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Short communication

Morphological and histological characterization of production structures, storage and distribution of venom in the parasitic wasp *Bracon vulgaris*

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ABSTRACT

It was described the morphology and histological composition of the structures related to production, storage and distribution of *Bracon vulgaris* venom, a wasp that parasite their hosts after the inoculation of a venom which causes irreversible paralysis. Were found 22 glandular filaments, coated with secretory epithelium associated with a reservoir coated internally by a chitin layer and externally by striated muscular fibers. A valve mediates the passage of the toxin to venom duct towards the parasitoids sting.

Insect toxins are currently identified as potential molecules with pharmacological use (Moreau and Guillot, 2005; Hoshina et al., 2013). Moreover, when isolated and accurately identified, the venom of an insect can be the basis for developing effective and selective insecticides, encouraging pest control programs (Beckage and Gelman, 2004). The wasp venom, being a multiple compound, can cause various effects to the affected host, including, cytolytic and neurotoxic effects (Ergin et al., 2006; Er et al., 2011). Thus, the identification of secretory structures, isolation of toxins and then predict their major proteins are of great value to understand the effects of the interaction toxin/host; in example, Cunha et al. (2005) and Wang et al. (2008) reported, respectively, the anticonvulsant and anti-tumor action of peptides isolated from the venom of the *Polybia* genus wasp.

Bracon vulgaris Ashmead (Hymenoptera: Braconidae) is a parasitoid wasp whose adult females parasitize their hosts by venom inoculation, not yet precisely identified, while taking oviposition. The interaction with the venom leads the host to irreversible paralysis, preventing their development and, subsequently, causing their death (Alves et al., 2014). Therefore, it is assumed that this wasp toxin may represent a potentially valuable source on the development of molecules with biological activity for the control of herbivorous, since, naturally, the accomplished parasitic rates by this wasp is greater or equal to 57% of mortality (Toscano and Carvalho, 2000).

However, to use this venom is necessary to previously know the venom apparatus of this species to understand how does the production occur, storage and use of the toxin for these parasitoid wasps. So the aim of this study is to assess and describe the structural organization and the histological constitution of the structures involved in the secretion, storage and distribution of *B. vulgaris* venom.

For morphological analysis of the venom apparatus of *B. vulgaris*, adult females (n = 30) 5 days of age, mated, and fed without parasitism experience were immobilized at low temperature (4 °C) and subsequently dissected, with the assistence of an ophthalmic tweezers under a stereomicroscope to access the formation and location of the structures that make up the venom apparatus of this parasitoid wasp. The structures were then shot with iSight 8MP

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camera, and the measurements of length and diameter was performed using the program Image J V1.49p. Subsequently, the portion containing the venom reservoir, glandular filaments and the venom duct were isolated and fixed in 10% formalin for 24 h, dehydrated in increasing ethanol baths (70, 80 and 95%) for 10 min each. Finally, the material was embedded in historesin and the obtained cuts with 3 μm were subjected to staining techniques by Toluidine blue (generally colored) and Xylidine ponceau (total protein). Histological analysis was performed using a light microscope, Leica DM 500, and the images obtained by the LAS EZ Version 2.0.0 ICC50 program.

The venom apparatus of *B. vulgaris* is located internally in the posterior region of the abdomen of adult females. It is formed by an oval morphology venom reservoir surrounded by 22 elongated gland filaments in the form of tentacles, from this reservoir, follows a duct that communicates with the Dufour gland duct, which is anastomosed to form the venom duct, which flows into the sting (Fig. 1A). The venom reservoir had the following measures of 1.7 mm of length and 0.9 mm of diameter, while the glandular filaments measured approximately 2.5 mm long and 0.5 mm of diameter and ranging from 3.2 to 1.4 mm in length and 0.2 to 0.1 mm in diameter (Fig. 1B). The Dufour gland presented measures of 4.2 mm in length and 0.9 mm in diameter (Fig. 1C). According to the literature, the reservoir is an organ of storage and distribution of the toxin, while this gland is related to tagging the host that were already infected (Abdalla and Cruz-Landim, 2001; Moreau et al., 2009) (Fig. 1C).

The histological analysis showed that the venom reservoir of *B. vulgaris* presents internally coated by an internal helicoidal chitin layer and externally by a thick layer of striated muscular fibers longitudinally oriented, in the central region of the structure can be viewed the lumen, region of venom storage (2A and 2B). The glandular filaments have become coated with a single layer of epithelium composed of cubic secreting cells with spherical nucleus (Fig. 2C). The secreted product is then released in the central lumen through dense vesicles (Fig. 2C). *B. vulgaris* venom reservoir in its end portion has a valve made of muscular tissue (Fig. 2D and E) which mediates the passage of the toxin in the reservoir, to the venom duct (Fig. 2D), which in turn, also presents externally a thick muscle layer.

Analyzing the venom apparatus is of great importance to correlate the toxin production process with the wasp parasitism behavior: if they cause temporary or irreversible paralysis in their hosts and how they parasitizes, whether internally or externally (Barbalho and Penteado-Dias, 1997; Quicke, 1997); as well to elucidate the evolutionary paths followed by different taxas, as the morphology, insertion and arrangement of glandular filaments and venom reservoir, for example, are widely divergent in this group of insects (Peydró et al., 1996; Barbalho and Penteado-Dias, 1997; Vardal, 2006; Moreau et al., 2009).

Histological evaluation of the venom apparatus revealed it to be of ectodermal origin, type I (Edson and Vinson, 1979; Quicke, 1997), where the thick outer layer of striated muscular fiber and the chitin helix of the venom reservoir function combines to allow the passage of the toxin into the sting. The high amount of glandular filaments observed in *B. vulgaris*, 22, may be an evolutionary advantage, which could explain the success of parasitism of this species when compared to other wasps that compete with this by the host (Ramalho et al., 2009), presumably the amount of glandular filaments as well as a venom reservoir with a large capacity of storage, can promote more efficient paralyzing responses by presenting synthesis property and/or more quickly venom replacement.

The reduced body size, usually presented by the parasitoid wasps, is a limitation for researches focused on assessing the anatomy of their venom apparatus and then the applicability of its toxin. *B. vulgaris*, for example, measures approximately 3.29 mm in length and about 0.00512 μg/ml of toxic peptides were collected on each female (author's observations). This obstacle justify the wide divergence found on the number of researches performed with histological evaluations of venom-secreting structures in other larger venom producing animals (Taib and Jarrar, 1993; Kovoor and Muñoz-Cuevas, 2000; Jarrar and Al-Rowaily, 2008; Antoniazzi et al., 2009; Dehghani et al., 2010; Giannotti et al., 2013).

The scarcity of scientific bases with morphological and histological data of venom apparatus in parasitoid wasps disfavors the discovery of innovatives and potential molecules, as the toxins, on these Hymenoptera, constitutes a physiological mechanism developed over evolutionary course to evade the immune defenses of their insects hosts, thus presenting, patterns of specialization for

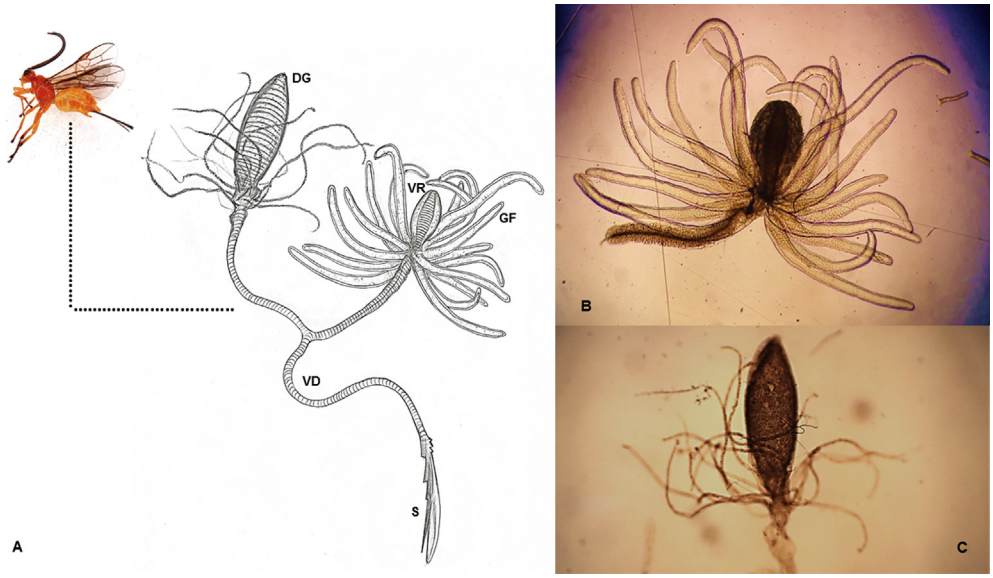

Fig. 1. Anatomy of *B. vulgaris* venom apparatus. A. Representation of the venom apparatus. B. Venom reservoir (VR) – 1.7 mm × 0.9 mm; and glandular filaments (GF) ≅ 2.5 mm × 0.5 mm. C. Dufour Gland (DG) – 4.2 mm × 0.9 mm. VD = venom duct and S = sting.

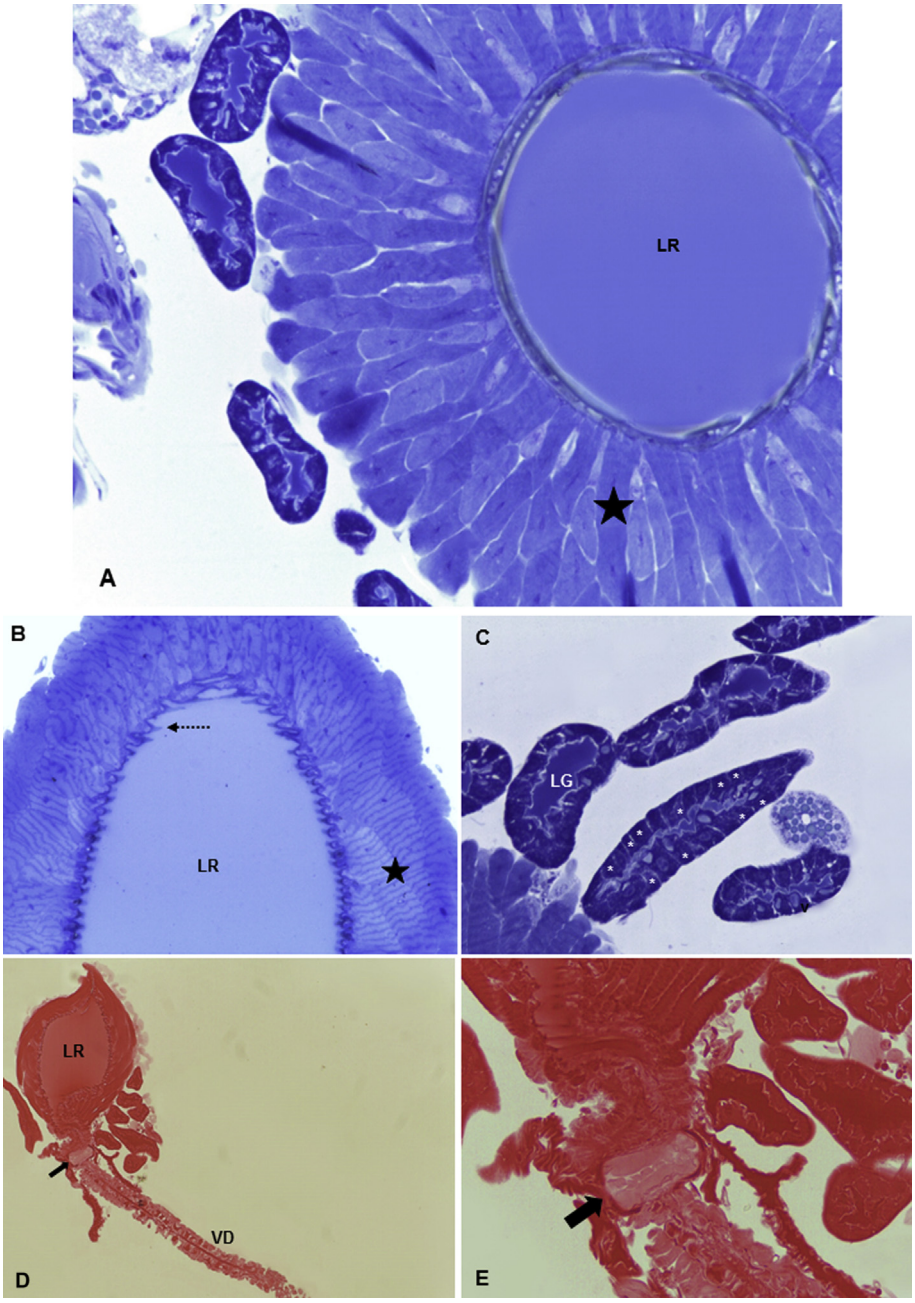

Fig. 2. Histology of production structures, toxin storage and distribution in the parasitoid wasp *Bracon vulgaris*. A. General provision of glandular filaments around the venom reservoir. Star – external muscle fibers. Toluidine blue, 100 μm. B. Venom reservoir. Internally coated by a helicoidal chitin layer (dashed arrow). LR – Reservoir's lumen, star – external muscle fibers. Toluidine blue, 20 μm. C. Glandular filaments. Epithelial (asterisk) arranged to circulate around the glandular filament, the secreted product is then released into the central lumen (LG). Presence of vacuoles (V) and cubic secreting cells (arrowhead). Toluidine blue, 20 μm. D. Muscular tissue valve (arrow) mediates the passage of the toxin, contained in the lumen of the reservoir (LR) the venom duct (VD). Xylidine ponceau, 100 μm. E. Highlight of the muscular valve (arrow). Xylidine ponceau, 20 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

immobilization and/or death of these (Beckage and Gelman, 2004; Moreau et al., 2009). Moreover, the lack of knowledge concerning the constitution of histological secreting venom cells and its correct location, may cause mechanical damage when the structures are handled incorrectly, as proposed Giannotti et al. (2013), becoming therefore necessary to previously know the organizational structure and composition of the venom apparatus in the studied body.

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References

Abdalla, F.C., Cruz-Landim, C., 2001. Dufour glands in the hymenopterans (Apidae, Formicidae, Vespidae): a review. Rev. Bras. Biol. 61 (1), 95–106.

Alves, T.J.S., Wanderley-Teixeira, V., Teixeira, A.A.C., Silva-Torres, C.S.A., Malaquias, J.B., Pereira, B.F., Cunha, F.M., 2014. Parasitoid-host interaction: sensory structures involved in the parasitism behavior of *Bracon vulgaris* (Hymenoptera: Braconidae). Anim. Biol. 64 (4), 365–381.

Antoniazzi, M.M., Pedroso, C.M., Knysak, I., Martins, R., Guizze, S.P.G., Jared, C., Barbaro, K.C., 2009. Comparative morphological study of the venom glands of the centipede *Cryptops iheringi, Otostigmus pradoi* and *Scolopendra viridicornis*. Toxicon 53, 367–374.

Barbalho, S.M., Penteado-Dias, A.M., 1997. Análise morfológica do aparelho de veneno nos Braconidae cyclostome (Hymenoptera). Rev. Bras. Zool. 14, 65–71.

Beckage, N.E., Gelman, D.B., 2004. Wasp parasitoid disruption of host development: implications for new biologically based strategies for insect control. Annu. Rev. Entomol. 49, 299–330.

Cunha, A.O.S., Mortari, M.R., Oliveira, L., Carolino, R.O.G., Coutinho-Netto, J., Santos, W.F., 2005. Anticonvulsant effects of the wasp *Polybia ignobilis* venom on chemically induced seizures and action on GABA and glutamate receptors. Comp. Biochem. Physiol. 141, 50–57.

Dehghani, H., Sajjadi, M.M., Parto, P., Rajaian, H., Mokhlesi, A., 2010. Histological characterization of the special venom secretory cells in the stinger of rays in the northern waters of Persian Gulf and Oman Sea. Toxicon 55, 1188–1194.

Edson, K.M., Vinson, S.B., 1979. A comparative morphology of the venom apparatus of female braconids (Hymenoptera: Braconidae). Can. Entomol. 111, 1013–1024.

Er, A., Uçkan, F., Rivers, D.B., Sak, O., 2011. Cytotoxic effects of parasitism and application of venom from the endoparasitoid *Pimpla turionellae* on hemocytes of the host *Galleria mellonella*. J. Appl. Entomol. 135 (3), 225–236.

Ergin, E., Uçkan, F., Rivers, D.B., Sak, O., 2006. In vivo and in vitro activity of venom from the endoparasitic wasp *Pimpla turionellae* (L.) (Hymenoptera: Ichneumonidae). Arch. Ins. Biochem. Physiol. 61 (2), 87–97.

Giannotti, K.C., Sesso, A., Grego, K.F., Fernandes, W., Cardoso Jr., R.P., Camargo, G.G., Carneiro, S.M., 2013. Viperid venom glands with defective venom production. Morphological study. Toxicon 70, 32–33.

Hoshina, M.M., Santos, L.D., Palma, M.S., Marin-Morales, M.A., 2013. Cytotoxic, genotoxic/antigenotoxic and mutagenic/antimutagenic effects of the venom of the wasp *Polybia paulista*. Toxicon 72, 64–70.

Jarrar, B.M., Al-Rowaily, M.A., 2008. Histology and histochemistry of the venom apparatus of the black scorpion *Androctonus crassicauda* (Olivier, 1807) (Scorpiones: Buthidae). J. Venom. Anim. Toxins Incl. Trop. Dis. 14 (3), 514–526.

Kovoor, J., Muñoz-Cuevas, A., 2000. Comparative histology of the venom glands in a Lycosid and several Oxyopid spiders (Araneae). Ekológica 19 (3), 129–140.

Moreau, S.J.M., Guillot, S., 2005. Advances and prospects on biosynthesis, structures and functions of venom proteins from parasitic wasps. Insect Biochem. Mol. Biol. 35, 1209–1223.

Moreau, S.J.M., Vinchon, S., Cherqui, A., Prévost, G., 2009. Components of *Asobara* venoms and their effects on hosts. Adv. Insect Physiol. 70, 217–232.

Peydró, R.J., Garí, J.V.F., Martos, C.G., Marí, J.M., 1996. Comparative morphology of the venom apparatus in some subfamilias of braconid wasps (Hymenoptera: Braconidae). Boln. Asoc. Esp. Ent. 20, 149–159.

Quicke, D.L.J., 1997. Parasitic Wasp, first ed. Chapman & Hall, Cambridge, p. 470.

Ramalho, F.S., Wanderley, P.A., Malaquias, J.B., Souza, J.V.S., Rodrigues, K.C.V., Zanuncio, J.C., 2009. Effect of temperature on the reproduction of *Bracon vulgaris*, Ashmead (Hymenoptera: Braconidae), a parasitoid of the cotton boll weevil. Entomol. News 120, 476–487.

Taib, N.T., Jarrar, B.M., 1993. Histological and histochemical characterization of the venom apparatus of Palestine yellow scorpion, *Leiurus quinquestriatus* Hemprich & Ehrenberg 1828. Trop. Zool. 6, 143–152.

Toscano, L.C., Carvalho, S.L., 2000. Parasitismo em *Anthonomus grandis* Boheman, 1843 por *Bracon vulgaris*, Ashmead em cultura de algodão sem medidas de controle na região de Ilha solteira – SP. Braz. J. Ecol. 12, 123–127.

Vardal, H., 2006. Venom gland and reservoir morphology in cynipoid wasps. Arthropod. Struct. Dev. 35, 127–136.

Wang, K.R., Zhang, B.K., Zhang, W.J.X., Li, J., Wang, R., 2008. Antitumor effects, cell selectivity and structure–activity relationship of a novel antimicrobial peptide *Polybia*-MPI. Peptides 29, 963–968.