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MORPHOLOGY AND ULTRASTRUCTURE OF THE SPERMATOZOA OF *Scaptotrigona xanthotricha* MOURE (HYMENOPTERA, APIDAE, MELIPONINI)

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ABSTRACT

The spermatozoa of *Scaptotrigona xanthotricha*, a stingless bee under extinction in some Brazilian states, are described. Seminal vesicles of adult males were dissected and processed for light and transmission electron microscopy. The spermatozoa were long (about 90 μm) and slender. The head consisted of an acrosome formed by an acrosomal vesicle covering the *perforatorium* and the nucleus. The latter was homogeneous, compact and about 11 μm long. The flagellum consisted of an axoneme, with the typical 9+9+2 microtubule arrangement that began just below the nuclear base. The two mitochondrial derivatives were asymmetric in length and diameter, and had two accessory bodies. The nucleus was attached to the flagellum by a centriolar adjunct. These results indicate that the spermatozoa of *S. xanthotricha* are similar to those of other Meliponini but differ markedly from other insect groups. The ultrastructure of hymenopteran spermatozoa may be a useful character for phylogenetic studies.

Key words: Apoidea, axoneme, mitochondrial derivatives, sperm, stingless bees

INTRODUCTION

The genus *Scaptotrigona* contains about 24 species distributed throughout the Neotropical region [24]. Eight species have been described in Brazil, although the true number of species is probably much greater [33]. Despite the abundance of this genus, the species *S. xanthotricha* is under extinction in some Brazilian states such as Pará and São Paulo [23].

Recently, studies of the morphology of the male reproductive apparatus and sperm of some insects have contributed to our understanding of the relationships among these groups [1,4,6-9,14]. For Hymenoptera, the descriptions suggest that the morphological variability in the male reproductive structures is sufficient to provide a character matrix for cladistic analysis [32]. However, the number of these studies are small compared to the large hymenopteran diversity [2,3,5-13,15-22,25-31,34-38]. To date, no phylogenetic studies using reproductive parameters have been done for the Hymenoptera, except for a short report on the corbiculated tribes of Apinae, *i.e.* Apini, Euglossini, Bombini and Meliponini [U.Z., Doctoral thesis, Estudo estrutural e ultraestrutural dos espermatozóides nas tribos Apini, Bombini, Euglossini e Meliponini (Hymenoptera: Apinae), com considerações filogenéticas. State Univ. Campinas (UNICAMP), Campinas 2003].

Although the sperm of some Meliponini have been studied in detail [36,37], in this study, we describe the morphology and ultrastructure of the spermatozoa of *S. xanthotricha* to provide additional information that may be useful for taxonomic and phylogenetic analyses.

MATERIAL AND METHODS

Adult males of *S. xanthotricha* were obtained from colonies kept in the Central Apiary of the Federal University of Viçosa, MG, Brazil.

Light microscopy

Seminal vesicles were dissected and squashed on clean glass microscope slides, followed by spreading and fixation with 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.2. After drying at room temperature, the slides were observed with an Olympus BX60 photomicroscope equipped with a phase contrast lens to allow measurement of the spermatozoa. To measure the nucleus, some of these preparations were stained for 15 min with 4,6-diamino-2-phenylindole (DAPI; 0.2 μg/mL in PBS), washed, and mounted with Vectashield. The preparations were then examined with an epifluorescence microscope Olympus BX60 equipped with a BP360-370 nm excitation filter.

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Approximately 50 spermatozoa and nuclei were analyzed and measured using the software Image Pro-Plus and the lengths were expressed as the mean of the total number examined.

Transmission electron microscopy

The seminal vesicles were dissected and fixed for 3 h in 2.5% glutaraldehyde, 0.2% picric acid, 3% sucrose and 5 mM $CaCl_2$ in 0.1 M cacodylate buffer, pH 7.2. The material was post-fixed in 1% osmium tetroxide, dehydrated in acetone and embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and lead citrate and observed with a Zeiss LEO 906 transmission electron microscope operated at 60-80 kV.

RESULTS

The spermatozoa of *S. xanthotricha* were long (mean length: 90 µm) and slender, and consisted of a head, with an acrosome and nucleus, and a flagellum that included an axoneme, a centriolar adjunct, two mitochondrial derivatives and two accessory bodies (Fig. 1A-D).

The acrosome included the acrosomal vesicle and the *perforatorium* (Fig. 1C, E-F) arranged in a bilayered pattern. The acrosomal vesicle covered the *perforatorium* until the latter penetrated a short cavity in the anterior nuclear tip (Fig. C). Between the acrosomal vesicle and the anterior tip of the nucleus, there was a granular, electrondense ring that also surrounded the *perforatorium* (arrow in Fig. 1C). The acrosomal vesicle was cone-shaped and, in transversal sections, was circular at the tip but triangular near the nucleus (Fig. 1E-F). The *perforatorium* was rod-shaped and appeared circular in transversal sections (Fig. 1F).

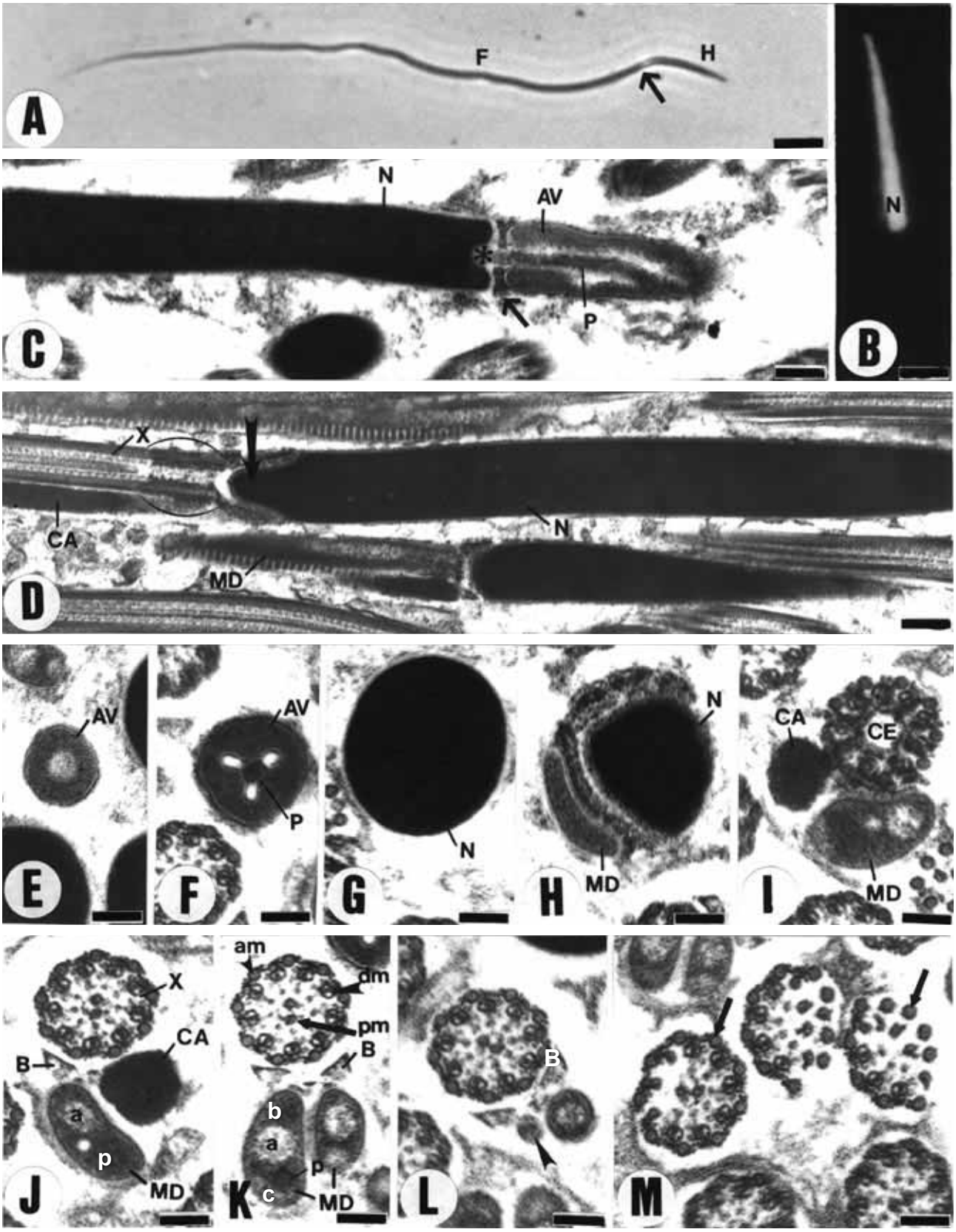
The nucleus was homogeneous and compact, and measured about 11 µm in length (Fig. 1A-C, G). A short cavity present at the anterior tip was penetrated by the *perforatorium* (asterisk in Fig. 1C). The nucleus also projected posteriorly, above the centriole (arrow in Fig. 1D). In transversal sections, the nucleus appeared circular, except in the basal region (compare Fig. 1G and H).

The centriolar adjunct was a rod-shaped structure that appeared roughly circular in transversal sections (Fig. 1I, J). This adjunct began at the centriolar level (Fig. 1I), ran laterally to the axoneme and the larger mitochondrial derivative (Fig. 1D-J) and ended just above the anterior tip of the smaller mitochondrial derivative.

The mitochondrial derivatives were elongated structures that ran parallel to the axoneme and accessory bodies. The larger mitochondrial derivative began at the nuclear base (Fig. 1H), while the smaller began after the posterior tip of the centriolar adjunct. These derivatives also terminated unequally, with the smaller terminating first (Fig. 1L). In transversal sections, the derivatives were ellipsoidal, with the larger being about twice the size of the smaller (Fig. 1K). In cross-sections, the derivatives showed various regions, namely a central region (a), which wais approximately circular and had a low electrondensity compared to the surrounding matrix (b) and the cristae (c). A paracrystalline region (p) was seen only in the large derivative (Fig. 1J-K).

The accessory bodies were seen as triangular structures between the axoneme and the two mitochondrial derivatives (Fig. 1J-K).

Figure 1. A-B. Phase contrast micrograph of a spermatozoon and the DAPI-stained head region, respectively. The arrow indicates the limit of the head (H) and flagellum (F). Bar: A- 5 µm; B- 1.90 µm. **C.** Transmission electron micrograph (TEM) of a longitudinal section of the anterior sperm tip, showing an acrosomal vesicle (AV), the *perforatorium* (P) and the nucleus (N). The arrow indicates the granular ring and the asterisk indicates the short nuclear cavity into which the *perforatorium* fits. Bar: 0.13 µm. **D.** TEM of a longitudinal section of the nuclear-flagellar transition showing the nuclear projection (**arrow**) terminating adjacent to the centriolar region (parenthesis) of the axoneme (**x**). The centriolar adjunct (CA) and the mitochondrial derivative (MD) are indicated. Bar: 0.20 µm. **E-M.** TEM of transverse sections of sperm at different levels. **E-F.** anterior and posterior sections of the acrosome, respectively. Note the *perforatorium* (P) separated from the acrosomal vesicle (AV) by an electronlucid layer. **G-H.** Nucleus and nuclear-flagellar transition, showing the nuclear projection (N) running laterally to the initial tip of the mitochondrial derivative (MD). **I.** Anterior section of the flagellum showing the centriolar region of the axoneme (CE), beside the mitochondrial derivative (MD) and centriolar adjunct (CA). **J.** The flagellum initially consists of the axoneme (AX), only one mitochondrial derivative (MD), a centriolar adjunct (CA) and an accessory body (B) located between the axoneme and the mitochondrial derivative. **K-L.** Posteriorly, two mitochondrial derivatives and two accessory bodies accompany the axoneme and the smaller one, finish first (arrowhead). Observe the axoneme made up of accessory, doublet and central microtubules (**am**, **dm** and **cm**, respectively). **M.** Final portion of flagellum showing the gradual disorganization of the axoneme. Observe the accessory microtubules as the last to be lost at the axoneme tip. Bars: (E-M) 77 nm.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The axoneme had a 9 + 9 + 2 microtubular arrangement with intertubular material (Fig. 1I-M). This structure began as an atypical centriole, without the central microtubules (Fig. 1I), and terminated below the other flagellar structures (Fig. 1M). In the final region, the two central microtubules terminated first, followed by nine doublets and, finally, the accessory microtubules (Fig. 1M).

DISCUSSION

The morphology of *S. xanthotricha* spermatozoa agreed with that described for the Meliponini [6-8,36,37], which vary in length from 80 to 300 μm [36,37]. This species, together with *S. postica* [37], have the shortest spermatozoa of this tribe, and this may indicate that the size of these cells is conserved within this genus. In *S. xanthotricha*, the spermatozoa are released from the testicles in sheaths, but gradually become detached during sexual maturation, as frequently occurs in the Aculeata [25].

The acrosome frequently projects beyond the *perforatorium* to produce a short, curved projection in *S. postica* [6,37]*, Nannotrigoga punctata* [37] and *Apis mellifera* [5,12,16,30-31]. In *S. xanthotricha,* the acrosome has no projection, as is also the case in most Meliponini [36,37].

The accessory microtubules were the first to terminate at the flagellar tip as also occurs in other Meliponini species [36,37]. This characteristic is apparently common in the Aculeata [3,36-38]. However, in the parasitic families Icheneumonidae [26] and Chalcidoidea [17-18,21] the accessory microtubules terminate above the central tubules and doublets. As suggested by Zama *et al.* [37], we also suspect that the pattern of microtubule termination at the flagellar tip could be used as a character in phylogenetic studies. Conversely, in the Formicidae, the microtubules terminate almost simultaneously [22,34].

The mitochondrial derivatives are another important interspecific character. In *S. xanthotricha*, these structures are somewhat asymmetrical in diameter, a condition similar to that of most Meliponini [37]. However, they differ from derivatives that are approximately equal in diameter [37]. The mitochondrial derivatives in the Formicidae [22,34] and Chalcidoidea [21] usually have approximately the similar diameter. Also, in the Chalcidoidea [21] and Scelionidae [20], the mitochondrial derivatives spiral around the axoneme and, in the latter family, only one derivative is found.

In conclusion, the spermatozoa of *S. xanthotricha* were similar to those of other Meliponini, but differed markedly from those of other insect groups. This finding provides further evidence for the potential usefulness of spermatozoon morphology and ultrastructure in phylogenetic analyses.

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