



Sperm of Doradidae (Teleostei: Siluriformes)

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ABSTRACT

Spermatic characteristics were studied in 10 species representing several distinct groups within the catfish family Doradidae. Interestingly, different types of spermatogenesis, spermiogenesis and spermatozoa are correlated with intrafamilial groups previously proposed for Doradidae. Semi-cystic spermatogenesis, modified Type III spermiogenesis, and biflagellate sperm appear to be unique within Doradidae to the subfamily Astrodoradinae. Other doradid species have sperm with a single flagellum, cystic spermatogenesis, and spermiogenesis of Type I (*Pterodoras granulosus*, *Rhinodoras dorbignyi*), Type I modified (*Oxydoras kneri*), or Type III (*Trachydoras paraguayensis*). Doradids have an external mode of fertilization, and share a few spermatic characteristics, such as cystic spermatogenesis, Type I spermiogenesis and uniflagellate sperm, with its sister group Auchenipteridae, a family exhibiting sperm modifications associated with insemination and internal fertilization. Semi-cystic spermatogenesis and biflagellate spermatozoa are also found in Aspredinidae, and corroborate recent proposals that Aspredinidae and Doradoidea (Doradidae + Auchenipteridae) are sister groups and that Astrodoradinae occupies a basal position within Doradidae. The co-occurrence in various catfish families of semi-cystic spermatogenesis and either biflagellate spermatozoa (Aspredinidae, Cetopsidae, Doradidae, Malapturidae, Nematogenyidae) or uniflagellate sperm with two axonemes (Ariidae) reinforces the suggestion that such characteristics are correlated. Semi-cystic spermatogenesis and biflagellate sperm may represent ancestral conditions for Loricarioidei and Siluroidei of Siluriformes as they occur in putatively basal members of each suborder, Nematogenyidae and Cetopsidae, respectively. However, if semi-cystic spermatogenesis and biflagellate sperm are ancestral for Siluriformes, cystic spermatogenesis and uniflagellate sperm have arisen independently in multiple lineages including Diplomystidae, sister group to Siluroidei.

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1. Introduction

Doradidae is a family of freshwater catfishes endemic to South America that comprises about 90 valid extant species and one fossil species arranged in 31 genera. Doradids commonly exhibit a row of thorny scutes along the lateral line, and are promptly diagnosed by the presence of the infranuchal scute, a laminar bone co-formed by fusion of an expanded ossicle of the lateral line canal and an ossified ligament extending from beneath the posterior nuchal plate to the tip of the first rib (Birindelli et al., 2008; Birindelli, 2010).

Doradidae often is separated into two major groups, one with simple barbels and more or less depressed head, and the other with fimbriate barbels and relatively deep head (Kner, 1853; Sabaj and Ferraris, 2003; Birindelli and Sousa, 2010). Doradids with sim-

ple barbels are non-monophyletic and include the most basal taxa according to both morphological and molecular cladistic analyses summarized below.

In the first cladistic analysis of intrafamilial relationships Higuchi (1992, unpublished Ph.D. Dissertation; cladogram and synapomorphies published in Pinna de, 1998) used morphological characteristics to support the monophyly of the family, and recovered *Wertheimeria* and *Franciscodoras*, respectively, as successive sister groups to all other doradids. For the remaining taxa Higuchi (1992) recognized three monophyletic subfamilies in an unresolved trichotomy: “Doradinae”, “Platydoradinae”, and Astrodoradinae, the lattermost formally named and diagnosed in Higuchi et al. (2007).

Moyer et al. (2004) subsequently used mitochondrial and nuclear DNA sequence data to examine phylogenetic relationships among doradids. Their topology conflicted with the supra-generic classification proposed by Higuchi (1992), however, their molecular analysis did not include several key genera (e.g., *Centrochir*, *Franciscodoras*, *Kalyptodoras* and *Wertheimeria*). Only one of the intra-familial groups proposed by Higuchi (1992), Astrodorad-

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inae, was supported as monophyletic, and Astrodoradinae and Acanthodoras were recovered as deep lineages forming a basal trichotomy with a third group comprising all other doradids in their analysis.

In a separate cladistic study based on morphology Birindelli (2006 unpublished Ph.D. Dissertation) recovered a new topology wherein *Kalyptodoras* and *Wertheimeria* formed a basal trichotomy with a clade containing all other doradid genera. Birindelli's (2006) study supported Higuchi's (1992) subfamilial group "Platydoradinae" as sister to Astrodoradinae + Doradinae. Later, Birindelli (2010, unpublished Ph.D. Dissertation) expanded his original study to include all genera of Auchenipteridae plus several additional catfish families as outgroups. His new study recovered *Kalyptodoras* + *Wertheimeria* as basal, sister to *Franciscodoras* + a clade containing the remaining doradid taxa analyzed. Within the remaining taxa, a clade composed of *Acanthodoras*, *Agamyxis* and two genera of Astrodoradinae was sister to a trichotomy formed by *Centrochir*, *Platydoras*, and a clade subdivided into three informally named tribes: "Pterodoradini" sister to "Rhinodoradini" + "Doradini". Finally, Sousa (2010, Unpublished Ph.D. Dissertation) used morphology to investigate phylogenetic relationships of Astrodoradinae. Sousa's analysis recovered *Anadoras* as the most basal member of Astrodoradinae, and expanded the subfamily to include *Acanthodoras* and *Agamyxis*.

Although the phylogenies differed, those studies have hypothesized several lineages within Doradidae. Morphological analyses consistently recover *Franciscodoras*, *Kalyptodoras* and *Wertheimeria* as the most basal doradids with the latter two as sister taxa in Birindelli (2010). Morphological and molecular analyses identify *Acanthodoras* and Astrodoradinae as deep lineages, and the two appear to be closely related based on morphology (Birindelli, 2010; Sousa, 2010). Most of the more derived taxa group into three lineages, two with simple barbels ("Pterodoradini", "Rhinodoradini"), and one inclusive of all fimbriate barbel genera. The monophyly of doradids sharing fimbriate barbels is well supported by morphological (Higuchi, 1992; Birindelli, 2006; 2010; Sousa, 2010) and molecular (Moyer et al., 2004) data. However, the sister group relationship between the fimbriate-barbel clade and *Oxydoras*, a genus with simple barbels, is only supported by the morphological studies.

A particularity of the Doradidae is the presence of an elastic-spring apparatus formed by a special arrangement of the parapophyses of the fourth vertebra (i.e., Müllerian rami), gas (swim) bladder, and associated muscles and ligaments (see Sabaj and Ferraris, 2003 and Birindelli et al., 2009 for review). This particularity is shared with the South American Auchenipteridae and with the African Mochokidae. According to Pinna de (1998) and Birindelli (2010), the South America families Doradidae and Auchenipteridae constitute a monophyletic group assembled in the superfamily Doradoidea, and Doradoidea with the African Mochokidae form the suborder Doradoidei. The occurrence of a similar elastic-spring apparatus in Ariidae has been used to suggest a sister group relationship with Doradoidei (Mo, 1991; Lundberg, 1993; Royero, 1999). Friel (1994) alternatively proposed Aspredinidae as the sister group to the Doradoidea (Doradidae + Auchenipteridae) based on phylogenetic analysis of morphological data; his hypothesis was later supported by phylogenetic analyses of molecular data (Hardman, 2005; Sullivan et al., 2006). Aspredinidae is alternatively considered a member of the otherwise Asian Sisoroidea (Chen, 1994; Pinna de, 1993, 1996, 1998; Diogo et al., 2002, 2003; Birindelli, 2010). A molecular phylogeny by Sullivan et al. (2008), however, recovered Sisoroidea as a monophyletic group restricted to the Asian Akysidae, Amblycipitidae and Sisoridae, and again placed Aspredinidae sister to South American Doradoidea.

Studies of phylogenetic relationships within and between families of Siluriformes have been based on bony and/or soft anatomy and molecular sequence data. It is known that sexual characteristics pertaining to spermatogenesis and spermiogenesis, as well as sperm morphology, may yield phylogenetically informative characters useful for cladistic analyses (Jamieson, 2009).

In an attempt to evaluate their phylogenetic significance, characteristics of spermatogenesis, spermiogenesis, and/or sperm ultrastructure of 10 representative species of Doradidae are described herein and discussed with respect to previously hypothesized relationships: within Doradidae, between Doradidae and purported sister group Auchenipteridae, between Doradoidea (Doradidae + Auchenipteridae) and purported sister group Aspredinidae, and between Doradoidea and purported related group Ariidae.

2. Materials and methods

2.1. Examined material

Museum specimens were examined from ichthyological collections at the Academy of Natural Sciences of Philadelphia (ANSP); Laboratório de Biologia de Peixes, Departamento de Morfologia, Universidade Estadual Paulista, Campus de Botucatu (LBP); and Museu de Zoologia da Universidade de São Paulo (MZUSP).

Descriptions of spermatogenic characteristics are based on analyses at the ultrastructural level of testis from adult males of *Anadoras weddellii* (LBP 672), *Amblydoras* sp. (ANSP 167626), *Wertheimeria maculata* (MZUSP 93658), *Franciscodoras marmoratus* (MZUSP 84224), *Kalyptodoras bahiensis* (MZUSP 100737), *Acanthodoras cataphractus* (MZUSP 6831), *Pterodoras granulatus* (LBP 4322), *Oxydoras kneri* (LBP 4323), *Rhinodoras dorbignyi* (LBP 4326) and *Trachydoras paraguayensis* (LBP 5627).

2.2. Preparation of specimens for observation of spermatogenic characteristics

Live specimens were anesthetized with 0.1% benzocaine and euthanized (according to institutional protocols and approval) for removal of the testis. Gonad fragments from freshly sacrificed fish were fixed overnight in 2% glutaraldehyde and 4% paraformaldehyde in 0.1 M Sorensen phosphate buffer, pH 7.4. The material was post-fixed in the dark for 2 h in 1% osmium tetroxide in the same buffer, stained in block with aqueous solution of 5% uranyl acetate for 2 h, dehydrated in acetone, embedded in araldite, and sectioned and stained with a saturated solution of uranyl acetate in 50% ethanol and lead citrate. Electron micrographs were obtained using a Phillips-CM 100 transmission electron microscope.

"Dead" specimens from ichthyological collections (i.e., previously fixed in 10% formalin and conserved in 70% ethanol) were dissected and the removed testis gradually rehydrated in a decreasing ethanol concentration (60%, 50%, 40% . . . distilled water). Once rehydrated the material was re-fixed and prepared for observation as described for the live specimens.

Instances when the condition of the testis did not permit complete or accurate observations (e.g., previously fixed museum specimens) are noted as "not available" (NA).

3. Results

Various features of spermatogenesis, spermiogenesis and spermatozoa are summarized for the doradids analyzed herein and compared to other catfishes in Table 1.

Table 1
Comparison of spermatic characteristics in Doradidae to other catfishes (Siluriformes). Data compiled from this study and Burns et al. (2002: Auchenipteridae), Burns et al. (2009: Ariidae, Auchenipteridae and Nematogenyidae), Parreira et al. (2009: Auchenipteridae), Quagio-Grassiotto et al. (2001: Diplomystidae), Shahin (2006: Malapteruridae), Spadella et al. (2006: Aspredinidae and Cetopsidae), Spadella et al. (2007: Callichthyidae). Doradid subgroups adopted from Birindelli et al. (2009).

| Family | Subgroup | Genus species | Maxillary barbel | Fertilization | Spermatogenesis | Spermiogenesis | | | |
|-----------------------------------|--------------------------------------|-----------------------------------|---|-------------------------|--|---------------------------|---|------------------------------|----------------------|
| | | | | | | Type | Chromatin | Nuclear rotation | Centriole migration |
| Doradidae | “Basal” | <i>Acanthodoras cataphractus</i> | Simple | External | NA | NA | NA | NA | NA |
| Doradidae | “Basal” | <i>Franciscodordas marmoratus</i> | Simple | External | NA | NA | NA | NA | NA |
| Doradidae | “Basal” | <i>Kalyptodoras bahiensis</i> | Simple | External | NA | NA | NA | NA | NA |
| Doradidae | “Basal” | <i>Wertheimeria maculata</i> | Simple | External | NA | NA | NA | NA | NA |
| Doradidae | Astrodoradinae | <i>Anadoras weddellii</i> | Simple | External | Semicystic | Type III modified | Diffuse, homogenous, irregular outline | Absent | Present ^a |
| Doradidae | Astrodoradinae | <i>Amblydoras sp.</i> | Simple | External | NA | NA | NA | NA | NA |
| Doradidae | Clade 3 | <i>Pterodoras granulosus</i> | Simple | External | Cystic | Type I | Diffuse, homogenous, circular outline | Complete | Present |
| Doradidae | Clade 4 | <i>Rhinodoras dorbignyi</i> | Simple | External | Cystic | Type I | Diffuse, homogenous, circular outline | Complete | Present |
| Doradidae | Clade 5 | <i>Trachydoras paraguayensis</i> | Fimbriate | External | Cystic | Type III | Diffuse, homogenous, circular outline | Absent | Absent |
| Doradidae | | <i>Oxydoras kneri</i> | Simple | External | Cystic | Type I modified | Diffuse, homogenous, circular outline | Complete | Absent ^a |
| Auchenipteridae | | <i>Trachelyopterus spp.</i> | Simple | Internal (inseminating) | Cystic | Type I | Diffuse, homogenous, circular outline | Complete | Present |
| Aspredinidae | | <i>Bunocephalus amazonicus</i> | Simple | External | Semicystic | Type III modified | Diffuse, homogenous, irregular outline | absent | Present ^a |
| Callichthyidae | Corydoradinae | <i>Corydoras flaveolus</i> | Simple | External | Semicystic | Type III modified | Diffuse, homogenous, irregular outline | Absent | Absent |
| Ariidae | | <i>Genidens genidens</i> | Simple | External | Semicystic | Type I modified | Diffuse, homogenous, circular outline | Complete | Absent ^a |
| Nematogenyidae | | <i>Nematogenys inermis</i> | Simple | External | Semicystic | Type III modified | Diffuse, homogenous, circular outline | absent | Present ^a |
| Malapteruridae | | <i>Malapterurus electricus</i> | Simple | External | Semicystic | NA | Diffuse, homogenous, circular outline | NA | NA |
| Cetopsidae | | <i>Cetopsis coecutiens</i> | Simple | External | Semicystic | Type III | Diffuse, homogenous, circular outline | Absent | Absent |
| Diplomystidae | | <i>Diplomystes mesembrinus</i> | Simple | External | Cystic | Type I | Diffuse, homogenous, circular outline | Present | Present |
| Genus species | Spermatozoa | | | | | | | | |
| | Nucleus | Chromatin | Nuclear fossa | Centrioles | Midpiece | Mitochondria | Cytoplasmic canal | Flagella | |
| <i>Acanthodoras cataphractus</i> | Subspherical, tip slightly flattened | Highly condensed, homogenous | Present (moderately deep, includes proximal centriole and most of distal centriole) | Almost perpendicular | Slightly asymmetric, contains mitochondria, vesicles and cytoplasmic canal | NA | NA | 1 (1 axoneme), fins lacking | |
| <i>Franciscodordas marmoratus</i> | Ovoid, tip flattened | Highly condensed, homogenous | Present (moderately deep, includes proximal centriole and most of distal centriole) | Almost perpendicular | Shorter, asymmetric, contains mitochondria, few vesicles and cytoplasmic canal | NA | NA | 1 (1 axoneme), fins lacking | |
| <i>Kalyptodoras bahiensis</i> | Ovoid, tip weakly flattened | Highly condensed, homogenous | Present (moderately deep, includes proximal centriole and most of distal centriole) | Almost perpendicular | Longer, asymmetric, contains mitochondria and cytoplasmic canal | NA | NA | 1 (1 axoneme), fins lacking | |
| <i>Wertheimeria maculata</i> | Ovoid, tip extremely flattened | Highly condensed, homogenous | Present (moderately deep, includes proximal centriole and most of distal centriole) | Almost perpendicular | Shorter, asymmetric, contains mitochondria, few vesicles and cytoplasmic canal | Very elongate | NA | 1 (1 axoneme), fins lacking | |
| <i>Anadoras weddellii</i> | Bell-shaped | Highly condensed, homogenous | Present ^a (very deep, includes centrioles, mitochondria and initial segment of flagella) | Parallel | Long, contains mitochondria, vesicles and two cytoplasmic canals | Ovoid (slightly elongate) | Results from simultaneous projection of nucleus and cytoplasm toward flagella and migration of centrioles | 2 (2 axonemes), fins lacking | |
| <i>Amblydoras sp.</i> | Bell-shaped | Highly condensed, homogenous | Present (very deep, includes centrioles and mitochondria) | Parallel | Long, filled with vesicles | Ovoid (slightly elongate) | NA | 2 (2 axonemes), fins lacking | |
| <i>Pterodoras granulosus</i> | Ovoid, tip flattened | Highly condensed, homogenous | Present (moderately deep, includes proximal centriole and most of distal centriole) | Almost perpendicular | Slightly asymmetric, contains mitochondria, vesicles and cytoplasmic canal | Oblong | Results from projection of cytoplasm towards flagellum | 1 (1 axoneme), fins lacking | |

Table 1 (Continued)

| Genus species | Spermatozoa | | | | | | | |
|----------------------------------|--------------------------------------|------------------------------|---|----------------------|---|-------------------------|---|------------------------------|
| | Nucleus | Chromatin | Nuclear fossa | Centrioles | Midpiece | Mitochondria | Cytoplasmic canal | Flagella |
| <i>Rhinodoras dorbignyi</i> | Subspherical, tip slightly flattened | Highly condensed, homogenous | Present (moderately deep, includes proximal centriole and most of distal centriole) | Almost perpendicular | Asymmetric, contains mitochondria, vesicles and cytoplasmic canal | Elongate | Results from projection of cytoplasm towards flagellum | 1 (1 axoneme), fins lacking |
| <i>Trachydoras paraguayensis</i> | Spherical | Highly condensed, homogenous | Absent | Oblique | Asymmetric, contains mitochondria, large vesicles and cytoplasmic canal | Elongate | Results from projection of midpiece vesicles towards flagellum | 1 (1 axoneme), fins lacking |
| <i>Oxydoras kneri</i> | Spherical | Highly condensed, homogenous | Present (moderately deep, includes proximal centriole and most of distal centriole) | Perpendicular | Slightly asymmetric, contains mitochondria, abundant vesicles and cytoplasmic canal | Elongate | Results from projection of cytoplasm towards flagellum | 1, fins lacking |
| <i>Trachelyopterus</i> spp. | Elongate | Highly condensed, homogenous | Present | Oblique | Peculiar | Very elongate | Results from projection of cytoplasm towards flagellum | 1 (1 axoneme) |
| <i>Bunocephalus amazonicus</i> | Bell-shaped | Flocculent | Present ^a (deep, includes centrioles) | Parallel | Long, contains mitochondria, vesicles and two cytoplasmic canals | Elongate | Results from simultaneous projection of nucleus and cytoplasm toward flagella and migration of centrioles | 2 (2 axonemes) |
| <i>Corydoras flaveolus</i> | NA | NA | Present ^a (shallow, not occupied by centrioles) | Parallel | NA | NA | NA | 1 (1 axoneme), fins lacking |
| <i>Genidens genidens</i> | | Highly condensed, homogenous | Present (deep, includes centrioles and mitochondria) | Parallel | Short, symmetric, contains mitochondria | Elongate | Absent | 1 (2 axonemes), fins lacking |
| <i>Nematogenys inermis</i> | Subspherical | Highly condensed, homogenous | Absent | Parallel | Symmetric, contains mitochondria and vesicles | Elongate | Absent | 2 (2 axonemes) |
| <i>Malapterurus electricus</i> | Subspherical | Highly condensed homogenous | Present | Parallel | Long, contains mitochondria, vesicles and two cytoplasmic canals | Elongate | Present | 2 (2 axonemes) |
| <i>Cetopsis coecutiens</i> | Subspherical | Highly condensed homogenous | Present ^a (deep, includes centrioles) | Parallel | Short, symmetric, contains mitochondria and vesicles | Rounded | Present | 2 (2 axonemes) |
| <i>Diplomystes mesembrinus</i> | Subspherical | Flocculent | Present (deep, includes centrioles and start of axoneme) | Perpendicular | Short | Single, large, C-shaped | Absent | 1 (1 axoneme) |

NA indicates comparative data not available.

^a Indicates modification from classic Type I or III spermiogenesis.

3.1. *A. weddellii* and *Amblydoras* sp. (Figs. 1–4)

3.1.1. Spermatogenesis

In *A. weddellii* spermatogenesis is semi-cystic. In this kind of spermatogenesis, although spermatogonia proliferation and meiotic divisions of the spermatocytes occur inside the spermatocysts (Fig. 1A), spermatid differentiation is extra-cystic and occurs outside the cysts in the luminal compartment of the testis (Fig. 1B). In the luminal compartment, clusters of spermatids recently released from the cysts remain connected to one another by cytoplasmic bridges. Spermatids gradually lose those connections and differentiate. Spermatid differentiation is not synchronous and cells in distinct phases of development can be seen together in the luminal compartment (Fig. 1C). Spermatozoa are also present in the luminal compartment (Fig. 1A). Information on spermatogenesis in *Amblydoras* is not available.

3.1.2. Spermiogenesis

In *A. weddellii* spermiogenesis is a modification of Type III. In the early spermatids (Fig. 2A and B), the cytoplasm symmetrically encircles the nucleus, which displays diffuse homogenous chromatin and has an irregular outline. The centriolar complex lies medially to the nucleus and is anchored to the plasma membrane. The centrioles are lateral and parallel to one another (Fig. 2A–C). Both centrioles differentiate into basal bodies, and each centriole forms one flagellum. Centrioles start their migration toward the nucleus, carrying along the plasma membrane and the initial segments of the flagella, which invaginate. Two independent cytoplasmic canals, a space between each flagellum and the plasma membrane, are then formed. A depression is formed in the nuclear outline at the level of the centrioles (Fig. 2A and B). The nucleus does not rotate in relation to the flagellar axis. Instead, in a suggested coordinated movement, the basal region of the nucleus is projected in the direction of the initial segment of the flagella while the centrioles continue their migration inside the nuclear fossa. Consequently, the nucleus takes on a bell shape in which the initial segments of the flagella, each with individualized cytoplasmic canals, are housed in a very deep nuclear fossa (Fig. 2C, E, G). The cytoplasm, which initially accumulates in the region surrounding the centrioles (Fig. 2A and B), moves toward the segments of the flagella located just outside of the nuclear fossa, forming the midpiece (Fig. 2C, E, G). The midpiece contains two cytoplasmic canals with the flagella, mitochondria and vesicles (Fig. 2D–H). Mitochondria are included inside the nuclear fossa (Fig. 2F). Information on spermiogenesis of *Amblydoras* is not available.

3.1.3. Spermatozoa

Spermatozoa of *A. weddellii* and *Amblydoras* are quite similar: the conical-trunk nucleus is bell shaped and contains highly condensed homogeneous chromatin interspersed by electron-lucent areas, and is surrounded by a narrow strip of cytoplasm with no organelles. Nucleus has about 2.0 μm in height by 1.4 μm in width at the base and 0.6 μm in width at the tip in *A. weddellii*, vs. 2.1 μm in height by 1.4 μm in width at the base and 0.6 μm in width at the tip in *Amblydoras* (Fig. 3A, D and E; Fig. 4F). The centrioles are lateral and parallel to one another, and are located internally to the nucleus at the tip of the very deep nuclear fossa. The centrioles are covered by electron dense material and fastened to one another, to the nuclear envelope at the nuclear fossa, and to the plasma membrane by deep stabilization fibrils (Fig. 3A, D, G; Fig. 4C). The spermatozoa have two flagella and two independent cytoplasmic canals extending internally from the tip of the nucleus to the terminal end of the midpiece (Fig. 3A, B, D, H–K; Fig. 4D and E). The slightly elongated mitochondria are located mainly near the base of the nucleus, but also are found internally in the deep

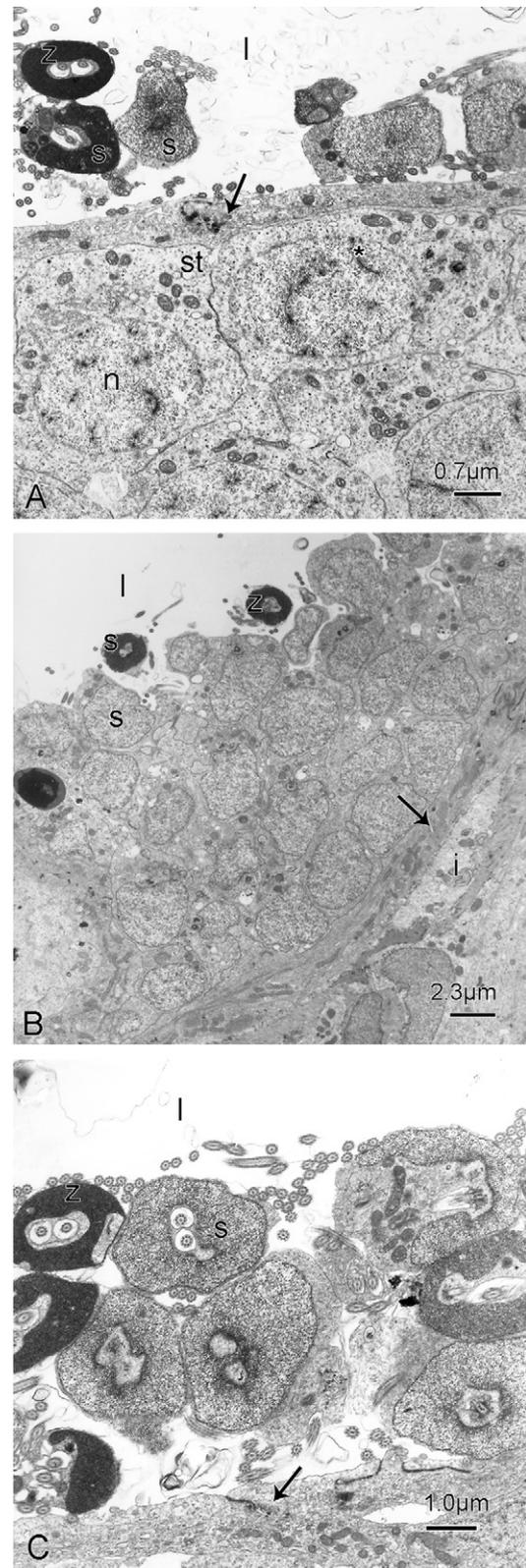


Fig. 1. Semi-cystic spermatogenesis in *Anadoras weddellii* (Astroderadinae). Cysts are formed by the germ cells surrounded by the cytoplasmic process (arrow) of the Sertoli cells (A): in this kind of spermatogenesis, only spermatogonia and spermatocytes (st) are inside the cysts whereas the spermatids (s) and spermatozoa (z) are found in the luminal compartment (l). n: nucleus; asterisk: synaptonemal complex. (B) A cluster of early spermatids (s) recently released from a cyst rests upon the germinal epithelium (arrow). i: interstitial compartment; l: luminal compartment; z: spermatozoa. (C) Spermatic cell differentiation is asynchronous and occurs in the luminal compartment (l) outside the cysts. s: spermatids; z: spermatozoa; arrow: cytoplasmic projections of Sertoli cells limiting a cyst.

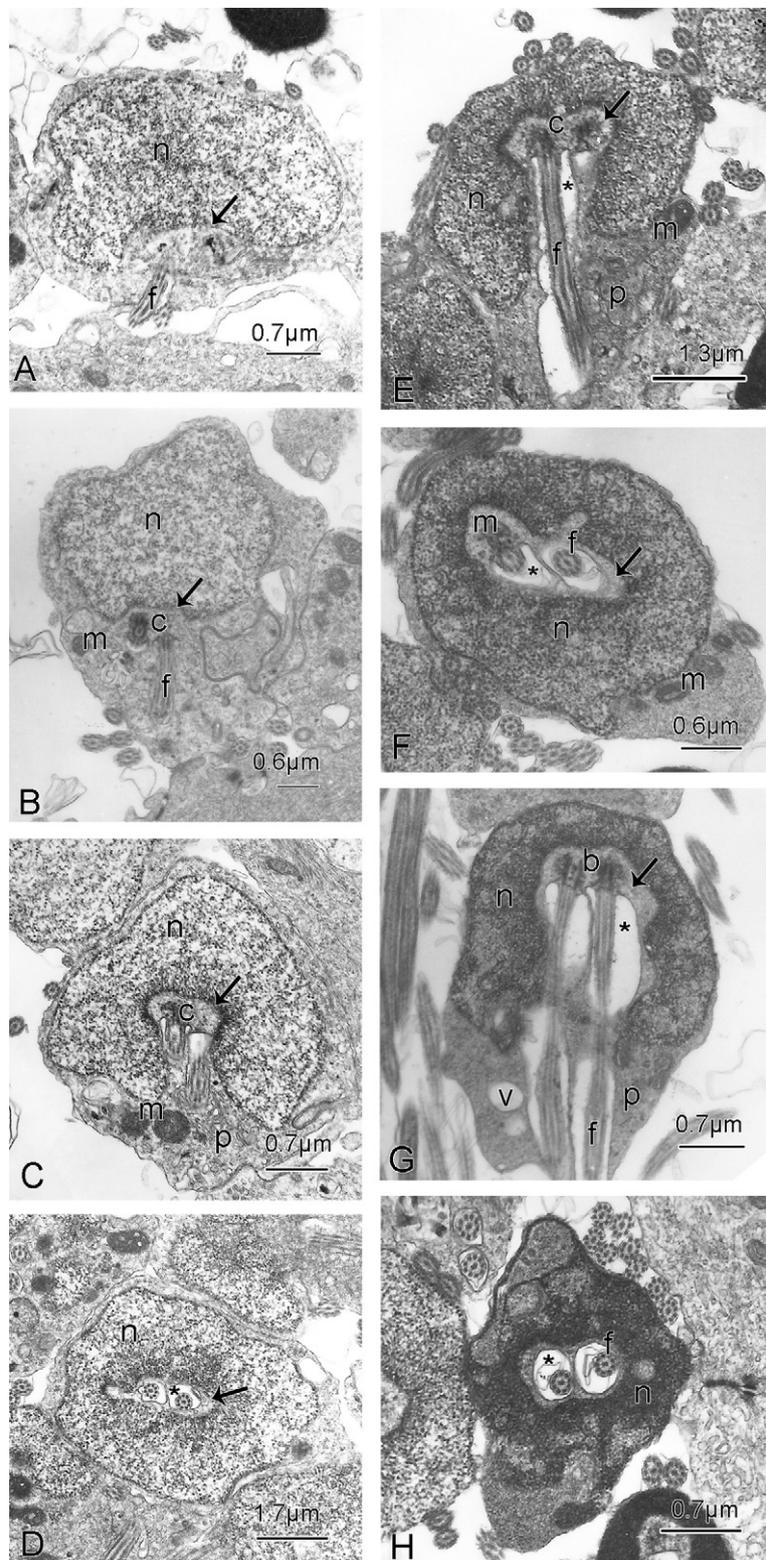


Fig. 2. Spermiogenesis in *A. weddellii*. (A and B) In early spermatids the centrioles (c) with the two forming flagella (f) are medially located in relation to the nucleus (n). m: mitochondria; arrow: nuclear fossa. (C, E, G) Spermatids in longitudinal sections. Note the centrioles (c) inside the nuclear fossa (arrow) moving in the direction of the tip of the nucleus (n) and forming the two cytoplasmic canals (asterisk). At the same time, the base of the nucleus and the cytoplasm project toward the flagella (f) forming the midpiece (p). m: mitochondria; v: vesicles. (D, F, H) Corresponding cross-sections of the same type of spermatids at the nuclear (n) region. Note the two flagella (f) and the respective two cytoplasmic canals (asterisk) inside the nuclear fossa (arrow). m: mitochondria.

nuclear fossa (Fig. 3D, H, I; Fig. 4A and E). The midpiece is filled with vesicles interspaced by a thin layer of cytoplasm, and has a cytoplasmic sleeve at the terminal end (Fig. 3A, B, D, J, K). Each flagellum contains a classic axoneme (9+2) (Fig. 3C, F; Fig. 4H).

Data on the limiting plasma membrane and midpiece of *Amblydoras* are not available because the specimens were obtained from ichthyological collections and the gonads were not properly preserved.

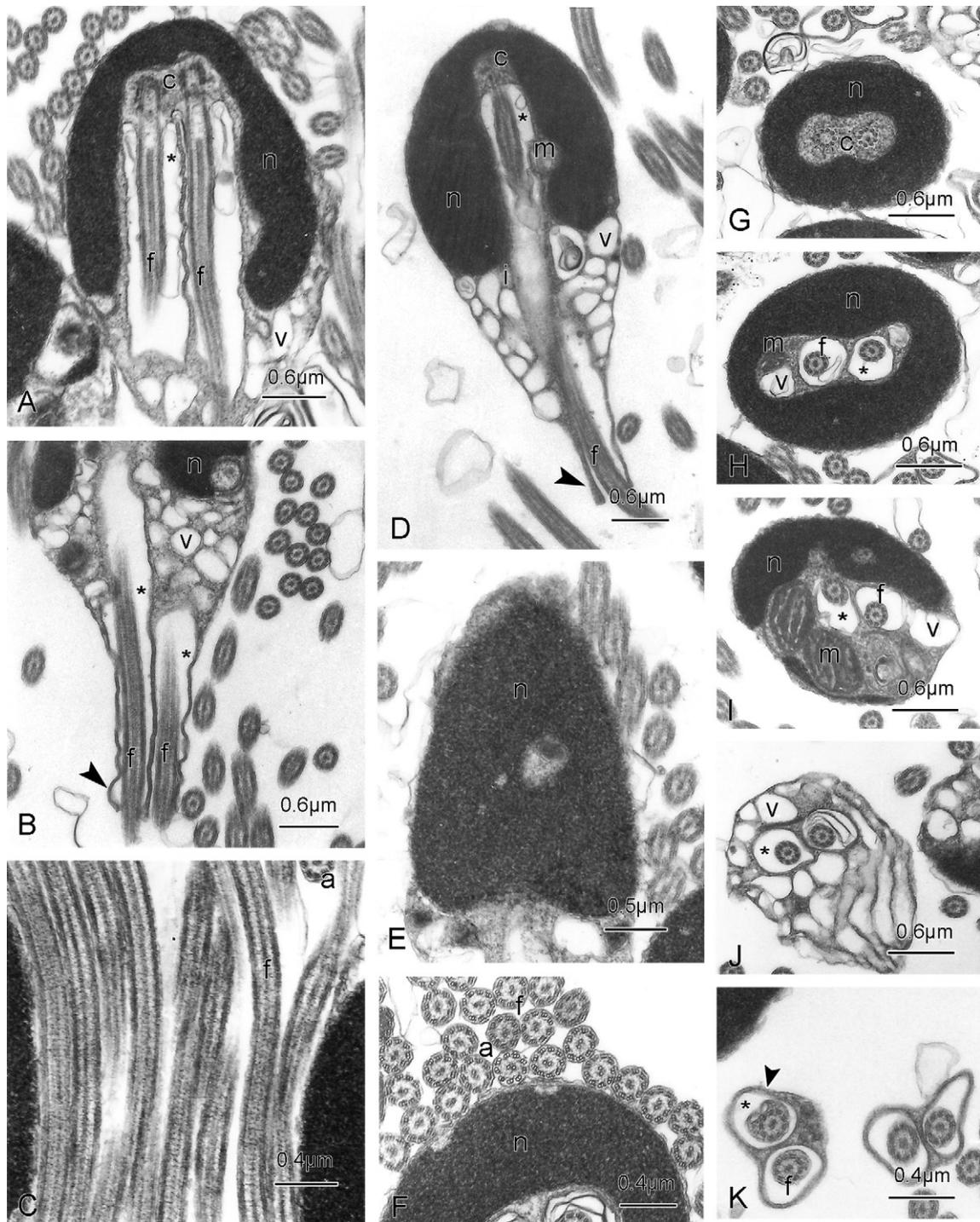


Fig. 3. Spermatozoon of *A. weddellii*. (A) The bell shaped nucleus (n) of this sperm in a medial/longitudinal section has a horseshoe shape with the centrioles (c) at the tip and an internal, large nuclear fossa (arrow) that settles the initial segments of the two flagella (f) and respective cytoplasmic canals (asterisk). v: vesicles. (B) Seen in a medial/longitudinal section, the midpiece is completely full of vesicles (v) and ends in a long cytoplasmic sleeve (arrow head) surrounding the two flagella (f). asterisk: cytoplasmic canals; n: nucleus. (C) Flagella (f) in a longitudinal view. (D) Longitudinal section along the sperm in which only one of the flagella (f) can be seen. n: nucleus; m: mitochondria; v: vesicles; arrow head: cytoplasmic sleeve; asterisk: cytoplasmic canals; c: centriolar complex. (E) In an external view, the nucleus (n) has a conical outline. (F) Axoneme (a) seen in cross-sectioned flagella. (G–I) Cross-sections in different levels of the nucleus. (G) The centrioles (c) in the tip of the nucleus (n). (H) In the middle region of the nucleus (n) the two flagella (f) and respective cytoplasmic canals (asterisk) are seen within the nuclear fossa. Note that some vesicles (v) and mitochondria (m) can also be found inside the nuclear fossa. (I) Most the mitochondria (m) are located at the base of the nucleus (n) around the two flagella (f) and respective cytoplasmic canals (asterisk). (J) In a cross to oblique section the vesicles (v) that fill the midpiece expose an elongated form. f: flagella; asterisk: cytoplasmic canals. (K) In a cross-section at the end of the midpiece, the cytoplasmic sleeves (arrow head) are seen surrounding the flagella (f) forming respective cytoplasmic canals (asterisk).

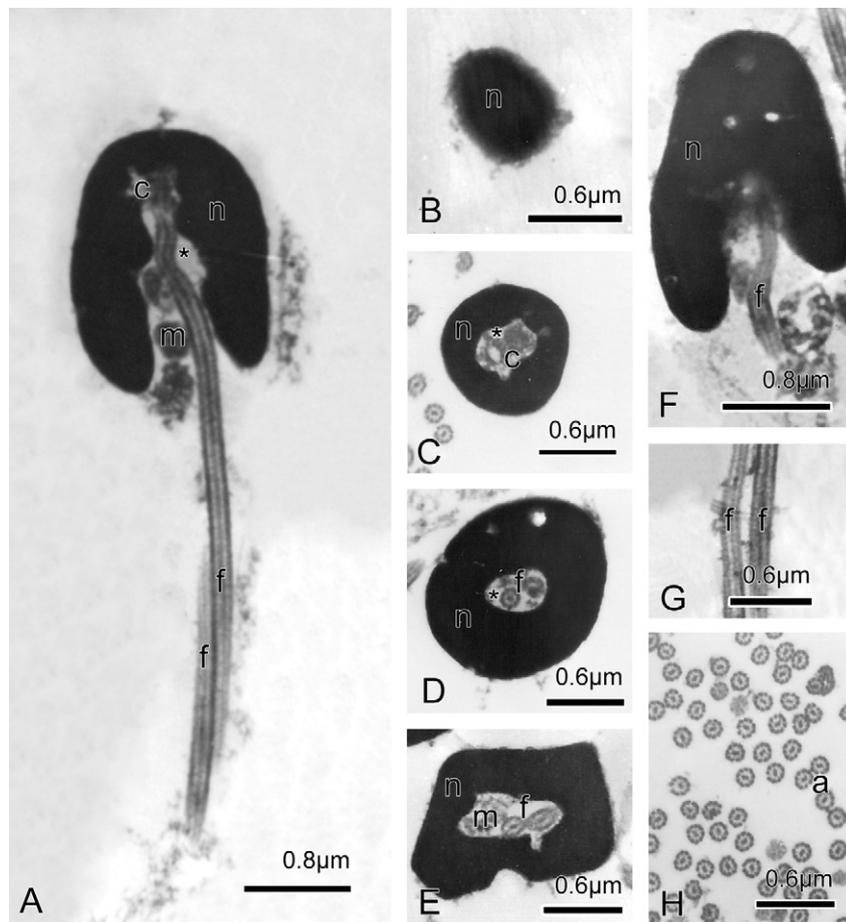


Fig. 4. Spermatozoon of *Amblydoras* sp. (specimen from zoological collection). (A) The bell shaped nucleus (n) of this sperm in a medial/longitudinal section has a horseshoe shape with the centrioles (c) at the tip and an internal, deep nuclear fossa (arrow) that settles the initial segments of the flagella (f) and some mitochondria (m). (B–E) In cross sections in different levels of the nucleus (n), the centrioles (c) and flagella (f) can be seen inside the nuclear fossa (asterisk). (F) In an external view, the nucleus (n) has a trunk-conical outline. f: flagella. (G–H) Note that the flagella (f) in a longitudinal or cross view are always in pars. a: axoneme.

3.2. *W. maculata*, *F. marmoratus* and *K. bahiensis* (Fig. 5)

Information on spermatogenesis and spermiogenesis are not available because the samples had only spermatozoa.

3.2.1. Spermatozoa

In the spermatozoa of *W. maculata*, *F. marmoratus* and *K. bahiensis* the nucleus has an ovoid shape with a flattened tip, contains highly condensed homogeneous chromatin, and is surrounded by a narrow strip of cytoplasm with no organelles (Fig. 5A, D, G). The tip of the nucleus is more flattened in *W. maculata* than in *F. marmoratus* and *K. bahiensis*. Nucleus has about 1.2 μm in height by 1.7 μm in width in *W. maculata*, 1.2 μm by 1.6 μm in *F. marmoratus*, and 1.3 μm by 1.6 μm in *K. bahiensis*. In all three species the nuclear outline that faces the midpiece has a medial and moderately deep depression, the nuclear fossa (Fig. 5A, D, G). The proximal centriole is anterior and almost perpendicular to the distal centriole. The centrioles are covered by electron dense material and fastened to one another. The proximal centriole and most of the distal centriole are inside the nuclear fossa (Fig. 5A, D, G). The midpiece contains the mitochondria, vesicles and the cytoplasmic canal in which lies the initial segment of the single flagellum (Fig. 5A–C, E, F, H). The midpiece is slightly asymmetric due to the unequal distribution of mitochondria and vesicles. In *W. maculata*, mitochondria seem to be very elongated and form a ring surrounding the cytoplasmic canal (Fig. 5B). Vesicles are mainly accumulated at the periphery and at the terminal regions of the midpiece (Fig. 5A, B, C, E, F). The

flagellum contains a classic axoneme (9+2) (Fig. 5I). Despite information on the limiting plasma membrane and midpiece structures such as mitochondria, data on the vesicles and cytoplasmic canal in *K. bahiensis* are not available because the gonads of the museum specimens were not properly preserved. The midpiece itself seems to be longer in *K. bahiensis* (Fig. 5 G, H, I) than in *W. maculata* and *F. marmoratus*.

3.3. *O. kneri* (Figs. 6 and 7)

3.3.1. Spermatogenesis

In *O. kneri*, spermatogenesis occurs inside the cysts. At the end of the differentiation process, spermatozoa are released into the luminal compartment of the testis (Fig. 6A).

3.3.2. Spermiogenesis

In *O. kneri* spermiogenesis is a modification of Type I. In the early spermatids (Fig. 7A) the cytoplasm symmetrically encircles the nucleus, which displays diffuse homogeneous chromatin and has a circular outline. The centriolar complex lies laterally to the nucleus and is anchored to the plasma membrane. The proximal centriole is anterior and perpendicular to the distal centriole. The distal centriole differentiates into the basal body and forms the single flagellum. The nucleus rotates toward the centriolar complex (Fig. 7B) with nuclear rotation of 90° considered complete. A depression is newly formed in the nuclear outline at the level of the centriolar complex that penetrates it (Fig. 7C). Simultane-

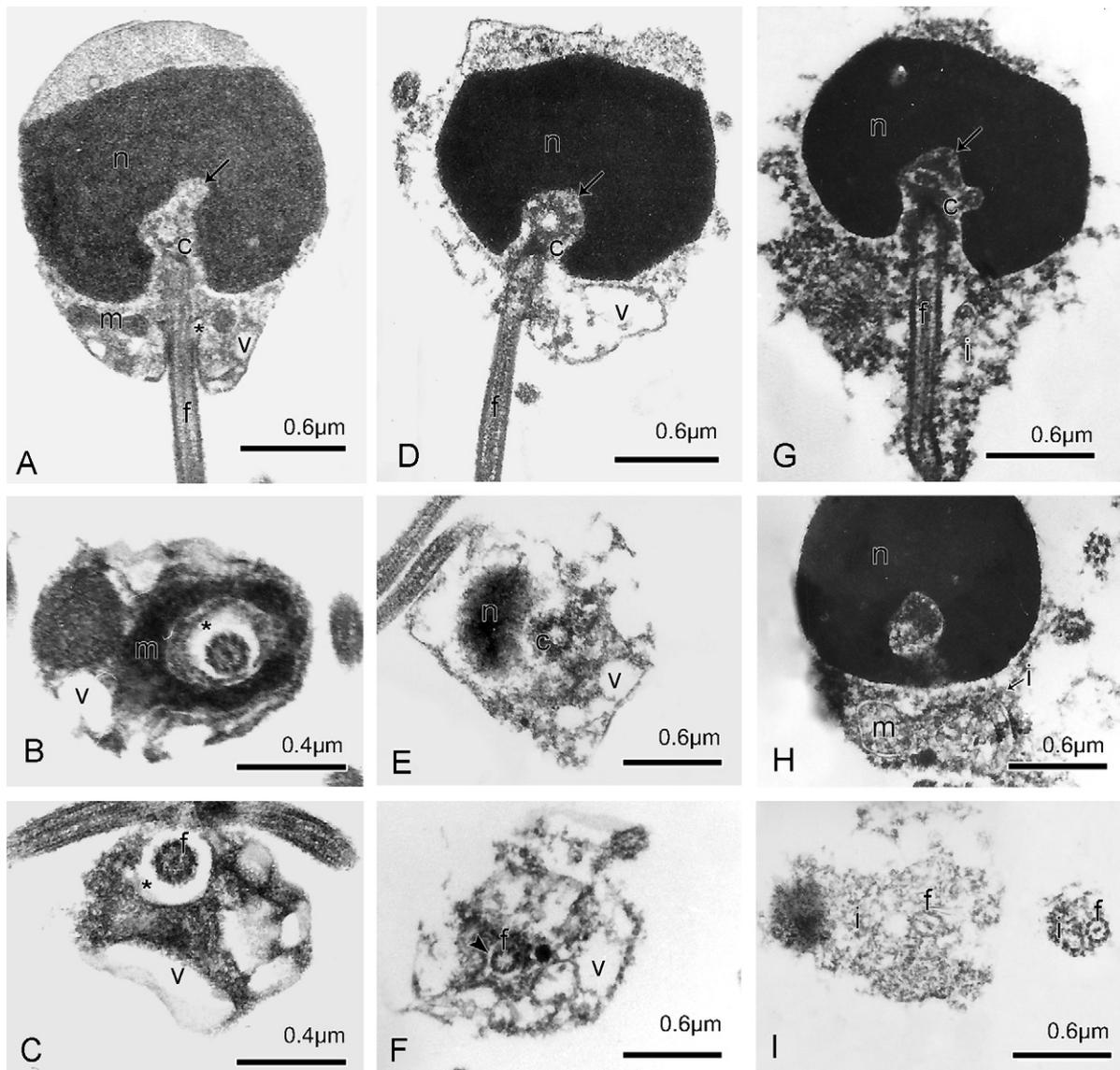


Fig. 5. Spermatozoa of *Wertheimeria maculata*, *Franciscodoras marmoratus* and *Kalyptodoras bahiensis* (specimens recovered from zoological collections). (A) Medial/longitudinal section from the spermatozoon of *W. maculata* exposing the nuclear fossa (arrow), the slightly eccentric position of the flagellum (f) in relation to the nucleus (n) and the also slightly asymmetric midpiece with few vesicles (v). c: centriolar complex; m: mitochondria. Note that the tip of the nucleus is flattened. (B and C) Cross sections of different levels of the midpiece. Note the cytoplasmic canal (asterisk) in which lies the initial segment of the flagellum (f), the ring shape and localization of the mitochondria (m) and vesicles (v). (D) Medial/longitudinal section from the spermatozoon of *F. marmoratus* exposing the nuclear fossa (arrow), the slightly eccentric position of the flagellum (f) in relation to the nucleus (n) and the also slightly asymmetric midpiece with few vesicles (v). c: centriolar complex. Note that the tip of the nucleus is flattened. (E–F) Cross sections at different levels of the midpiece. Only one of the centrioles (c) is visible and some vesicles (v). n: nucleus; arrow head: cytoplasmic canal. (G) Medial/longitudinal section from the spermatozoon of *K. bahiensis* exposing the nuclear fossa (arrow) and the slightly eccentric position of the flagellum (f) in relation to the nucleus (n). c: centriolar complex; i: midpiece. (H and I) Longitudinal and cross sections of the midpiece (i) at the base of the nucleus (n), at its middle region and at the terminal end. f: flagellum; m: mitochondria.

ous to nuclear rotation, the cytoplasm projects in the direction of the initial segment of the flagellum forming the cytoplasmic canal and midpiece (Fig. 7A–C). The midpiece contains the mitochondria, forming vesicles and cytoplasmic canal housing the initial segment of the flagellum (Fig. 7B and C).

3.3.3. Spermatozoon

In the spermatozoon of *O. kneri* the spherical nucleus (about 1.5 μm in diameter) contains highly condensed homogeneous chromatin interspersed by electron-lucent areas, and is surrounded by a narrow strip of cytoplasm with no organelles (Fig. 7D and E). In the nuclear outline that faces the midpiece there is a medial and moderately deep depression, the nuclear fossa (Fig. 7D–F). The proximal centriole, initially anterior and perpendicular to distal

one, attains an oblique acute angle to the distal centriole. The centrioles are covered by electron dense material and are fastened to one another, to the nuclear envelope at the nuclear fossa, and to the plasma membrane by stabilization fibrils. The proximal centriole and most of the distal centriole are inside the nuclear fossa (Fig. 7F and G). The midpiece contains the mitochondria, abundant vesicles and the cytoplasmic canal in which lies the initial segment of the flagellum. The midpiece is slightly asymmetric due to the unequal distribution of mitochondria and vesicles. The mitochondria are elongated and mainly accumulated in the larger portion of the midpiece. Vesicles are elongated and mainly concentrated at the periphery and at the terminal regions of the midpiece (Fig. 7H–K). The single flagellum contains a classic axoneme (9+2) (Fig. 7L).

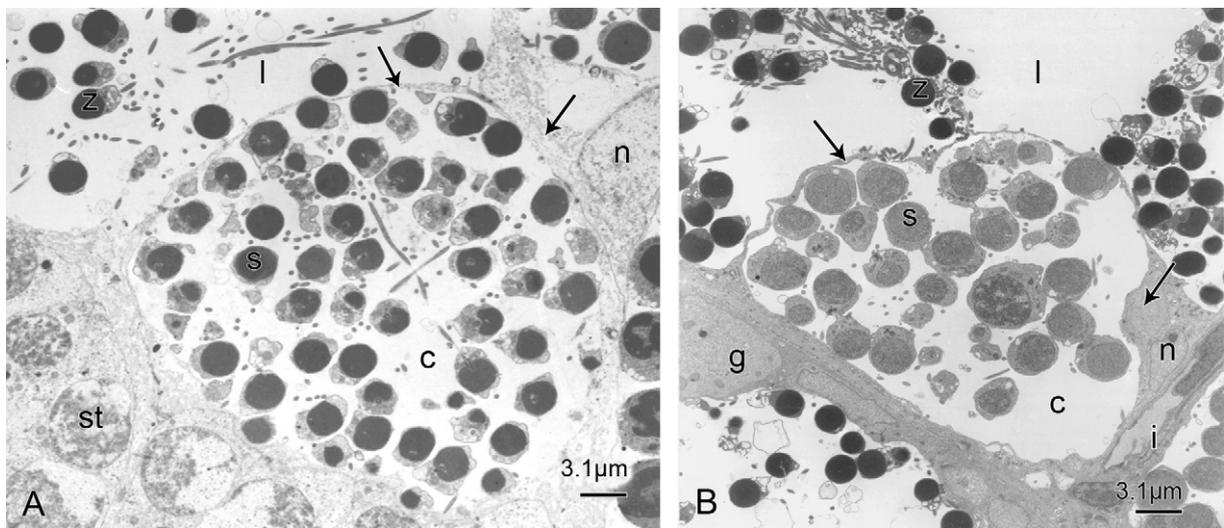


Fig. 6. Cystic spermatogenesis in (A) *Oxydoras kneri* and (B) *Trachydoras paraguayensis*. In the germinal epithelium, spermatogonia (g), spermatocytes (st) and spermatids (s) are always found inside the cysts (c) whereas spermatozoa (z) are in the luminal compartment (l). In the cysts, the differentiating germ cells are surrounded by the cytoplasmic process of the Sertoli cells (arrow). n: Sertoli nucleus; i: interstitial tissue.

3.4. *A. cataphractus*, *P. granulatus* and *R. dorbignyi* (Fig. 8)

Information on spermiogenesis in *A. cataphractus* is not available. In *P. granulatus* and *R. dorbignyi*, as in *O. kneri*, spermatogenesis is cystic and spermiogenesis is Type I.

3.4.1. Spermatozoa

In the spermatozoa of *A. cataphractus*, *P. granulatus* and *R. dorbignyi* the nucleus contains highly condensed homogeneous chromatin and is surrounded by a narrow strip of cytoplasm with no organelles (Fig. 8A, E, I). The nucleus is flattened at the tip and assumes an ovoid shape in *P. granulatus* (about 1.2 μm in height by 1.8 μm in width) vs. almost spherical in *A. cataphractus* (about 1.2 μm in height by 1.3 μm in width) and in *R. dorbignyi* (about 1.4 μm in height by 1.3 μm in width). In all three species the nuclear outline that faces the midpiece has a medial and moderately deep depression, the nuclear fossa (Fig. 8A, E, I). The proximal centriole is anterior and almost perpendicular to the distal centriole. The centrioles are covered by electron dense material and fastened to one another. The proximal centriole and most of the distal centriole are inside the nuclear fossa (Fig. 8A, B, E, I). The midpiece contains the mitochondria, vesicles and the cytoplasmic canal in which lies the initial segment of the flagellum (Fig. 8C–D, F–H, J–L). The midpiece is slightly asymmetric due to the unequal distribution of mitochondria and vesicles. The asymmetry of the midpiece is more accentuated in *R. dorbignyi*. Mitochondria are oblong in *P. granulatus* and elongated in *R. dorbignyi*. Vesicles are mainly concentrated at the periphery and at the terminal regions of the midpiece (Fig. 8D, G, K). The single flagellum contains a classic axoneme (9+2) (Fig. 8L). Information on the limiting plasma membrane and midpiece, especially from the mitochondria, of *A. cataphractus* are not available because the gonads were not properly preserved in the museum specimens.

3.5. *T. paraguayensis* (Figs. 6 and 9)

3.5.1. Spermatogenesis

In *T. paraguayensis*, spermatogenesis occurs inside the cysts. At the end of the differentiation process spermatozoa are released into the luminal compartment of the testis (Fig. 6B).

3.5.2. Spermiogenesis

In *T. paraguayensis*, spermiogenesis is Type III. In the early spermatids (Fig. 9A) the cytoplasm symmetrically encircles the nucleus, which displays diffuse homogeneous chromatin and has a circular outline. The centriolar complex lies medially to the nucleus and is anchored to the plasma membrane. The proximal centriole is anterior and oblique to the distal centriole (Fig. 9B and C). The distal centriole, differentiated into the basal body, remains associated with the plasma membrane and forms the single flagellum. The nucleus does not rotate in relation to the flagellar axis, and a nuclear fossa is not formed (Fig. 9A–C). Most of the cytoplasm concentrates in the region surrounding the centriolar complex, forming the midpiece which contains the mitochondria (Fig. 9A–C). Progressively formed in the midpiece terminal portion, vesicles enlarge, project toward and surround the initial segment of the flagellum, forming a cytoplasmic canal (Fig. 9B and C).

3.5.3. Spermatozoon

In the spermatozoon of *T. paraguayensis*, the spherical nucleus (1.68 μm in diameter) contains highly condensed homogeneous chromatin interspersed by electron-lucent areas, has no nuclear fossa, and is surrounded by a narrow strip of cytoplasm with no organelles (Fig. 9D and E). The centrioles remain near the nucleus. They are covered by electron dense material and are fastened to one another, to the nuclear envelope, and to the plasma membrane by stabilization fibrils (Fig. 9F). The proximal centriole is anterior and oblique to the distal centriole (Fig. 9F). The flagellum is slightly eccentric to the nuclear axis (Fig. 9D). The midpiece contains the mitochondria and large vesicles that project and surround the initial segment of the flagellum to form the cytoplasmic canal (Fig. 9F–L). The midpiece is asymmetric due to the unequal distribution of mitochondria and vesicles. Most of the midpiece is composed of the vesicles interspaced by a thin cytoplasmic layer. Vesicles have different dimensions and formats (Fig. 9G–L). The single flagellum contains a classic axoneme (9+2) (Fig. 9M).

4. Discussion

4.1. Spermatogenesis

Two types of spermatogenesis are found among the five species of Doradidae analyzed herein: cystic (sensu Grier, 1981) and semi-

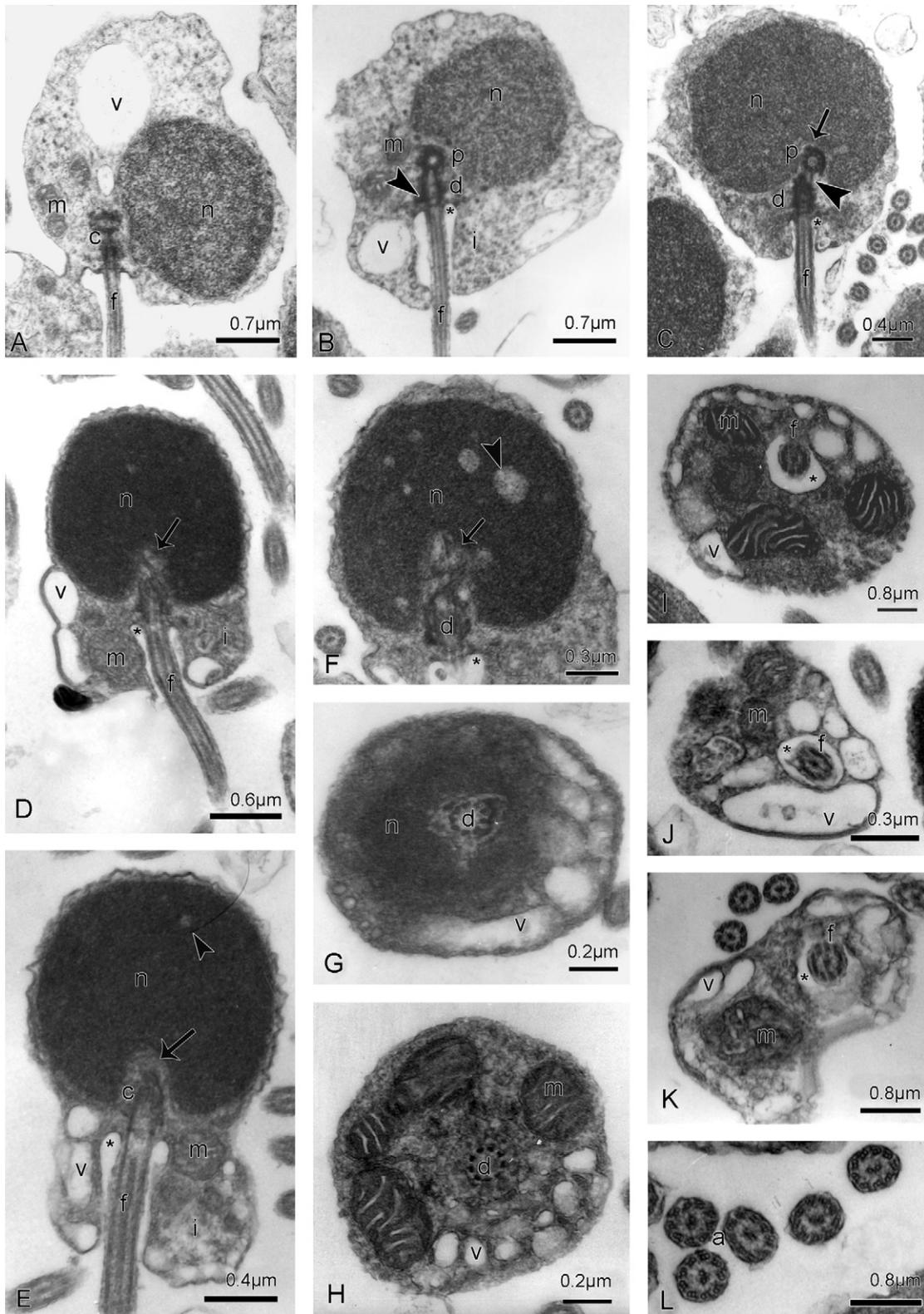


Fig. 7. Spermiogenesis and spermatozoon of *O. kneri*. (A–C) Longitudinal sections of early to late spermatids. Note that the centriolar complex (c) initially lateral to the nucleus (n) assumes a medial position at the end of the nuclear rotation. The cytoplasm moves in the direction of the flagellum (f) giving rise to the midpiece and the cytoplasmic canal (asterisk). Mitochondria (m) and forming vesicles (v) are found in the midpiece (i) and in the centriolar complex. The proximal centriole (p) is anterior and perpendicular to the distal centriole (d). Note the electron dense material surrounding the centrioles and the fibrils (arrow head) that fasten these to the nucleus and to the plasma membrane, also seen in the sperm. (D and E) Medial/longitudinal sections of spermatozoa exposing the insertion of the centriolar complex (c) in the nuclear fossa (arrow), the initial segment of the flagellum inside the cytoplasmic canal (asterisk), and the localization of mitochondria (m) and vesicles in the midpiece (i): n: nucleus. (F) Medial/longitudinal section of the nucleus (n). Note the electron dense material (arrow head) interspersed through the highly condensed chromatin, the ramified nuclear fossa (arrow), the distal centriole (d) partially inside the fossa. (G–K) Cross sections at different levels of the sperm, from the base of the nucleus (n) to the midpiece end. Note the distal centriole (d) that gives rise to the flagellum (f), the cytoplasmic canal (asterisk), and the form and localization of the mitochondria (m) and vesicles (v). (L) Flagella in cross sections exposing the axoneme (a) formed by the central pair of microtubules and the nine peripheral doublets of microtubules.

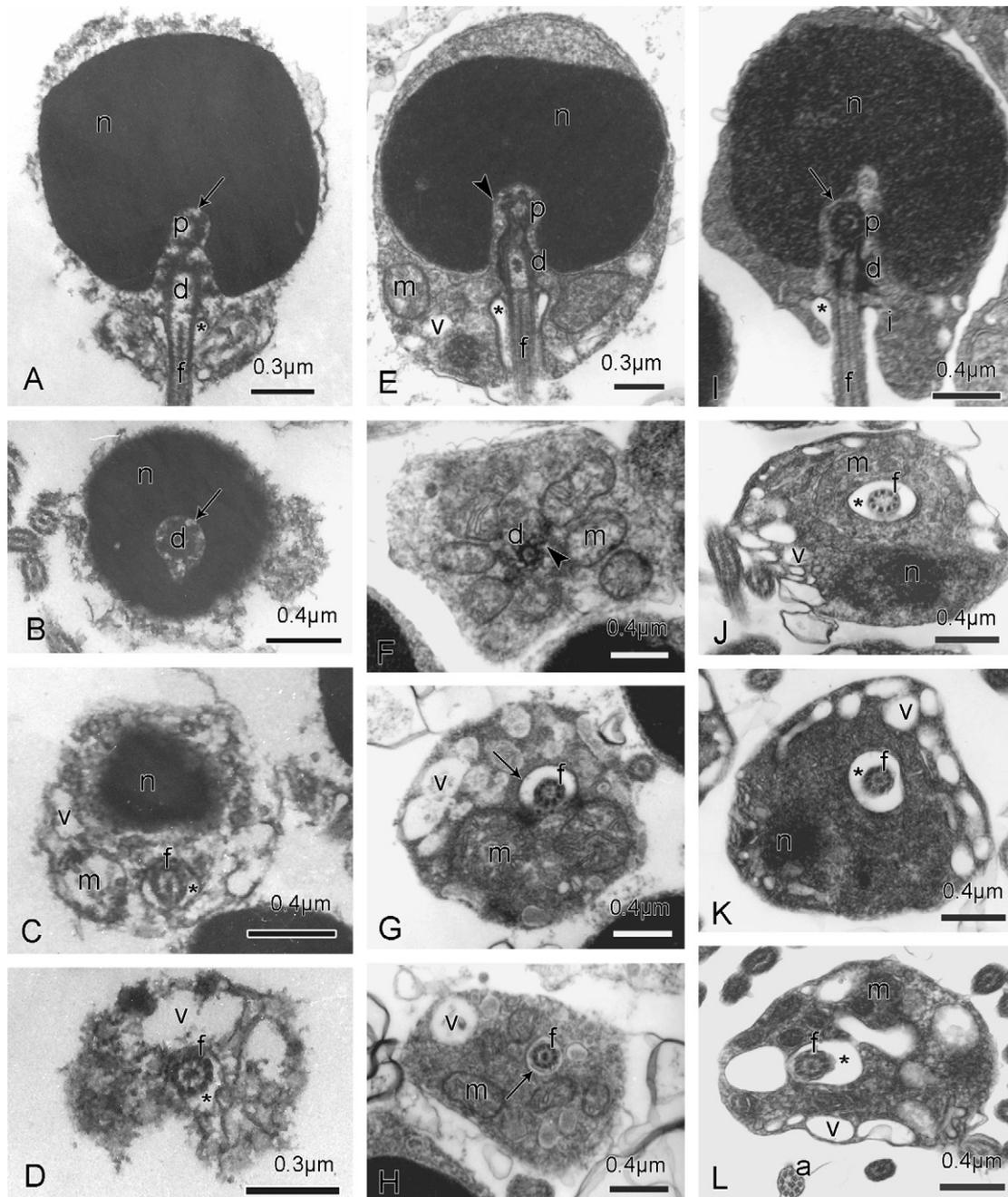


Fig. 8. Spermatozoa of *Acanthodoras cataphractus* (specimen recovered from zoological collections), *P. granulosa* and *R. dorbignyi*. (A) Medial/longitudinal section from the spermatozoon of *A. cataphractus* exposing the nuclear fossa (arrow), the slightly eccentric position of the flagellum (f) in relation to the nucleus (n) and the also slightly asymmetric midpiece. d: distal centriole; p: proximal centriole; asterisk: cytoplasmic canal. Note that the tip of the nucleus is slightly flattened. (B–D) Cross sections of the nucleus (n) and at different levels of the midpiece, on the base and under the nucleus. Note the distal centriole (d) inside the nuclear fossa (arrow), the cytoplasmic canal (asterisk) in which is the initial segment of the flagellum (f), the mitochondria and the vesicles (v). (E) Medial/longitudinal section from the spermatozoon of *P. granulosa* exposing the nuclear fossa (arrow head), the slightly eccentric position of the flagellum (f) in relation to the nucleus (n) and the also slightly asymmetric midpiece. d: distal centriole; m: mitochondria; v: vesicles (v). Note that the tip of the nucleus is flattened. (F–H) Cross sections at different levels of the midpiece. Note the several oblong mitochondria (m) mainly accumulated around the distal centriole (d), the cytoplasmic canal (asterisk) in which is the initial segment of the flagellum (f), and the vesicles (v). (arrow head) fibrils. (I) Medial/longitudinal section from the spermatozoon of *R. dorbignyi* exposing the nuclear fossa (arrow) and the slightly eccentric position of the flagellum (f) in relation to the nucleus (n). asterisk: cytoplasmic canal; d: distal centriole; p: proximal centriole. (J–L) Cross sections at different levels of the midpiece, from the base of the nucleus (n) to the midpiece end. Note the initial segment of the flagellum (f) inside the cytoplasmic canal (asterisk), and the localization of the elongate mitochondria (m) and the vesicles (v) in the midpiece. Note the flagellum in cross sections exposing the axoneme (a) formed by the central pair of microtubules and the nine peripheral doublets of microtubules.

cystic (sensu Mattei, 1993). In the cystic type, the entire process from spermatogonia proliferation, through meiosis to spermatid differentiation, occurs totally inside the cysts, in the germinal epithelium. In semi-cystic spermatogenesis, spermatogonia proliferation and meiotic divisions occur inside the cysts, whereas spermatid differentiation occurs outside the cysts, in the luminal

compartment of the testis. Cystic spermatogenesis is characteristic of most Siluriformes (Burns et al., 2009), whereas the semi-cystic type of development has been previously documented only in Aspredinidae and Cetopsidae (Spadella et al., 2006), Malapteruridae (Shahin, 2006), Callichthyidae (Spadella et al., 2007), and Ariidae and Nematogenyidae (Burns et al., 2009). In Doradidae spermatoge-

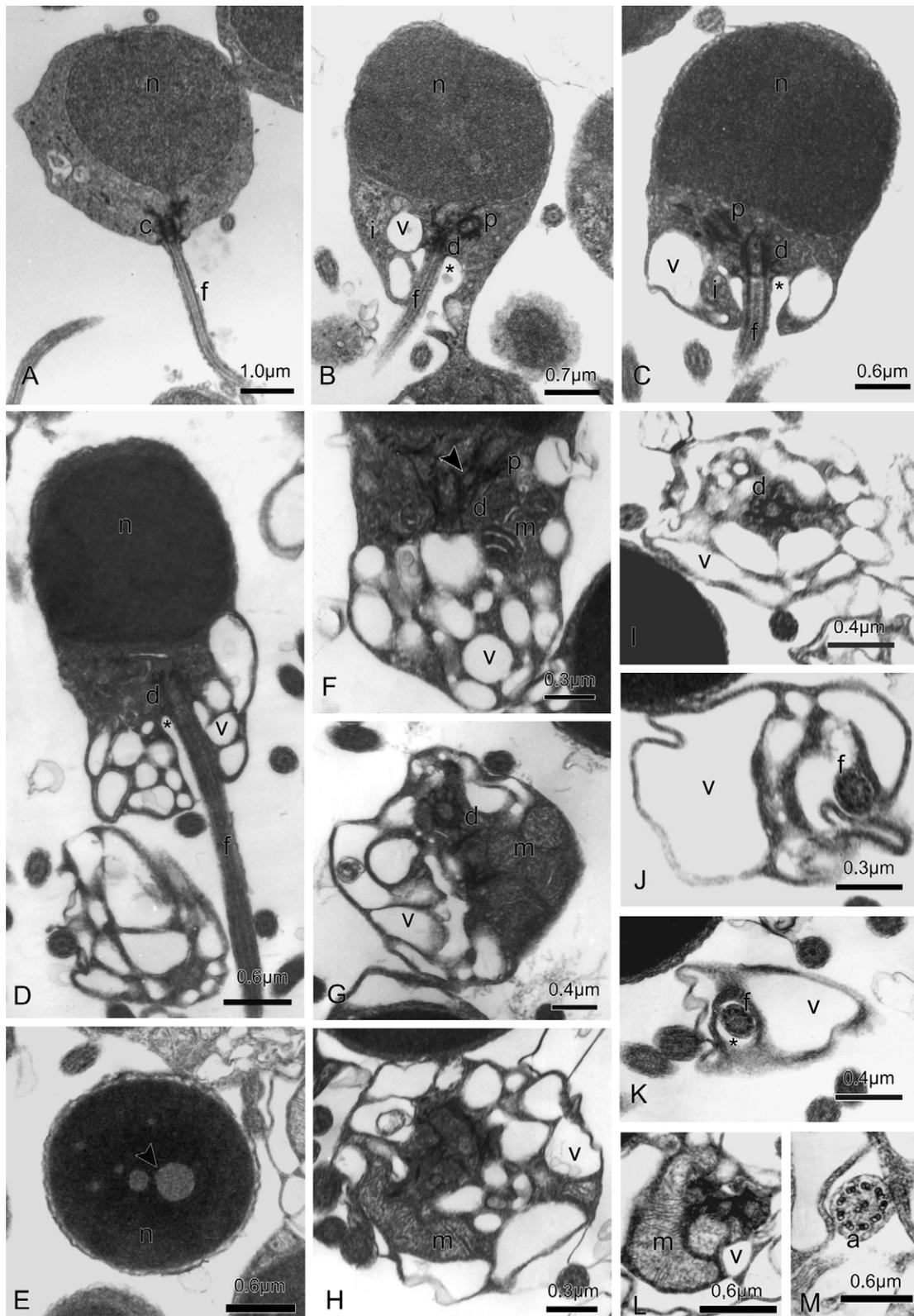


Fig. 9. Spermiogenesis and spermatozoon of *T. paraguayensis*. (A–C) Longitudinal sections of early to late spermatids. Note that the position of the centriolar complex (c) is always medial to the nucleus (n) and the nuclear rotation does not occur. The cytoplasm moves in the direction of the centriolar complex and the forming vesicles (v) enlarge and project in the direction of the flagellum (f) giving rise to the midpiece (i) and the cytoplasmic canal (asterisk). In the centriolar complex the proximal centriole (p) is anterior and oblique to the distal (d). (D) Medial/longitudinal section of spermatozoa exposing the slightly eccentric position of the flagellum (f) and the asymmetric midpiece with abundant vesicles (v). d: distal centriole; n: nucleus; asterisk: cytoplasmic canal. (E) Cross section of the nucleus (n). Note the electron lucent areas (arrow head) interspersed through the highly condensed chromatin. (F) Detail of the centriolar complex. Note that the proximal centriole (p) is anterior and oblique to the distal (d), these are fastened to one another, to the nuclear envelope and plasma membrane by fibrils and covered by electron dense material (arrow head). m: mitochondria. (G–K) Cross sections of different levels of the midpiece, from the base of the nucleus (n) to the midpiece end. Note the distal centriole (d) that gives rise to the flagellum (f), the cytoplasmic canal (asterisk), and form and localization of the mitochondria (m) and vesicles (v). (L) Detail of the elongated and ramified mitochondria (m). (M) Flagellum in cross section exposing the axoneme (a) formed by the central pair of microtubules and the nine peripheral doublets of microtubules.

nesis in *A. weddellii*, subfamily Astrodoradinae, is also semi-cystic. In species for which spermatogenesis is semi-cystic, the spermatids present centrioles parallel to each other. Each centriole gives rise to one axoneme resulting in a biflagellate sperm except in two known cases. In *Corydoras flaveolus* (Callichthyidae: Corydoradinae) spermatogenesis is semi-cystic, but sperm have only one axoneme and a single flagellum (Spadella et al., 2007). In the ariid *Genidens genidens* sperm have two axonemes, but they share the same flagellar membrane and form a single flagellum (Burns et al., 2009). The co-occurrence of semi-cystic spermatogenesis and sperm with two axonemes in six families of Siluriformes suggests that the two characteristics are related (Burns et al., 2009). The four other species of Doradidae analyzed herein, *O. kneri*, *P. granulosis*, *R. dorbignyi* and *T. paraguayensis*, all have cystic spermatogenesis.

4.2. Spermiogenesis

Spermiogenesis in Siluriformes may be of Type I (sensu Mattei, 1970) or Type III (sensu Quagio-Grassiotto and Oliveira, 2008). Slight variations of these two types also are found. There is no register of Type II spermiogenesis in Siluriformes (Burns et al., 2009). In Type I spermiogenesis (Mattei, 1970) the centrioles that initially have a lateral position migrate in the direction of the nucleus. As they are anchored at the plasma membrane, the migration pulls the membrane and forms an invagination that gives rise to the cytoplasmic canal. The developing flagellum settles into the interior of the recently formed canal. The nucleus rotates 90° in relation to the flagellar axis, and the flagellum from a lateral position stays medial to the nucleus. In the region of the nucleus that faces the centrioles a depression is formed, the nuclear fossa, which totally or partially houses the centrioles. In Type III spermiogenesis (Quagio-Grassiotto et al., 2005; Quagio-Grassiotto and Oliveira, 2008), at the beginning of the differentiation process, the centrioles are anchored at the plasma membrane in a position medial to the nucleus. The centriolar migration does not occur and neither does the nuclear rotation. The cytoplasmic canal may or may not be formed. When it does occur, the formation of the cytoplasmic canal is due to the movement of the midpiece cytoplasm in the direction of the initial segment of the flagellum. Alternatively, it may be due to the formation of vesicles at the midpiece terminal end that project in the direction of the initial segment of the flagellum.

Variations in Type III spermiogenesis are found in Callichthyidae, subfamily Corydoradinae (Spadella et al., 2007). Here the centriolar complex is strongly eccentric in relation to the nucleus. Consequently flagellum development also occurs in an eccentric position. The centrioles do not migrate and the nuclear rotation does not occur. A shallow nuclear fossa is formed, but the centrioles stay outside.

T. paraguayensis has a classical spermiogenesis of Type III in which the nuclear fossa is never formed and the cytoplasmic canal results from the projection of the midpiece vesicles in the direction of the initial segment of the flagellum. Spermiogenesis has peculiar characteristics in the two other doradids examined herein.

Spermiogenesis in *A. weddellii* is a variation of Type III (i.e., Type III modified). The initial position of the centrioles is medial to the nucleus, and the absence of nuclear rotation characterizes spermiogenesis as Type III. The formation of the nuclear fossa and the cytoplasmic canal are due to the simultaneous projection of the nucleus and cytoplasm toward the initial segments of the flagella and to the migration of the centrioles forward towards the tip of the nucleus.

P. granulosis and *R. dorbignyi* have a classical spermiogenesis of Type I in which nuclear rotation is complete and centriolar migration occurs. Spermiogenesis in *O. kneri* is a variation of Type I, in which the nuclear rotation is complete; however, the centrioles do not migrate. In *O. kneri* the nuclear fossa is formed by the projec-

tion of the nucleus toward the centrioles, whereas the cytoplasmic canal results from the projection of the cytoplasm toward the initial segment of the flagellum.

The different types of spermiogenesis, Types I and II (Mattei, 1970) and Type III (Quagio-Grassiotto et al., 2005; Quagio-Grassiotto and Oliveira, 2008) characterize the extremes. As previously noted by Mattei (1970), variations in these processes are conducive to the formation of intermediate types of sperm, mainly considering the orientation of the flagellum in relation to the nucleus.

4.3. Sperm

Sperm of Doradidae analyzed herein can be separated into three morphotypes on the basis of ultrastructural characteristics. The spermatozoa of *A. weddellii* and *Amblydoras* represent the first morphotype and differ from all others by having: a bell-shaped nucleus with a deep nuclear fossa, centrioles parallel to one another, a long midpiece, and, most interestingly, two flagella.

The second morphotype is represented by spermatozoa in *Acanthodoras*, *Franciscodoras*, *Kalyptodoras*, *Wertheimeria*, *Oxydoras*, *Pterodoras* and *Rhinodoras*, wherein the nucleus is spherical to ovoid with flattened tip, nuclear fossa is present, centrioles are perpendicular or nearly so, midpiece is relatively short, and a single flagellum with one axoneme is present.

Although museum collections yield specimens that are inappropriate for complete analysis of sperm formation and morphology, they do provide opportunities to make important observations in rare taxa such as *Franciscodoras*, *Kalyptodoras* and *Wertheimeria*. For example, the nuclear and flagellar characteristics remain sufficiently clear for morphological analysis, even though midpiece structures, such as mitochondria and vesicles, do not. Preservation of specimens from museum collections (i.e., 70% alcohol) may result in cell dehydration, which is detectable as a reduction in the dimension of the cellular structures such as the nucleus. Thus, sperm of *Wertheimeria* and *Franciscodoras*, both from museum collections, share the same type of nucleus (i.e., ovoid, flattened at tip), format of the nuclear fossa (moderately deep), position of centrioles relative to each other (nearly perpendicular), and apparently the general aspect of the midpiece (short, asymmetric). The sperm of *W. maculata* and *F. marmoratus* differ from that of *A. cataphractus* mainly by having a shorter midpiece and more accentuated flatness of the nucleus. In the sperm of *K. bahiensis*, the nucleus is not remarkably flattened and has an intermediate shape between distinctly flattened (e.g., *W. maculata*, *F. marmoratus*, *P. granulosis*) and spherical (*O. kneri*, *T. paraguayensis*) or subspherical (*A. cataphractus*, *R. dorbignyi*). Sperm of *O. kneri* and *R. dorbignyi* were very well preserved as they were collected fresh, and are quite similar, sharing nuclear characteristics and the same kinds of midpiece and organelles such as mitochondria and vesicles.

The sperm of *T. paraguayensis* represents the third morphotype and is relatively unique among doradids. It differs from all other unflagellate doradid sperm by having a spherical nucleus that lacks a nuclear fossa, centrioles obliquely oriented in relation to one another, and relatively large vesicles in the midpiece. These differences arise from their spermiogenesis, viz the ontogeny.

The spermatid characteristics of Doradidae are of interest when compared to the separation of the family into two groups based on simple vs. fimbriate maxillary barbels (see Sabaj and Ferraris, 2003 and Birindelli and Sousa, 2010 for review). Substantial differences in spermatid characteristics noted for doradids with simple barbels further corroborate the non-monophyly of this group (Higuchi, 1992; Moyer et al., 2004; Birindelli, 2006, 2010). The unique sperm morphotype of *T. paraguayensis*, the only fimbriate-barbel doradid examined, distinguishes it from doradids with simple barbels. Additional fimbriate-barbel taxa should be analyzed to deter-

mine if the spermatic characteristics of *T. paraguayensis* are more widespread in this group.

Spermatic patterns tend to be constant within families (Baccetti et al., 1984; Quagio-Grassiotto et al., 2003; Quagio-Grassiotto and Oliveira, 2008; Burns et al., 2009) or subfamilies (Spadella et al., 2007, 2009). The types of spermatogenesis and spermiogenesis and the ultrastructural differences found in the sperm of the Astrodoradinae corroborate the distinctiveness of this subfamily as previously proposed by Higuchi (1992), Birindelli (2006), and Higuchi et al. (2007). Specifically semi-cystic spermatogenesis and modified Type III spermiogenesis (both confirmed for *Anadoras weddellii*), and biflagellate sperm (confirmed for *A. weddellii* and *Amblydoras*) may be diagnostic characteristics unique within Doradidae to Astrodoradinae. Spermatic characteristics of *A. cataphractus* (e.g., nucleus subspherical, centrioles perpendicular, single flagellum), however, do not corroborate its close relationship with *Anadoras* and *Amblydoras* (e.g., nucleus bell-shaped, centrioles parallel, two flagella) supported by phylogenetic analyses of bony and soft anatomy (Birindelli, 2010; Sousa, 2010). Their morphological studies also recover *Acanthodoras* and *Agamyxis* as sister taxa, a relationship not supported by the molecular data (Moyer et al., 2004). Spermatic characteristics in *Agamyxis* should be analyzed to help resolve this conflict.

4.4. Doradoidea vs. Aspredinidae

Friel's (1994) phylogenetic analysis of morphological data recovered Aspredinidae as the sister group of Doradoidea (Doradidae + Auchenipteridae), a relationship further corroborated by molecular data (Hardman, 2005; Sullivan et al., 2006). The sperm of the aspredinid, *Bunocephalus amazonicus* (Spadella et al., 2006) and of the doradids, *A. weddellii* and *Amblydoras*, subfamily Astrodoradinae, are very similar, remarkably so with respect to the bell-shaped nucleus. Few differences include the pattern of chromatin condensation (highly condensed and homogenous in *A. weddellii* and *Amblydoras*, vs. flocculent in *B. amazonicus*), mitochondrial shape (ovoid in *A. weddellii* and *Amblydoras*, vs. elongated in *B. amazonicus*), and details of midpiece structures such as vesicles. In addition to sperm characteristics, *A. weddellii* and *B. amazonicus* share the same type of spermatogenesis (semi-cystic) and spermiogenesis (Type III modified with centriole migration and formation of deep nuclear fossa). The similarities in spermatogenesis, spermiogenesis and spermatozoa shared among the Astrodoradinae (*A. weddellii* and *Amblydoras*) and the Aspredinidae (*B. amazonicus*) are consistent with the hypothesis that the two families are closely related and suggest that Astrodoradinae may occupy a basal position within Doradidae.

4.5. Doradidae vs. Auchenipteridae

Various authors have long recognized Auchenipteridae as the sister group of Doradidae (Pinna de, 1998; Sullivan et al., 2006; Birindelli, 2010), and together they form the superfamily Doradoidea. Auchenipteridae are inseminating (Meisner et al., 2000) and have highly modified sperm associated with their internal mode of fertilization. Descriptions of sperm in Auchenipteridae are restricted to the genus *Trachelyopterus* and species *T. lucenai* (Burns et al., 2002), *T. galeatus* (Parreira et al., 2009), and *T. striatulus* (Burns et al., 2009). The sperm of all three species are very similar to one another by having an elongated nucleus and peculiar midpiece. As auchenipterid sperm are highly modified, they share with doradid sperm only a few characteristics such as the homogeneous and highly condensed pattern of chromatin condensation and single flagellum (Astrodoradinae excluded). Auchenipteridae also exhibits cystic spermatogenesis and Type I spermiogenesis

(Burns et al., 2009), conditions shared with several species of Doradidae.

4.6. Doradidae vs. Ariidae

Early hypotheses of interfamilial relationships within Siluriformes proposed Ariidae as closely related to Doradidae (Royero, 1987; Mo, 1991; Lundberg, 1993; Pinna de, 1998). Comparison of spermatozoa in the Doradidae analyzed herein and Ariidae (Burns et al., 2009: *G. genidens*) provide no compelling new evidence for their close relationship. Spermatic characteristics in the ariid *G. genidens* are most similar to that of Astrodoradinae as both share semi-cystic spermatogenesis and sperm with highly condensed, homogenous chromatin, deep nuclear fossa, parallel centrioles, and two axonemes (but forming only one flagellum in *Genidens* vs. two in Astrodoradinae; flagellar fins lacking in both cases).

4.7. An end note

Spermatic characteristics have been little used in the cladistic analysis of Teleostei. Available data show that the fine structure of the sperm in Ostariophysi is very conservative within genera and often similar among confamilial genera (see Burns et al., 2009 for review). Nevertheless, conspicuous intrafamilial differences are apparent among the doradids analyzed herein (Table 1) and may prove a rich source of characteristics for diagnosing particular taxa and subgroups within the family. More and more the suspicion that spermatic characteristics are phylogenetically informative has attracted the attention of systematists and spermatozoologists alike. Thus the co-occurrence of two axonemes (or of two flagella) and semi-cystic spermatogenesis in many families of Siluriformes is thought to be a correlated feature of sperm formation (Burns et al., 2009). An intriguing question is whether semi-cystic spermatogenesis and two axonemes, as found in Ariidae, Aspredinidae, Cetopsidae, Doradidae (Astrodoradinae), Malapteruridae and Nematogenyidae, represent ancestral spermatic characteristics for Siluriformes or has evolved independently in multiple lineages.

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