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The sperm structure of the Scraptiidae (Coleoptera; Tenebrionoidea)

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ABSTRACT

The sperm ultrastructure of two members of the Scraptiidae *Anaspis pulicaria* and *A. lurida* was studied. The results confirm the general organization of the sperm in the superfamily Tenebrionoidea. The sperm bundles at the end of the spermiogenesis show the same peculiar antiparallel distribution at the two opposite poles of the germ cyst, observed in other Tenebrionoidea. The sperm have a bi-layered acrosome, a long cylindrical nucleus with two infoldings at its basal region, two elliptical equal mitochondrial derivatives and two triangular accessory bodies. The flagellar axoneme has the common 9 + 9 + 2 microtubular pattern that at the tail end results disorganized. All these sperm characteristics are quite similar to those found in Pythidae, a closely related family, according to molecular data.

1. Introduction

Tenebrionoidea is one of the largest and most complex superfamilies of Coleoptera, with about 30 families and more than 30.000 species described so far (Crowson, 1955, 1966; Abdullah, 1973; Lawrence, 1977; Pollock, 1995). According to molecular studies on the superfamily, it was suggested that it is a monophyletic group, and within the group can be recognizable some clades with the basal position of Ripiphoridae-Mordellidae-Meloidae assemblage (Lawrence and Newton, 1995; Beutel and Friedrich, 2005; Levkaničová, 2009). However, studies by Zhang et al. (2018) and McKenna et al. (2019) based on extensive gene sampling, including nuclear protein-coding genes, have supported a close relationship between Ripiphoridae and Mordellidae but have not validated the positioning of Meloidae close to the families mentioned above. An ultrastructural study on the spermatozoa of these tenebrionoid families has reached a similar conclusion, supporting the validity and relevance of spermatological studies in insect phylogeny investigations. Ripiphoridae and Mordellidae share some important cytological characteristics indicative for their positioning at the base of the whole family and are not closely related to Meloidae (Dias et al., 2022).

According to Dias et al. (2022), members of the tenebrionid families studied so far, except for the basal Ripiphorid-Mordellid clade, share the uncommon feature of spermatozoa which do not maintain a simple orientation within the germ cyst, as it usually occurs in insects. In such families in fact, sperm are distributed into two bundles at the opposite sites of the cyst, in an antiparallel sperm bundle array. The only other insect group showing a similar pattern are the Hemiptera *Planococcus citri* and *Kerria chinensis*. However, the separation of sperm in two sperm bundles at the end of spermatogenesis is realized by a different mechanism from that in Tenebrionoidea (Seubparu et al., 2018; Bongiorni et al., 2004). The presence of such a peculiar antiparallel position of sperm at the end of spermiogenesis was confirmed in several tenebrionoid families, as Tenebrionidae (Baccetti et al., 1973; Dias et al., 2012), Oedemeridae (Dias et al., 2022), Ciidae (Folly et al., 2021), and Pythidae (Dias et al., 2021) suggesting that this character could be an apomorphy shared by the whole superfamily.

The present study dealing with another family of Tenebrionoidea, the Scraptiidae, which has not yet been investigated, could provide more data to support such a hypothesis. Scraptiidae is a small family of beetles, common on flowers (Ruzzier, 2016). They comprise about 500 species and 35 genera distributed worldwide (Ślipiński et al., 2011). They are known as false flower beetles, with some species resembling those of Mordellidae. In the past, the genus *Anaspis* was ascribed to this group, but later classified as belonging to Scraptiidae (Buck, 1954). The family is more closely related to Pythidae, recently studied (Dias et al., 2021). A comparison between the sperm structure and spermiogenesis of these families could confirmed their relationship.

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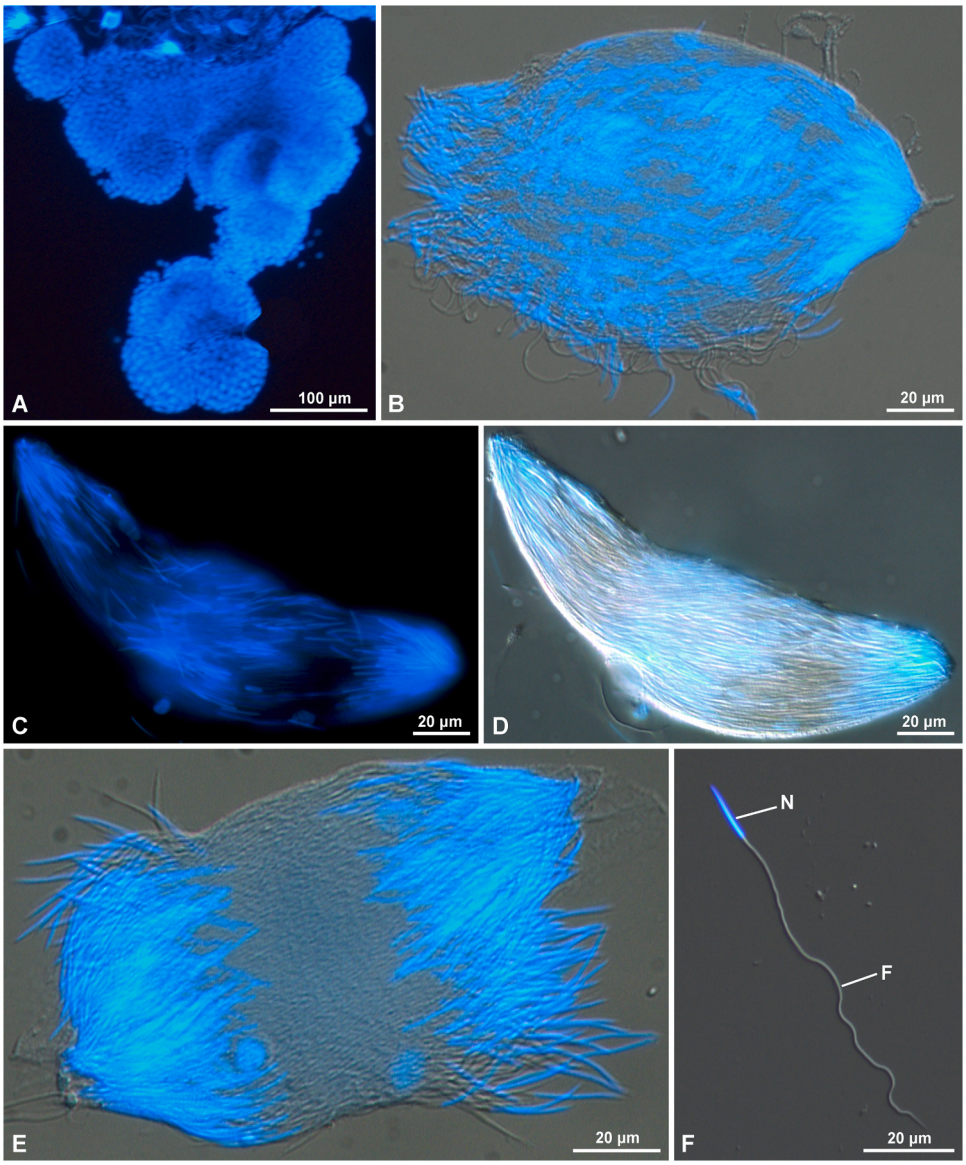

Fig. 1. *A. pulicaria*, Development and migration of spermatids in cysts. A) Hoechst staining of a group of germ cysts. Note the globular shape of the structures with several nuclei. B) Germ cyst with early spermatids at the beginning of the migration of germ cells to the opposite poles. C-D) Intermediary stage of the sperm migration to the opposite poles of the cyst. Some cells are still in the center of the complex. E) Advanced stage of a cyst showing the sperm nuclei arranged at the two opposite poles. F) Isolated sperm. B, D, E and F) Merge between Hoechst staining and phase-contrast photographs. N, nucleus; F, flagellum.

2. Material and methods

2.1. Light and epifluorescence microscopic preparations

Males of *Anaspis pulicaria* and *A. lurida* were dissected in 0.1 M phosphate buffer pH 7.2 with 3 % sucrose (PB) to remove the genital system. The cysts were removed from the testes, and the sperm, taken from the deferent duct and seminal vesicles, were spread over histological slides. In order to visualize sperm nuclei, a drop of 1 ug/mL of the DNA-specific dye Hoechst in 0.1 M PB was added and the resulting samples were finally covered with a glass coverslip. Fluorescence observations of the labelled samples were carried out with a Leica DMRB light microscope equipped with a UV light source, fluorescein, UV filters, and a Zeiss AxioCam Mrc5 digital camera.

2.2. Transmission electron microscopy (TEM)

Adult males were dissected in PB to isolate the testes and deferent ducts. The material was fixed in 2.5 % glutaraldehyde in PB overnight. After careful rinsing, the material was post-fixed in 1 % osmium tetroxide for 2 h. After rinsing, the material was dehydrated with ethanol series (50–100 %), transferred to propylene oxide, and finally embedded in a mixture of Epon-Araldite resins. Ultrathin sections were obtained with a Reichert Ultracut ultramicrotome, routinely stained with uranyl acetate and lead citrate, and observed with a TEM Philips CM10 operating at 80 kV electron accelerating voltage.

3. Results

The sperm of *Anaspis pulicaria* and *A. lurida* are long cells of about 85–90 µm in length, with a 14–15 µm long nucleus (Fig. 1F). During the spermatogenesis, round early germ cysts, about 6.0–7.0 µm in diameter, are visible after immunofluorescence in the apical region of the testes (Fig. 1A). Further, during spermiogenesis, early spermatids 5.0 µm X 7.5 µm large are visible. In cross-section, each cyst contains about 500 spermatid cells, corresponding to nine (2^9) cell divisions. Spermatids

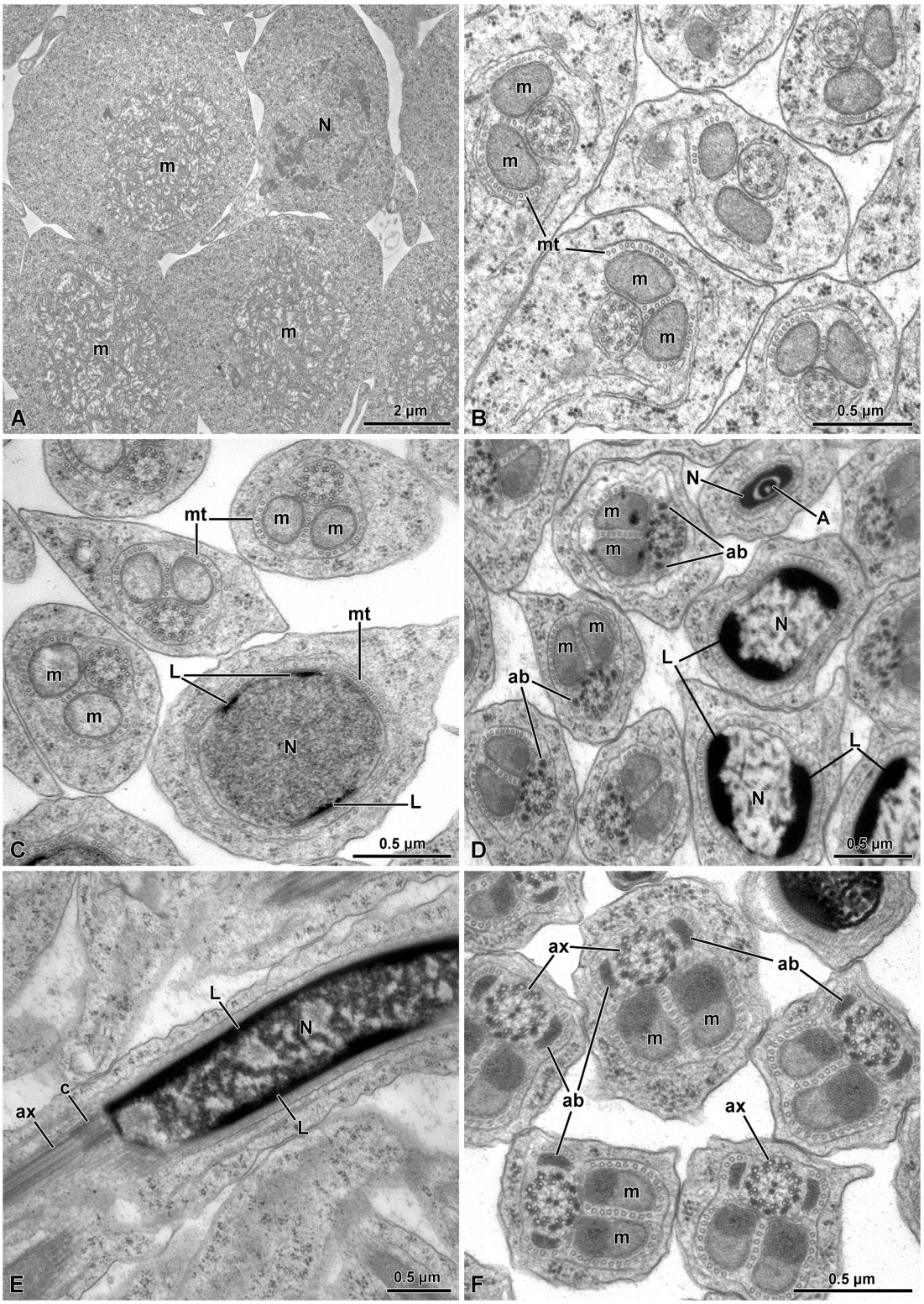

Fig. 2. *A. pulicaria* A) Cross-section of early spermatids with the evident "nebenkerns" (m) and nucleus (N). B-C) Cross-sections through early spermatids flagella. The mitochondrial derivatives (m) are elliptical and surrounded by a layer of microtubules (mt). The nucleus (N) has a chromatin uniformly distributed and two opposite electron-dense laminae (L). D) Cross sections of the more advanced stage of spermiogenesis with the nuclei (N) showing two opposite large electron-dense layers (L) of chromatin. The mitochondrial derivatives (m) are enlarged. Note on the upper side a cross-section of the acrosome (A) inserted in the nuclear apex and the two small dots at the opposite sides of the axoneme representing the accessory bodies (ab). E) Longitudinal section of an almost mature spermatid with the nucleus (N) showing the two opposite dense layers of chromatin (L). Beneath the nucleus, a centriole (c) and an axoneme (ax) are visible. F) Cross section of mature spermatids flagella with the two large mitochondrial derivatives (m) showing a crystallization in the region facing the axoneme (ax). Lateral to the axoneme, two kidney-shaped accessory bodies (ab) are visible.

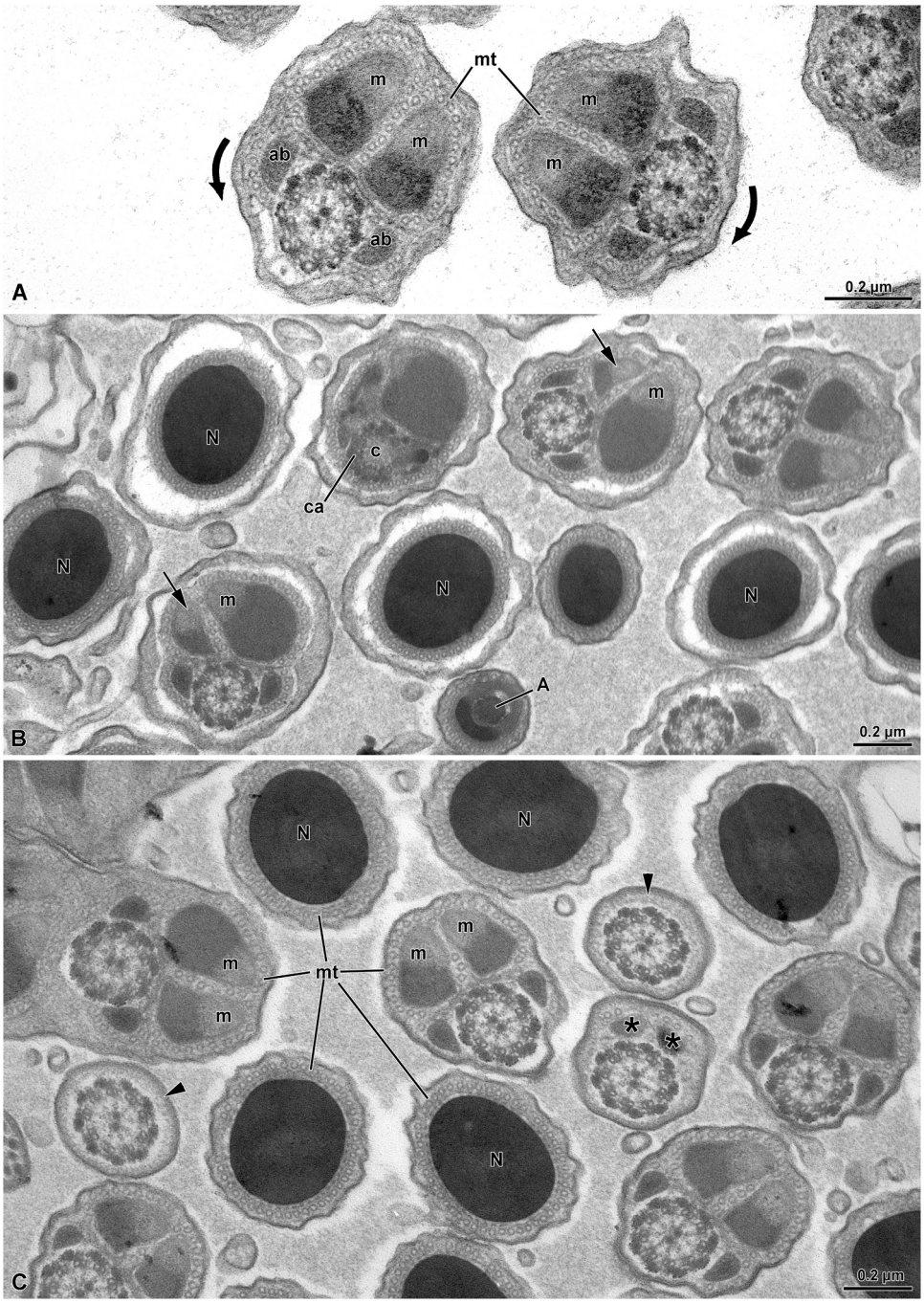

Fig. 3. *A. pulicaria* A) Cross-section of two flagella of mature spermatids, each coming from opposite poles of the germ cyst. Note the clockwise orientation of the right axoneme and the anticlockwise orientation of the left axoneme, respectively (see arrows). ab, accessory bodies; m, mitochondrial derivatives; mt, microtubules. B-C) Cross-sections of mature spermatids showing the nuclei (N), the centriolar region (c) with around the centriolar adjunct material (ca), a large mitochondrial derivative (m), and a smaller one (arrowheads). They become of the same size in a more posterior region. Toward the tail end, the mitochondrial derivatives reduce their size (asterisk) and then disappear (arrowheads). All the structures are surrounded by microtubules (mt). A, acrosome.

show large intercellular bridges and are provided with nuclei about 3.0 µm in diameter with dispersed chromatin. In the cytoplasm of each cell, a large "nebenkern," 3.0 µm in diameter, is observed (Fig. 2A). In a more advanced stage of maturation, the spermatid cysts elongate up to 120 µm in length, and spermatid nuclei are scattered along the cyst length, with several assembled at the opposite apices of the cyst (Fig. 1B–D). In cross-section, the spermatid has decreased its diameter, and the nucleus became elongated with the chromatin condensed along two opposite sides of the nuclear envelope. Indeed, an unusual longitudinally polarized (Fig. 2 C-E) initiation of chromatin condensation is observed, that starts at the nuclear periphery and proceeds towards the inner part until final condensation is achieved in the mature sperm (Fig. 3 B-C). All around the spermatid components, a layer of microtubules is evident (Fig. 2B–D, F). Beneath the nucleus, a centriole is evident, surrounded by scanty dense material of the centriole adjunct (Fig. 2E).

Further along spermiogenesis, the spermatids become distributed in two bundles on the opposite poles of the cyst. This is quite evident after fluorescence preparations (Fig. 1E). At this stage, the cysts are fusiform and reach about 150 µm in length. A cross-section through the spermatids at this stage of maturation shows the complex organization of

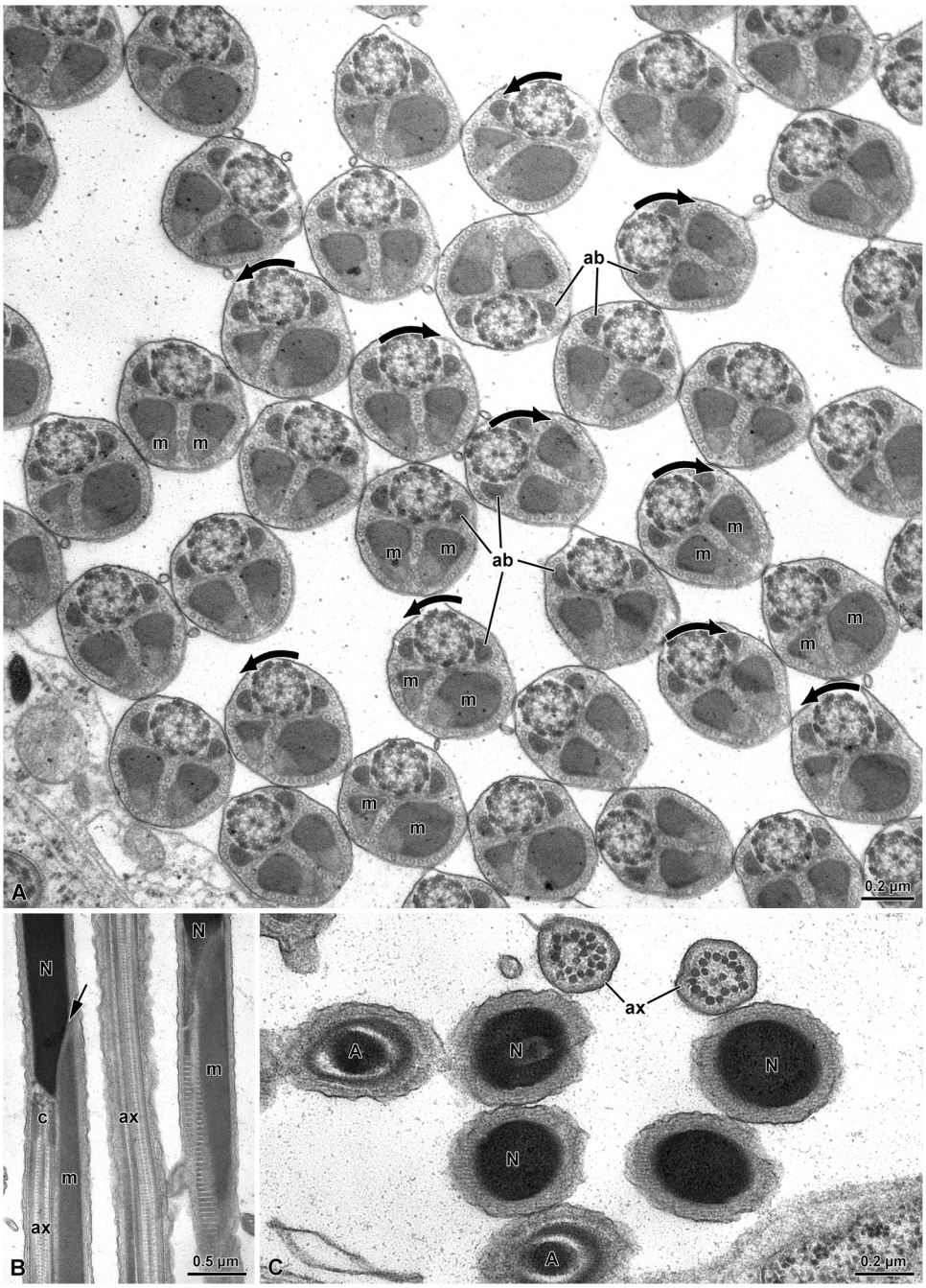

Fig. 4. *A. pulicaria* A) Cross-section through the middle region of the cyst with mature spermatids coming from opposite poles (see arrows). ab, accessory bodies; m, mitochondrial derivatives. *A. lurida* B) Longitudinal section of the centriolar region of mature spermatids showing the centriole (c), the flagellar axoneme (ax), and the insertion of one mitochondrial derivative (m) in a nuclear (N) infolding (arrow). C) Cross-section of mature spermatids showing the acrosome (A), the nuclei (N), and the tail end with the disorganization of the axoneme (ax).

these cells. The nucleus has become cylindrical with a compact chromatin material uniformly distributed (Fig. 3B, C). Apically it has a short conical acrosome with a well-evident dense perforatorium (Fig. 2C). The nuclear posterior region shows two infoldings where the two mitochondrial derivatives are housed. At the opposite position is placed the centriole surrounded by the dense material of the centriole adjunct and the flagellar axoneme (Figs. 3B, 4B). In *A. lurida*, the two mitochondrial derivatives have a different size at this level, with the one on the left side of axoneme sections, larger than the opposite. Posteriorly, however, the two structures become of equal size as in the other species (Fig. 3B). A cross-section through the flagellum shows a 9 + 9 + 2 axoneme flanked by two small accessory bodies, which have increased their size from the early spermatids (Fig. 2C) to the more advanced stage of spermiogenesis (Fig. 3A). At this latter stage of maturation, they have a banana-shape in the cross-section (Fig. 2F) and are surrounded by some microtubules. Later, they increase their size and become triangular (Figs. 3A, 4A). Beneath the axoneme, two similar triangular mitochondrial derivatives are visible, with their largest area facing the axoneme filled with a dense matrix. They are surrounded by a layer of microtubules up to the end of spermatid maturation. Between the mitochondrial derivatives and the axoneme, remnants of the cistern surrounding the flagellar axoneme are present (Fig. 4A). The flagellar posterior end is characterized by the disorganization of the axoneme with the microtubular doublets having the two tubular components separated; the mitochondrial derivatives

progressively decrease their size and then disappear, as also occurs to the accessory bodies (Fig. 4C).

The most interesting feature of a sperm cross-section cut at sperm bundle middle length, is the antiparallel distribution of the sperm starting from the two extremities of the cyst. Looking at the flagellar axonemes (Fig. 4A), the clockwise or anticlockwise orientation of the dynein arms of microtubule doublets is evident, indicating that the sperm observed belong to two sperm bundles with opposite orientation.

4. Discussion

The general organization of the *A. pulicaria* and *A. lurida*, confirms that the two species of Scraptiidae share the sperm characteristics of other members of Tenebrionoidea (Jamieson et al., 1999; Dias et al., 2022). First of all, the sperm bundles show the peculiar antiparallel distribution, which could represent a synapomorphy of all families of the superfamily with the only exception of the basal clade Ripiphoridae-Mordellidae (Dias et al., 2022). This unusual characteristic is the consequence of a process occurring during spermiogenesis when spermatids elongate and their nuclei migrate to opposite poles of the cyst (Dias et al., 2012, 2013a, 2015, 2021, 2022). The process has already been described in some families of Tenebrionoidea such as the Tenebrionidae, *Tenebrio molitor*, *Tribolium castaneum* and *Lagria villosa* (Nardi et al., 2013; Dias et al., 2012, 2013a, 2015, 2022), the Pythidae *Pytho depressus* (Dias et al., 2021) and the Ciidae *Cerasis cornifer* (Folly et al., 2021). The two species studied here have the same sperm structural characteristics as the sister group Pythidae (Dias et al., 2021). As in this family, the sperm share the same length and a similar shape of their conical acrosome and the cylindrical nucleus other than their mitochondrial derivatives and the accessory bodies (Dias et al., 2021). As in Pythidae and other Tenebrionoidea (Dias et al., 2013b, 2015, 2021, 2022), the species examined show the posterior nuclear region with two infoldings where the anterior end of the two mitochondrial derivatives are hosted. Beneath the nucleus, a centriole is present, surrounded by a scanty material of the centriole adjunct. Posterior to the region, a typical axoneme $9 + 9 + 2$ microtubular pattern is evident. The axoneme maintains a regular structure up to the flagellar end, where it loses the regular organization, as it occurs in the sperm flagella of many insects (Jamieson et al., 1999; Dallai, 2014). Among Tenebrionoidea, only Mordellidae show a flagellar posterior end provided with only accessory tubules that make the whole region stiff and immotile (Dias et al., 2022). The mitochondrial derivatives of the two species examined here have the region facing the axoneme with a dense matrix crystallized, as in other Tenebrionoidea (Dias et al., 2022). The two structures are triangular, very similar to those in the Pythidae (Dias et al., 2021). The mitochondrial derivatives can have a different shape in other Tenebrionoidea, such as in *T. molitor* and *Tr. castaneum* (Baccetti et al., 1973; Dias et al., 2012, 2015). The size of mitochondrial derivatives is an important parameter to characterize the Coleoptera such as Curculionoidea (Burrini et al., 1988; Dallai et al., 1998; Alzahrani et al., 2013), Chrysomeloidea (Baccetti and Daccordi, 1988) and Scarabaeoidea (Werner and Simmons, 2011). The two mitochondrial derivatives in these groups have different shapes and sizes, being elliptical or roundish, small or large. The structure and shape of the accessory bodies are also important structures in distinguishing the different families of Tenebrionoidea and Cucujoidea (Jamieson et al., 1999; Dias et al., 2022). The variations of these structures deal with their size, and in some groups, such as Curculionidae, they form a remarkable feature called “puff,” which apparently is a portion of accessory bodies with a less compact structure (Burrini et al., 1988; Dallai et al., 1998). As it was well ascertained (Jamieson et al., 1999; Dallai, 2014), the accessory bodies originate from the centriole adjunct material placed beneath the nucleus and extend along the flagellum on both sides of the axoneme, accompanying the two mitochondrial derivatives. In the Scraptiidae studied the accessory bodies have a somewhat triangular shape at the end of sperm maturation. Also these structures are very similar to those described in Pythidae (Dias et al., 2021); on the contrary, they are quite different from those described in other Tenebrionoidea such as Meloidae and Tenebrionidae (Nardi et al., 2013; Dias et al., 2012, 2015, 2022) that have an elliptical or roundish shape.

In conclusion, the ultrastructural study performed on the two species of Scraptiidae supports the close relationship of this family with that of Pythidae. Such results align with the results of molecular data by Zhang et al. (2018) and McKenna et al. (2019). These families belong to the same lineage of Tenebrionoidea and are well separated either by the basal Ripiphoridae-Mordellidae-clade and from Meloidae and Tenebrionidae. Our results need to be extended to other families of Tenebrionoidea before establishing the relationships between the numerous families of the large superfamily. The present data, even though preliminary, also confirm that sperm ultrastructure can significantly contribute to investigations on insect relationships (Jamieson et al., 1999; Dallai, 2014).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

No data was used for the research described in the article.

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