Micron 43 (2012) 695–704

Contents lists available at SciVerse ScienceDirect

Micron

journal homepage: www.elsevier.com/locate/micron

Review

Morphology of the male reproductive system and spermatozoa in *Centris* Fabricius, 1804 (Hymenoptera: Apidae, Centridini)

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ARTICLE INFO

Article history:
Received 19 September 2011
Received in revised form 26 January 2012
Accepted 27 January 2012

Keywords:
Bees
Ultrastructure
Seminal vesicle
Testes

ABSTRACT

The genera *Centris* and *Epicharis* constitute the Centridini and are widely distributed in the Neotropical region. *Centris* is also found in the southern portion of the Neartic region, although both genera are more abundant in the humid tropical regions. To describe the structure of the male reproductive system and spermatozoa, light and transmission electron microscopy were used. The male reproductive system of *Centris* sp. is formed by a pair of testes, a pair of deferent ducts, a pair of seminal vesicles, a pair of accessory glands and an ejaculatory duct connected to the external genitalia, the aedeagus. In this species, testes and the pre-vesicular deferens ducts as well as the seminal vesicles are encapsulated in a single conjunctive capsule, the scrotal membrane. Each testis consists of four testicular follicles, made up of cysts with up to 64 germinative cells. Histologically, the seminal vesicles are formed by a simple cylindrical epithelium, basal membrane and muscular tunic. The spermatozoa of *Centris analis*, *C. fuscata*, *C. tarsata* and *Centris* sp. are morphologically similar. They have two easily distinguishable regions: the head and flagellum. The head is formed by the two-layer acrosome, the linear nucleus and the flagellum, the centriole adjunct, the axoneme of pattern 9 + 9 + 2 microtubules, two asymmetric mitochondrial derivatives and two accessory bodies. These *Centris* species share various morphological characteristics of the male reproductive system and spermatozoa with the other bees previously described, indicating that several characteristics are synapomorphic for the family Apidae. Studies on the morphology of the male reproductive system and spermatozoa in Hymenoptera have demonstrated the diversity of the information provided by these reproductive structures, which can be used in taxonomy studies and the phylogeny of this important group of insects.

Contents

1. Introduction

The wasps, Ampulicidae, Heterogynaidae, Sphecidae and Crabronidae, and bees form the monophyletic group Apoidea (Melo, 1999; Melo and Gonçalves, 2005), with more than 25,000

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0968-4328/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.micron.2012.01.013

species described (Hanson and Menke, 2006) and distributed throughout nearly all terrestrial ecosystems (Michener, 2000). The bees are the group with the most number of Apoidea, comprising around 16,000 species in approximately 500 genera, although the estimated number of species may be higher (Bohart, 1970; Michener, 2000; Griswold et al., 2006).

The genera *Centris* Fabricius, 1804 and *Epicharis* Klug, 1807 constitute the Centridini (Michener, 2000) and are widely distributed in the Neotropical region. *Centris* is also found in the southern portion of the Neartic region, although both genera are more abundant in the humid tropical regions (Silveira et al., 2002; Moure and Melo, 2007a, b). The Centridini tribe bees, with approximately 270 described species, are among the main pollinators in the Neotropics and are one of the groups of non-corbiculate bees most intensively studied in this region (Griswold et al., 2006).

The genus *Centris* comprises numerous species of solitary bees, characterized by their sturdy and hairy appearance, and ranging from medium- to large-sized in a variety of colours (Michener, 2000; Silveira et al., 2002). This genus is composed of several sub-genera, whose phylogenetic relationships have been studied by Ayala (1998), who recognized the existence of three groups: (1) the Centris group, constituting the subgenera *Acritocentris*, *Centris* (*sensu stricto*), *Exallocentris*, *Paracentris*, *Xanthemisia* and *Xerocentris*; (2) the Melacentris group, constituting the subgenera *Melacentris*, *Ptilocentris*, *Wagenknechtia*, *Ptilotopus*, *Aphemisia* and *Schistemisia*; and (3) the Trachina group, constituting the subgenera *Hemisiella*, *Heterocentris*, *Trachina* and *Paremisia*. According to Silveira et al. (2002), Ayala's proposal (1998) agrees partially with the phylogenetic relationships suggested by Michener (1951). However, many aspects of the systematics of this group remain controversial, including the recognition of some of the subgenera.

The most recent study on *Centris* phylogeny was conducted by Vivallo (2010). In this study, the phylogenetic relationships between the Centridini, Ericrocidini and Rhathymini tribes were evaluated. The cladistic analysis, with 216 characteristics of the external morphology of males and females including the male genitalia and sting, showed that the three tribes are monophyletic, although only Centridini and Ericrocidini form a natural group. The internal phylogenetic relationships of Centridini and Ericrocidini showed that all the genera and subgenera are monophyletic, except for *Centris* (*Melacentris*), which is paraphyletic in relation to *Centris* (*Aphemisia*), and *Centris* (*Paracentris*), which is paraphyletic in relation to *Centris* (*Penthemisia*) and *Centris* (*Xanthemisia*). The author suggested that these last two subgenera should be considered together with *Centris* (*Aphemisia*). Another suggestion was to create a new subgenus to include the Hyptidis group species, which belongs to an independent strain close to *Centris* (*Wagenknechtia*).

The male reproductive system in the Hymenoptera is composed of a pair of testes connected to the external medium through genital ducts, which are frequently associated with accessory glands. The seminal vesicles represent specialized areas of the deferent ducts, which store the spermatozoa produced in the testes until their release during copulation (Cruz-Landim, 2008). Ferreira et al. (2004) compared the morphology of the male reproductive system of 51 bee species, which belong to six families based on the classification by Michener (1965), and identified four types (I, II, III and IV) according to the anatomical differences of the reproductive structures. This grouping of families based on these characteristics shows that they are potentially useful in studies of the phylogenetic relationships in these bees.

In insects, including Hymenoptera, characteristics obtained from the spermatozoa structure are used in phylogenetic studies (Baccetti, 1970; Dallai, 1974; Jamieson, 1987; Quicke et al., 1992; Jamieson et al., 1999). The morphology of the Hymenoptera spermatozoa is known in various groups (Newman and Quicke, 1999a, b, 2000; Lino-Neto et al., 1999, 2000a; Lino-Neto and Dolder, 2001b; Zama et al., 2005b, 2007; Mancini et al., 2006, 2009; Moya et al., 2007; Brito et al., 2009; Araújo et al., 2010a, b; Moreira et al., 2010; Oliveira et al., 2010). In bees, the most detailed studies are restricted to the Apidae (Lino-Neto et al., 2000b; Zama et al., 2001, 2004, 2005a; Báo et al., 2004; Araújo et al., 2005c; Badke et al., 2005; Fiorillo et al., 2009), some Halictidae (Fiorillo et al., 2005), Megachilidae and Andrenidae (Quicke et al., 1992).

This study describes for the first time the structure and ultrastructure of organs of the internal male reproductive system and spermatozoa in *Centris*, aiming to contribute to the knowledge of reproductive biology, as well as to provide information on the systematic studies of this group.

2. Materials and methods

Adult males of *Centris* (*Heterocentris*) *analis* Fabricius, *C.* (*Trachina*) *fuscata* Lepeletier, *C.* (*Hemisiella*) *tarsata* Smith and *Centris* sp. were collected on flowers at the campus of the Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil.

In this study, the results on the morphology and structure of the male reproductive system refer to those of the species *Centris* sp. The results on the ultrastructure of the testes, seminal vesicles and spermatozoa refer to those of all the four species studied.

2.1. Light microscopy

To describe the general morphology of the male reproductive system of the *Centris* sp., adult males were dissected in a buffer solution of 0.1 M sodium cacodylate, pH 7.2, and this system was removed, photographed in a Zeiss stereoscope microscope (SPEM 2000C0).

To describe the structure of the male reproductive system of the *Centris* sp., adult males were dissected and this system removed, fixed in 2.5% glutaraldehyde solution in a buffer solution of 0.1 M sodium cacodylate, pH 7.2, for 12–24 h, dehydrated in solutions of increasing ethanol concentrations and then infiltrated and embedded in glycolmetacrylate resin (Historesin, Leica). Semi-thin sections of 2–5 μm thick were stained with 1% toluidine blue-sodium borate and haematoxylin & eosin and mounted with Entelan (Merck). The analysis and photographs were made with an Olympus BX-60 microscope.

To determine the total size and nucleus of the spermatozoa of the four *Centris* species, adult males were dissected, the seminal vesicles removed and opened, and the spermatozoa extracted and spread on histological slides, fixed with solution of 4% (w/v) paraformaldehyde in 0.1 M sodium phosphate buffer, pH 7.2, for 15 min, washed in running water and dried at room temperature. The slides were examined and the spermatozoa were photographed in an Olympus BX-60 photomicroscope equipped with phase contrast. To determine the size of the nuclei, some of these preparations were stained for 15 min with 0.2 mg/ml 4,6-diamino-2-phenylindole (DAPI) in PBS, washed in distilled water and mounted with 50% sucrose. The analysis and photographic records were made in an epifluorescence microscope (Olympus, BX-60), equipped with a BP360–370 nm excitation filter. All the measurements were obtained using the software Image Pro-Plus, version 4.5 (Media Cybernetics Inc., MD, USA) and the lengths were averaged from the total number of spermatozoa analyzed.

2.2. Transmission electron microscopy

To analyze the ultrastructure of the testes, seminal vesicles and spermatozoa of the four *Centris* species, adult males were dissected and the testes and seminal vesicles separated and fixed for 24 h in a solution of 2.5% glutaraldehyde, 0.2% picric acid, 3% sucrose and 5 mM $CaCl_2$ in 0.1 M sodium cacodylate buffer at pH 7.2. The

material was post-fixed in a 1% osmium tetroxide solution for 2 h, dehydrated in solutions of increasing acetone concentrations, and then infiltrated and embedded in epoxy resin (Epon 812). Ultrathin sections were contrasted with 3% uranyl acetate and 2% lead citrate, and photographed in a transmission electron microscope, JEOL 1011, operating at 80 kV.

3. Results

The internal male reproductive system of *Centris* sp. is formed by a pair of testes, a pair of deferent ducts, a pair of seminal vesicles, a pair of accessory glands and an ejaculatory duct (Figs. 1A and B and 2A). Each testis consists of four fusiform follicles (or seminiferous tubules) (Fig. 1B), which are filled with the testicular cysts formed by the cystic cells, involving up to 64 germinative cells (Fig. 3A). From each follicle arisen an efferent duct and the four ones fuse together into the deferent duct (Fig. 1B).

Each deferent duct is partially differentiated into a seminal vesicle. Thus, each deferent duct is divided into seminal vesicle and pre- and post-vesicular regions (Figs. 1B and 2B and C). The post-vesicular regions merge into the tubular ejaculatory duct, which is connected to the external genitalia, the aedeagus (Figs. 1A and 2A, B and D). In this species, the two testes, the pre-vesicular deferent ducts and seminal vesicles are enveloped by a single conjunctive capsule, the scrotal membrane (Figs. 1A and B and 2A).

The seminal vesicles are specialized tubular-coiled dilated regions of the deferent ducts, where the spermatozoa are stored until copulation. Histologically, they are composed of simple epithelium, with cylindrical cells of spherical and basal nuclei, basal lamina and muscular tunic (Figs. 2C and 3C). The nuclei of the epithelial cells present a de-condensed chromatin and large nucleoli (Fig. 3B and C). A well-developed Golgi complex is found next to the nuclei (Fig. 3B and C). Many mitochondria are observed in the apical cytoplasm of the epithelial cells, while microvilli are seen at the apical plasma membrane (Fig. 3B and D). Between the adjacent epithelial cells, septate junctions are observed (Fig. 3C). The basal plasma membrane presents hemidesmosome-type junctions and various invaginations, in which the thick basal membrane appears to be inserted (Fig. 3C).

The accessory glands have a tubular shape and are slightly dilated in the further most anterior region, merging into the post-vesicular deferent ducts (Figs. 1A and 2A and D). Associated with the reproductive system, the musculature fixes it to the internal abdominal wall (Fig. 2A, B and D).

The spermatozoa of *Centris analis*, *C. fuscata*, *C. tarsata* and *Centris* sp. are morphologically similar. These cells are elongated, almost uniform in diameter throughout its length, except at both ends, where they are more tapered (Fig. 4A). They have two easily distinguishable regions, the head and flagellum. The head is formed by a two-layer acrosome and the linear nuclei (Figs. 1C and 4B–H). The flagellum is formed by the centriole adjunct, the axoneme of 9+9+2 microtubules pattern, two asymmetric mitochondrial derivatives and two accessory bodies (Figs. 1D–F and 4N–P). The spermatozoon of *Centris* (*Hemisiella*) *tarsata* is 540 mm long, and the nucleus and flagellum are 42 and 498 mm long, respectively (Fig. 4A and B).

The acrosome is formed by a cone-shaped acrosomal vesicle that covers the perforatorium up to the anterior end of the nucleus (Figs. 1C and 4C). The posterior end of the acrosomal vesicle is chamfered and juxtaposed to the anterior end of the nucleus (Fig. 4C and E). At the base of the acrosome, the perforatorium appears inserted into a short nuclear cavity (Figs. 1C and 4C and G). In cross sections of the acrosome, one can see that the acrosomal vesicle is electron-dense and ellipsoidal-shaped, while the perforatorium is circular. Between these two structures lies an electron-lucid region (Fig. 4D). In the acrosome–nucleus transition, the ends of each structure appear in the same section, indicating that they are symmetrical and chamfered (Fig. 4E). The part of the perforatorium inserted into the nucleus remains circular but its diameter is gradually reduced like an inverted cone (Fig. 4C, G and H).

The nucleus is dense and elongated (Figs. 1C and 4B and I) and is found in some individuals with completely compact chromatin, and in others, with several de-compacting areas identified both in cross-sections (Fig. 4F, J and K) and in longitudinal sections (Fig. 4I). The most posterior region of the nucleus is laterally projected towards the anterior end of the larger mitochondrial derivative, with which it associates through the centriole adjunct, finally becoming aligned and partially inserted into the centriolar region (Figs. 1D and 4L).

The centriolar region is located immediately below the nucleus and laterally to the centriole adjunct and to the larger mitochondrial derivative. It consists of nine accessory microtubules surrounding nine pairs of microtubules (Figs. 1D and 4M).

The centriole adjunct is electron-dense, cone-shaped with a very pointed anterior end, and located between the posterior region of the nucleus and the anterior region of the larger mitochondrial derivative (Fig. 1D). It lies parallel to the latter structure and to the axoneme, finally becoming juxtaposed to the smaller mitochondrial derivative (Fig. 1D). In cross sections, this is initially circular and with a quite reduced area (Fig. 4N), later turning into a larger area with a triangular shape (Fig. 4O).

The mitochondrial derivatives are asymmetrical in length and diameter (Fig. 1D and F). The larger mitochondrial derivative starts before and next to the nuclear projection (Figs. 1D and 4L), around the centriole adjunct (Fig. 4N and O), ending after the smaller one at the end of the flagellum (Figs. 1F and 4Q and R). In cross sections, the anterior part of the larger mitochondrial derivative, at the height of the centriolar region, is more or less circular (Fig. 4M), while its middle portion is pyriform with an area almost two times larger than the anterior part (Fig. 4P). The larger mitochondrial derivative presents the following four regions in its median portion: (1) the electron-dense amorphous region (proximal to the axoneme), (2) the electron-lucid amorphous region (central), (3) the paracrystalline region (distal to the axoneme) and (4) the region with mitochondrial cristae (near the outer mitochondrial membrane) (Fig. 4P). The smaller mitochondrial derivative starts subjacent to the posterior end of the centriole adjunct (Fig. 1D). In cross sections, it has a roughly circular shape and, unlike the larger one, does not have a paracrystalline region, only the other three regions (Fig. 4P).

The axoneme has a 9+9+2 microtubule arrangement (Fig. 4P). This starts from the anterior region of the flagellum (Figs. 1D and 4M), becoming gradually disorganized at the end where the central and pair microtubules are initially lost, followed by each of the accessories (Fig. 4S).

The accessory bodies are electron-dense elongated structures that begin and end in different locations of the flagellum. One starts below the centriolar region, together with the beginning of the larger mitochondrial derivative and ends immediately before its end (Figs. 1D and F and 4M and R), while the other begins only after the end of the centriole adjunct, with the beginning of the smaller mitochondrial derivative, and ends immediately before its end (Fig. 1D and F). In cross sections, they exhibit a triangular shape and are located between the mitochondrial derivatives and axoneme, but not between the axoneme and centriole adjunct (Fig. 4P).

4. Discussion

The general morphological structure of the male reproductive system of the *Centris* species studied in this work is similar to

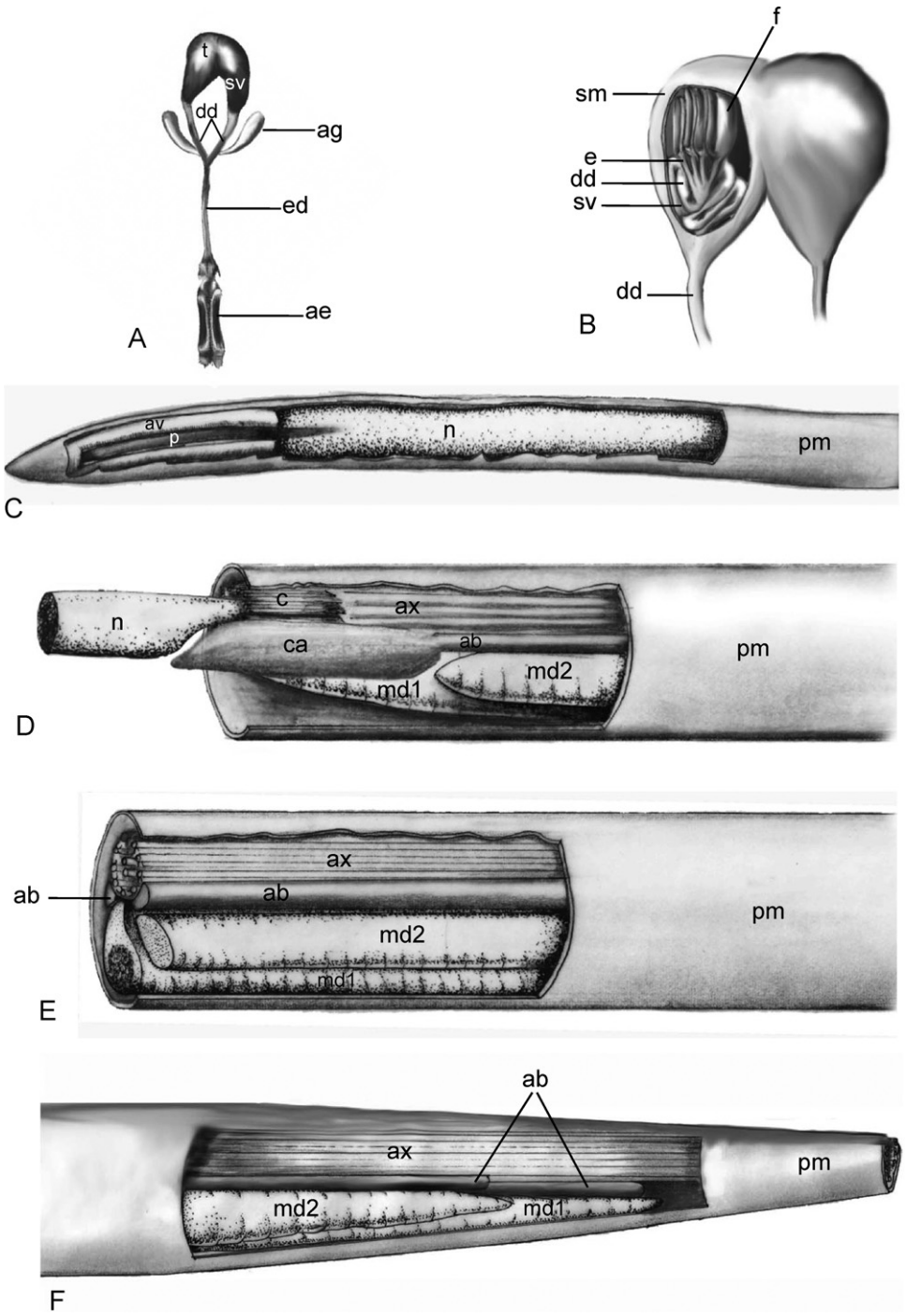

Fig. 1. Schematic representation of the anatomical structure of the male reproductive system of *Centris* sp. (A and B) and the sperm ultrastructure of *C. analis*, *C. fuscata*, *C. tarsata* and *Centris* sp. (C–F). (A) Schematic representation of the general morphology. (B) Schematic representation of the interior of the region surrounded by the scrotal membrane, where the testicular follicles, efferent ducts, pre-vesicular deferent ducts and seminal vesicles can be seen. (C) Head region; (D) head flagellum transition region; (E) flagellum medium region; (F) posterior flagellum region. ae, aedeagus; ab, accessory body; ag, accessory gland; av, acrosomal vesicle; ax, axoneme; ca, centriole adjunct; c, centriolar region; dd, deferent duct; e, efferent duct; ed, ejaculatory duct; f, testicular follicle; md1, larger mitochondrial derivative; md2, smaller mitochondrial derivative; n, nucleus; p, perforatorium; pm, plasma membrane; sm, scrotal membrane; sv, seminal vesicle; t, testis.

that described in several other Hymenoptera species (Dirks and Sternburg, 1972; Dallacqua and Cruz-Landim, 2003; Ferreira et al., 2004; Bushrow et al., 2006; Fiorillo et al., 2008, 2009; Gracielle et al., 2009; Araújo et al., 2010a, b; Moreira et al., 2008, 2010).

The internal male reproductive system of *Centris* sp. is similar to that of *C. violacea* (Ferreira et al., 2004). Based on the classification by Ferreira et al. (2004), the reproductive system of these two species are of type II, as they present both testes and the two deferent ducts (pre-vesicular and seminal vesicle) enveloped by a single scrotal membrane, forming only one globular unit. On the other hand, in *C. fuscata*, *C. tarsata* and *C. vittata* (Ferreira et al., 2004), the reproductive system is of type III, presenting each testis and deferent duct (pre-vesicular and seminal vesicle) enveloped by a separate scrotal membrane to form two globular units. The type II reproductive system is considered the anatomical standard, a phylogenetic intermediary to types I and III, indicating a tendency for the separation of the units enveloped by the scrotal membrane with a greater development of the ejaculatory duct (Ferreira et al.,

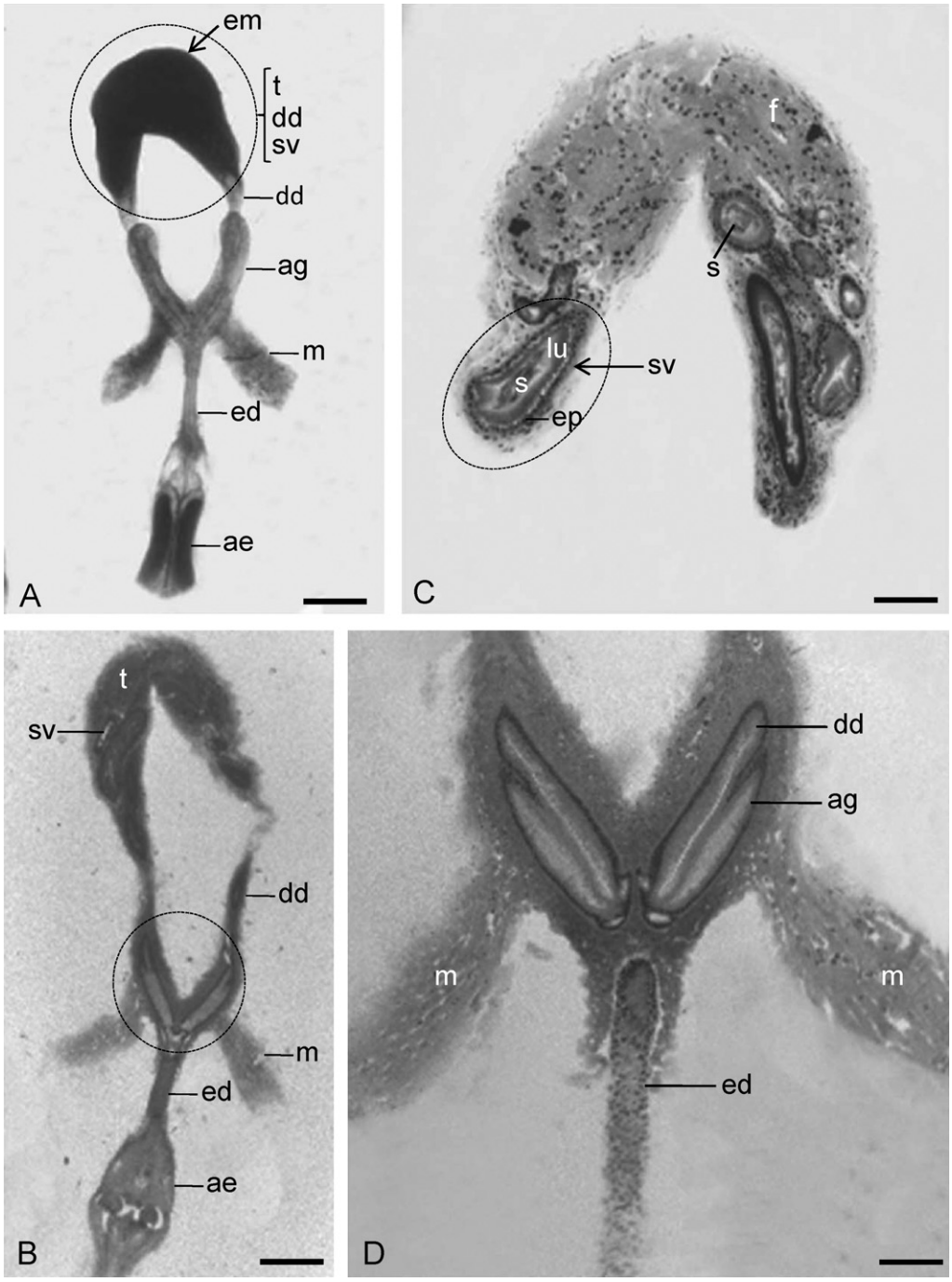

Fig. 2. Light micrographs of the general morphology (A) and semi-thin sections of the male reproductive system (B–D) of *Centris* sp. ae, aedeagus; ag, accessory gland; dd, deferent duct; ed, ejaculatory duct; sm, scrotal membrane; ep, epithelium; f, testicular follicle; lu, lumen; m, muscle; s, sperm; sm, scrotal membrane; sv, seminal vesicle; t, testis. Scale bars: (A) and (B) = 333 μm; (C) = 125 μm; (D) = 85 μm.

2004). The occurrence of these two types of system in *Centris* is not surprising, especially for being subgenera of different groups: *C. fuscata*, *C. tarsata* and *C. vittata* are included in the group Trachina, while *C. violacea* belongs to the group Melacentris (Ayala, 1998). *Centris* sp. may belong to the group Melacentris (*sensu* Ayala, 1998). This grouping according to the anatomical differences of the male reproductive system shows that these characteristics are potential objects for phylogenetic studies.

Except for *Apis mellifera* (Apinae) (Louveaux, 1977) and *Hypanthidium foveolatum* (Megachilinae) (Gracielle et al., 2009), which have around 200 and 28 follicles per testis, respectively, other Apidae studied have three or four follicles per testis. For instance, *Centris*, as well as other Apinae (Ferreira et al., 2004; Lima et al., 2006; Fiorillo et al., 2009; Brito et al., 2010), Anthophorinae (Araújo et al., 2010b), Melittinae and some Megachilinae (Roig-Alsina and Michener, 1993; Ferreira et al., 2004), present four follicles per testis. On the other hand, Andreninae, Colletinae, Halictinae, the majority of the Megachilinae (Ferreira et al., 2004) and the wasps Crabronidae (Zama et al., 2007; Moreira et al., 2008) and Vespidae (Dirks and Sternburg, 1972; Brito et al., 2005; Bushrow et al., 2006; Araújo et al., 2010a) present three follicles per testis. In Formicidae, this number ranges from 1 to 25 (Wheeler and Krutzsch, 1992) and in parasitic wasps (Fiorillo et al., 2008; Moreira et al., 2010) there is only one follicle per testis.

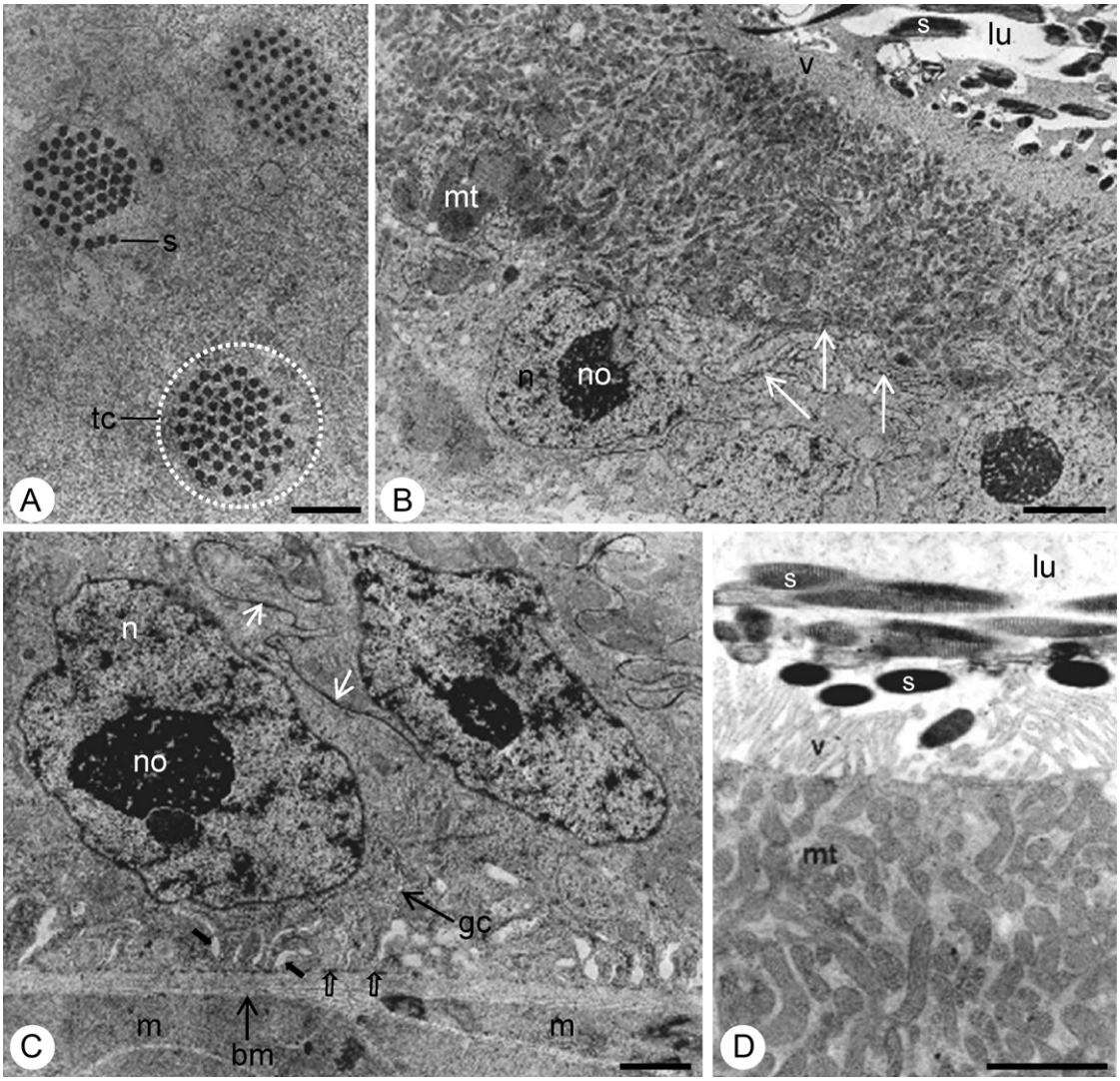

Fig. 3. Transmission electron micrographs of the testes (A) and seminal vesicles (B–D) of *C. analis*, *C. fuscata*, *C. tarsata* and *Centris* sp. (A) Cross section of the testis. (B) Section of the simple cylindrical epithelium of the seminal vesicle. (C) Basal region of two epithelial cells separated from the muscular tunic (m) by the basal membrane (bm). (D) Increase in the apical region of the cell shown in (B). bm, basal membrane; gc, Golgi complex; lu, lumen; m, muscular tunic; mt, mitochondria; n, nucleus; no, nucleole; s, sperm; tc, testicular cyst; v, microvilli; white arrows, septate junctions; black full arrows, basal plasma membrane invaginations; black empty arrows, hemidesmosome-type junctions. Scale bars: (A) and (B) = 2 μm; (C) = 1 μm; (D) = 0.5 μm.

Organization of cysts in the testicular follicles is a common feature of insects. However, the number of spermatozoa formed by cysts varies considerably. In Hymenoptera, during spermatogenesis, only two spermatids are formed from each haploid spermatogonium (Cruz-Landim, 2008). If all the spermatids are viable and transformed into spermatozoa during spermiogenesis, the maximum number of spermatozoa per cysts is the same as that of the spermatids, determined by the number of mitotic cycles through which that single initial spermatogonium underwent. Thus, one can determine the number of mitotic cycles from the number of spermatids and/or spermatozoa formed. In some groups of Hymenoptera, such as Meliponini (Conte et al., 2005; Lino-Neto et al., 2008) and *A. mellifera* (Cruz-Landim, 2001), half of the spermatids are eliminated during spermiogenesis. Thus, in each cyst, the maximum number of spermatozoa found corresponds to half the final number of cells that have developed in synchrony within the cysts from a single spermatogonium.

The presence of up to 64 spermatozoa per cyst, as in *Centris*, is also observed in other Apidae such as Euglossini (Zama et al., 2005a), Bombini (Zama, 2003) and Xylocopini (Fiorillo et al., 2009). However, in the Anthidiini *H. foveolatum* (Gracielle et al., 2009) and in the Apidae Meliponini, up to 128 spermatozoa per cyst are observed (Cruz-Landim, 2001; Zama et al., 2001; Lino-Neto et al., 2008), indicating that 64 spermatozoa per cyst is the basal (plesiomorphic) condition for the bees. In the Crabronidae *Trypoxylon* (Moreira et al., 2008; Araújo et al., 2009) and *Microstigmus* (Zama et al., 2007) up to 32 and 64 spermatozoa are found per cyst, respectively, while in the Sphecidae *Sceliphron* (Zama et al., 2005b) and in the Vespidae *Myschocyttarus* (Brito et al., 2005) and *Polistes* (Araújo et al., 2010a), 128 spermatozoa per cyst are found.

The seminal vesicles in *Centris* are specialized coiled-tubular dilated regions of the deferent ducts, similar to that observed in most bees (Ferreira et al., 2004; Gracielle et al., 2009), except in Meliponini, where they form a spherical structure (Dallacqua and Cruz-Landim, 2003; Ferreira et al., 2004; Araújo et al., 2005a, b; Brito et al., 2010). The histological structure of the seminal vesicles of *Centris* sp. is quite similar to that described for *A. mellifera* (Cruz-Landim and Cruz-Höfling, 1969a, b) and Meliponini *Melipona bicolor bicolor* Lepeletier (Dallacqua and Cruz-Landim, 2003), *Scaptotrigona xanthotricha* Moure (Araújo et al., 2005a, b), *Partamona helleri* Friese (Banin et al., 2005), *Melipona mondury* Smith (Lima et al., 2006) and *Friesella schrottky* Friese (Brito et al.,

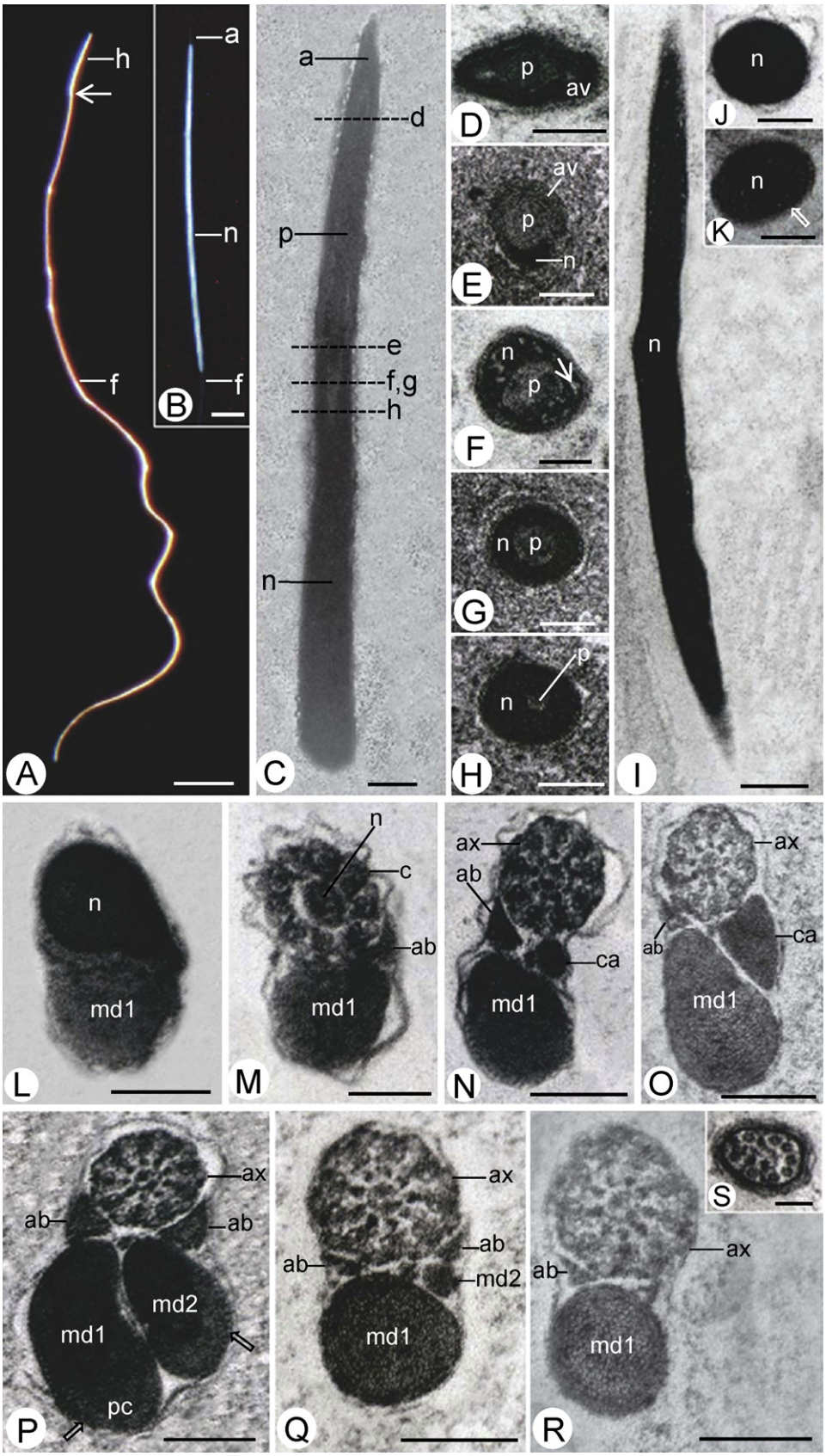

Fig. 4. Light (A and B) and transmission electron (C–S) micrographs of the sperm of *Cenris analis*, *C. fuscata*, *C. tarsata* and *Centris* sp. (A) Phase contrast. The white arrow shows the boundary between the head and flagellum. (B) Nucleus stained with DAPI. (C) Longitudinal section of the head region. Note the posterior end of the perforatorium inserted into the cavity of the anterior nucleus region. (D–H) Cross sections at various levels from the anterior towards the posterior end of the head indicated by dotted lines in (C). (D) Acrosome region where the ellipsoidal acrosome vesicle, perforatorium and the electron-lucid layer separating these two structures (seen as two white points between them) can be seen. (E) Boundary between the end of the acrosomal vesicle and the start of the nucleus. (F–H) Region where the perforatorium is inserted into the

2010). However, the authors describe the occurrence of numerous lipid vesicles and myelin structures in the epithelial cells, indicative of autophagic activity of the epithelium that becomes more abundant in more mature individuals (Cruz-Landim and Cruz-Höfling, 1969a, b; Dallacqua and Cruz-Landim, 2003; Araújo et al., 2005a, b; Banin et al., 2005). The absence of these structures in *Centris* sp. indicates a morpho-functional difference of this organ, since in many solitary bees the spermatozoa production is continuous and the individuals copulate several times during adulthood. On the other hand, in social bees such as the ones previously mentioned, the spermatozoa production occurs only once and the males mate only once. The ultrastructural characteristics, de-condensed chromatin, large nucleoli, many mitochondria, a developed Golgi complex, many microvilli, septate junctions between adjacent cells, hemidesmosome-type junctions and several invaginations in the basal membrane of the epithelial cells of the seminal vesicle indicate that they may be absorptive, perform transport and have high metabolic activity. Some of these characteristics are also present in other Hymenoptera, such as in the bees *A. mellifera* (Cruz-Landim and Cruz-Höfling, 1969a, b), *M. bicolor bicolor* (Dallacqua and Cruz-Landim, 2003), *Xylocopa frontalis* (Fiorillo et al., 2009) and *Pegoscapus* wasp genus (Chalcidoidea) (Fiorillo et al., 2008).

The ultrastructure of the spermatozoa with a two-layer acrosome (acrosomal vesicle and perforatorium), as in *C. analis*, *C. fuscata*, *C. tarsata* and *Centris* sp., is a synapomorphic characteristic of all the bees studied (Cruz-Höfling et al., 1970; Quicke et al., 1992; Lino-Neto et al., 2000b; Zama et al., 2001, 2004, 2005a; Zama, 2003; Báo et al., 2004; Araújo et al., 2005c; Badke et al., 2005; Fiorillo et al., 2005, 2009; Gracielle et al., 2009), as well as of the other groups of Aculeata: Sphecidae (Zama et al., 2005b), Crabronidae (Araújo et al., 2009), Formicidae (Thompson and Blum, 1967; Wheeler et al., 1990; Lino-Neto and Dolder, 2002; Moya et al., 2007) and Vespidae (Mancini et al., 2006, 2009). In most of these Aculeata, in cross sections of the acrosome, the acrosomal vesicle is electron-dense and ellipsoidal, while the perforatorium is circular. However, in Meliponini (Zama et al., 2001, 2004; Araújo et al., 2005c; Badke et al., 2005) and Formicidae (Lino-Neto and Dolder, 2002; Moya et al., 2007), the acrosomal vesicle observed in cross section is circular, acquiring a triangular shape as it approaches the nucleus. On the other hand, in *A. mellifera*, there is an exclusive projection of the acrosomal vesicle at the anterior end, where it is very long and slender, and the perforatorium is rectangular in cross sections (Cruz-Höfling et al., 1970; Peng et al., 1993). This characteristic can be considered an autapomorphy for this species.

Nuclei with altered chromatin compaction, as observed in these *Centris* species, have been described for some meliponids, where it is compressed into flakes, giving the nucleus a loose chromatin appearance (Zama et al., 2004; Badke et al., 2005). Numerous paracrystalline incrustations were observed in the sperm nucleus of Halictidae (Fiorillo et al., 2005), while *Exomalopsis auropilosa* Spinola (Exomalopsini) and *Paratetrapedia* sp. Michener and Moure (Tapinotaspidini) present small clear or electron-lucid regions (Báo et al., 2004). Similar changes have also been observed in Formicidae (Lino-Neto and Dolder, 2002; Moya et al., 2007). However, in these Aculeata, in contrast to *Centris*, changes in chromatin pattern (homogeneous and compact) are observed in all individuals, indicating the typical morphological aspect of the nucleus in these species. However, in *Centris*, this compaction variation occurs in different individuals of the same species, indicating a relationship with some of their physiological conditions.

In Aculeata, the asymmetrical projection on the posterior part of the nucleus aligned with the centriolar region is a synapomorphic characteristic. In most cases, the posterior end of this projection is juxtaposed to the centriolar region, as in Apidae (Zama et al., 2001, 2004, 2005a; Zama, 2003; Báo et al., 2004; Araújo et al., 2005c; Gracielle et al., 2009), Bethylidae (Oliveira et al., 2010), Crabronidae (Araújo et al., 2009), Formicidae (Lino-Neto and Dolder, 2002; Moya et al., 2007), Halictidae (Fiorillo et al., 2005) and Sphecidae (Zama et al., 2005b), or inserted into the centriolar region, as in *A. mellifera* (Apidae) (Lino-Neto et al., 2000b), *X. frontalis* (Apidae) (Fiorillo et al., 2009), *Microstigmus arlei* and *M. nigrophthalmus* (Crabronidae) (Zama et al., 2007), *Agelaia vicina* and *Vespa crabro* (Vespidae) (Mancini et al., 2006, 2009) and in the *Centris* species studied in this work.

The centriole adjunct in *Centris* is similar to that of *A. mellifera* (Lino-Neto et al., 2000b). In these bees, this structure is cone-shaped and anteriorly circular in cross section, posteriorly acquiring a triangular shape. In other bees, the centriole adjunct appears as an electron-dense rod continually triangular in cross sections (Zama et al., 2001, 2004, 2005a; Báo et al., 2004; Araújo et al., 2005c; Badke et al., 2005; Fiorillo et al., 2005, 2009; Gracielle et al., 2009). In *Centris* and other Apidae studied, the centriole adjunct begins adjacent to the posterior region of the nucleus, extending parallel to the anterior axoneme regions and to the larger mitochondrial derivative, ending over the anterior end of the smaller mitochondrial derivative. This characteristic is synapomorphic of the family.

The mitochondrial derivatives asymmetric in diameter and length and only the largest one presenting a paracrystalline region located distally in relation to the axoneme, as observed in *Centris*, occur in most aculeatan (Lino-Neto et al., 2000b; Zama et al., 2001, 2004, 2005a, b; Zama, 2003; Báo et al., 2004; Araújo et al., 2005c; Badke et al., 2005; Fiorillo et al., 2005, 2009; Gracielle et al., 2009; Araújo et al., 2009; Mancini et al., 2006, 2009). Ants (Wheeler et al., 1990; Lino-Neto and Dolder, 2002; Moya et al., 2007) and the wasp *Prorops nasuta* (Chrysidoidea: Bethylidae) (Oliveira et al., 2010) are exceptions, since the mitochondrial derivatives are symmetrical in cross sections and the paracrystalline material occurs in both. In *Centris* and in almost all the Hymenoptera studied, the smaller mitochondrial derivative ends before the larger one. Vespidae *V. crabro* (Mancini et al., 2009) and Eulophidae *Melittobia hawaiiensis* and *M. Australia* (Parasitic) (Brito et al., 2009) are the only exceptions so far, since it is the larger mitochondrial derivative that ends before the smaller one. In addition, the parasitic wasps *Trissolcus basalis* and *Telenomus podisi* (Platygastroidea) (Lino-Neto and Dolder, 2001b) are the only species so far that have only one mitochondrial derivative.

The axoneme of *Centris* and most Aculeata studied become gradually disorganized at the end, where the central microtubules initially end, followed by the doublets, and finally, by each of the accessories (Lino-Neto et al., 2000b; Zama et al., 2001, 2004, 2005a, b, 2007; Báo et al., 2004; Badke et al., 2005; Fiorillo et al., 2005, 2008; Mancini et al., 2006, 2009; Araújo et al., 2009; Gracielle et al., 2009; Oliveira et al., 2010). On the other hand, in parasitic wasps (Lino-Neto et al., 1999, 2000a; Lino-Neto and Dolder, 2001a; Fiorillo et al.,

nucleus. (F and G) Same cross section level indicated in (C), while in (F), the nucleus exhibits a de-compacted chromatin region (white arrow). (I) Longitudinal and (J and K) cross sections of the nucleus. (K) Various de-compacted chromatin points (white empty arrows). Cross sections of the transition region between the head and flagellum (L–O) and of the flagellum region (P–S). (M) Note the anterior projection of the nucleus inserted into the centriolar region. (P) Median flagellum region. The paracrystalline material region occurs only in the larger mitochondrial derivative, while the mitochondrial cristae region (black empty arrows) occurs in both mitochondrial derivatives. (Q–S) Flagellum end regions. The smaller mitochondrial derivative finalizes before the larger one and the accessory microtubules are the last to finalize. a, acrosome; ab, accessory body; av, acrosomal vesicle; ax, axoneme; ca, centriole adjunct; c, centriolar region; f, flagellum; h, head; md1, larger mitochondrial derivative; md2, smaller mitochondrial derivative; n, nucleus; p, perforatorium; pc, paracrystalline material. Scale bars: (A) = 25 μm; (B) = 4 μm; (C) and (I) = 0.5 μm; (D), (E), (G), (H), (K–M), (P–Q) = 0.2 μm; (F), (R–S) = 0.1 μm; (J), (M–O) = 0.3 μm.

2008; Brito et al., 2009; Moreira et al., 2010) the accessory microtubules end before the microtubules of the nine doublets. Of all the hymenopteran species studied so far, only the ants of the genus *Pseudomyrmex* display an axoneme with a 9+9+1 microtubule arrangement (Moya et al., 2007), which differ from the axonemal pattern 9+9+2 observed in insects in general.

The accessory bodies in *Centris*, as also observed in other aculeatan species (Lino-Neto et al., 2000b; Zama et al., 2001, 2004, 2005a, b, 2007; Báo et al., 2004; Araújo et al., 2005c, 2009; Fiorillo et al., 2005, 2009; Mancini et al., 2006, 2009; Moya et al., 2007; Gracielle et al., 2009), are elongated structures, triangular in cross sections, and located between the axoneme and the mitochondrial derivatives, but not between the axoneme and the centriole adjunct. In Hymenoptera, these structures are well conserved and exceptionally large in the *Centris* species.

The *Centris* species analyzed in this study share several morphological characteristics of the male reproductive system and spermatozoa with the other bees previously described, indicating that several characteristics are synapomorphic for the Apidae family. This information reinforces the suggestion by Melo and Gonçalves (2005) that all bees belong to the Apidae family.

The morphological studies of the male reproductive system and spermatozoa in Hymenoptera demonstrate the diversity of information provided by these reproductive structures and their validity in the elucidation of taxonomy and phylogenetic issues remaining in this important group of insects.

Acknowledgments

The authors thank the Centre for Microscopy and Microanalysis of the Federal University of Viçosa (UFV) and the University of Campinas (UNICAMP). This research was funded by CAPES, CNPq, FAPEMA and FAPEMIG.

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