convoluted gland, which in turn empties into the reservoir. The convoluted gland is located on the dorsum of the reservoir, a unique condition within the Hymenoptera (Hölldobler & Wilson, 1990).

The primary function of the poison gland is the production of formic acid (in Formicinae), or venom used in the predation and defense for other ants (Hölldobler & Wilson, 1990). The main components of Ponerinae ant venom are proteins, alkaloids, terpenoids and hydrocarbon which have different functions. In *Dinoponera australis* Emery (Fowler, 1985) there are 75 proteins and some peptides (dinoponeratoxins) with biological activities (Johnson *et al*., 2010). In *Paraponera clavata* (Fabricius, 1775) and *Pachycondyla goeldii* (Forel, 1912) some substances named poneratoxins have antimicrobial properties (Orivel *et al*., 2001; Orivel & Dejean, 2001; Zelezetsky *et al*., 2005).

The venom composition may vary among the different species and subfamilies of ants (Lima & Brochetto-Braga, 2003). The venom is synthesized by secretory cells where it is stored as secretory granules prior to its release into the gland lumen and reservoir, suggesting that the secretion initially

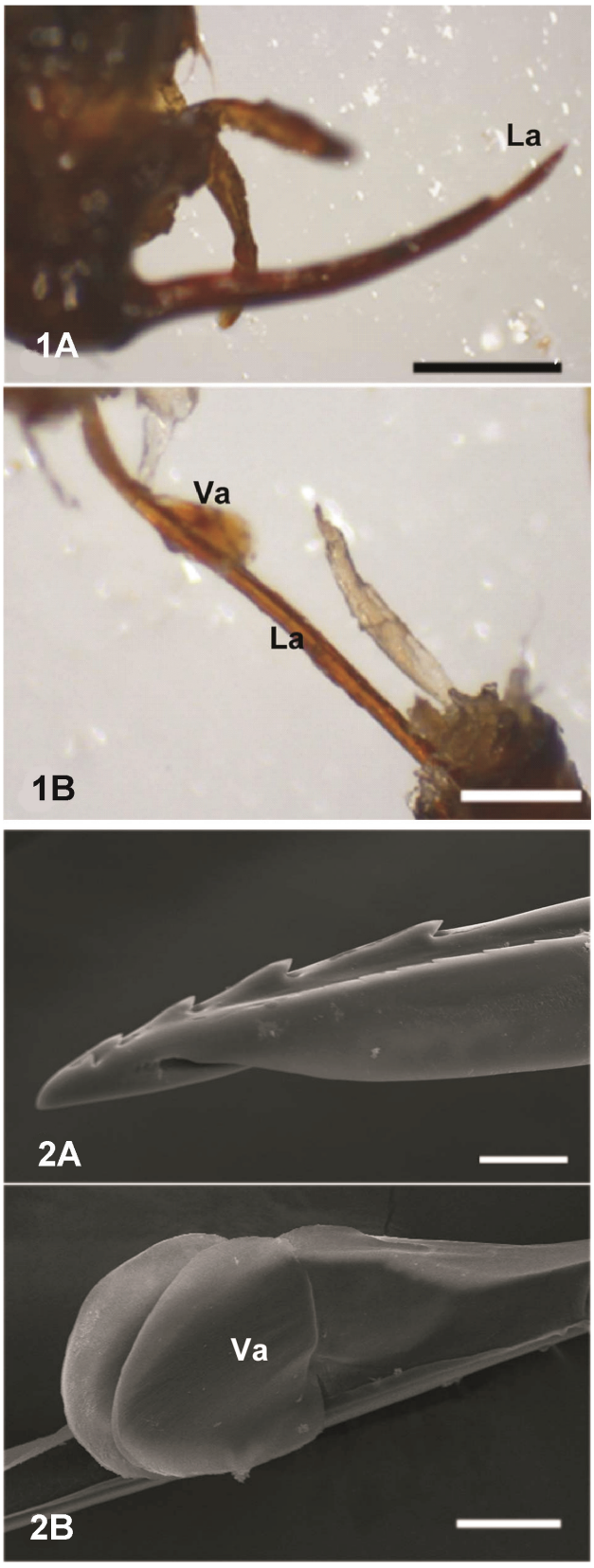

Figure 1 - Sting apparatus of *Dinoponera quadriceps*. (A) Sting in the abdomen showing the lancet La). (B) Sting pulled apart from the abdomen showing internal lancet (La) and proximal valve (Va). Scale Bars = 100 µm.

Figure 2 - Scanning electron micrographs of the lancet of *Dinoponera quadriceps*. A) distal end showing the serrated surface. B) Detail of the valve with two subunits. Scale bars = 30 100 µm .

produced may have a different composition of that released as venom (Cruz-Landim *et al*., 1967; Nunes & Camargo-Mathias, 2005; Ortiz & Camargo-Mathias , 2006). Another factor that may affect the venom composition in ants of a same species is the ecological niche, since these ants may have different diets as well as environmental conditions or some level of speciation (Hölldobler & Wilson, 1990).

Dinoponera quadriceps (Santschi, 1921) has an aggressive and predatory behavior, using its functional sting to inject the venom (Caetano *et al*., 2002; Hölldobler & Wilson, 1990). The distribution of this species is restricted to the semiarid region of Northeastern Brazil (Hölldobler & Wilson, 1990), and there are no studies on the sting apparatus structure, neither on the associated exocrine glands. The chemical composition of the venom of four populations of *D. quadriceps* showed differences among them (Cologna *et al*., 2013), including the three populations used in this study. For that reason, the present article compares the structure of the exocrine glands associated with the sting apparatus in different populations of *D. quadriceps* found in three regions of the Brazilian semiarid.

Material and methods

Areas

Specimens of *D. quadriceps* were collected at the municipalities of Feira de Santana (12°16'S – 38°56'W), Manoel Vitorino (14°8'S – 40°13'W) and Caetité (14°5'S – 42°28'W'), state of Bahia, Brazil. The climate in these areas is semiarid according to Koeppen's climate classification, defined as BSwh (high temperature and evaporation in summer), presenting average annual temperatures between 27-29ºC and annual rainfall < 800 mm with rains concentrated between January and March (Köeppen, 1948; Miller, 1971).

Ants

The ants were manually collected, in January 2010, following the identification and excavation of the their nests in natural environment (study granted SISBIO/MMA 20549-1) and transferred to the Laboratory of Animal Study at Bahia State University, Campus VI, municipality of Caetité, Bahia, Brazil.

Three ants samples from each area studied were preserved in 70% ethanol and transferred to the Myrmecology Laboratory for taxonomic identification. Voucher specimens were deposited in the Formicidae Collection of the Myrmecology Laboratory in the Cocoa Research Center (CEPEC/CEPLAC) in Ilhéus (Bahia), assigned as 5592 to 5595.

Light Microscopy

Five workers from the three ant population were dissected in presence of 125 mM NaCl and the sting apparatus, venom and Dufour glands transferred to 10% formalin. These structures were photographed in stereomicroscope and then dehydrated in gradual ethanol series and embedded in historesin (Leica). Slices of 3 µm thick were stained with hematoxylin and eosin and analyzed in light microscope.

Scanning electron microscopy

For the ultra-morphology analyses, the sting of three ants from each region was dissected, dehydrated in a gradual ethanol series, transferred to hexametildisilazane (MDS) for five min, air dried, gold covered (20 nm), and analyzed in a

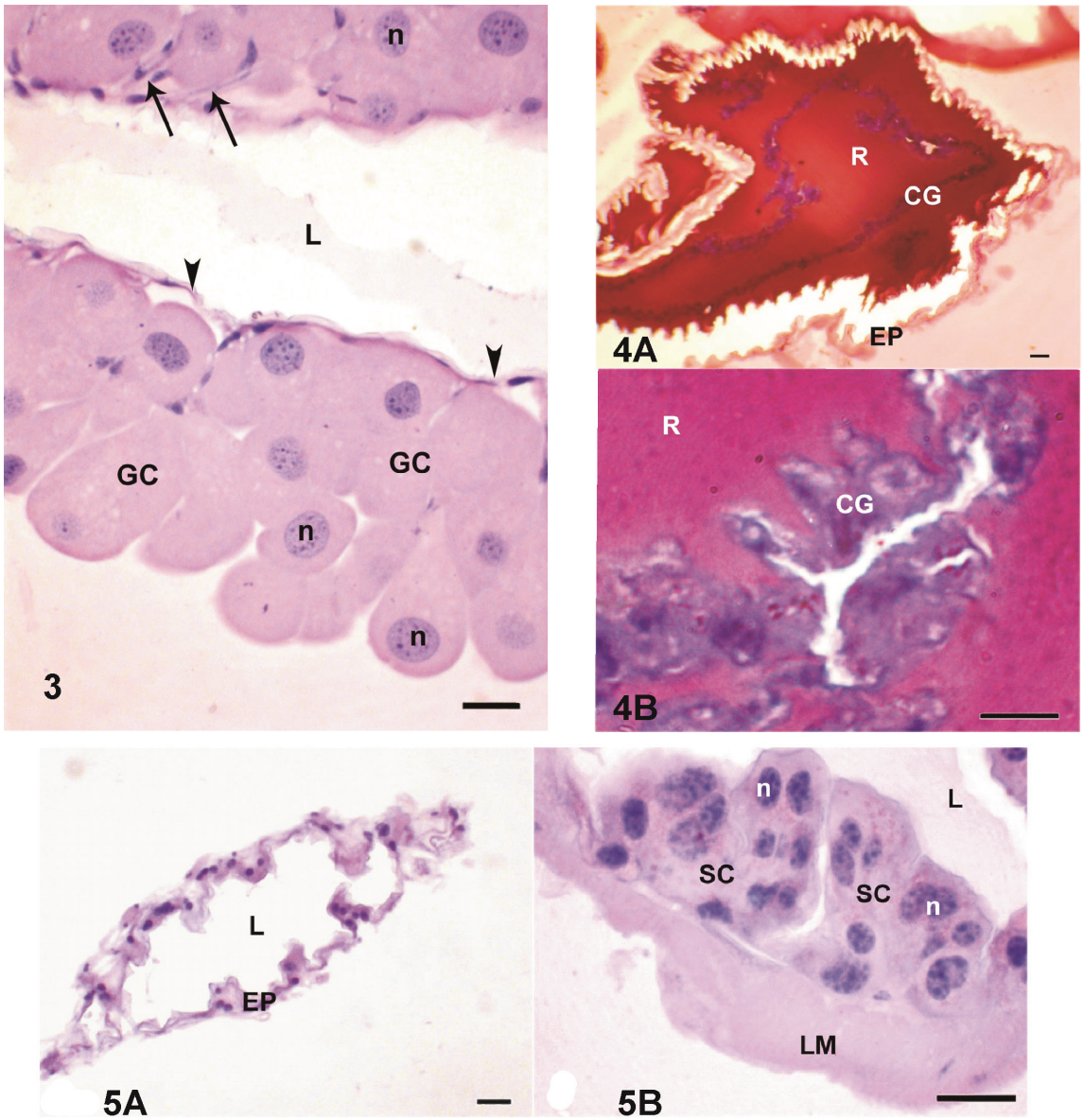

Figure 3 - Histological section of the secretory portion of the venom gland of *Dinoponera quadriceps* showing well-developed glandular cells (GC) with spherical nucleus (n). Note the excretory canaliculi (arrows) within the cell projecting into the gland lumen (L), which is coated with a thin layer of flattened cells (arrowheads). Scale bar = 10 µm.

Figure 4 - Histological sections of the venom reservoir of *Dinoponera quadriceps*. A) reservoir (R) showing the flattened epithelium (EP) lining the lumen filled with acidophilic content and the convoluted gland (CG). B) The convoluted gland (CG) epithelium with basophilic cells in contact with acidophilic venom stored in the reservoir (R). Scale bars = 10 µm.

Figure 5 - Histological sections of the Dufour gland of *Dinoponera quadriceps*. A) Gland wall with cubic epithelium (EP) delimiting the lumen (L). B) Secretory cells (SC) with granular cytoplasm and small nucleus (n). Note a longitudinal muscle layer (LM). Scale bars = 10 µm.

LEO VP 1430 scanning electron microscope at the Nucleus of Microscopy and Microanalysis, Federal University of Viçosa, state of Minas Gerais, Brazil.

Results

The representatives of *D. quadriceps* workers from Caetité, Feira de Santana and Manoel Vitorino showed similar anatomy and histology of the sting apparatus, as well as in the venom and Dufour glands.

The sting, venom and Dufour glands were located at the terminal region of the abdomen. In the arrow-shaped sting there are three parts that could be characterized as: a stylet, a superior portion and a pair of lancets in the lower portion (Figures 1A and 1B). The distal end of the lancets had a short serrated area (Figure 2A), whereas in the proximal end occurred two valves, one in each lancet, belonging to the lancets' basal segment (Figures 1B and 2B).

In the proximal end of the sting, a duct connects the venom gland reservoir, and another connects to the Dufour gland. The secretory portion of the venom gland has two cylindrical and elongated tubules with a blind end, which are joined, forming a short filament opening into an enlarged reservoir with a yellow content.

The secretory epithelium of the venom gland has a single layer of globular cells with homogeneous acidophilic cytoplasm and a large spherical nucleus with decondensed chromatin and several nucleoli (Figure 3). The secretory cells

release their secretion via excretory canaliculi, in a narrow lumen limited by a thin layer of flattened cells (Figure 3).

The wall of the venom gland reservoir has a single layer of flattened cells lined by a thick cuticle (Figure 4A). Inside the venom gland reservoir was found a convoluted gland in contact with secretion (venom) stored in the reservoir (Figure 4A). The convoluted gland is formed by a single layer of cubical cells with the cytoplasm strongly basophilic and the nucleus with decondensed chromatin, contrasting with the acidophilic content of the venom gland reservoir (Figure 4B).

The Dufour gland has a finger-shaped opening toward to the sting, near to the venom gland duct. The epithelium of the Dufour gland showed short folds formed by a single layer of cubic cells with a granular acidophilic cytoplasm and nucleus with some granules of condensed chromatin (Figures 5A and 5B). Externally, the epithelium of the Dufour gland was lined by a layer of longitudinal muscles (Figure 5B).

Discussion

This is the first description of the morphology of the sting apparatus and the associated exocrine glands in *D. quadriceps*. The results obtained here are similar to those found in other Ponerinae species (Schoeters & Billen, 1995; Nunes & Camargo-Mathias, 2005; Ortiz & Camargo-Mathias, 2006). However, differences in the morphology of the sting apparatus between species of the same genera have been used as features for the taxonomic differentiation in several ant subfamilies (Kugler, 1978, 1980, 1994; Lacau *et al*., 2008).

In the sting of *D. quadriceps* the lancets have a distal end with a serrated surface, which may be important injecting venom into the prey, similarly to what was described in the sting of other ants (Hermann, 1971). At the proximal end of each lancet there are valves with similar sizes and connected to a pair of muscles. The valve act as a pump to inject the venom and the Dufour gland compounds (Snodgrass, 1956). The sting apparatus was also described for *Dinoponera grandis* (Guérin-Méneville, 1838) without the report of the occurrence of a serrated distal end and the valves (Hermann *et al*., 1984). However these authors analyzed the sclerotized venom apparatus under low resolution and the presence of the above structures found in *D. quadriceps* should be further studied.

The venom gland has secretory cells with excretory canaliculi opening into the gland's lumen, characterizing these cells as class III, according to the classification of Noirot and Quennedey (1991). This organization was also identified in the venom gland of *Dinoponera australis* (Schoeters & Billen, 1995).

The gland which is presumably responsible for venom production in *D. quadriceps* is the venom gland plus the convoluted gland. Convoluted gland with similar organization to that of *D. quadriceps* was described in the Ponerini *D. australis* (Schoeters & Billen, 1995), *Neoponera villosa* (Fabricius, 1804) (Henrique, 2000, unpublished data), *Pachycondyla striata* Fr. Smith, 1858 (Ortiz & Camargo-Mathias, 2006) and *Ectatomma quadridens* (Fabricius, 1793) (Nunes & Camargo-Mathias, 2005).

The convoluted gland of *D. australis* releases substances directly into the venom reservoir (Schoeters & Billen, 1995), which may also occur in the convoluted gland of *D. quadriceps*, whose cells are in direct contact with the venom. The convoluted gland, besides releasing secretions in the venom gland reservoir, may also store proteins for later use. Thus some content from the venom gland reservoir may also be captured by the convoluted gland and remain stored in the cytoplasm of its cells, so the secretions would be used by the ant when injected by the sting (Ortiz & Camargo-Mathias, 2006).

The venom stored in the reservoir of *D. quadriceps* has basic properties due to its affinity to the eosin (acid), whereas the Dufour gland secretion presents an acidic nature. Unlike what was found by the Cassier *et al*. (1994) for Aculeata, we did find in the *D. quadriceps* that the venom gland is acidic and the gland Dufour is basic.

Conclusion

The structure of the sting, venom gland and Dufour gland is similar in *D. quadriceps* from different populations of the semiarid Northeastern Brazil and with other Ponerinae ant species.

Acknowledgements

The authors thank to the Nucleus of Microscopy and Microanalysis of Federal University of Vicosa, for its technical support with regard to the use of scanning electron microscope; to the graduate program in Biotechnology of State University of Feira de Santana, for its logistical support; and to Brazilian research agencies CAPES, CNPq, FAPEMIG and PRONEX-FAPESB, for the financial support. Authors are grateful to Prof. Jacques Hubert Charles Delabie for identification of ant species.

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