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Morphology and Histochemistry of the Venom Apparatus in Different Castes of the Ant *Ectatomma vizottoi* (Formicidae: Ectatomminae)

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Abstract

The elimination of toxins via a venom gland by some ant species is a component of a larger mechanism for capturing prey and defense. The present study describes the morphology and histochemistry of the venom apparatus of different castes of the ant *Ectatomma vizottoi* Almeida, 1987. Morphologically, the venom apparatus of queens, gynes and workers of *E. vizottoi* are similar and composed of the sting apparatus and three distinct portions: two secretory portions (convoluted gland and secretory filament), and a storage portion (reservoir) - a hollow sting apparatus covered in the terminal portion of the reservoir by a sclerotized cuticle. The venom gland of *E. vizottoi* is longer in queens and gynes than in workers. Furthermore, the epithelium of the convoluted gland is taller in the glands of queens and gynes than in workers, which may be indicative of greater toxin synthesis, or may be related to different body sizes of the castes. The morphology and histology of the venom apparatus reflect those of a generalist, while the histochemical tests indicated that this structure has the same chemical content of lipids, proteins and polysaccharides among queens, gynes, and workers. Images obtained by confocal microscopy and scanning electronic microscopy reveal a muscle layer surrounding the reservoir that is interlaced with secretory filaments, which fixes, in a manner, the filaments in place. This musculature serves to eject stored venom, which likely leads to greater success in defense or foraging.

Introduction

The sting apparatus is an important structure of species of Aculeata (stinging Hymenoptera); its effectiveness is reflected in the thousands of species that mimic the species who possess it (Grimaldi & Engel, 2005). The sting apparatus originally evolved to capture prey, however, some species only use it for defense. Ants use the sting apparatus to inject venom while hunting for food or defending their colonies (Grimaldi & Engel, 2005).

The hymenopteran sting apparatus has two main portions associated with exocrine glands called the Dufour gland (Dufour, 1841) and the venom gland (Abdalla & Cruz-Landim, 2001; Gullan & Cranston, 2014). The first is commonly described as a single epithelial sac enveloped by muscle, tracheoles and nerves that, in ants, opens at the base of the venom apparatus with the release of its contents not occurring together with venom (*e.g.* Hermann & Blum, 1967a,b; Landolt & Akre, 1979; Billen, 1987; Abdalla & Cruz-Landim, 2001). On the other hand, the venom gland has an ectodermal origin and its organization is the same in all Hymenoptera studied so far, with a muscular-chitinous structure that ends in a venom sac that opens at the base of the sting apparatus, which allows venom release instantaneously with a sting (Abdalla & Cruz-Landim, 2001; Britto & Caetano, 2005; Gullan & Cranston, 2014).

Venom glands of several species of the basal ant subfamilies have already been studied, including members of

Ponerinae [*Dinoponera australis* Emery, 1901 (Schoeters & Billen, 1995), *Neoponera (=Pachycondyla) villosa* (Fabricius, 1804) (Henrique et al., 2011) and *Pachycondyla striata* Smith, 1858 (Ortiz & Camargo-Mathias, 2006)] and Paraponerinae [*Paraponera clavata* (Fabricius, 1775) (Hermann & Blum, 1966)]. There have also been studies with derived ants, such as species of Dolichoderinae [*Azteca* Forel, 1878, *Bothriomyrmex* Emery, 1869, *Dolichoderus* Lund, 1831, *Iridomyrmex* Mayr, 1862 and *Tapinoma* Förster, 1850 (Billen, 1986a)]; Formicinae [*Camponotus* Mayr, 1861, *Cataglyphis* Förster, 1850, *Formica* Linnaeus, 1758, and *Lasius* Fabricius, 1804 (Schoeters & Billen, 1998)]; Amblyoponinae [*Amblyopone* Erichson, 1842, *Mystrium* Roger, 1862, *Onychomyrmex* Emery, 1895 and *Prionopelta* Mayr, 1866 (Schoeters et al., 1999)]; and Myrmicinae [*Myrmica rubra* (Linnaeus, 1758) (Billen, 1986b)]. However, to our knowledge, there have been only two studies of the venom gland in Ectatomminae; one with *Rhytidoponera* sp. (Robertson, 1968) and one with *Ectatomma brunneum* Smith, 1858 (= *quadridens*) (Nunes & Camargo-Mathias, 2005).

The genus *Ectatomma* is endemic to the Americas and is distributed from Mexico to Argentina, with 14 extant species that are important for natural biological control of pests in tropical agriculture because they are predators (Fernández, 1991; Delabie et al., 2007; Bolton, 2016). Thus, the study of the biology of species of this genus is indispensable for understanding their intra- and interspecific interactions.

Behavioral differences between queens and virgin gynes of *E. vizottoi* Almeida, 1987 have been documented in laboratory conditions. The queens preproduce and are the only caste able to produce viable eggs, while virgin gynes perform activities similar to workers, such as foraging and brood care (Vieira et al., 2012). The species remains poorly studied, with only aspects of its behavior, immature stages and occurrence in Brazil being reported (*e.g.* Martins et al., 2006; Vieira et al., 2007, 2009, 2010, 2012; Lima & Antonialli-Junior, 2013). Since these ants are generalists, although preying mostly on other ants (Lima & Antonialli-Junior, 2013), and the queens apparently do not prey frequently, it is expected that there would be differences in the morphology of the venom apparatus among castes. Thus, the aim of this study was to describe the morphology and histochemistry of the venom apparatus of the workers and queen castes, including gynes, of *E. vizottoi*.

Material and Methods

In order to acquire queens, gynes and workers of *E. vizottoi*, three colonies of this species located on the campus of Universidade Estadual de Mato Grosso do Sul in Dourados, Mato Grosso do Sul, Brazil (22º 13′ 16″ S; 54º 48′ 20″ W) were excavated and 66 ants collected with tweezers. The ants were transported to the Scanning Electron Microscopy and Histology laboratories of the Biology Department, Bioscience Institute, Universidade Estadual Paulista (UNESP), Rio Claro, São Paulo, Brazil where the present study was performed. The collection and transport of specimens were authorized by the Biodiversity Authorization and Information System (SISBIO) license No. 3187422).

The ants were counted and, after assessment of ovaries and spermathecae, were identified as workers (n = 36), queens (n = 20), and gynes (n = 10). For venom gland extraction, ants were cold-anesthetized at 4 ºC for 2 min and then placed in Petri dishes with a saline solution for insects (0.128 M NaCl, 0.016 M Na_2HPO_4, and 0.019 M KH_2PO_2 at pH 7.2). The venom apparatuses were dissected under a stereomicroscope with the aid of dissecting forceps and microsurgical scissors.

Morphology
Harris hematoxylin technique and aqueous eosin (according to Junqueira & Junqueira, 1983)

Twelve venom glands (six of workers, four of queens and two of gynes) were used for morphological assessment using light microscopy. After dissection, the venom glands were immediately fixed in 4.0% paraformaldehyde for 2 h, and rinsed twice in phosphate buffer (0.1 M, pH 7.4). The material containing the venom glands was dehydrated in a graded ethanol series (70, 80, 90, and 95%) for 15 min in each concentration. The dehydrated material was then embedded in resin for 24 h and, finally, transferred to plastic molds previously filled with resin and catalyst. After polymerization, the material was sectioned at 4 μm thickness with the aid of a Leica RM 2145 microtome. Histological sections were hydrated for 1 min in distilled water, stained with hematoxylin for 10 min and then immersed in a cuvette with water for 4 min. The samples were then rinsed with tap water to allow the reaction to occur. The sections were then stained with eosin for 5 min and washed with tap water. After the slides with the sections were dried, they were covered with Canada balsam and cover slips, and examined and photographed under a Motic BA 300 photomicroscope connected to a computer.

Scanning electron microscopy (SEM)

For scanning electron microscopy (SEM), a total of six venom glands of workers were mounted and fixed in Karnovsky's solution for 24 h, dehydrated in a series of acetone solutions from 50 to 95%, and washed in two baths of 100% acetone for 5 min each. The material was critical point dried and sputter-coated with carbon-palladium. Images were obtained with a PHILIPS SEM 505 scanning electron microscope.

Histochemistry
Bromophenol blue technique (Pearse, 1985)

Twelve venom glands (six of workers, four of queens and two of gynes) were used for detection of total protein. After extraction, the venom glands were fixed with 4%

paraformaldehyde in 0.1 M Phosphate Buffered Saline (PBS) for 24 h. Histological sections, as previously described, were stained with bromophenol blue for 1 h at room temperature and immersed in an aqueous solution of 0.5% acetic acid for 5 min and tertiary butyl alcohol for 5 min. Slides were cleared in xylol and mounted in Canada balsam for later examination and photographic documentation using a Motic BA 300 photomicroscope connected to a computer.

PAS/Alcian blue technique (Junqueira & Junqueira, 1983)

Another set of venom glands (six of workers, four of queens and two of gynes) were used for polysaccharide detection. After extraction, the venom glands were fixed with Bouin solution. Histological sections, as previously described, were then stained with Alcian blue (pH 2.5) for 30 min. The material was then washed with distilled water and immediately transferred to a solution of 0.4% periodic acid for 5 min and then the Schiff's reagent for 30 min (in the dark). The material was washed with a sulfurous solution for 1 min and then with tap water for 5 min.

Slides were dried, cleared in xylol, and mounted in Canada balsam for later examination and photographic documentation using a Motic BA 300 photomicroscope connected to a computer.

Nile blue technique (Lison, 1960)

Twelve venom glands (six of workers, four of queens and two of gynes) were used for lipid detection. After extraction, the venom glands were fixed with calcium formol. Histological sections were stained with Nile blue for 5 min at 37 °C, washed in tap water and immersed in 1% acetic acid for 1 min. After drying, the sections were mounted in glycerinated gelatin and covered with a cover slip for observation and photographic documentation using a Motic BA 300 photomicroscope connected to a computer.

Immunofluorescence

Twelve venom glands (six of workers, four of queens and two of gynes) were used for reservoir musculature study. After extraction, the venom glands were fixed with 4% paraformaldehyde for 24 h. The whole mounted was then washed twice for 5 min with PBS. The sections were then permeabilized with Triton X-100 (0.1%) for 30 min and incubated with PBS + Bovine Serum Albumin (BSA), for blocking non-specific staining, for 2 h. The samples were then incubated in Alexa Fluor 488 phalloidin (5 µL of Alexa Fluor phalloidin + 200 µL de PBS + 2 µL de BSA) (Molecular Probes, USA) for 30 min at room temperature and in the dark for F-actin detection. The material was then washed twice for 5 min each with PBS. Next, the samples were stained with 4',6-diamidine-2'-phenylindole dihydrochloride (DAPI, Molecular Probes, USA) (1:500) for 10 min. The material was washed numerous times in PBS buffer, after which it was mounted with ProLong® Gold antifade reagent and analyzed in whole mounts using a Leica TCS SP5II confocal laser scanning microscope (CLSM), with the fluorescent images being obtained using a laser in the wavelengths of 405 nm and 488 nm. Optical sections were obtained in suitable sectioning stepsize of the Z axis (0.63 µm and 0.67 µm). Different modes of the CLSM were used in the confocal analysis, including maximal projection.

Results

Morphology

The venom apparatuses of *E. vizottoi* queens, gynes and workers are similar and composed of three distinct portions and the sting apparatus: two secretory portions - a convoluted gland (Figs 1, 2B-C, 5C-D) and the secretory filament coming out of it (Figs 1, 2D-E, 3A, and E-F, 6B and E); and a storage portion - a sac-shaped translucent reservoir (Figs 1, 2A and E, 3A-F, 5C-D). The hollow sting apparatus is involved in the terminal portion of the reservoir by a sclerotized cuticle (Figs 1, 2F). The length of the sac was found to vary among castes with it being 1.650 ± 0.34 µm in queens/gynes and 1.500 ± 0.13 µm in workers.
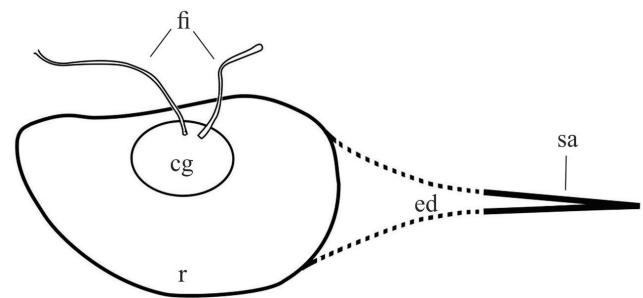

Fig 1. Schematic drawing of the venom apparatus of *Ectatomma vizottoi* with secretory filament (fi), reservoir (r), convoluted gland (cg), excretory duct (ed), and a hollow sting apparatus (sa) involved by a sclerotized cuticle. Scale = 1mm."

The convoluted glands of the three castes are oval in shape and located in the internal part of the reservoir (Figs 1, 2B-C, 5C-D). The wall of the reservoir is delimited by simple epithelium lined by a cuticle (Fig 2A), with taller epithelium in the glands of queens and gynes than in workers (Figs 2B and E).

The epithelium comprises columnar cells in the bifurcation of the initial portion of the convoluted gland, in which its basal domain is narrow, and its lateral domain is high and becomes completely columnar in the convoluted gland and internally lined by a cuticle (Fig 2B). There is a granular secretion in the lumen (Figs 2B-C).

The two of secretory filaments are composed of cuboidal cells and are elongated in shape, with no secretion observed in their lumen (Fig 2D). The nucleus of the secretory cells can be seen in the secretory filament in queens and gynes (Fig 2E).
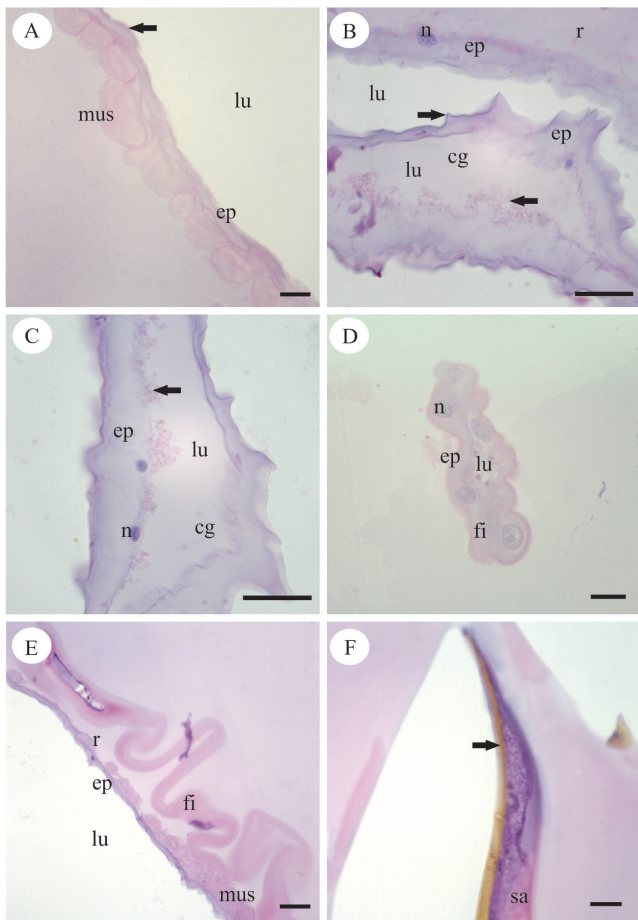
Fig 2. Photomicrographs of the venom apparatus of queens, gynes and workers of the ant *Ectatomma vizottoi*. A – Detail of the reservoir wall showing the simple cuboidal epithelium (ep), the cuticle (arrow) toward the lumen (lu) and the musculature (mus) that involves the reservoir in worker ants. B-C – Detail of the convoluted gland (cg) present in the venom gland of queens showing its thick epithelium (ep), lumen (lu) with secretion (arrow), cuticle covering the inside (arrow), reservoir (r) with simple cuboidal epithelium (ep), and its respective elongated nucleus (n) in a queen. D – Detail of the secretory filament (fi), showing the secretory epithelium (ep), its nucleus (n) and its lumen (lu) in a worker. E – Detail of the reservoir wall (r) of the venom gland of a queen, and its simple epithelium (ep), lumen, (lu), secretory filament (fi) and musculature (mus) covering the epithelium in a gyne. F – The hollow sting apparatus (sa) of a worker covered by a cuticle (arrow). Scale of all the images = 20 μm.

Table 1. Results of the histochemical tests on the venom gland of queens, gynes and workers of the ant *Ectatomma vizottoi*.

Portion (epithelium)	Bromophenol blue (proteins)		Nile blue (lipids)		PAS (polysaccharides)	
	Queen/ gyne	Worker	Queen/ gyne	Worker	Queen/ gyne	Worker
reservoir	+++	+++	+++	+++	+++	+++
convoluted gland	++	++	++	++	++	++
secretory filament	+++	+++	+++	+++	+++	+

(+++) strongly positive; (++) moderately positive; (+) weakly positive.

Overall, the nucleus of the epithelium of the reservoir is cylindrical, with musculature externally (Figs 2D, 3B-D) and a thin layer of cuticle internally, where a secretion (probably venom) was observed in the lumen. Well-developed muscle tissue is present in the proximal region of the duct, which conducts venom to the sting apparatus and to the remaining part of the reservoir. In general, the muscle tissue is in circular layers surrounding the reservoir (Figs 3B-D).

Histochemistry

The reservoir, convoluted gland, secretory filaments, and stinger of the venom apparatus all had the same chemical content in queens, gynes and workers, which reacted positively for lipids, proteins, and polysaccharides (Table 1).

The reservoir epithelium of queens, gynes and workers stained strongly for lipids (Figs 4A-B), proteins (Fig 4D), and polysaccharides (Fig 4E). The convoluted gland, however, exhibited a moderate reaction for lipids, proteins, and polysaccharides in gynes, queens and workers (Fig 4C).
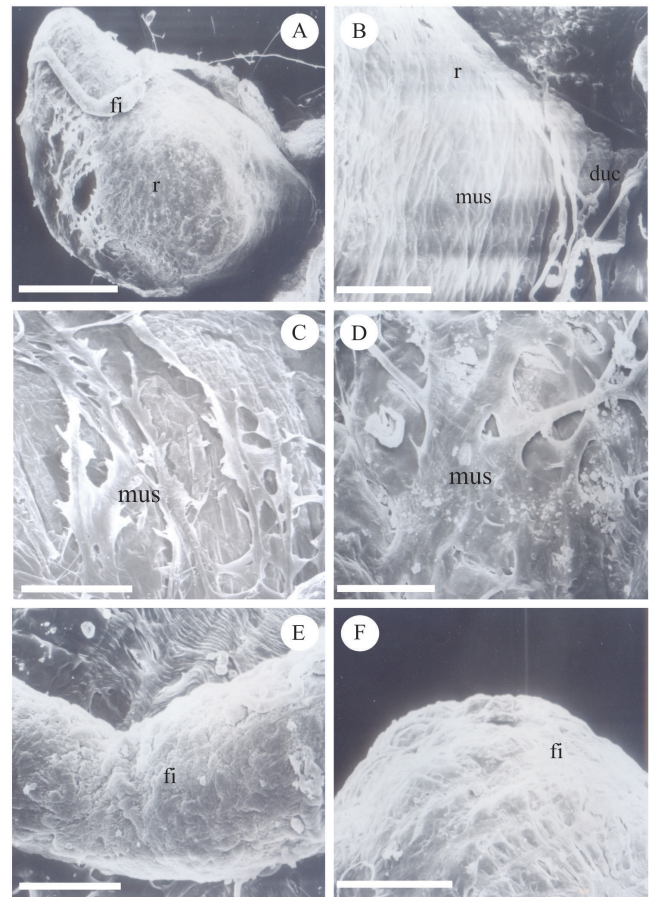

Fig 3. Scanning electron micrographs of the venom apparatus of workers of the ant *Ectatomma vizottoi*. A – Venom gland reservoir (r) and secretory filament (fi) that originates in the convoluted gland. Scale = 0.05 mm. B – Reservoir epithelium (r) covered by thick muscles (mus) close to the region of the duct (duc) that carries venom to the sting apparatus. Scale = 0.1 mm. C-D – Detail of the musculature (mus) that surrounds the reservoir epithelium. Scale = 0.05 mm and 0.1 mm. E-F – Detail of the secretory filament (fi) that originates in the convoluted gland. Scale = 0.05 mm and 0.1 mm.

The secretory filaments were strongly positive for all histochemical tests (Figs 4B and D) (Table 1); their lumen possessed a lipid secretion (Fig 4B). Polysaccharide secretion was observed inside the sting apparatus (Fig 4F).

Immunofluorescence

The venom apparatus of workers stained positively (green) for filaments of actin (striated muscle), which surround the reservoir (Figs 5A and C-D, 6A and D), with the muscles interlacing with the secretory filaments (Figs 5C and F, 6D).
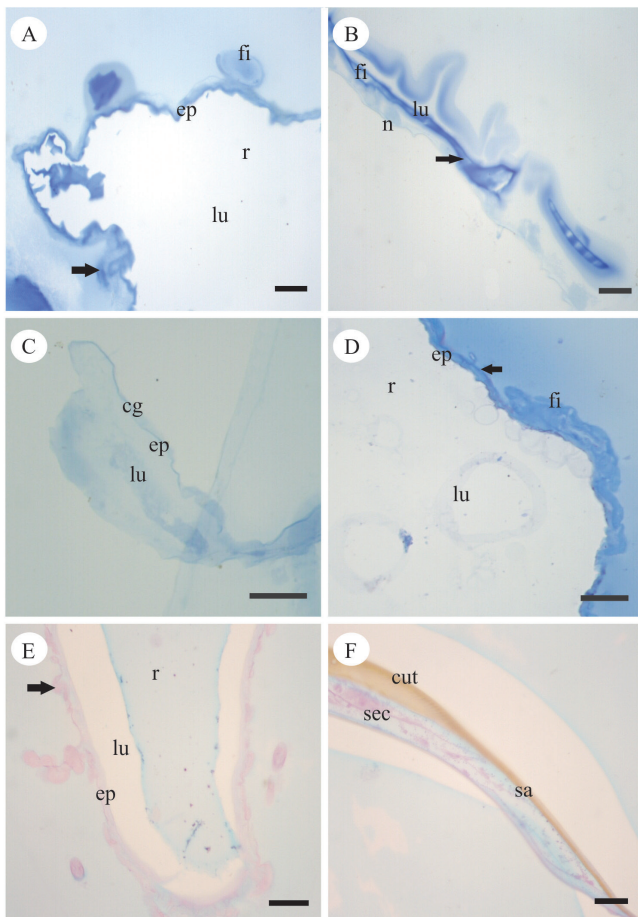

Fig 4. Histochemistry of the venom apparatus of queens, gynes and workers of *Ectatomma vizottoi*. A – Detail of the reservoir wall (r) showing the simple cuboidal epithelium (ep), the reservoir lumen (lu) and the musculature (arrow) that covers the reservoir epithelium and the secretory filament (fi) in a worker. Reaction: Nile blue for lipid detection. B – Detail of the secretory filament (fi), its columnar epithelium (arrow), and its nucleus (n). A large quantity of secretion is present inside the lumen (lu) of the filament of a worker ant. Reaction: Nile blue. C – Detail of the convoluted gland (cg) of a queen showing its thick epithelium (ep) and its lumen (lu). Reaction: Nile blue. D – Detail of the reservoir wall (r) showing the simple epithelium (ep), the reservoir lumen (lu) and the musculature (arrow), that covers the reservoir epithelium and the secretory filament (fi) in a queen. Reaction: Bromophenol blue for protein detection. E – View of the reservoir wall (r) showing the simple epithelium (ep), the reservoir lumen (lu) and the musculature (arrow), that covers the reservoir epithelium in a worker. Reaction: PAS for polysaccharide detection. F – Sting apparatus (sa) covered by cuticle (cut), showing the hollow interior with secretion granules (sec), in a worker. Reaction: PAS for polysaccharide detection. Scale of all the images = 30 μm.
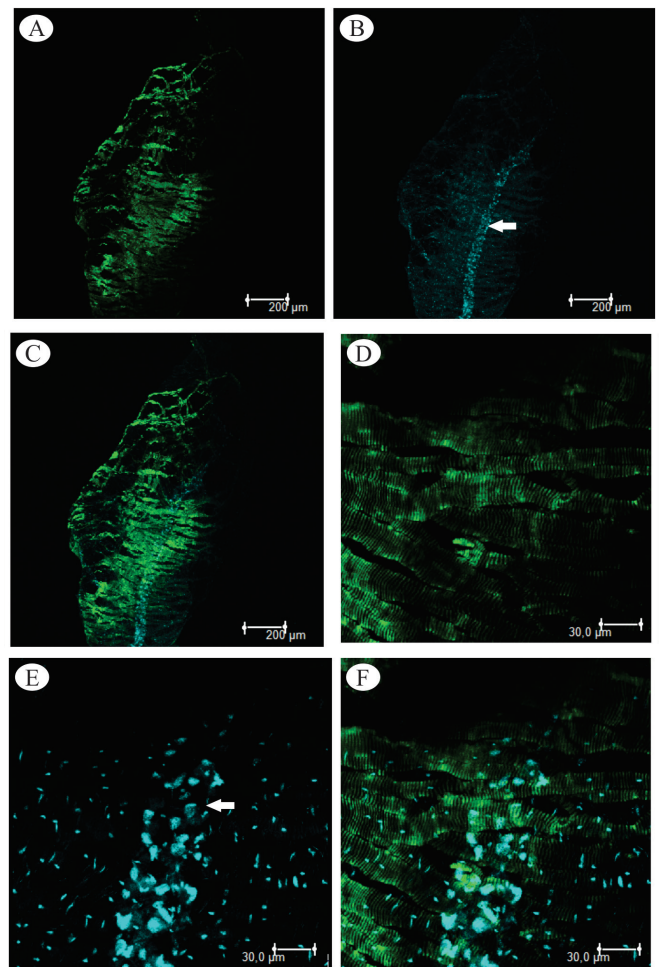

Fig 5. Confocal laser-scanning micrographs of the venom apparatus of *Ectatomma vizottoi* workers. A and D – Detail of the striated musculature surrounding the reservoir with the actin filaments stained (green). B and E – Detail of the nuclei (blue) of the reservoir (spherical), of the secretory filament (arrow) (voluminous nucleus) and of the musculature (elongated). C and F – Maximal projection showing the musculature (green) and the nuclei (blue) of the reservoir (spherical), of the secretory filament (voluminous rounded) and of the musculature (elongated).

The reservoir exhibited small spherical nuclei (blue) (Figs 5B-E, 6B), typical of simple epithelium. The secretory filaments exhibited voluminous rounded nuclei (arrow, blue) (Figs 5B-C and E-F, 6B) typical of columnar cells. The muscle had elongated nuclei (blue) (Figs 5E-F).

Discussion

In general, the venom apparatuses of queens, gynes and workers of *E. vizottoi* are similar and have three distinct portions in addition to the sting apparatus: the secretory portion (composed of one secretory filament, which is distally bifurcated); the internal secretory portion, represented by the convoluted gland; and the storage portion, represented by the sac-shaped reservoir with a proximally located excretory duct. These structures are similar to the structures previously described for Ectatomminae in *E. brunneum* (Nunes &
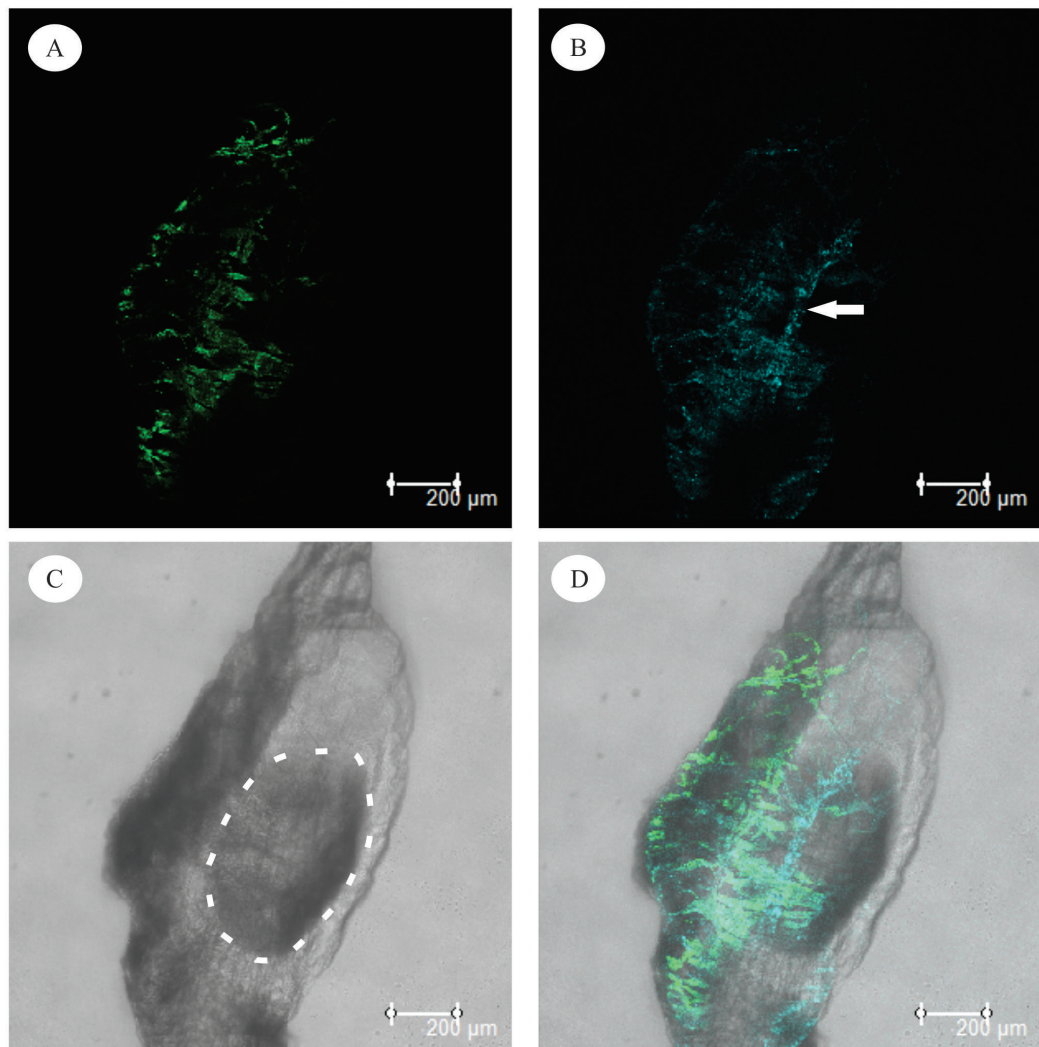
Fig 6. Confocal laser-scanning micrographs of the venom apparatus of *Ectatomma vizottoi* workers. A – General view of venom gland reservoir with staining (green) of the actin filaments (musculature) surrounding it. B – General view of the venom gland reservoir with staining (blue) of the reservoir nucleus, of the musculature and of the secretory filaments (arrow) of the convoluted gland. C – View of the reservoir without staining (DIC), with detail of the convoluted gland (dashed circle). D – Maximal projection of the reservoir showing the convoluted gland (dark structure inside the reservoir) (DIC) and the musculature – actin filaments (stained green), and nucleus (stained blue).

Camargo-Mathias 2005), as well as for Ponerinae in *N. villosa* (Henrique et al., 2011) and *P. striata* (Ortiz & Camargo-Mathias, 2006), and Paraponerinae in *P. clavata* (Hermann & Blum, 1966). Despite *E. vizottoi* frequently preying on other ant species (Lima & Antonialli-Junior, 2013), its venom apparatus does not seem to be different from those species that are generalist predators and can forage solitarily and prey on other arthropods, including other ants (Young & Hermann, 1980; Lachaud et al., 1984; Overal, 1986; Giannotti & Machado, 1992; Medeiros & Oliveira, 2009), and so its feeding preference is not decisive for its morphology.

The portions of the venom apparatus of *E. vizottoi* are also similar to that of other ants, such as the genera *Azteca*, *Bothriomyrmex*, *Dolichoderus, Iridomyrmex* and *Tapinoma* of the Dolichoderinae (Billen, 1986a), and *Camponotus, Cataglyphis*, *Formica* and *Lasius* of the Formicinae (Schoeters & Billen, 1998), which are phylogenetically closely related to Ectatomminae (Moreau & Bell, 2013).

On the other hand, Schoeters et al. (1999) reported that ants of the Amblyoponinae, which are phylogenetically distant from Ectatomminae, (*i.e. Stigmatomma reclinatum* (Mayr, 1879), *Mystrium camillae* Emery, 1889, *Prionopelta kraepelini* Forel, 1905 and *Onychomyrmex hedleyi* Emery, 1895) do not have a convoluted gland, suggesting some variation in the venom apparatus among ant species.

We found that the structures of the venom apparatus of queens and gynes of *E. vizottoi* are larger than those of workers and have taller convoluted gland epithelium, which is indicative of greater toxin synthesis. Thus, the results demonstrate that different castes have different venom apparatus morphology and probably different toxicity. However, these differences in sizes may simply be proportional to body size, since queens are noticeably larger than workers in this species, as has been previously described by Vieira et al. (2009). However, Billen (1986b) did not notice significant differences among the venom apparatus of the different castes

of *M. rubra*, except for the size of the reservoir, which was smaller in queens. Therefore, there seems to be no consistent pattern concerning venom apparatus size among ants. In the genus *Ectatomma*, workers and gynes are likely capable of performing the behaviors of defense and hunting. Vieira et al. (2012) described a system of functional monogyny for *E. vizottoi* with only one fertile queen and the virgin gynes performing tasks inherent to the behavioral repertoire of workers, which would require a functional venom apparatus in queens and gynes as reported in this study.

A simple epithelium delimits the reservoir of the venom apparatus of *E. vizottoi*, which is also covered by a thick cuticle, identical to what was observed for *E. brunneum* (Nunes & Camargo-Mathias, 2005) and similar to that of *P. striata* (Ortiz & Camargo-Mathias, 2006) and *N. villosa*, which have a reservoir with thin walls covered internally by a thick cuticle (Henrique et al., 2011). Some ants of the Dolichoderinae also have a thin layer of epithelium on the wall of the reservoir (Billen, 1986a). Moreover, Billen (1986b) noted that the reservoir of *M. rubra* (Myrmicinae) is covered by a thin glandular epithelium composed of a layer of secretory cells constituting the highest part of the wall (polygonal cells with large, rounded nuclei), and by a thin cuticle. This suggests that the reservoir of the venom apparatus of ants of the genus *Ectatomma* has a simple epithelium that does not have a secretory role, as reported by Billen (1986b) for *M. rubra*, which has secretory cells but lacks a convoluted gland for venom secretion.

The thin cuticle might function to protect the reservoir against the action of the venom synthetized in the convoluted gland and the secretory filaments, as reported by Schoeters and Billen (1995) for *D. australis*. Thus, even though Myrmicinae, Dolichoderinae and Ectatomminae ants are phylogenetically close, and despite of some similarities of their venom apparatuses, they exhibit differences in their reservoirs, which in some cases are significant. In the same way, ants of the genus *Ectatomma* share features with previously studied phylogenetically distant ants, even in some cases belonging to another clade, but sharing similar behaviors or habits and have evolved a sting apparatus (see Touchard et al. (2016) regarding the relationships among stinging and non-stinging subfamilies).

The convoluted gland of *E. vizottoi* is oval in shape with columnar cells, covered by a thin cuticle, and has secretory granules in its lumen and a secretory filament in its wall with cuboidal cells. Schoeters and Billen (1995) found an epithelial layer surrounding the convoluted gland of *D. australis*, which was composed of flattened cells with elongated nuclei. Hermann and Blum (1966) reported that in *P. clavata* the convoluted gland and free venom secretory filaments seem to be anatomically part of the same gland with columnar and cuboidal cells.

On the other hand, the convoluted gland of *E. vizottoi* differs from that of *D. australis*, which is a flat-sac covered by epithelium with an elongate invaginated projection of the excretory tube in the venom apparatus reservoir that forms a structure similar to an umbrella inside the reservoir (Schoeters & Billen, 1995). Moreover, the convoluted gland of *P. striata* is formed by polyhedral cells and also resembles an umbrella (Ortiz & Camargo-Mathias, 2003, 2006). Thus, the convoluted gland of *E. vizottoi* differs from the oval-shaped convoluted gland with columnar and cuboidal cells of the more phylogenetically distant Ponerinae (i.e. *Dinoponera* and *Pachycondyla* Smith, 1858) and from the more phylogenetically close Myrmicinae (*M. rubra*), which does not have a convoluted gland (Billen, 1986b).

Therefore, the convoluted gland of Ectatomminae seems to have some characteristics similar to more derived ants and others similar to more basal groups. Columnar cells have a high secretory capacity (Junqueira & Carneiro, 2008), suggesting that the predatory nature of this species requires a venom apparatus with a high capacity for toxin secretion.

The venom apparatus and the epithelium of the reservoir in queen, gynes and workers of *E. vizottoi* reacted positively for lipids, proteins, and polysaccharides, while the convoluted gland exhibited only a moderate reaction. This differs from the filaments, which reacted positively to all histochemical tests and possessed a lipid secretion in its interior, and granules and polysaccharide secretion in the sting apparatus. Thus, secretory filaments seem to be responsible for the absorption of lipids, proteins, and polysaccharides from the hemolymph. Consequently, these substances can be metabolized into venom by the convoluted gland, as this gland possessed a smaller quantity of these substances compared to the secretory filaments.

The histochemical techniques applied in this study revealed no protein inside the reservoir, but peptides may yet be part of the secretion. However, in ponerine ants, such as *P. striata* and *N. villosa*, there are proteins in the secretion stored inside the reservoir, in the cytoplasm of cells of the convoluted gland and in the cytoplasm of the cells of the epithelium (Ortiz & Camargo-Mathias, 2003; Henrique et al., 2011). The presence of peptides (the dominant component in animal toxins) has previously been reported in the venom of Amblyoponerinae, Dorylinae, Ectatomminae, Myrmeciinae, Myrmicinae, Paraponerinae, Ponerinae and Pseudomyrmecinae (Cologna et al., 2013; Touchard et al., 2015, 2016). Moreover, the presence of peptides in the venom of the wasp *Polybia paulista* (Ihering, 1896) was recently reported by Leite et al. (2015). Furthermore, it is known that ant toxins vary according to species and potent neurotoxic peptides have been isolated from conspecifics of *E. vizottoi*, for example for *Ectatomma tuberculatum* (Olivier, 1792) and *E. brunneum* (Aili et al., 2014). We think that toxin variation among the castes of *E. vizottoi* might occur since the castes of this predatory ant perform different behavioral tasks, with gynes performing tasks similar to workers and queens only laying eggs. More specific chemical techniques may confirm differences in venom toxins among the different castes of this species.

It is noteworthy that, using confocal microscopy, the present study documented the occurrence of a layer of circular muscles surrounding the reservoir and, for the first time, that these layers interlace with the secretory filaments, resulting in a manner of fixing them in position. In other studies, such as for the ant *P. clavata*, for example, the outer surface of the venom apparatus reservoir is slightly crinkled, although the inner surface possesses wavy projections (Hermann & Blum, 1966). Schoeters et al. (1999) noted that in *S. reclinatum* there is a series of muscles that run parallel to the longitudinal axis of the reservoir. Nunes and Camargo-Mathias (2005) found the wall of the reservoir of *E. brunneum* to be externally covered by muscle and internally by a cuticle. These observations suggest that the circular musculature that involves the venom apparatus reservoir of *E. vizottoi*, and interlaces with the secretory filaments, has a role in ejecting stored venom, which would presumably lead to greater success in stinging for defense or hunting by workers and gynes of this species. For *Rhytidoponera* sp. (Ectatomminae) a short length of filament is incorporated in the reservoir wall, which can also ensure direct control over expansion and contraction of the sac (Robertson, 1968).

Conclusions

The venom apparatus of *E. vizottoi* differs from some ant subfamilies but is similar to that of others. It also differs in size among castes, being longer and with taller epithelium of the convoluted gland in queens and gynes than in workers, which suggests differences in venom composition among the castes or may only be related to body size. The morphology and histology of the venom apparatus reflect that of a generalist species, and the histochemical test showed the same chemical content for lipids, proteins and polysaccharides among castes.

Confocal microscopy revealed layers of musculature surrounding the reservoir and interlaced with the secretory filaments, which fixes, in a manner, the filaments in place. This musculature serves to eject stored venom, which likely leads to greater success in defense or foraging.

Acknowledgements

Authors' contribution

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