Structural and ultrastructural characterization of male reproductive tracts and spermatozoa in fig wasps of the genus Pegoscapus (Hymenoptera, Chalcidoidea)

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Abstract

The three Pegoscapus species present the same internal reproductive tract features comprising testes with a single testicular tubule, seminal vesicles, vasa deferentia, accessory glands and an ejaculatory duct. The seminal vesicle shows two morphologically distinct portions although they do not resemble the separate chambers found in other Chalcidoidea. The anterior portion of the seminal vesicle shows a prominent epithelium and stores the mature spermatozoa, while the posterior region is formed by a thicker muscular sheath that participates on ejaculation. The sexual maturation in Pegoscapus is achieved at emergence, when the testicular degeneration occurs. The spermatozoa of Pegoscapus reveal a basic structure similar to that of other Chalcidoidea. In Pegoscapus sp1. and Pegoscapus sp2. they present the same features, whereas Pegoscapus tonduzi comprises some different characteristics. It measures approximately 160 μm in Pegoscapus sp1. and Pegoscapus sp2., while in P. tonduzi the spermatozoa measure about 360 μm. The extracellular sheath thickness is another difference among the species. While Pegoscapus sp1. and Pegoscapus sp2. show a thick extracellular sheath, in P. tonduzi this sheath is very thin resulting in a large space intervening between the extracellular sheath and the nucleus. Despite these differences, the three species analyzed share some characteristics that allow the establishment of an identity to the spermatozoon of the genus Pegoscapus: the seminal vesicle not divided in chambers; the absence of acrosomal structures in the spermatozoa; the length of the extracellular sheath; the central microtubules being the firsts to terminate in the sequence of microtubular cutoff at the final axonemal portion.

Keywords: Ultrastructure; Spermatozoa; Reproductive tract; Pegoscapus; Chalcidoidea; Ficus pertusa; Ficus obtusifolia; Ficus citrifolia

1. Introduction

Fig wasp is a term applied to chalcid wasps that exclusively breed in figs, which are the enclosed inflorescence of fig trees. The members of the family Agaonidae are the only among fig wasps which pollinate figs (Boucek, 1993). The interaction between figs (Ficus: Moraceae) and fig pollinating wasps (Agaonidae, Chalcidoidea) represents perhaps the most tightly integrated pollination mutualism that is known (Ramirez, 1970, Wiebes, 1979, Weiblen, 2002 and Cook and Rasplus, 2003). Ficus is one of the most diverse genera of flowering plants (Berg and Wiebes, 1992 and Harrison, 2005). The nearly 750 described species of Ficus (Berg, 1989) occur worldwide in tropical and subtropical regions, and are considered "keystone" species in tropical forests due to their continual production of fruit, which is essential to a large number

of frugivores (Korine et al., 2000). Figs depend on female wasps to pollinate the flowers and thereby initiate seed production (Herre and West, 1997 and Herre, 1999). The mated female wasps, in turn, rely on the developing fig inflorescence to breed their offspring, since each wasp larva consumes the contents of one would-be seed.

The observation that related species of wasps generally pollinate related species of figs has led to the proposal of strict-sense coevolution between the two groups (Ramirez, 1974, Wiebes, 1979, Wiebes, 1982 and Berg and Wiebes, 1992). However, the existing classifications of figs and their pollinators are based on characters that are often intimately involved in their mutualistic interactions. Therefore, the apparent congruence observed in their current classifications might simply reflect reciprocal adaptations leading to convergent evolution (Van Noort and Compton, 1996). Fortunately, other data can provide independent characters for reconstructing phylogenies and rigorously testing evolutionary hypotheses concerning figs and their pollinators.

Systematic work on both Ficus and its associated insects is of fundamental importance to ecological and evolutionary studies. In this sense, the Ficus taxa of the Asian-Australian and African regions have been revised albeit the identity of the taxa is still problematical (Berg, 1989). Even in relation to fig wasps, systematics of Neotropical species is controversial. The classification of some fig wasp species is uncertain and the determination of host plants is unclear (Wiebes, 1995).

The superfamily Chalcidoidea is one of the most species and biologically diverse group of insects (Grissell and Schauff, 1997). It accounts for roughly one-third of the world's parasitic species of Hymenoptera (LaSalle and Gauld, 1991). In spite of its considerable importance to applied entomology, the knowledge of evolutionary relationships among chalcidoids is still in its infancy (Grissell and Schauff, 1997); as a consequence, the pattern of relationships is vague and the placement of several subfamilies and genera is a subject of disagreement. According to Heraty et al. (1997) new character systems are needed to resolve the relationships among families and subfamilies of Chalcidoidea. However, very little has so far been done and the pattern of relationships is still vague for most groups (Grissell and Schauff, 1997), perhaps because their morphological plasticity and extreme reduction in size.

Several of these uncertainties about the evolutionary relationships within the fig wasps and within Chalcidoidea probably will be resolved with the inclusion of new morphological characters, and the analyses of sperm ultrastructure may reveal helpful data (Carcupino et al., 1995 and Jamieson et al., 1999). Indeed, some recent studies have demonstrated that the structure and ultrastructure of spermatozoa of Chalcidoidea furnish sufficient variations to provide additional characters for cladistic analysis (Hogge and King, 1975, Quicke et al., 1992,

Lino-Neto et al., 1999, Lino-Neto et al., 2000a and Lino-Neto and Dolder, 2001b), in accordance with other works addressing the sperm ultrastructure of varied Hymenoptera (Dallai and Afzelius, 1995, Jamieson et al., 1999, Lino-Neto et al., 2000b, Lino-Neto and Dolder, 2001a, Lino-Neto and Dolder, 2002, Zama et al., 2001, Zama et al., 2004, Zama et al., 2005a, Zama et al., 2005b, Zama et al., 2007, Báo et al., 2004, Araújo et al., 2005b and Fiorillo et al., 2005).

In the present study, we describe the structure and ultrastructure of the male reproductive tract and the spermatozoa in genus Pegoscapus, providing some additional data about the reproduction of the group besides revealing some data that may be useful for future studies in taxonomy and phylogeny of Agaonidae, especially within Chalcidoidea.

2. Materials and methods

Pupae and adult virgin males of Pegoscapus sp1., pollinator of Ficus obtusifolia; Pegoscapus sp2., pollinator of Ficus pertusa and Pegoscapus tonduzi which pollinates Ficus citrifolia (Pereira et al., 2000, referred to as F. eximia) were obtained from strangler figs (subgenus Urostigma, section Americana) collected in the vicinity of the Campinas State University campus, Campinas, SP and São Paulo University campus, Ribeirão Preto, SP, Brazil, between February 1997 and February 2005. Ripe figs were collected from the trees, cut in half, and placed in Petri dishes to allow the wasps to emerge.

2.1. Transmission electron microscopy

The seminal vesicles were dissected and fixed for 4 h in a solution containing 2.5% glutaraldehyde, 3% sucrose, 0.2% picric acid and 5 mM CaCl2 in 0.1 M sodium cacodylate buffer at pH 7.2. After rising in buffer, they were post-fixed with 1% osmium tetroxide in the same buffer for 1 h. Dehydration was carried out in acetone, followed by embedding in Epon 812 resin. Ultrathin sections were stained with uranyl acetate and lead citrate and observed using a Jeol 1011 transmission electron microscope, operating at 80 kV.

For detection of basic proteins, the ethanolic phosphotungstic acid (E-PTA) method was applied. Seminal vesicles were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.2, for 24 h at 4 °C. After washing in the same buffer and dehydrating in alcohol, the material was treated en bloc with a solution of 2% PTA in absolute ethanol for 2 h at room temperature and embedded in Epon 812 resin. Ultrathin sections were observed unstained and partly stained with uranyl acetate and lead citrate.

2.2. Scanning electron microscopy

Spermatozoa collected from the seminal vesicle were spread on a coverglass slip, fixed in 2.5% glutaraldehyde, dehydrated in acetone, dried at the critical point and sputter-coated with gold. They were observed using LEO VP1430 and Jeol 840A scanning electron microscopes.

2.3. Light microscopy

The reproductive tract of the specimens was dissected and photographed. The material processed for transmission electron microscopy was also sectioned for light microscopy. Semi-thin sections were stained with toluidine blue and photographed with an Axiophot Zeiss Microscope equipped with a Zeiss Axiocam MRc digital camera and the Axiovision 4.5 software.

Dissected seminal vesicles were smeared on clean glass microscope slides, to release the sperm within it, and fixed in solution of 4% (w/v) paraformaldehyde in 0.1 M sodium phosphate buffer, pH 7.2. After drying at room temperature, the preparations were observed in a photomicroscope (Olympus, BX60), equipped with phase contrast.

To access nucleus measurements, some of these preparations were stained during 15 min with 0.2 μg/ml 4,6-diamino-2-phenylindole (DAPI) in PBS, washed, and mounted with Vectashield. They were then examined in an epifluorescence microscope (Olympus, BX60) equipped with a BP 360–370 nm excitation filter.

3. Results

3.1. Reproductive tract

The three species analyzed do not present any difference in their reproductive tracts. The anatomy of the internal reproductive tract of the Pegoscapus adult male comprises: paired degenerated testes with reduced volume, seminal vesicles, vasa deferentia, accessory glands and an ejaculatory duct (Fig. 1A and B). Each testis contains a single testicular tubule (seminiferous tubule) that forms a projection into the seminal vesicle (Fig. 1A, B and D). The anterior portion of the seminal vesicle stores the mature spermatozoa until copulation and

then the posterior region empties into the vas deferens (Fig. 1B–D). Accessory glands join the vasa deferentia near their ends and, posteriorly, the paired vasa deferentia join to form the ejaculatory duct (Fig. 1A and B).
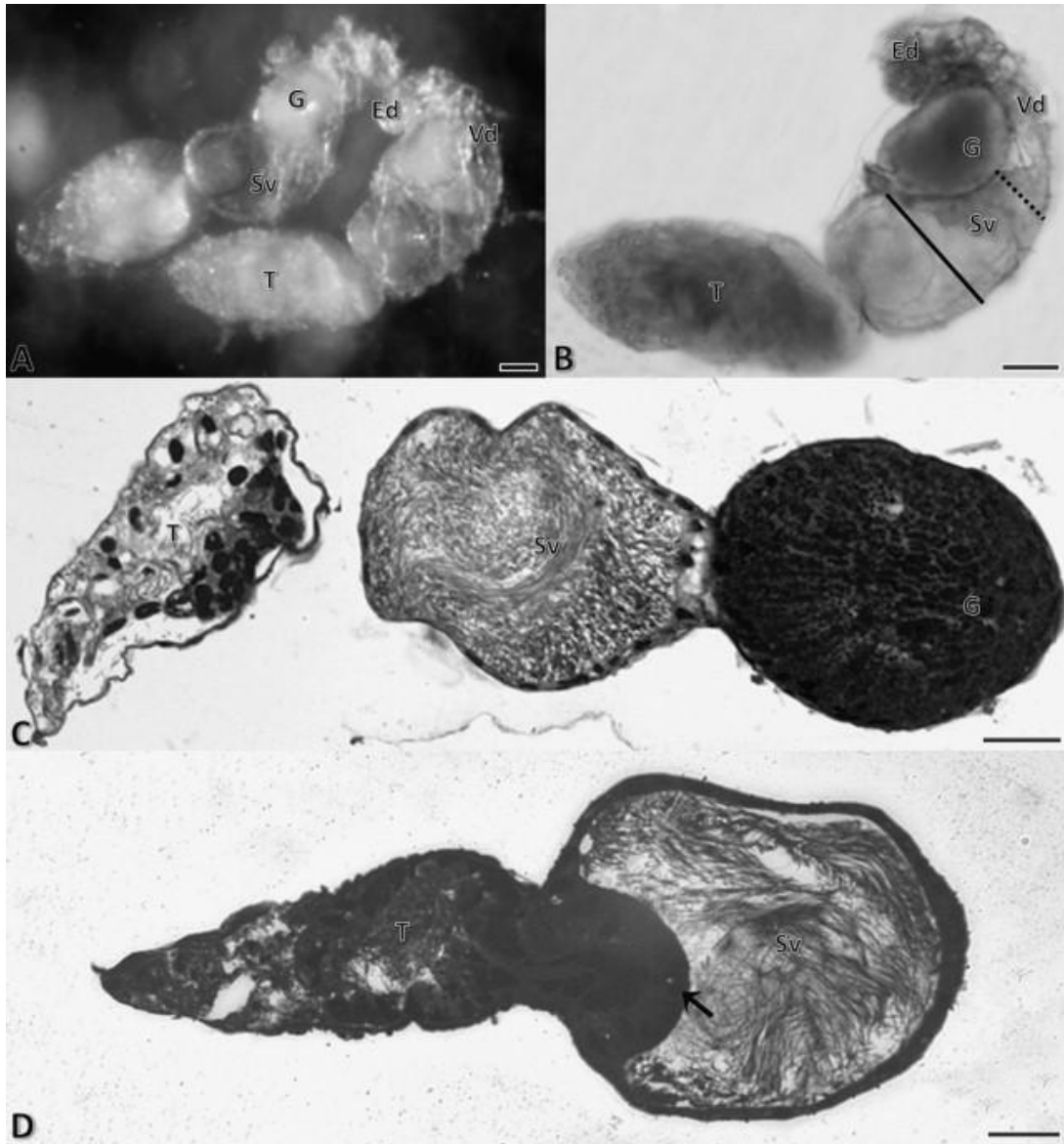
Fig. 1. Internal reproductive tract of pupae (A and B) and adult (C and D) males of Pegoscapus. (A) The complete reproductive tract presenting voluminous testes full of sperm cysts. (B) Single unit of the tract showing the localization of anterior (line) and posterior (dotted line) portions of the seminal vesicle. (C) Section of the reproductive tract showing the testes presenting a degenerated appearance. (D) Section of the testis/seminal vesicle interface. Arrow indicates the testis projection into the seminal vesicle. Ed, ejaculatory duct; G, accessory gland; Sv, seminal vesicle; T, testis; Vd, vas deferens. Scale bars: (A and B) 50 μm; (C and D) 20 μm.

The sexual maturation of Pegoscapus, which occurs close to the emergence, involves the cessation of spermiogenesis, the migration of spermatozoa to the seminal vesicles and the testicular degeneration. Before emergence, the wasp males present the testes full of spermatozoa, being more voluminous than the seminal vesicles, which are empty (Fig. 1A and B). In contrast, in newly emerged males the seminal vesicle lumen is filled with spermatozoa immersed in an amorphous material and the testes reveal a degenerated appearance (Fig. 1C and D). Thus, migration of the spermatozoa and degeneration of testes result in seminal vesicles larger than the testes after the emergence (Fig. 1C and D).

The seminal vesicle is a specialized region consisting of a globular enlargement of the anterior region of the vas deferens (Fig. 1A and B). It presents two distinct portions (Fig. 1B) comprising a double-layered wall: an inner epithelium and an outer muscular sheath (Fig. 2A–C). The anterior portion bears a thin muscular layer (Fig. 2A and B), while the posterior region consists of a narrow epithelium and a thicker muscular sheath (Fig. 2C). The epithelium comprises a single layer of polarized columnar cells covered with microvilli-like projections at their luminal surface (Fig. 2B). These cells present some septate junctions in the contacts between the neighboring cells (Fig. 2A). The nucleus is irregularly shaped and it is located at the cell basal region (Fig. 2A and B). In the luminal region, the spermatozoa are stored individually and dispersed in an amorphous material of median electron density and no sperm bundles can be observed (Fig. 2A–C).
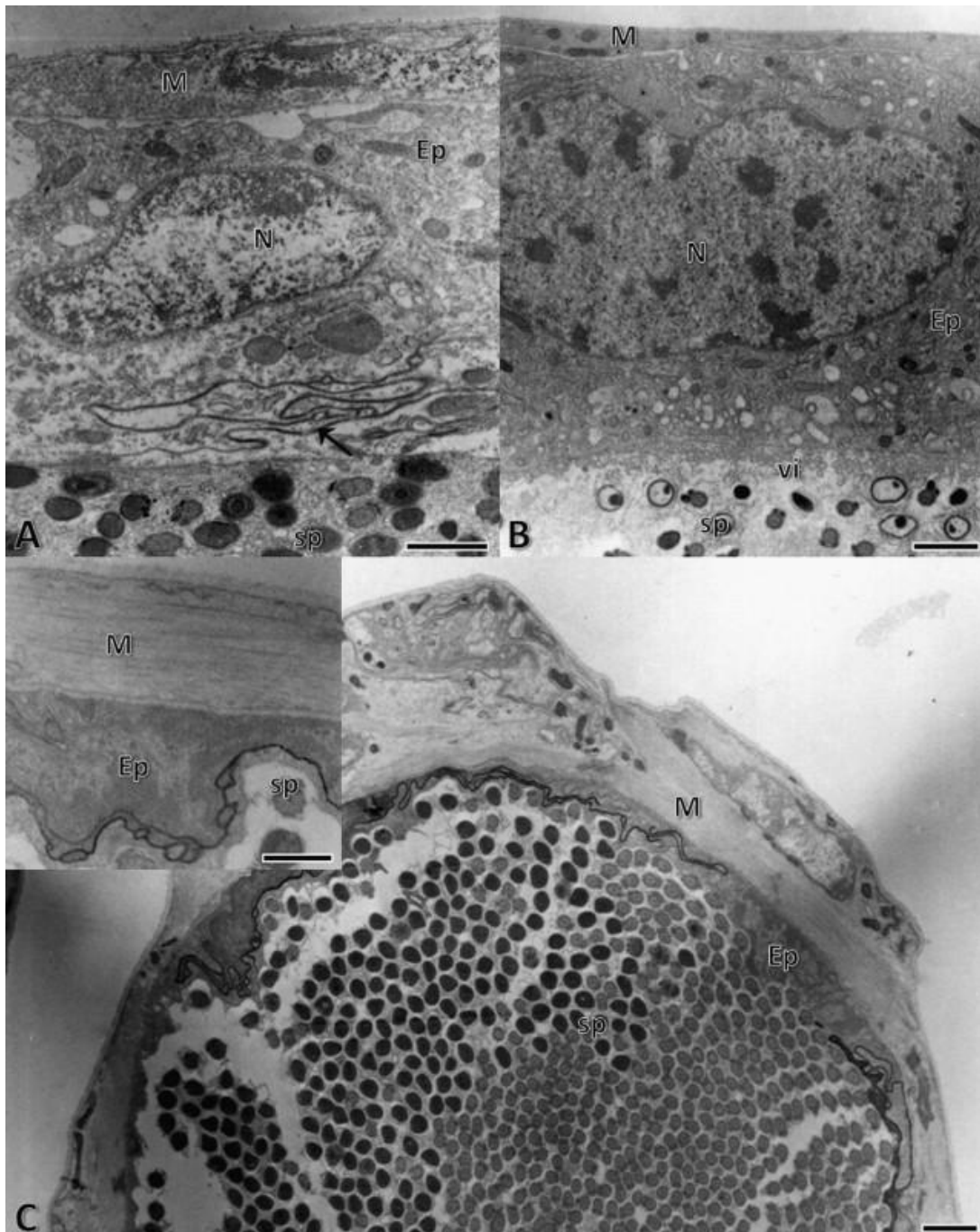
Fig. 2. Ultrastructure of the seminal vesicle in Pegoscapus. (A and B) Sections of the anterior portion of the seminal vesicle wall. Arrow indicates some septated junctions. (C) Section of the posterior portion of the seminal vesicle. (Inset) Thicker muscular sheath at this region. Ep, epithelial region; M, muscular sheath; N, nucleus; sp, spermatozoa; vi, microvilli-like projections. Scale bars: (A–C) 1 μm; inset 0.5 μm.

3.2. Spermatozoa

The spermatozoon of Pegoscapus is represented diagrammatically in Fig. 3. The spermatozoa are long and slender cells. In P. tonduzi they measure approximately 360 μm (Fig. 4A), while in Pegoscapus sp1. and Pegoscapus sp2. spermatozoa measure about 160 μm (Fig. 4B). The spermatozoon is helicoidally twisted (Fig. 4 and Fig. 5) and comprises a head

region consisting of an extracellular sheath and the nucleus (Fig. 4H–S) and a flagellar region that includes axoneme, centriolar adjunct and paired mitochondrial derivatives (Fig. 5A–L).
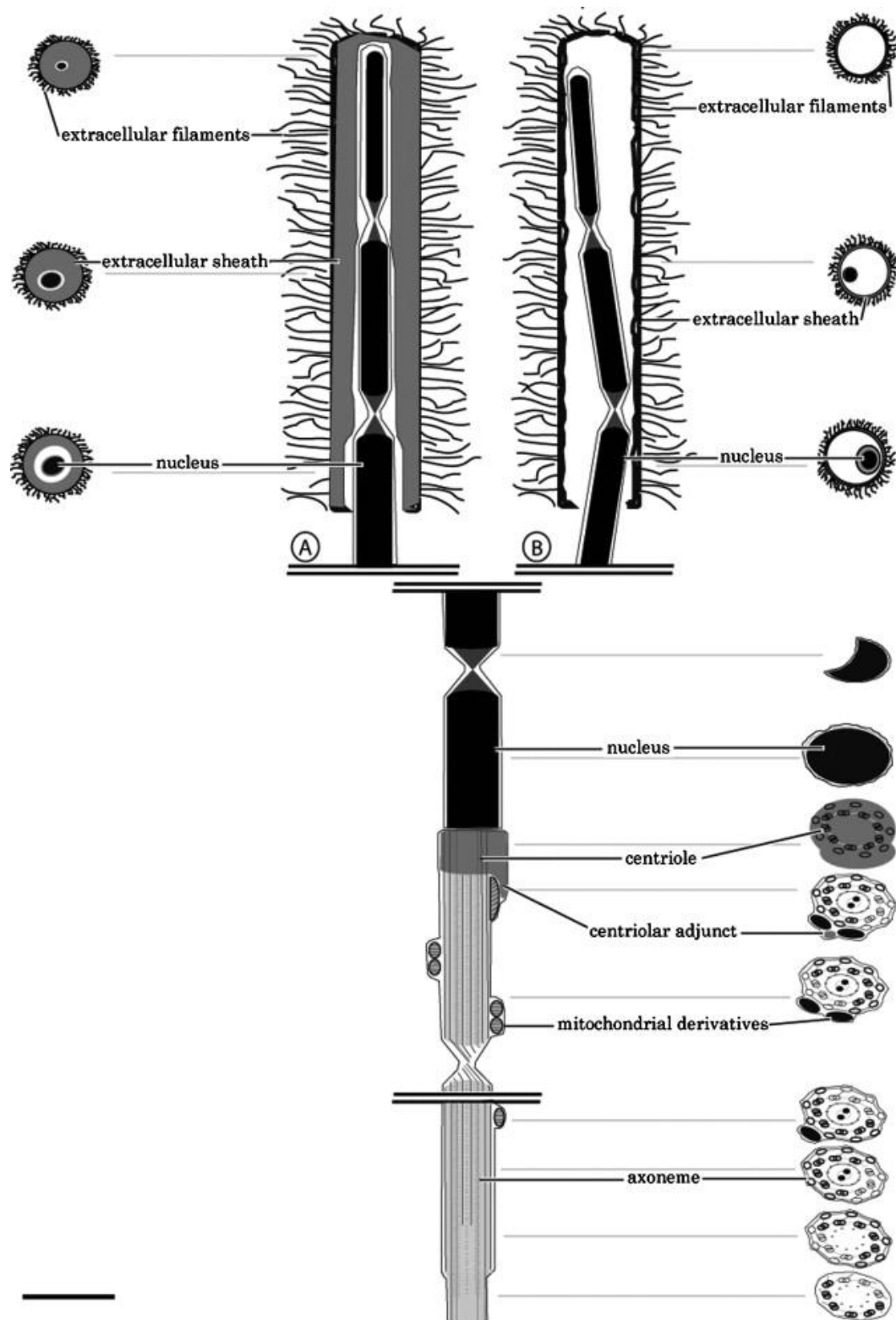
Fig. 3. Diagram of the spermatozoon of Pegoscapus and corresponding transverse section. In the head region it is emphasized the pattern difference in (A) Pegoscapus sp1 and Pegoscapus sp2. in relation to (B) Pegoscapus tonduzi. Scales of various components are only approximate. Scale bar, 2 μm.
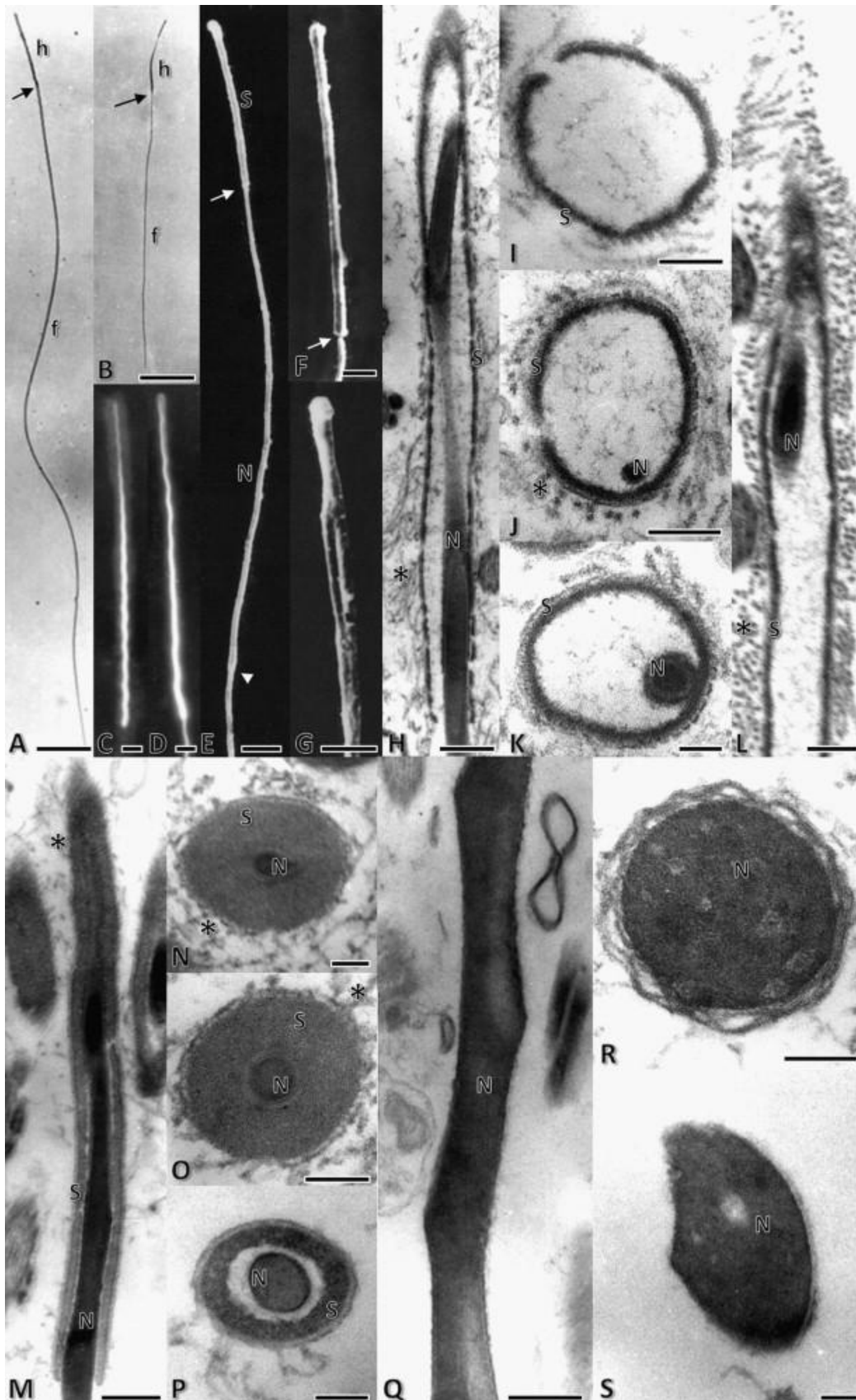
Fig. 4. Light (A–D) and electron (E–S) micrographs of Pegoscapus spermatozoa. (A and B) Phase contrast micrographs of spermatozoa. The arrows indicate the limit between head (h) and flagellum (f). (C and D) DAPI stained fluorescence micrographs of the nucleus. (E–G) Scanning electron micrographs of the head region. Arrows indicate the beginning of the extracellular sheath and the arrowhead, the limit between nucleus and flagellum. (H–P) Longitudinal (H, L and M) and transversal (I–K, N–P) sections of the head

region showing the nucleus surrounded by the extracellular sheath. Note the difference in extracellular sheath thickness between P. tonduzi (H–L) and the other Pegoscapus species (M–P). Asterisk indicates the filaments irradiating from this sheath. (Q–S) Longitudinal (Q) and transversal (R and S) sections of the nucleus. The nucleus appears circular (R), but it is irregularly shaped (S) in some portions due to its twist. f, flagellar region; h, head region; N, nucleus; S, extracellular sheath. Species: (A, C and E–L) P. tonduzi; (B, D and Q) Pegoscapus sp1.; (M–P, R–S) Pegoscapus sp2. Scale bars: (A and B) 25 µm; (C–E) 2 µm; (F and G) 1 µm; (H, M and Q): 0.4 µm; (I–J, L) 0.2 µm; (K, N–P, and R–S) 0.1 µm.
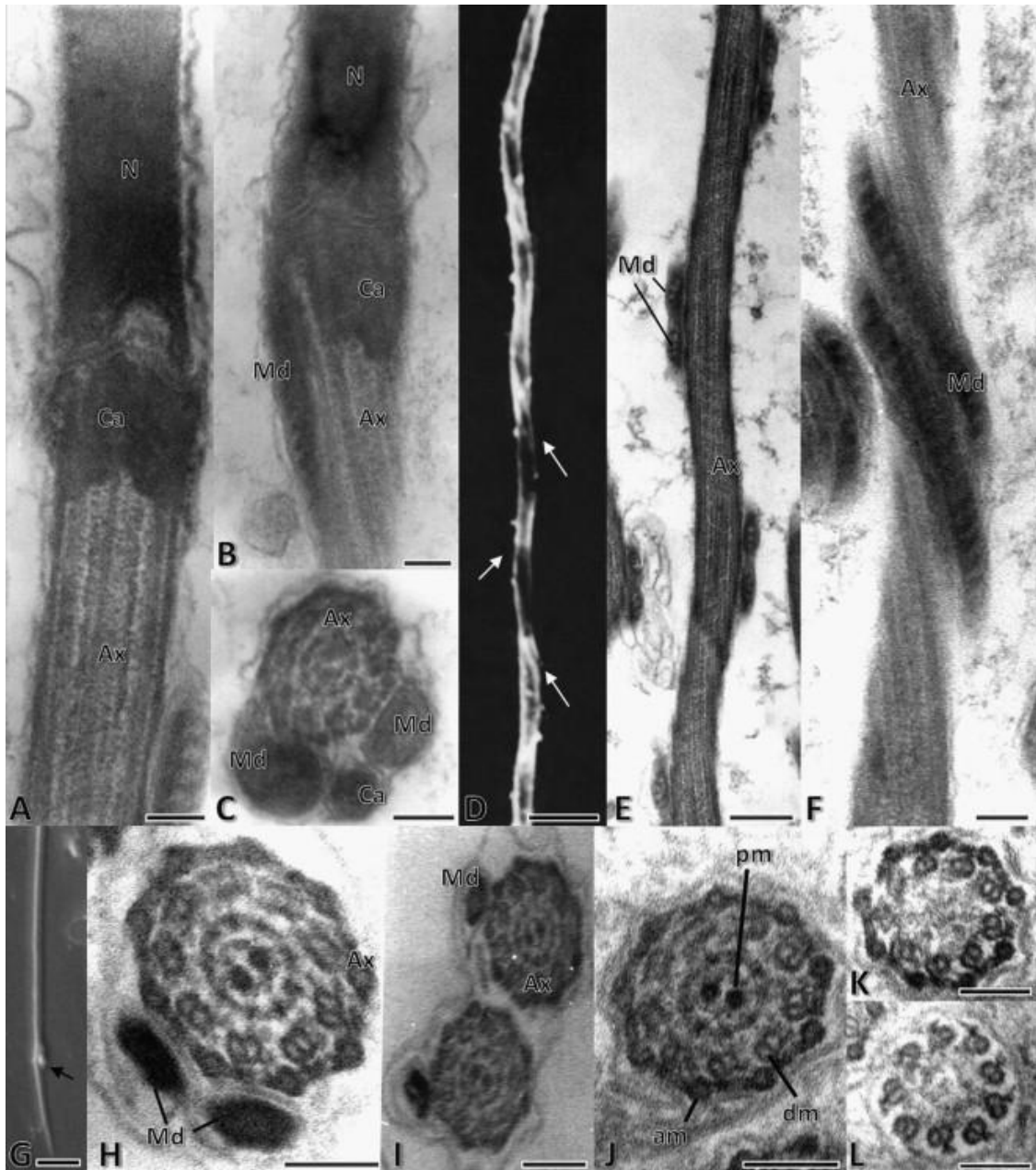
Fig. 5. Electron micrographs of the flagellar region of Pegoscapus spermatozoa. (A and B) Longitudinal sections of nucleus-flagellum transition region showing the centriolar adjunct anteceding the mitochondrial derivatives and the axoneme. (C) Transverse flagellar section showing the centriolar adjunct tip with both mitochondrial derivatives. (D) Scanning electron micrograph of the flagellar region. Arrows indicate the mitochondrial derivatives around the axoneme. (E and F) Longitudinal flagellar sections showing the mitochondrial derivatives coiling regularly around the axoneme. (G) Scanning electron micrograph of the flagellar extremity. Arrow indicates the longer mitochondrial derivative tip. (H) Transverse flagellar section. (I–L) Transverse flagellar sections showing the posterior tip, where one mitochondrial derivative ends before the other and axoneme gradually disorganizes. am, accessory microtubules; Ax, axoneme; Ca, centriolar adjunct; dm, microtubule doublets; Md, mitochondrial

derivatives; N, nucleus; pm, central microtubule pair. Species: (A–D, F–G) P. tonduzi; (E) Pegoscapus sp2.; (H–L) Pegoscapus sp1. Scale bars: (A–C, F, H–L) 0.1 µm; (D and G) 1 µm; (E) 0.3 µm.

The anterior region of the spermatozoon is made up of an extracellular sheath with 10 µm in length that covers the anterior portion of the nucleus (Fig. 4E–P). Due to the presence of this sheath, the anterior portion of the spermatozoon is larger than the others portions, as better revealed by scanning electron microscopy images (Fig. 4E and F). The extracellular sheath, from which innumerable filaments irradiate (Fig. 4H and L), varies in thickness among Pegoscapus species, being thicker in Pegoscapus sp1. and Pegoscapus sp2. (Fig. 4M–P) than in P. tonduzi (Fig. 4H–L). In transverse sections, this difference results in a larger space intervening between the extracellular sheath and the nucleus in P.tonduzi (Fig. 4H–L). The classical acrosomal structures (acrosomic vesicle and perforatorium) are absent in Pegoscapus species analyzed herein.

The nucleus possesses an elongated and electron-dense appearance (Fig. 4Q–S) measuring about 30 µm when stained with DAPI in light microscopy observations (Fig. 4C and D). It is helicoidally twisted and this twisting is more prominent in P.tonduzi (Fig. 4C). The nucleus is circular in transverse sections (Fig. 4R); however it is irregularly shaped due to its twist in some sections (Fig. 4S). Cross-sections also show that the nucleus narrows along its length in direction of its anterior tip (Fig. 4J, K, N–P). In E-PTA treatment, the spermatozoan nucleus appeared completely negatively stained whereas the extracellular sheath was positively (Fig. 6A–E), which can be clearly revealed by the increased electron density at the thicker extracellular sheath of Pegoscapus sp1. and Pegoscapus sp2. (Fig. 6A and C).
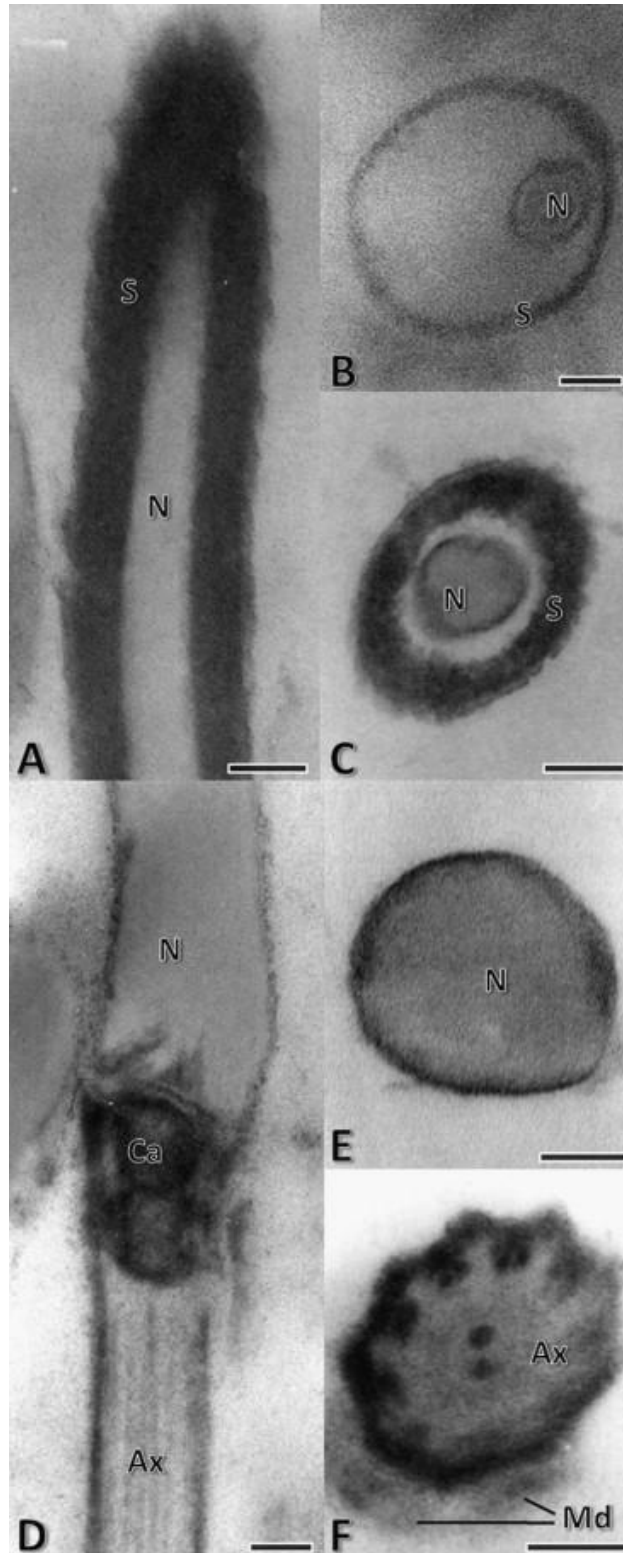
Fig. 6. Electron micrographs of the spermatozoa of Pegoscapus stained with E-PTA. (A) Longitudinal and (B, C and E) transversal sections of the head region showing the extracellular sheath positively stained and the negative nucleus. (D) Longitudinal and (F) transversal sections of the flagellar region with the centriolar adjunct and the axonemal microtubules positively stained. Ax, axoneme; Ca, centriolar adjunct; Md, mitochondrial derivatives; N, nucleus; S, extracellular sheath. Species: (A, C–F) Pegoscapus sp1.; (B) P. tonduzi. Scale bars: (A–F) 0.1 μm.

The nuclear posterior truncated extremity is attached to the flagellum by a small, uniformly compact and electron-dense centriolar adjunct measuring about 200 nm in length (Fig. 5A and B). The centriolar adjunct presents a posterior projection that can be observed in transverse sections together with the beginning of the mitochondrial derivatives (Fig. 5C). This structure is E-PTA positively stained (Fig. 6D). The centriole is probably localized immediately above the nuclear tip, covered by the centriolar adjunct until the beginning of the axoneme (data not shown).

The axoneme follows the typical 9 + 9 + 2 pattern of microtubule arrangement (Fig. 5H–J). The microtubules are twisted and the spiraling is clearly evident in transverse sections, since not all of the doublets can be simultaneously sectioned at perfect right angles (Fig. 5H–L). The microtubules are E-PTA positive, while the intertubular material is negative (Fig. 6F). In the final posterior region of the sperm, the axoneme becomes gradually disorganized, with the central microtubules terminating first and the nine doublets last (Fig. 5J–L).

The mitocondrial derivatives of Pegoscapus coil around the twisted axoneme (Fig. 5D–F). In longitudinal sections as well as in scanning electron micrographs, they can be seen coiling regularly around the axoneme with a periodicity about 2-µm long (Fig. 5D and E). They are alike in diameter and placed very close to the axoneme (Fig. 5H). Anteriorly, the mitochondrial derivatives begin together in contact with the posterior extremity of the centriolar adjunct (Fig. 5B and C). Although their anterior extremities are coincident, the posterior extension of the mitochondrial derivatives varies slightly between each other, and the longest one terminates about 3 µm above the axoneme tip (Fig. 5G, I and J).

4. Discussion

The process of sexual maturation in Pegoscapus agreed with those observed for some Hymenoptera (Araújo et al., 2005a and Boomsma et al., 2005). The spermatogenesis occurs only during the pupation and ceases at the sexual maturity, which for some Chalcidoidea is reached at the male emergence. The process of spermatic maturation occurs at the testicular tubule and after the emergence, the mature spermatozoa are located at the seminal vesicle and the testes have a degenerated appearance.

The morphology of the male reproductive apparatus in Pegoscapus is similar to that of other Chalcidoidea (Damiens and Boivin, 2005). In fact, the general morphology of the apparatus has been maintained throughout the order Hymenoptera, but the number of testicular tubules per testis varies considerably among hymenopteran groups. The occurrence

of three tubules was reported for some bee families (Ferreira et al., 2004 and Araújo et al., 2005a) whereas other bees present four tubules per testis, such as Mellitidae, some Megachilidae and Apidae s. stricto (Roig-Alsina and Michener, 1993 and Ferreira et al., 2004), with the exception of Apis mellifera with about 250 tubules (Chapman, 1998). On the other hand, this number varies from 1 to 25 in Formicidae (Wheeler and Krutzsch, 1992). Interestingly, in Pegoscapus only one tubule is observed per testis, such as in all Chalcidoidea species observed to date (Lino-Neto, personal communication).

In almost all Chalcidoidea the seminal vesicle comprises two separate chambers, which can also be observed in Spalangia cameroni (Gerling and Legner, 1968) and Trichogramma evanescens (Damiens and Boivin, 2005). Baer and Boomsma (2004) named these chambers as: sperm reservoir (the upper part) and ejaculatory section (the lower part). However, in Pegoscapus, the seminal vesicle is not divided in separate chambers, but it shows two morphologically distinct portions sharing similarities with the previously described chambers. The anterior portion stores the mature spermatozoa and participates in the reabsorption and digestion of defective cells (spermiophagy) and sperm fluid, as reported for other species (Viscuso et al., 1999, Dallacqua and Cruz-Landim, 2003 and Araújo et al., 2005a). On the other hand, the posterior seminal vesicle portion acts on the ejaculation (Gerling and Legner, 1968 and Baer and Boomsma, 2004). The presence of this second portion, probably guarantees that only a little amount of the stored spermatozoa is ejaculated per sexual copulation. This restraint would be essential to provide each male with the potential to mate with many females in rapid succession (King, 1987).

The basic structure of the spermatozoa of Pegoscapus is similar to that of other Chalcidoidea (Wilkes and Lee, 1965, Hogge and King, 1975, Quicke et al., 1992, Lino-Neto et al., 1999, Lino-Neto et al., 2000a and Lino-Neto and Dolder, 2001b) whose nucleus is twisted into a helix and the mitochondrial derivatives coil around the twisted axoneme.

The spermatozoa of Pegoscapus sp1. and Pegoscapus sp2. present the same features and there are no sperm characters that could distinguish these species. On the other hand, in P. tonduzi the spermatozoa comprise some characteristics that diverge from the sperm of other Pegoscapus species analyzed. The morphometric data show the first discordant character. The total length of Pegoscapus sp1. and Pegoscapus sp2. spermatozoa is about a half of the P. tonduzi sperm length. However, the head region shows the same length for the three species. This aspect is also observed in Trichogrammatidae (Lino-Neto et al., 2000a and Lino-Neto and Dolder, 2001b) where differences in sperm length are concentrated in the flagellar region.

The extracellular sheath thickness also differs among these Pegoscapus species. As occurs in Eurytomidae (Lino-Neto et al., 1999) and Trichogrammatidae (Lino-Neto et al., 2000a and Lino-Neto and Dolder, 2001b), Pegoscapus sp1. and Pegoscapus sp2. present a thick extracellular sheath that is juxtaposed to the nucleus. Conversely, in P. tonduzi this sheath is very thin resulting in a larger space intervening between the extracellular sheath and the nucleus, a characteristic that has not yet been observed for other Chalcidoidea.

The head region comprising the anterior portion of the E-PTA negative nucleus surrounded by an E-PTA positive extracellular sheath bearing some filaments is a common characteristic for chalcidoids (Quicke et al., 1992, Lino-Neto et al., 1999, Lino-Neto et al., 2000a and Lino-Neto and Dolder, 2001b). The acrosomal structures were not observed in Pegoscapus, as they were also not distinguished in Trichogramma pretiosum (Lino-Neto et al., 2000a). For the other Chalcidoidea already observed, the acrosomal structures are very small, measuring less than 2 µm (Quicke et al., 1992, Lino-Neto et al., 1999, Lino-Neto et al., 2000a and Lino-Neto and Dolder, 2001b).

In relation to the flagellar structures, Pegoscapus spermatozoon follows the pattern observed in the other Chalcidoidea already described, where the centriolar adjunct precedes both mitochondrial derivatives and the axoneme (symmetric pattern), attaching these structures to the posterior end of the nucleus (Quicke et al., 1992, Lino-Neto et al., 1999, Lino-Neto et al., 2000a and Lino-Neto and Dolder, 2001b). In contrast, most Hymenoptera possess an elongated centriolar adjunct located laterally to the axoneme and interposed between the nuclear base and the tip of one of the mitochondrial derivatives in an asymmetric pattern (Newman and Quicke, 1998, Newman and Quicke, 1999a, Newman and Quicke, 1999b, Lino-Neto et al., 2000b, Zama et al., 2001, Zama et al., 2004, Zama et al., 2005a, Zama et al., 2005b, Báo et al., 2004 and Fiorillo et al., 2005). Still, in these hymenopterans, the anterior extremities of the axonemal microtubules are parallel to the adjunct and not inserted into it, as in chalcidoids (Lino-Neto et al., 1999, Lino-Neto et al., 2000a and Lino-Neto and Dolder, 2001b). Moreover, the centriole is probably inserted into the centriolar adjunct in all Chalcidoidea, which is clearly observed in some species such as in Eulophidae representants Palmisticus elaeisis and Trichospilus diatraeae (Lino-Neto, personal communication). Unfortunately, for most chalcidoids including Pegoscapus, the high electron density of the centriolar adjunct makes centriole observation difficult.

The 9 + 9 + 2 microtubular pattern is conserved throughout Hymenoptera, but the sequence of their cutoff in the final axonemal portion differentiates the major groups. In all Aculeata, the central microtubules and the nine doublets terminate first, followed by the accessory microtubules (Lino-Neto et al., 2000b, Zama et al., 2001, Zama et al., 2004, Zama et

al., 2005a, Zama et al., 2005b, Zama et al., 2007, Báo et al., 2004, Araújo et al., 2005b, Fiorillo et al., 2005 and Mancini et al., 2006) although they all terminate approximately together in ants (Wheeler et al., 1990 and Lino-Neto and Dolder, 2002). In the Parasitica the nine doublets are the last microtubules to be lost at the flagellum tip (Newman and Quicke, 1998, Lino-Neto et al., 1999, Lino-Neto et al., 2000a and Lino-Neto and Dolder, 2001b). The other Chalcidoidea previously analyzed showed a similar pattern of microtubule cutoff, with the accessory microtubules terminating first (Lino-Neto et al., 1999, Lino-Neto et al., 2000a and Lino-Neto and Dolder, 2001b). Nevertheless, in Pegoscapus the central microtubules disappear prior to the others.

The mitochondrial derivatives in Pegoscapus are also very similar to those of the remaining chalcidoids. They are oval and alike in diameter, lying very close to the axoneme and coiling regularly around the axoneme (Quicke et al., 1992, Lino-Neto et al., 1999, Lino-Neto et al., 2000a and Lino-Neto and Dolder, 2001b). The coiling periodicity is the same as observed in the Eurytomidae Bephratelloides pomorum (Lino-Neto et al., 1999). However, in Trichogrammatidae (Lino-Neto et al., 2000a and Lino-Neto and Dolder, 2001b) this periodicity is a little different, with about 1.5 μm. The mitochondrial derivatives begin together, in a small distance from the nucleus and in contact with the posterior base of the centriolar adjunct.

Finally, there are two significant differences among these species: the sperm length and the extracellular sheath thickness. The sperm length shows large variations for Chalcidoidea and the thicker extracellular sheath, which is observed for Pegoscapus sp1. and Pegoscapus sp2., is a plesiomorphic character. For these reasons, these differences may not indicate the phylogenetic relationship among these Pegoscapus species. Considering these circumstances, additional research describing spermatozoal structure and ultrastructure of species of Pegoscapus genera are necessary to determine the real degree of variability in sperm ultrastructure between Pegoscapus, shedding light on the phylogenetic relationships of fig wasps. On the other hand, the three species analyzed in this study share various structural and ultrastructural characteristics of the male reproductive tracts and spermatozoa which allows the establishment of an identity to the genus Pegoscapus: the seminal vesicle not divided into chambers; the length of the extracellular sheath; the absence of acrosomal structures; the central microtubules being the firsts to terminate in the sequence of microtubular cutoff at the final axonemal portion. The establishment of such a pattern for the genus Pegoscapus will be useful for future phylogenetic studies in Chalcidoidea.

Acknowledgements

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