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Proventriculus of Cephalotes ants: A structural and comparative analysis

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ABSTRACT

The use of optic microscopy and scanning electron microscopy propitiated the comparative examination of the structure of the proventriculus bulb of *Cephalotes atratus*, *Cephalotes clypeatus* and *Cephalotes pusillus*. This portion of the digestive tract possesses highly sclerotized projections which act in the selection of victuals. This structure is of importance to phylogeny studies and thus was compared with others known species of ants in a dendrogram adapted from Eisner (1957). We did not detect differences among the three studied species in the nature of histochemistry and ultra morphology.

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1. Introduction

Adult hymenopterans are unique regarding their food habits, mostly feeding on liquids (Eisner, 1957). When workers forage to provide nutrients for the colony, part of the fluids collected does not pass directly to the midgut. Instead, it is first temporarily retained in the crop and passed slowly in small amounts to the midgut, via the proventriculus. This structure thus plays an important role in regulating the flow of food in the digestive tract (Eisner, 1957; Caetano, 1988; Bution and Caetano, 2005).

According to Caetano (1988), this organ is the most variable portion of the anterior intestine among Formicidae. In most cases, it is clearly divided into three regions: the bulb, located inside the crop; the neck, a thin tube connecting the former to the midgut, and finally, the stomodeal valve, located in the lumen of the anterior portion of the midgut (Chapman, 1975; Wigglesworth, 1974; Caetano, 1988; Bution and Caetano, 2005).

The bulb of the proventriculus, when present, is divided into sepals that are brought closer together by the pressure of liquids in the crop (passive process), or by the action of longitudinal and circular muscles (active process) (Eisner, 1957). In most ant species, the closed sepals of the bulb form a cross-shaped slit. The bulb is sclerotized and may present cuticular hairs that act as a filter. The contraction of circular muscles present in the neck of the proventriculus closes the passage to the midgut (Eisner, 1957). Thus, an adequate performance of the social role of the crop might be associated with the efficiency of the bulb of the proventriculus (Eisner, 1957; Wigglesworth, 1974; Caetano, 1984, 1988; Bution and Caetano, 2005).

In the ants examined to the present, the bulb of the proventriculus consists of four mobile sepals (Caetano, 1988; Tomotake, 1996). This quadripartite aspect seems to be constant in the Formicidae family (Tomotake, 1996). Eisner (1957), however, observed the presence of six mobile sepals in *Amblyopone australis*, the only one among Formicidae. Caetano (1984) reported the occurrence of five mobile sepals in males of the wasp *Myscho-cyttarus atramentarius.*

In members of the subfamilies Dolichoderinae, Pseudomyrmicinae, and Formicinae, the bulb of the proventriculus is highly elaborated; with a very thick dome with cuticular spine-shaped projections. The role of these structures is to prevent the passage of solid particles to the midgut (Eisner, 1957; Caetano, 1984; Roche and Wheeler, 1997; Bution and Caetano, 2005).

The neck of the proventriculus in ants is a thin tube connecting the proventriculus to the midgut, usually lined by two muscle layers, a longitudinal and a circular one (Caetano, 1984, 1988; Tomotake, 1996; Bution and Caetano, 2005). Eisner (1957) described three types of muscles in ants of the subfamily Formicinae while Caetano (1992) reported three longitudinal and two circular muscles in *Neoponera villosa*, subfamily Ponerinae.

The stomodeal valve is the last portion of the proventriculus and is located inside the midgut. It consists of a projection of the stomodeal epithelium that folds onto itself and prevents the reflux of foods to the midgut (Eisner, 1957; Wigglesworth, 1974; Caetano, 1984, 1988; Bution and Caetano, 2005, 2008; Bution et al., 2006, 2007).

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Several researchers have described a remarkable structural plasticity in the proventriculus of different genera of ants. Caetano (1984) described the complex mushroom-shaped proventriculus of ants of the tribe Cephalotini, a rigid and very sclerotized structure covered by dense layers of spines. Similar descriptions have been reported by Roche and Wheeler (1997) and Bution and Caetano (2005) in ultramorphological studies.

The structure and shape of this portion of the digestive tract indicate a distinct adaptation to the fluid diet of ants. The proventricular activity consists of rhythmic muscle contraction and distention, making this organ an excellent regulator of the digestive tract, in addition to working as a strong suction pump (Eisner, 1957).

Despite the conserved structure of the digestive tract, the proventriculus is an example of a highly variable structure among Formicidae. Darwin, in his theory of the origin of species, described that throughout evolution, structures that do not play a decisive role in natural selection may vary considerably regarding their morphology. Brown (1954) and Eisner (1957) pointed out that morphological analyses of the proventriculus might help in determining subfamilies and even genera among ants, and thus are of importance to phylogeny studies.

The use of optical microscopy combined with histological and histochemical techniques, in addition to those for scanning electron microscopy, allowed an analysis of the proventriculus of *Cephalotes atratus*, *Cephalotes clypeatus*, and *Cephalotes pusillus*. Thus, as a contribution for understanding this complex structure that plays an important role in the social and evolutionary behavior of Formicidae, in this study we examined the proventriculus of these three species of the tribe Cephalotini and compared them with other previously studied species, based on histochemical, ultramorphological, and evolutionary aspects.

2. Materials and methods

2.1. Materials

Large workers of *C. pusillus* and workers of *C. clypeatus* were collected from trees located at the UNESP/Rio Claro campus, while large workers of *C. atratus* were collected from trees located at the margins of the Jacaré-pepira River, in Brotas City, São Paulo State/Brazil. In this work, ten individuals of each species were analyzed.

2.2. Methods

2.2.1. Scanning electron microscopy (SEM)

The proventriculus was removed and fixed in Karnovsky fluid for 24 h, dehydrated in ascending alcohol series (70–100%), subjected to two acetone 100% baths of 15 min each and critical point dried (Balzers CPD 030). After dehydration the material was placed on aluminum supports attached with double-faced tape and sputter coated with gold (in sputtering Balzers SD 050). The proventriculus was examined with a Jeol P15 SEM and photographed on Neopan SS 120 film.

2.2.2. Histology

For analysis of the histology, the proventriculus was fixed in 4% paraformaldehyde for 2 h and then buffered in sodium phosphate pH 7.4 for 24 h. After this period, the material was passed through an alcohol series (70–95%) for 30 min. Later the proventriculus embedded in resin was soaked for 24 h in the refrigerator to be then included in polymeric resin.

Some slides were stained with Hematoxylin–Eosin (Junqueira and Junqueira, 1983) for analysis of the proventriculus.

2.2.3. Histochemistry

Part of the slides was stained with PAS (Junqueira and Junqueira, 1983) for neutral carbohydrates; Toluidine Blue (pH 3.5) (Junqueira and Junqueira, 1983) for acidic polysaccharides and DNA; Bromophenol Blue (Pearse, 1960) and Xilidine Ponceau (Junqueira and Junqueira, 1983), to detect protein compounds. All slides were examined with a Leica photomicroscope. The images were captured and digitalized with the software Leica Image Manager 50.

2.2.4. Dendrogram

A dendrogram adapted from Eisner (1957) is presented, evaluated as complementary analysis.

3. Results and discussion

In the description of the results of the present work, we did not detect differences among the three studied species in the nature of histochemistry and ultra morphology, thus they will be described together.

The morphological characters observed in the proventriculus of *C. atratus*, *C. clypeatus*, and *C. pusillus* allowed establish among similar characters in the examined species and some previously studied species in order to outline phylogenetic relationships.

Our findings confirm that the proventriculus is more than a connection between the crop and the midgut. It is a structure responsible for retaining liquid food that will remain in the social stomach or crop, transporting and controlling the feeding of nestmates, and thus playing an important social role (Eisner, 1957; Wigglesworth, 1974). The remarkable ability of food storage of the crop is believed to be the basis for the development of complex patterns of oral exchange of food (trophallaxis) among foraging workers and other members of the colony. This exchange is one of the bases in the social organization of ants (Wheeler, 1923; Wilson and Eisner, 1957; Eisner and Wilson, 1958; Kempf, 1951, 1973).

The proventriculus is also of considerable interest from a phylogenetic perspective. According to Eisner (1957), the presence of an elaborated bulb in the proventriculus can be considered an adaptive characteristic, as it is a refined structure specialized in retaining and selecting nutrients.

According to Eisner and Brown (1958), the primitive ant proventriculus (Subfamilies Myrmeciinae, Pseudomymecinae, Aneuretinae, Ponerinae, and Cerapachyinae) is thin-walled and flaccid, and therefore ill-adapted to dam the crop, requiring the support of sphincter muscles. For these authors, ants having such proventriculus have failed to develop castes capable of distending the crop and gaster. On the other hand, in specialized ants, such as Formicinae and Dolichoderinae, the proventriculus damming for long periods of time does not depend on sustained sphincter contraction. In these ants, the structure of the proventriculus is rigid and sclerotized, constructed to be impervious to leakages of food from the crop to the midgut (Eisner and Brown, 1958).

In *C. atratus*, *C. clypeatus*, and *C. pusillus*, the proventriculus bulb is a delicate mushroom-shaped structure (Fig. 1A), in agreement with the described by Caetano (1984) and Roche and Wheeler (1997). However, the homogeneity of the surface of the bulb disappears when the amorphous material covering this structure is not preserved or removed (Fig. 1B). The absence of this material reveals a striate surface covered with spines that might play a role in the filtration of solid particles (Fig. 1D) (Caetano, 1984, 1988; Roche and Wheeler, 1997; Bution and Caetano, 2005).

The efficiency of this filter seems to be maximized by the presence of the amorphous material that covers the entire surface