Morphology and ultrastructure of the venom gland in the ant *Brachyponera sennaarensis*

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ABSTRACT. We studied the morphology and ultrastructure of the venom gland of queens and workers of the samsum ant *Brachyponera sennaarensis*, which is known for its very painful sting. The general anatomical features of the gland are similar to those of other ants. The secretory cells do not have a granular endoplasmic reticulum, but contain numerous scattered free ribosomes and a well-developed Golgi apparatus. The convoluted gland represents an additional secretory tissue, in which the venom reaches its final toxicity. A considerable muscular supply surrounds the venom reservoir, and allows a forceful squeezing out of the venom during stinging. The ants have a mainly granivorous diet during the dry season, and feed on animal prey during the rainy season. For their prey hunting, and possibly also to protect the stored seeds against potential seed thieves, they need to rely on a powerful venom gland.

Keywords: morphology, ultrastructure, venom gland, *Brachyponera sennaarensis*

INTRODUCTION

Many hymenopteran insects are notorious for their painful sting, used for prey capture as well as for defence against larger opponents. The sting apparatus evolved from the ovipositor and therefore occurs in the female castes of queens and workers only. It is formed by a number of sclerotized elements that originate from modified abdominal sternites, such as the sting shaft and sting lancets, the furcula, and the quadrate, oblong, spiracular and triangular plates (Hermann & Blum 1978). These articulating cuticular elements are associated with powerful muscles that cause the mechanical manoeuverability of the sting and its insertion into the prey or enemy during the act of stinging. In some insects, such as Mutillidae (Hermann 1968) and Tiphiidae (Billen et al. 2017), the sting shows a conspicuous dorsal

curvature to allow more efficient penetration of their host insect. Besides the sclerotized parts, the sting apparatus also contains a glandular component, that is made up by the venom (= poison) gland and Dufour's gland, that both open through the sting shaft in ants (Billen 1987). In bees and wasps, the Dufour's gland bends downward when it approaches the sting base, and hence opens into the dorsal oviduct. The venom gland, however, always opens and releases its contents through the sting shaft (Billen 1987). This gland is the production site of the venom, that is injected into the prey or enemy, and that is formed by often complex cocktails of toxins (reviewed in Touchard et al. 2016). Its anatomy shows a compartmented organization that is an adaptation for storage of the toxic secretion it produces and to prevent self-intoxication of the ant against its own venom.

Species of the subfamily Ponerinae are usually known for their strong and painful sting. This can form a threat to humans when such species are accidentally introduced in new areas where they become invasive. A clear example is the samsum ant *Brachyponera sennaarensis*, that has recently expanded from its original distribution in the Eastern African savannas into the Middle East (Paknia 2006; Al-Khalifa et al. 2010; Wetterer 2013). In their search for humidity, the ants come into contact with humans, in whom the sting may cause anaphylactic shock (Al-Shawan et al. 2006; Al-Anazi et al. 2009), making this species a serious pest in Saudi Arabia (Al-Khalifa et al. 2015). On the positive side, samsum ant venom has a potential for medical applications, as it was found to have an anti-tumor effect on human breast carcinoma cells (Badr et al. 2012). We recently reported on the morphology and ultrastructure of several cephalic glands of *B. sennaarensis* (Billen & Al-Khalifa 2015, 2016, 2018), and in the present paper describe the structural characteristics of its venom gland.

MATERIAL AND METHODS

Workers and alate queens of *Brachyponera sennaarensis* were collected in May 2014 and May 2015 from two large colonies nesting between the root systems of date palm trees at Naa'm, Huata bani Tamim region in the south of Riyadh, Saudi Arabia. The venom glands were carefully dissected from the gaster and were fixed in cold 2% glutaraldehyde, buffered at pH 7.3 with 50 mM sodium cacodylate and 150 mM saccharose. After postfixation in 2% osmium tetroxide in the same buffer and dehydration in a graded acetone series, tissues were embedded in Araldite. Sections were produced with a Leica EM UC6 ultramicrotome: semithin sections with a thickness of 1 μm were stained with methylene blue and thionin and viewed with an Olympus BX-51 microscope, thin sections of 70 nm were double stained with lead citrate and uranyl acetate and examined with a Zeiss EM900 electron microscope. For SEM, dissected venom glands were fixed in 2% glutaraldehyde, critical point dried in a Balzers CPD 030 instrument, mounted on stubs, gold coated, and viewed with a JEOL JSM-6360 scanning microscope.
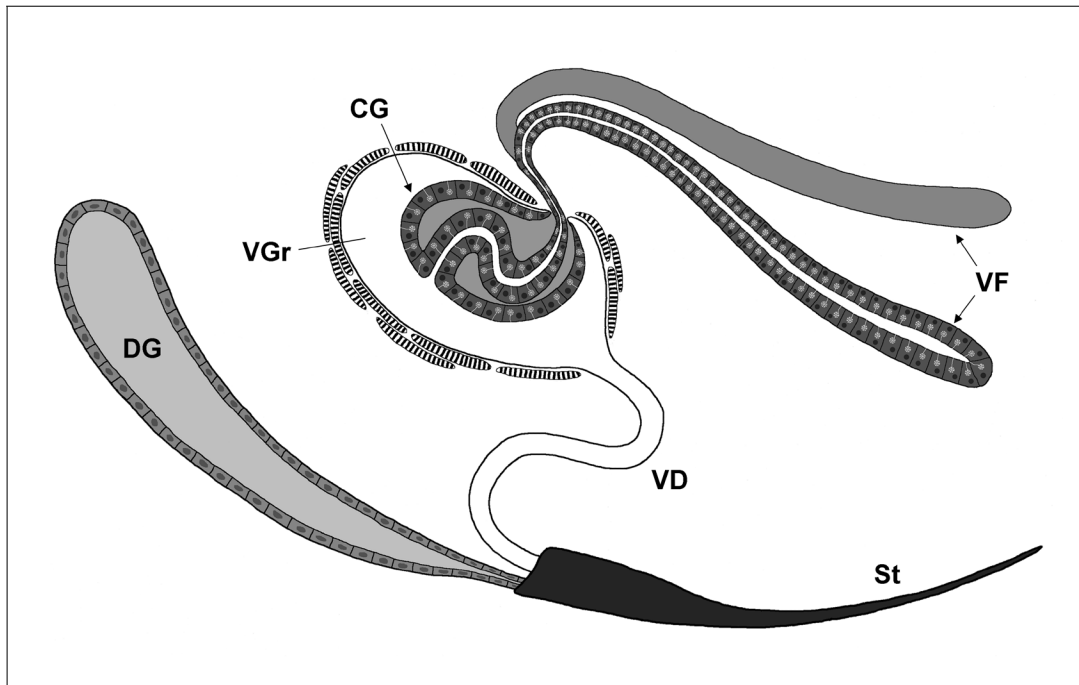

Fig. 1. Schematical representation of the sting (St) with its associated venom gland and Dufour's gland (DG). CG: convoluted gland, VD: venom duct, VF: venom gland filaments, VGr: venom gland reservoir.
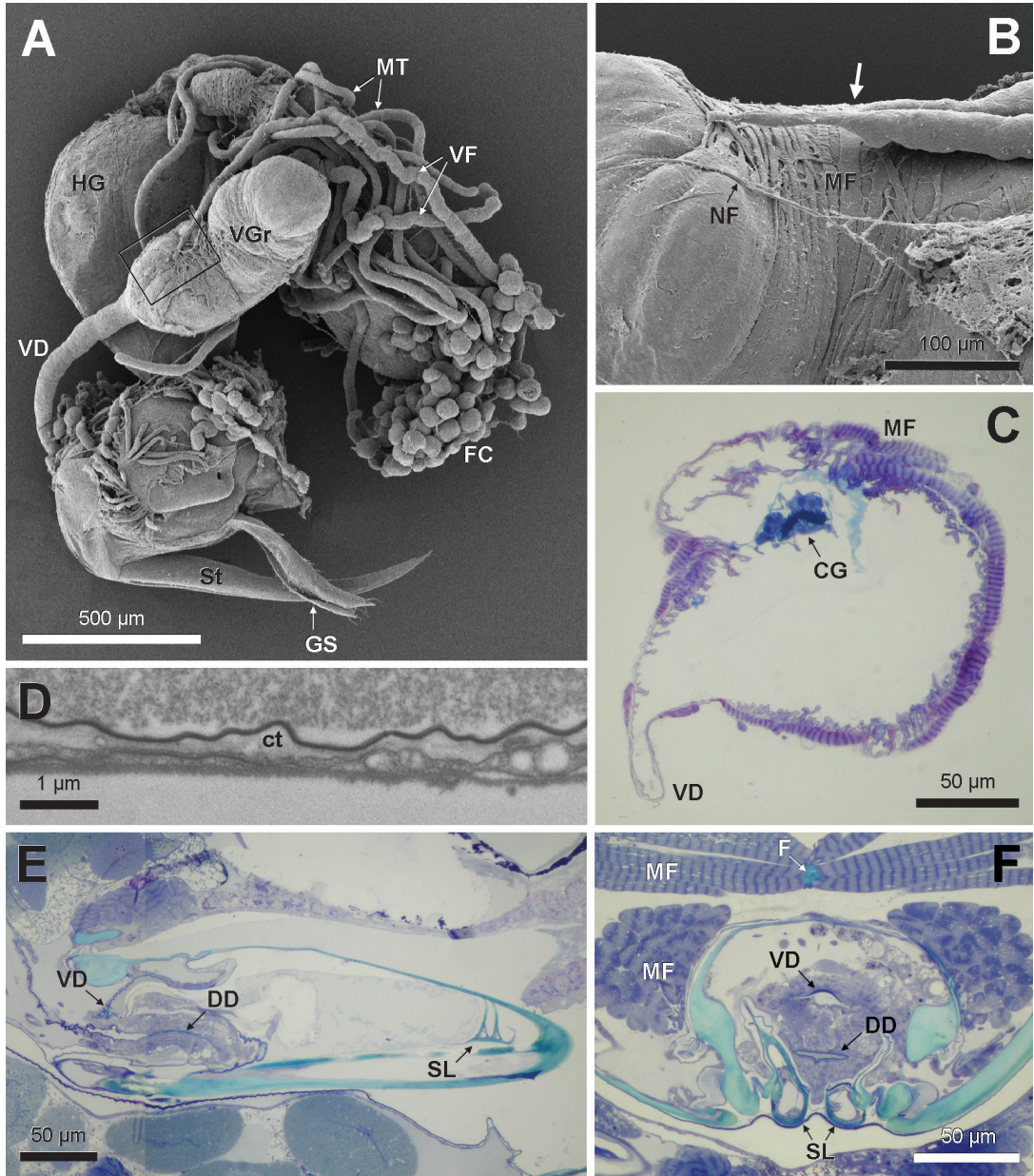
Fig. 2. A. Scanning micrograph of the sting apparatus with attached hindgut (HG) and Malpighian tubules (MT) of *B. sennaarensis* worker. The venom gland consists of two free filaments (VF), a reservoir (VGr) with an internalized convoluted gland (visible in 1C) and a venom duct (VD) that opens through the sting (St). **B**. Detail of framed area in 1A, showing how both filaments fuse into a considerably thinner unpaired filament prior to entering the reservoir sac (white arrow). **C**. Semithin section through venom reservoir and internalized convoluted gland (CG) in *B. sennaarensis* alate queen. Note conspicuous supply of muscle fibres (MF). **D**. Ultrastructural detail (worker) of very thin cuticle-lined reservoir wall. **E**,**F**. Longitudinal and transverse semithin sections through the sting base of a worker, showing opening of venom gland duct (VD) and Dufour gland duct (DD). ct: cuticle, F: furcula, FC: fat cells, GS: gonostyli, MF: muscle fibres, NF: nerve fibre, SL: sting lancets.
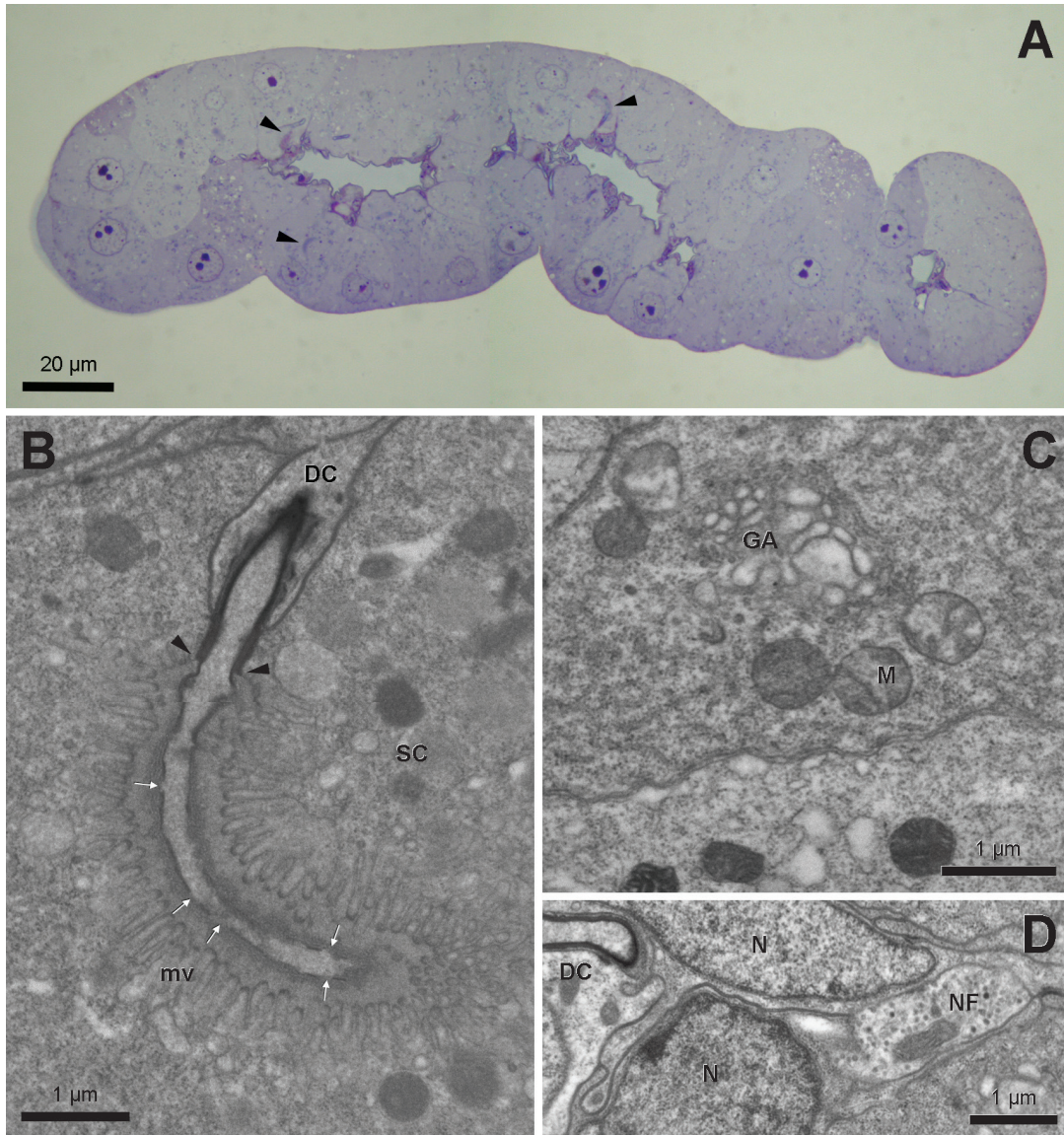
Fig. 3. A. Longitudinal semithin section through venom gland filament in worker with central lumen and sections through end apparatus (black arrowheads). **B**. Electron micrograph detail of end apparatus in worker. Note abrupt transition (arrowheads) of thick and continuous cuticle in duct cell (DC) into thin and discontinuous cuticle in secretory cell (SC). Small white arrows indicate disrupted epicuticular inner lining. **C**. Ultrastructure of secretory cell cytoplasm (queen) with granular appearance because of abundant free ribosomes, and with well-developed Golgi apparatus (GA) and mitochondria (M). **D**. Detail of nerve fibre (NF) wedged in between gland cells (queen). mv: microvilli, N: nucleus.
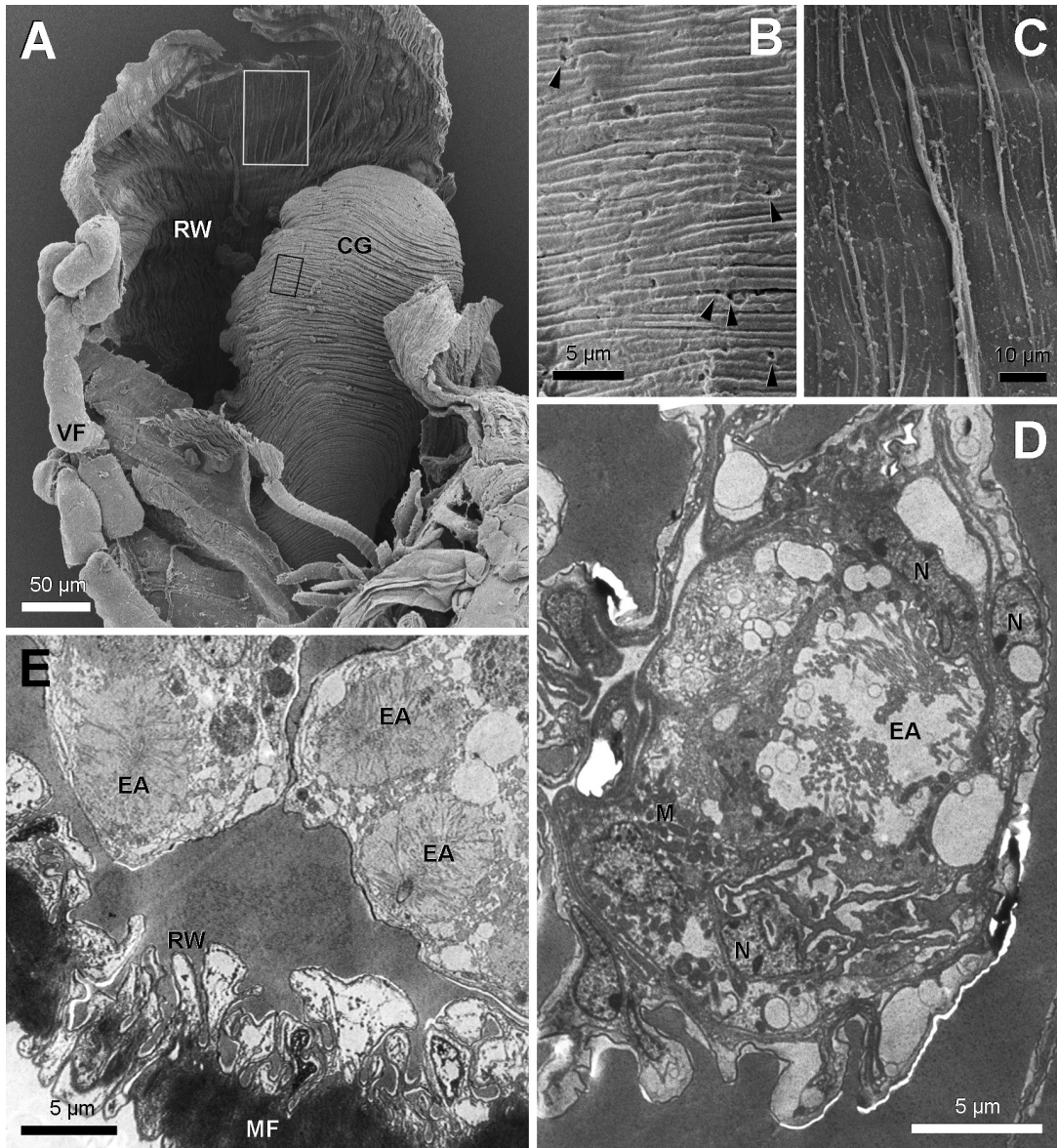
Fig. 4. Convoluted gland of *B. sennaarensis* worker. **A**. Scanning micrograph showing striated appearance of convoluted gland (CG) after tearing open venom reservoir (RW: reservoir wall). **B**. Detail of convoluted gland surface (of area indicated by black frame in 4A), arrowheads show openings of gland ducts. **C**. Detail of inner reservoir wall (of area indicated by white frame in 4A). **D**,**E**. Electron micrographs of central (D) and peripheral (E) portion of convoluted gland, showing presence of secretory cells with end apparatus (EA). M: mitochondria, MF: muscle fibres, N: nucleus, VF: venom filaments.

RESULTS

As in other stinging ants, the venom gland of both queens and workers is formed by two free filaments in which the initial venom synthesis starts, a reservoir sac that encloses the convoluted gland portion, and the venom duct, that opens through the sting (Fig. 1). The paired filaments have a length of approx. 1.5-2 mm and a diameter between 40 and 50 µm, and are situated in between the other abdominal organs with a close proximity to the fat body (Fig. 2A). Both filaments fuse together to become a single unpaired filament shortly before entering the reservoir sac. The unpaired filament is considerably thinner than the paired filaments and has a diameter of 10-15 µm (Fig. 2B). Upon entering the reservoir sac, it expands to form the convoluted gland portion, which is entirely invaginated inside the reservoir sac (Fig. 2C, 4A). The reservoir sac is surrounded by a thick muscular supply (Fig. 2C), while also an occasional nerve can be observed (Fig. 2B). The elongated reservoir sac has a length of approx. 700 µm and a width around 400 µm, and has a cuticle-lined epithelial wall with a thickness of less than 1 µm (Fig. 2D). The venom duct has a length of approx. 750 µm and a diameter around 50 µm and enters the sting base dorsally to the Dufour gland duct, both ducts are associated with bundles of muscle fibres (Fig. 2E,F).

The venom gland filaments consist of class-3 secretory cells (classification of Noirot & Quennedey 1974) that are arranged around a central cuticle-lined filament lumen, into which they open through their accompanying duct cells (Fig. 3A). Each duct cell connects with a secretory cell through the end apparatus, which is formed by a cuticular continuation of the ductule that is surrounded by a sheath of microvilli (Fig. 3B). The cuticular characteristics in the duct cell and the end apparatus differ considerably: in the duct cell, the cuticular lining is thick and continuous, whereas in the end apparatus it is thin and discontinuous (Fig. 3B). The cytoplasm contains a well-developed Golgi apparatus and numerous mitochondria. Endoplasmic reticulum could not be observed, but numerous free ribosomes and polysomes give the cytoplasm a fine granulated appearance (Fig. 3C). In between the secretory cells of the free filaments, tracheoles (not shown) and nerve fibres can be observed (Fig. 3D).

The ovoid convoluted gland is completely internalized inside the reservoir sac, and measures approx. 400x200 µm. Careful tearing open of the reservoir sac of critical point dried material allows observation of the convoluted gland with scanning microscopy (Fig. 4A). Its external surface appears striated, with irregularly scattered small pores with a diameter around 0.5 µm (Fig. 4B). The internal reservoir wall, in contrast, shows a less dense striation without any pores (Fig. 4C). As a continuation of the venom gland filaments, the bulk of the convoluted gland is made up of secretory tissue with class-3 gland cells with a clear end apparatus and their accompanying ducts. The ducts open both along the continuation of the internal lumen inside the convoluted gland lumen and along the periphery of the convoluted gland (Fig. 1, 4D,E).

DISCUSSION

The samsum ant *Brachyponera sennaarensis* has a bad reputation to humans because of its painful sting, caused by the toxic secretions that are produced in its venom gland (Orivel & Dejean 2001). Together with its Asian relative *B. chinensis*, it is on the list of the ant species with the most painful stings (Dib et al. 1995; Klotz et al. 2005; Schmidt 2016). *Brachyponera sennaarensis* displays a mainly granivorous diet during the dry season, whereas the ants feed on animal prey during the rainy season, when no seeds are available (Levieux & Diomande 1978; Lachaud & Dejean 1994; Mashaly et al. 2013). For prey hunting, the ants need to rely on a powerful venom gland, while they may also use it in the protection of stored seeds against mice or other potential seed thieves.

The majority of ant (as well as bee and wasp) species are characterized by a highly proteinaceous venom composition (Hermann & Blum 1978; O'Connor & Peck 1978; Edery et al. 1978). This is usually reflected in the presence of a well-developed granular endoplasmic reticulum

(RER) in the secretory cells (e.g. Owen & Bridges (1976) for *Apis mellifera*; Delfino et al. (1983) for *Polistes gallicus*; Billen (1990a) for *Myrmecia gulosa*). The presence of RER, however, is not an absolute prerequisite for venom toxicity, as some ants that are known for their extremely painful sting do not have such RER in their venom gland cells, but rather contain an abundance of scattered free ribosomes and numerous mitochondria (Billen (1990b) and Fox et al. (2010) for the fire ants *Solenopsis invicta* and *S. saevissima* [in which the venom is dominated by alkaloids: Brand et al. 1972], and Billen (unpubl. data) for the bullet ant *Paraponera clavata*). The secretory cells of *B. sennaarensis* in this regard resemble the venom gland of these species.

A peculiarity for the venom gland is its compartmented organization, in which the initial venom produced in the secretory filaments needs to pass through the convoluted gland portion, where additional toxins and other components are added before the venom reaches the venom reservoir in its final composition. This special anatomical arrangement is commonly found in ants (Callahan et al. 1959; Billen 1990a,b; Schoeters & Billen 1995a; Nunes & Camargo-Mathias 2005; Fox et al. 2010) and wasps (Schoeters & Billen 1995b; Petrocelli et al. 2014), but not in bees (Owen & Bridges 1976). The convoluted gland has been interpreted as an adaptation to prevent self-intoxication of the insect's internal tissues by its own venom, as the initially non-toxic secretion of the secretory filaments only reaches its final toxicity after passing through the convoluted gland (Schoeters & Billen 1995a; Ortiz & Camargo-Mathias 2005; Fox et al. 2010; Cardoso et al. 2014). Using histochemical analyses, Nunes & Camargo-Mathias (2005) showed in *Ectatomma quadridens* that the secretion becomes modified inside the reservoir with an increase of the concentration of proteic elements closer to the main excretory venom duct. The presence of cells with an end apparatus in the convoluted gland tissue clearly illustrates its secretory capacity, and hence its ability to modify the final secretory product that will be eventually stored in the reservoir. During the act of stinging, the venom will be forcefully emitted through the sting, with an important role for the extensive musculature that surrounds the venom reservoir. In this regard, the presence of nerve fibres presumably controls the muscular activity to force venom out through the sting.

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REFERENCES

Al-Anazi M, Al-Ashahrani M and Al-Salamah M, 2009. Black ant stings caused by *Pachycondyla sennaarensis*: a significant health hazard. Annals Saudi Medicine 29: 207 – 211.

Al-Khalifa MS, Ahmed AM, Mashaly AMA, Al-Mekhalfi FA, Khalil G, Siddiqui MI and Ali MF, 2010. Studies on the distribution of *Pachycondyla sennaarensis* (Hymenoptera: Formicidae: Ponerinae) in Saudi Arabia. 1. Ar-Riyadh Region. Pakistan Journal of Zoology 42: 707 – 713.

Al-Khalifa MS, Mashaly AMA, Siddiqui MI and Al-Mekhalfi FA, 2015. Samsum ant, *Brachyponera sennaarensis* (Formicidae: Ponerinae): Distribution and abundance in Saudi Arabia. Saudi Journal of Biological Sciences 22: 575 – 579.

Al-Shawan M, Al-Khenaizan S and Al-Khalifa MS, 2006. Black (samsum) ant induced anaphylaxis in Saudi Arabia. Saudi Medical Journal 27: 1761 – 1763.

Badr G, Garraud O, Al-Khalifa MS and Richard Y, 2012. Human breast carcinoma cells are induced to apoptosis by samsum ant venom through an IGF-1-dependant pathway, PI3K/AKT and ERK signaling. Cellular Immunology 273: 10 – 16.

Billen J, 1987. New structural aspects of the Dufour's and venom glands in social insects. Naturwissenschaften 74: 340 – 341.

Billen J, 1990a. Morphology and ultrastructure of the Dufour's and venom gland in the ant *Myrmecia gulosa* (Fabr.) (Hymenoptera: Formicidae). Australian Journal of Zoology 38: 305 – 315.

Billen J, 1990b. A survey of the glandular system of fire ants. In: Applied Myrmecology, A World Perspective (Vander Meer RK, Jaffe K and Cedeno A, eds), Westview Press, Boulder, San Francisco & Oxford, 85 – 94.

Billen J and Al-Khalifa MS, 2015. Morphology and ultrastructure of the pro- and postpharyngeal glands in workers of *Brachyponera sennaarensis*. Sociobiology 62: 270 – 275.

Billen J and Al-Khalifa MS, 2016. A novel intramandibular gland in the ant *Brachyponera sennaarensis*. Insectes Sociaux 63: 321 – 326.

Billen J and Al-Khalifa MS, 2018. Morphology and ultrastructure of the mandibular gland in the ant *Brachyponera sennaarensis* (Hymenoptera, Formicidae). Micron 104: 66 – 71.

Billen J, Justino CEL, Carnimeo FH and Noll FB, 2017. Morphology and ultrastructure of the Dufour gland of *Myzinum* sp. (Tiphiidae). Journal of Hymenoptera Research 55: 109 – 119.

Brand JM, Blum MS, Fales HM and MacConnell JG, 1972. Fire ant venoms: comparative analyses of alkaloidal components. Toxicon 10: 259 – 271.

Callahan PS, Blum MS and Walker JR, 1959. Morphology and histology of the poison glands and sting of the imported fire ant *Solenopsis saevissima* v. *richteri* Forel. Annals of the Entomological Society of America 52: 573 – 590.

Cardoso JS, Souza ALB, Serrão JE, Uetanabaro APT and Neto EMC, 2014. Structure of the sting apparatus and associated exocrine glands in *Dinoponera quadriceps* (Santschi, 1921) (Hymenoptera: Formicidae). Boletín de la Sociedad Entomológica Aragonesa 55: 289 – 293.

Delfino G, Marino Piccioli MT and Calloni C, 1983. Ultrastructure of the venom glands in *Polistes gallicus* (L.) (Hymenoptera Vespidae). Monitore Zoologico Italiano 17: 263 – 277.

Dib G, Guerin B, Banks WA and Leynadier F, 1995. Systemic reactions to the Samsum ant: An IgE-mediated hypersensitivity. Journal of Allergy and Clinical Immunology 96: 465 – 472.

Edery H, Ishay J, Gitter S and Joshua H, 1978. Venoms of Apidae (Bettini S, ed.), Springer, Berlin, Heidelberg, New York, 691 – 771.

Fox EGP, Bueno OC, Yabuki AT, de Jesus CM, Solis DR, Rossi ML and de Lima Nogueira N, 2010. General morphology and ultrastructure of the venom apparatus and convoluted gland of the fire ant, *Solenopsis saevissima*. Journal of Insect Science 10: 24.

Hermann HR, 1968. The hymenopterous poison apparatus. IV. *Dasymutilla occidentalis* (Hymenoptera: Mutillidae). Journal of the Georgia Entomological Society 3: 1 – 10.

Hermann HR and Blum MS, 1978. Venoms and venom apparatuses of the Formicidae: Myrmeciinae, Ponerinae, Dorylinae, Pseudomyrmecinae, Myrmicinae, and Formicinae. In: Arthropod Venoms (Bettini S, ed.), Springer, Berlin, Heidelberg, New York, 801 – 869.

Klotz JH, deShazo RD, Pinnas JL, Frishman AM, Schmidt JO, Suiter DR, Price GW and Klotz SA, 2005. Adverse reactions to ants other than imported fire ants. Annals of Allergy, Asthma & Immunology 95: 418 – 425.

Lachaud JP and Dejean A, 1994. Predatory behavior of a seed-eating ant: *Brachyponera senaarensis*. Entomologia Experimentalis et Applicata 72: 145 – 155.

Levieux J and Diomande T, 1978. La nutrition des fourmis granivores. II. Cycle d'activité et régime alimentaire de *Brachyponera senaarensis* (Mayr) (Hymenoptera, Formicidae). Insectes Sociaux 25: 187 – 196.

Mashaly AMA, Al-Mekhlafi FA and Al-Qahtani AM, 2013. Foraging activity and food preferences of the samsum ant, *Pachycondyla sennaarensis*. Bulletin of Insectology 66: 187 – 193.

Noirot C and Quennedey A, 1974. Fine structure of insect epidermal glands. Annual Review of Entomology 19: 61 – 80.

Nunes HN and Camargo-Mathias MI, 2005. Study of the venom glands in *Ectatomma quadridens* (Hymenoptera, Formicidae). Evolutionary hypothesis in the subfamily Ponerinae. Sociobiology 45: 949 – 966.

O'Connor R and Peck ML, 1978. Venoms of Apidae (Bettini S, ed.), Springer, Berlin, Heidelberg, New York, 613 – 659.

Orivel J and Dejean A, 2001. Comparative effect of the venoms of ants of the genus *Pachycondyla* (Hymenoptera: Ponerinae). Toxicon 39: 195 – 201.

Ortiz G and Camargo-Mathias MI, 2006. Venom gland of *Pachycondyla striata* worker ants (Hymenoptera: Ponerinae). Ultrastructural characterization. Micron 37: 243 – 248.

Owen MD and Bridges AR, 1976. Aging in the venom glands of queen and worker honey bees (*Apis mellifera* L.): some morphological and chemical observations. Toxicon 14: 1 – 5.

Paknia O, 2006. Distribution of the introduced ponerine ant *Pachycondyla sennaarensis* (Hymenoptera: Formicidae) in Iran. Myrmecologische Nachrichte 8: 235 – 238.

Petrocelli I, Turillazzi S and Delfino G, 2014. The venom apparatus in stenogastrine wasps: Subcellular features of the convoluted gland. Arthropod Structure and Development 43: 457 – 468.

Schmidt JO, 2016. The Sting of the Wild. Johns Hopkins University Press, Baltimore, MD, 257 pp.

Schoeters E and Billen J, 1995a. Morphology and ultrastructure of the convoluted gland in the ant *Dinoponera australis* (Hymenoptera: Formicidae). International Journal of Insect Morphology and Embryology 24: 323 – 332.

Schoeters E and Billen J, 1995b. Morphology and ultrastructure of a secretory region enclosed by the venom reservoir in social wasps (Hymenoptera, Vespidae). Zoomorphology 115: 63 – 71.

Touchard A, Aili SR, Fox EGP, Escoubas P, Orivel J, Nicholson GM and Dejean A, 2016. The biochemical toxin arsenal from ant venoms. Toxins, 8: 30.

Wetterer JK, 2013. Geographical spread of the samsum or sword ant, *Pachycondyla (Brachyponera) sennaarensis* (Hymenoptera: Formicidae). Myrmecological News 18: 13 – 18.