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Spermiogenesis and sperm ultrastructure in ten species of Loricariidae (Siluriformes, Teleostei)

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Abstract The current study describes the ultrastructural characteristics of spermatogenesis, spermiogenesis, and spermatozoa in specimens of siluriform taxa Neoplecostominae, Hypoptopomatinae, Otothyrinae, Loricariinae, and Hypostominae. Our data show that the characteristics of spermatogenesis and spermiogenesis and spermatozoa ultrastructure of Neoplecostominae are more common to Hypoptopomatinae and Otothyrinae than to Loricariinae and Hypostominae. Furthermore, Loricariinae and Hypostominae have more characteristics in common than with any other group of Loricariidae. These data reinforce the phylogenetic hypotheses of relationships among the subfamilies of Loricariidae. Considering the available data in Loricarioidei, Loricariidae presents ultrastructural characteristics of spermatogenesis and spermiogenesis that are also observed in Astroblepidae, its sister group. However, the most of the characteristics of spermatozoa ultrastructure found in Astroblepidae are also observed in Scoloplacidae, the sister group of a clade composed of Astroblepidae and Loricariidae.

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Introduction

Loricarioidei is the most diverse clade of the Siluriformes, endemic to South and lower Central America, and has long been recognized as a monophyletic group based on morphological and molecular data (see Sullivan et al. 2006—for review). Previously considered as the superfamily Loricarioidea (de Pinna 1998), Sullivan et al. (2006) acknowledge this group as a suborder and propose that Loricarioidei be placed as the sister group to all other Siluriformes, which are divided into Diplomystidae and Siluroidei.

Actually, Loricarioidei comprises six largely groups: Nematogenyidae, Trichomycteridae, Callichthyidae, Scoloplacidae, Astroblepidae, and Loricariidae. Loricariidae is the largest of these groups, distributed throughout the Neotropics (Reis et al. 2006). Phylogenetic studies using morphological (de Pinna 1998) and molecular (Sullivan et al. 2006) data suggest that Loricariidae is a sister group to the Astroblepidae. Loricariids are traditionally classified into seven subfamilies: Lithogeneinae, Delturinae, Neoplecostominae, Hypoptopomatinae, Otothyrinae, Loricariinae, and Hypostominae (Armbruster 2004; Reis et al. 2006; Chiachio et al. 2008).

In the current study, the ultrastructural characteristics of spermatogenesis, spermiogenesis, and spermatozoa of specimens of Neoplecostominae, Hypoptopomatinae, Otothyrinae, Loricariinae, and Hypostominae are described. The data obtained in the present study and those available for other Loricarioidei are compared in order to evaluate whether these reproductive ultrastructural characters are useful to better understanding the relationships among groups of this clade.

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Materials and methods

The specimens examined belong to the fish collection of the Laboratório de Biologia de Peixes (LBP), Departamento de Morfologia, Universidade Estadual Paulista, Campus de Botucatu.

Testes from adult males from representatives of subfamilies of Loricariidae were analyzed at the ultrastructural level, for the description of the spermatic characteristics.

This study was conducted with two adult males of each species of the following groups traditionally classified as subfamilies: (1) Neoplecostominae: *Kronichthys heylandi* (Boulenger 1900) (LBP 2122), and *Neoplecostomus paranensis* Langeani 1990 (LBP 3597); (2) Hypoptopomatinae: *Hypoptopoma guentheri* Boulenger 1895 (LBP 693); (3) Otothyrinae: *Corumbataia cuestae* Britski 1997 (LBP 1313, LBP 2001); *Hisonotus* sp. (LBP 1292, LBP 1999), and *Schizolecis guntheri* (Miranda-Ribeiro 1918) (LBP 2123); (4) Loricariinae: *Loricariichthys platymetopon* Isbrücker & Nijssen 1979 (LBP 1080); *Loricaria lata* Eigenmann & Eigenmann 1889 (LBP 2443); and *Farowella oxyrryncha* (Kner 1853) (LBP 2441); and (5) Hypostominae: *Hypostomus ancistroides* (Ihering 1911) (LBP 1376).

The specimens collected were anesthetized with 0.1% benzocaine and killed (according to the approved institutional ethical protocols) for the removal of the testes. Gonad fragments were fixed in 2% glutaraldehyde and 4% paraformaldehyde in 0.1 M Sorensen phosphate buffer, pH 7.4. The material was post-fixed in 1% osmium tetroxide, in the dark, for 2 h in the same buffer, contrasted 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% alcohol and lead citrate. Electromicrographs were obtained using a Phillips-CM 100 transmission electron microscope.

The characteristics of ultrastructure of the spermatogenesis, spermiogenesis, and spermatozoa present in at least one species analyzed were employed in the comparative analyses among them and with other groups of Loricariodei.

The measurements of length and width of nucleus, nuclear fossa, midpiece, cytoplasmic canal, and measurement of lateral projection lengths of the spermatozoa of all the loricariid analyzed are presented in Table 1.

Results

The particular ultrastructural characteristics observed in the spermatogenesis, spermiogenesis, and spermatozoa of each Loricariidae subgroup analyzed are presented.
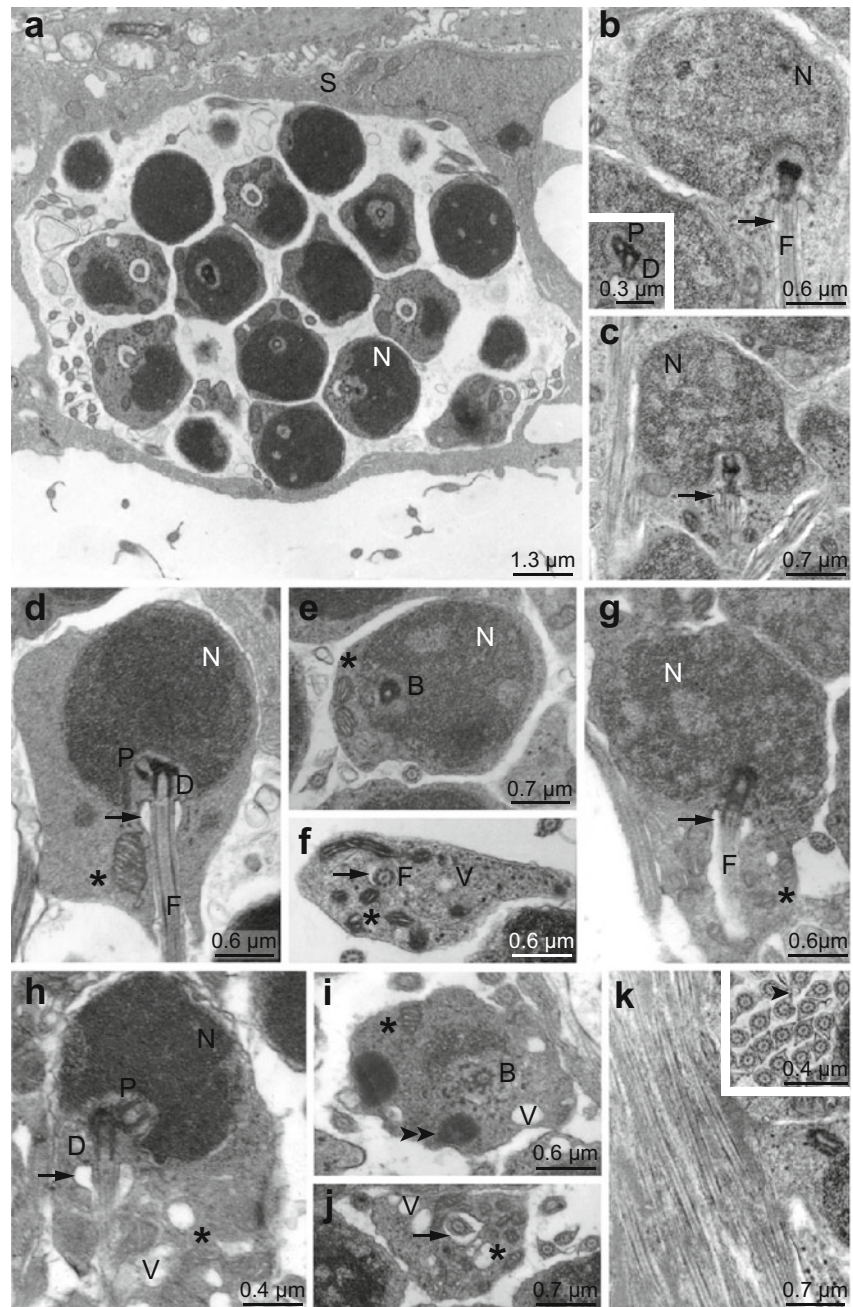
Neoplecostominae

Spermatogenesis

In the analyzed species of Neoplecostominae, spermatogenesis occurs inside the cysts. At the end of the differentiation process, spermatozoa are released into the luminal compartment of the testis (Fig. 1a).

Table 1 Spermatozoa dimensions present in the loricariids analyzed

Subfamilies	*Neoplecostominae*		Otothyrinae			*Hypoptopomatinae*	*Loricariinae*		*Hypostominae*
Species	*K. heylandi*	*N. paranensis*	*C. cuestae*	*Hisonotus* sp.	*S. guntheri*	*H. guentheri*	*L. platymetopon*	*Loricaria lata*	*H. ancistroides*
Structures									
Nucleus									
Length (μm)	1.5 (*n* = 7)	1.6 (*n* = 5)	1.6 (*n* = 8)	1.4 (*n* = 8)	1.6 (*n* = 6)	1.8 (*n* = 5)	1.9 (*n* = 10)	2.0 (*n* = 7)	2.0 (*n* = 6)
Width (μm)	1.5 (*n* = 7)	1.7 (*n* = 5)	1.4 (*n* = 7)	1.4 (*n* = 7)	1.6 (*n* = 6)	1.5 (*n* = 5)	2.0 (*n* = 10)	2.0 (*n* = 7)	2.3 (*n* = 6)
Nuclear fossa									
Length (μm)	0.7 (*n* = 7)	0.6 (*n* = 5)	0.6 (*n* = 6)	0.3 (*n* = 6)	0.3 (*n* = 4)	0.4 (*n* = 5)	Absent	0.3 (*n* = 7)	0.2 (*n* = 4)
Width (μm)	0.5 (*n* = 7)	0.5 (*n* = 5)	0.4 (*n* = 7)	0.4 (*n* = 7)	0.6 (*n* = 4)	0.5 (*n* = 5)	Absent	0.3 (*n* = 7)	0.3 (*n* = 4)
Midpiece									
Length (μm)	1.2 (*n* = 8)	1.0 (*n* = 7)	1.4 (*n* = 7)	1.4 (*n* = 6)	0.9 (*n* = 5)	1.2 (*n* = 4)	1.0 (*n* = 13)	1.5 (*n* = 8)	1.4 (*n* = 5)
Width (μm)	1.4 (*n* = 8)	1.8 (*n* = 7)	1.1 (*n* = 5)	1.6 (*n* = 5)	1.6 (*n* = 5)	2.0 (*n* = 4)	2.0 (*n* = 13)	1.7 (*n* = 8)	2.0 (*n* = 5)
Cytoplasmic canal									
Length (μm)	0.6 (*n* = 6)	0.5 (*n* = 6)	0.7 (*n* = 5)	0.3 (*n* = 5)	0.4 (*n* = 6)	0.4 (*n* = 4)	0.2 (*n* = 13)	0.8 (*n* = 6)	0.6 (*n* = 5)
Width (μm)	0.3 (*n* = 6)	0.4 (*n* = 6)	0.4 (*n* = 5)	0.3 (*n* = 5)	0.4 (*n* = 6)	0.3 (*n* = 4)	0.4 (*n* = 13)	0.6 (*n* = 6)	0.3 (*n* = 5)
Lateral projections									
Length (μm)	0.3 (*n* = 10)	0.4 (*n* = 7)	0.4 (*n* = 8)	0.2 (*n* = 9)	0.3 (*n* = 6)	0.2 (*n* = 5)	Absent	Absent	0.4 (*n* = 10)

The "*n*" shows the number of structures measured

Fig. 1 Spermiogenesis in Neoplecostominae. **a–f** *Kronichthys heylandi* and **g–k** *Neoplecostomus paranensis*. **a** Spermatids cyst; **b**, **c**, **g** early spermatids in longitudinal sections (*b-inset* centriolar complex arrangement); **d**, **e**, **h** late spermatids (longitudinal sections); **f**, **i**, **j** midpiece showing mitochondria, vesicles, and an electron-dense structure surrounded by membrane (cross-sections); **k** flagella exhibiting the formation of lateral projections (longitudinal and cross-sections (*inset*)). *B* basal body, *D* distal centriole, *F* flagellum, *N* nucleus, *P* proximal centriole, *S* Sertoli cell, *V* vesicles, *asterisk* mitochondria, *arrow* cytoplasmic canal, *arrowhead* lateral projections, *double arrowhead* electron-dense structure surrounded by membrane

Spermiogenesis

The description of the spermiogenesis process was based on the observation of spermatids from *Kronichthys heylandi* and *Neoplecostomus paranensis* (Fig. 1a–k). In the early spermatids of these taxa, the cytoplasm is symmetrically distributed around the nucleus, which has a circular outline and contains diffuse homogeneous chromatin. The centriolar complex lies laterally to the nucleus and anchors to the plasma membrane (Fig. 1b, g). The proximal centriole is lateral and in obtuse angle to the distal centriole (Fig. 1d, h). The distal centriole differentiates into the basal body and forms the flagellum.

The nuclear rotation occurs, and the flagellum is positioned medially to the nucleus (Fig. 1d). During the nuclear rotation, a depression is formed in the nuclear outline giving rise to the nuclear fossa. At the end of this process, the centriolar complex is totally inserted into the nuclear fossa (Fig. 1b-inset, d, h). Simultaneously with the nuclear rotation, the centrioles move toward the nucleus, bringing with it the plasma membrane and the initial segment of the flagellum, which invaginates (Fig. 1c, g, h). With this movement, the cytoplasmic canal, a space between the plasma membranes of the flagellar region and the midpiece, is formed. The midpiece contains mitochondria, vesicles, and an electron-dense structure sur-

rounded by membrane (Fig. 1f, i, j). The flagellar membrane forms two lateral projections or fins (Fig. 1k, k-inset).

Spermatozoa

The spermatozoa of Neoplecostominae are found in the luminal compartment of the testis, which in *N. paranensis* contains a high electron-dense secretion (Fig. 3i). Spermatozoa exhibit a spherical head, a symmetric midpiece, and one flagellum medially positioned in relation to the nucleus (Figs. 2a, f, g, 3a, e). The spherical nucleus contains highly condensed homogeneous chromatin interspersed with electron-lucent areas (Figs. 2b, c, 3b, c). The nuclear fossa is single, medially positioned and has a ramified shape. The centriolar complex is completely inserted into the nuclear fossa (Figs. 2d, e, g, 3d, e), and the centrioles are lateral and in obtuse angle to each

other. In *K. heylandi*, a set of microtubules irradiates from the proximal centriole in the direction of the nucleus, which invaginates (Fig. 2e). Several spherical to elongated mitochondria are randomly distributed throughout the midpiece and are mainly concentrated close to the nucleus and in the medial region. Vesicles are also found throughout the midpiece and are few in *K. heylandi* and several in *N. paranensis* (Figs. 2a, h, i, j, 3a, g). The medial flagellum contains a 9 + 2 axoneme and has two lateral projections (Figs. 2k, l, 3h, h-inset).

Hypoptopomatinae and Otothyrinae

Spermatogenesis

In *Hypoptopoma guentheri* (Hypoptopomatinae) and *Corumbataia cuestae* and *Hisonotus* sp. (Otothyrinae),

Fig. 2 Spermatozoa in *Kronichthys heylandi* (Neoplecostominae). **a** Longitudinal section, **b**, **c** head region, **d**, **e** centriolar complex arrangement, **f–h** spermatozoon longitudinal sections showing nuclear fossa, cytoplasmic canal, mitochondria, and vesicles in the midpiece, **i**, **j** midpiece cross-sections showing mitochondria and vesicles, **k**, **l** flagella in longitudinal and cross-sections. *B* basal body, *C* centriolar complex, *D* distal centriole, *E* electron-lucent area, *F* flagellum, *N* nucleus, *P* proximal centriole, *V* vesicles, *asterisk* mitochondria, *arrow* cytoplasmic canal, *arrowhead* lateral projections
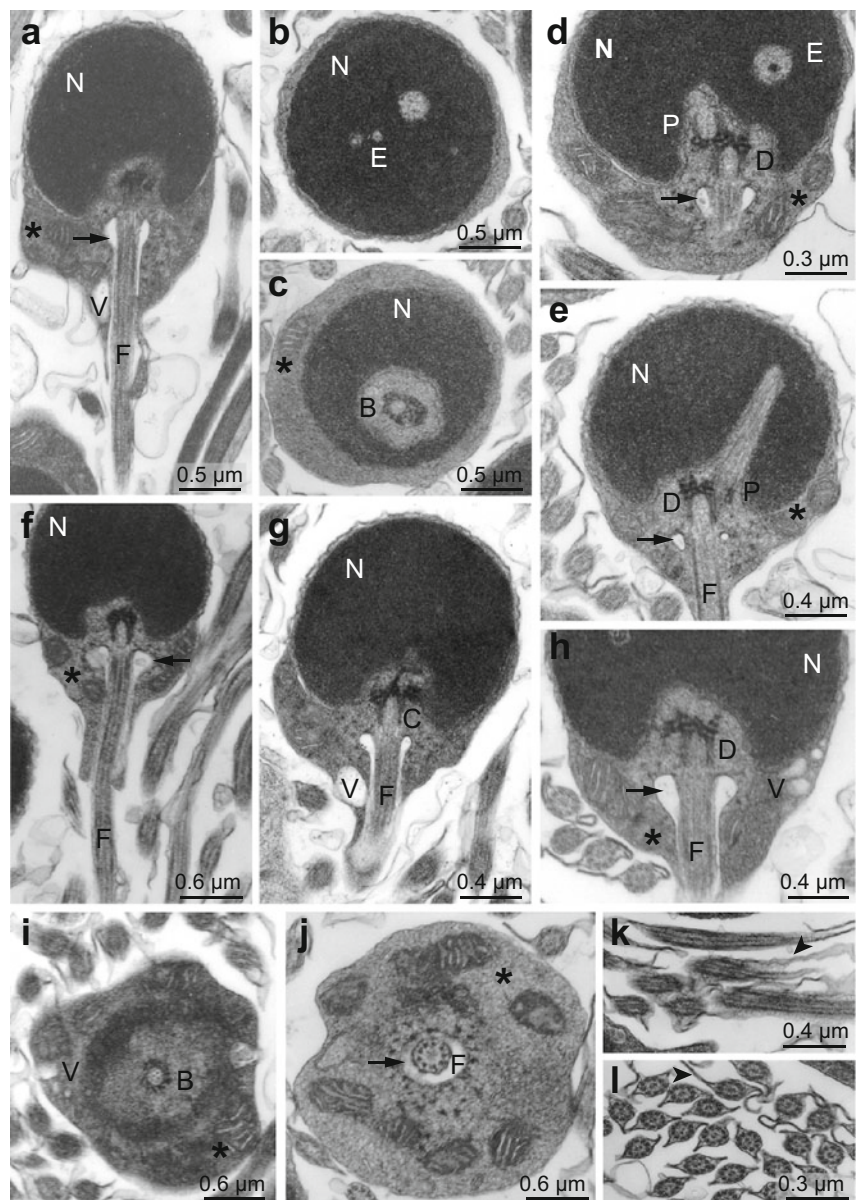
Fig. 3 Spermatozoa in *Neoplecostomus paranensis* (Neoplecostominae). **a** Spermatozoon in longitudinal section, **b**, **c** head cross- and longitudinal sections, **d** detail of centriolar complex arrangement, **e**, **g** spermatozoa in longitudinal and cross-sections showing mitochondria and vesicles in the midpiece, **h** flagella in longitudinal and cross-sections (*inset*), **i** spermatozoa in the luminal compartment of the testis surrounded by an electron-dense secretion. *B* basal body, *D* distal centriole, *E* electron-lucent area, *F* flagellum, *N* nucleus, *P* proximal centriole, *V* vesicles, *asterisk* mitochondria, *arrow* cytoplasmic canal, *arrowhead* lateral projections
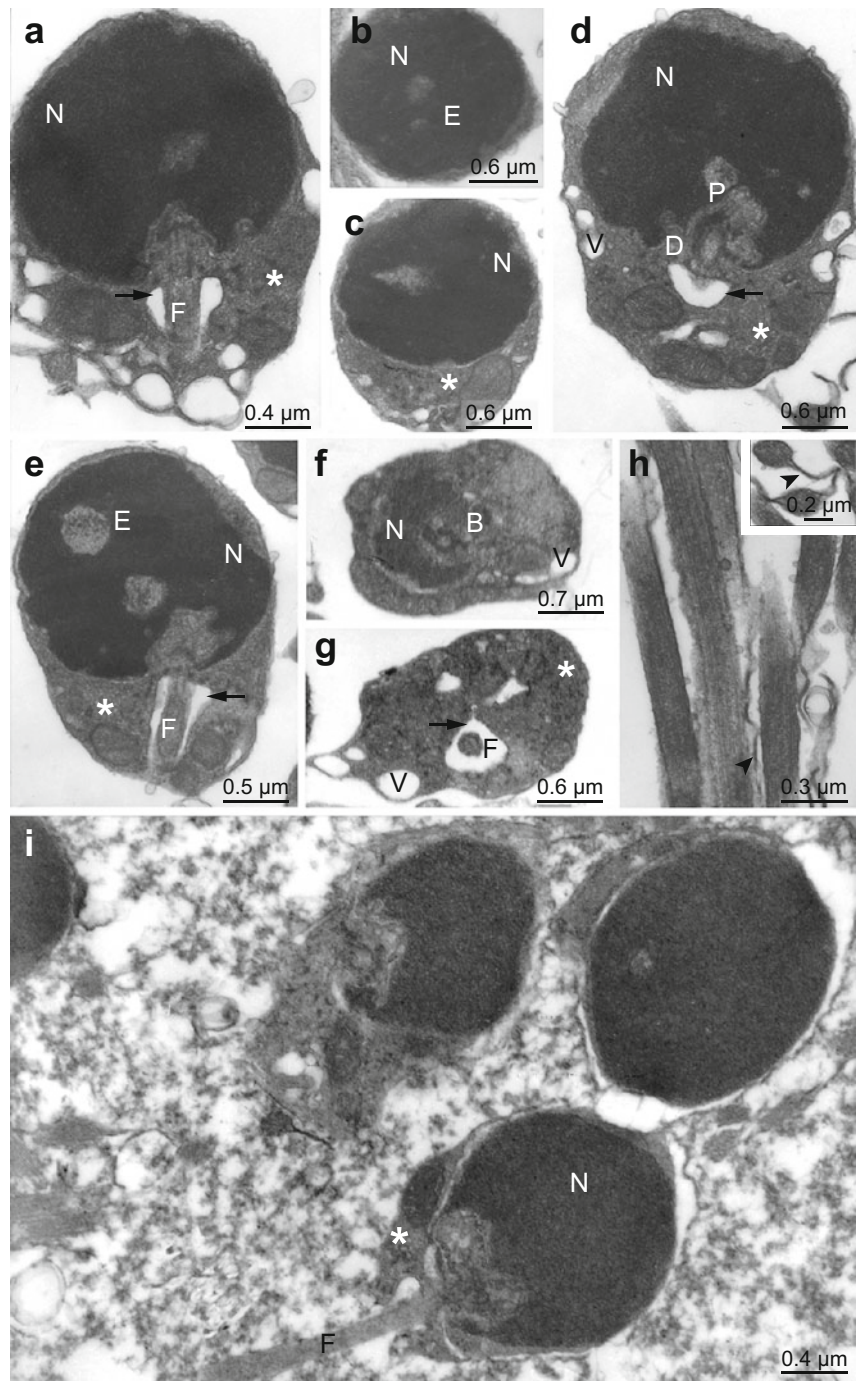

spermatogenesis occurs inside the cysts. At the end of the differentiation process, spermatozoa are released into the luminal compartment of the testis (Fig. 4a). Spermatogenesis and spermiogenesis of *Schizolecis guntheri* (Otothyrinae) remain unknown, since no spermatids were found in the testes of the specimen studied.

Spermiogenesis

In the early spermatids from the species analyzed, the cytoplasm is symmetrically distributed around the nucleus,

which contains diffuse homogeneous chromatin. The centriolar complex, with the proximal centriole perpendicular to the distal, lies laterally to the nucleus and is anchored to the plasma membrane. The flagellum development from the distal centriole takes place initially lateral to the nucleus (Fig. 4b, c-inset, f). The nuclear rotation occurs, and during this process, the nuclear fossa is formed in the nuclear outline. After nuclear rotation, the flagellum assumes a medial position to the nucleus, and the proximal and distal centrioles are totally inserted into the nuclear fossa (Fig. 4b, e, g). The centriolar complex moves toward

Fig. 4 Spermiogenesis in Oto-
thyrinae: **a–c**, **h** *Corumbataia
cuestae* and **d**, **e**, **i** *Hisonotus* sp.,
and Hypoptopomatinae:
f, **g** *Hypoptopoma guenteri*.
a Spermatids cyst, **b**, **f**, **g** early
spermatids (longitudinal sec-
tions), **c–e** late spermatids,
c detail of centriolar complex
arrangement (*inset*), **h**,
i midpiece showing mitochon-
dria and vesicles (cross-sec-
tions), **j** flagella in longitudinal
and cross-sections (*inset*).
D distal centriole, *F* flagellum,
N nucleus, *P* proximal centriole,
S Sertoli cell, *V* vesicles, *aster-
isk* mitochondria, *arrow* cyto-
plasmic canal, *arrowhead* lateral
projections, *double arrowhead*
electron-dense structure sur-
rounded by membrane
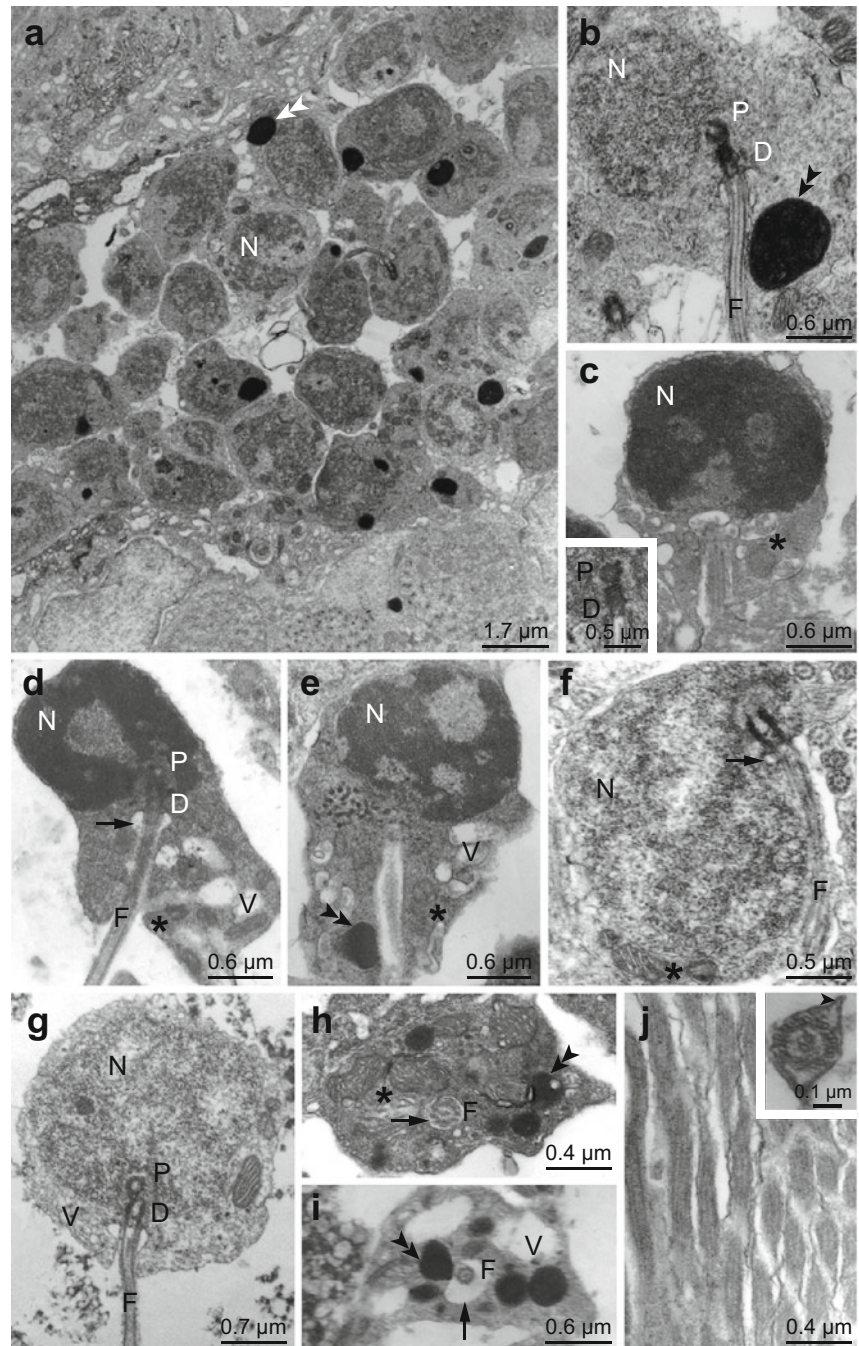
the nucleus bringing the plasma membrane and the initial
segment of the flagellum with it, forming the cytoplasmic
canal (Fig. 4b–g). The midpiece comprises the cytoplas-
mic canal, mitochondria, and the forming vesicles. In the
cytoplasm of the midpiece, the spermatids also have an
electron-dense structure surrounded by membrane
(Fig. 4a, b, h, i). The flagellum has a 9 + 2 axoneme, and
two lateral projections develop from the flagellar mem-
brane (Fig. 4j, j-inset).

Spermatozoa

In all four species studied, the spermatozoa are found in the
luminal compartment of the testis, which contains an elec-
tron-dense secretion. They have an ovoid head with an ovoid
nucleus in *C. cuestae* and *H. guentheri* and in *Hisonotus* sp.
In *S. guntheri*, the head is spherical and contains a spherical
nucleus. Spermatozoa also have a symmetric midpiece and
one flagellum medial to the nucleus (Figs. 5a, e, 6a, f). The

Fig. 5 Spermatozoa in Otothyrinae: **a–d** *Corumbataia cuestae* and **e–i** *Hisonotus* sp. **a**, **g** Spermatozoon in longitudinal sections exhibiting the centriolar complex arrangement, **b**, **c**, **f**, **h**, **i** midpiece showing cytoplasmic canal, mitochondria, and vesicles (longitudinal and cross-sections), **d**, **e** spermatozoon in longitudinal sections showing mitochondria and vesicles, **d** flagella in cross-sections showing classical axoneme (9 + 2) and lateral projections (*inset*). *D* distal centriole, *F* flagellum, *N* nucleus, *P* proximal centriole, *V* vesicles, *asterisk* mitochondria, *arrow* cytoplasmic canal, *arrowhead* lateral projections, *double arrowhead* electron-dense structure surrounded by membrane
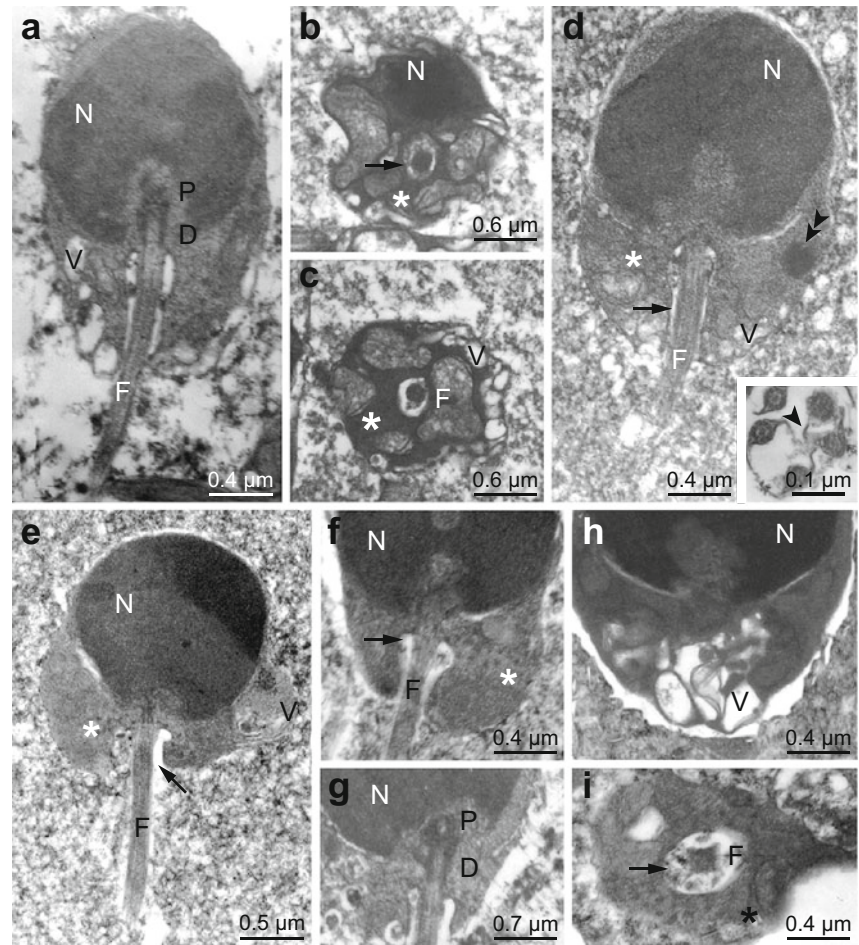
nucleus contains highly condensed homogeneous chromatin interspersed with electron-lucent areas (Figs. 5d, 6a–c). The nuclear fossa is single and medial (Figs. 5a, b, e, h, 6a, i). In the centriolar complex, the proximal centriole is perpendicular to the distal, and both of them lie within the nuclear fossa (Figs. 5a, g, 6i). Several mitochondria are randomly distributed throughout the midpiece, mainly concentrated on the medial region. Mitochondria are also found in the baso-lateral region of the nucleus in *H. guentheri* and *S. guntheri*, but not in *C. cuestae* and *Hisonotus* sp (Figs. 5a, d, e, 6a, f). In the midpiece, an electron-dense structure surrounded by membrane is observed in *C. cuestae* (Figs. 5b, c, f, h, i, 6c–e, h, i). Several vesicles interconnected to each other are observed in the basal region of the midpiece concentrated around the proximal part of the flagellum in *C. cuestae*, *H. guentheri*, and *S. guntheri* (Figs. 5b, 6d, g, i). In the midpiece of *Hisonotus* sp., few elongated vesicles are mainly found in the proximity of the nucleus and around the initial segment of the flagellum (Figs. 5e, 6h, i).The flagella have a 9 + 2 axoneme and two lateral projections (Figs. 5d-inset, 6j).

Despite many changes in the fixation procedures, the secretion present in the luminal compartment of the testis of Hypoptopomatinae and Otothyrinae seems to prevent the plasma membrane preservation. It is possible that the secretion precludes the infiltration of fixatives, resulting in an ill-preservation of the plasma membranes of the spermatozoa, as presented in Figs. 5 and 6.

Loricariinae

Spermatogenesis

In the three species of Loricariinae analyzed, spermatogenesis occurs inside the cysts with the release of spermatozoa into the luminal compartment of the testis at the end of the differentiation process (Fig. 7a).

Spermiogenesis

The spermiogenesis process in Loricariinae was based on the observation of spermatids of *Loricariichthys platymetopon* and *Farlowella oxyrryncha*, and *Loricaria lata* (Fig. 7a–l). In these species, two types of spermiogenesis are found. In early spermatids of *L. platymetopon*, the

Fig. 6 Spermatozoa in Hypop-
topomatinae: **a–e** *Hypoptopoma
guentheri* and Otothyrinae:
f, **j** *Shizolecis guntheri*.
a, **f** Longitudinal sections,
b, **c** head region,
d, **e**, **g**, **h** midpiece (longitudi-
nal and cross-sections) showing
mitochondria and vesicles,
i detail of nuclear fossa and
centriolar complex arrangement,
j flagella in cross-sections
exhibiting lateral projections.
B basal body, *D* distal centriole,
E electron-lucent area,
F flagellum, *N* nucleus,
P proximal centriole, *V* vesicles,
asterisk mitochondria, *arrow*
cytoplasmic canal, *arrowhead*
lateral projections
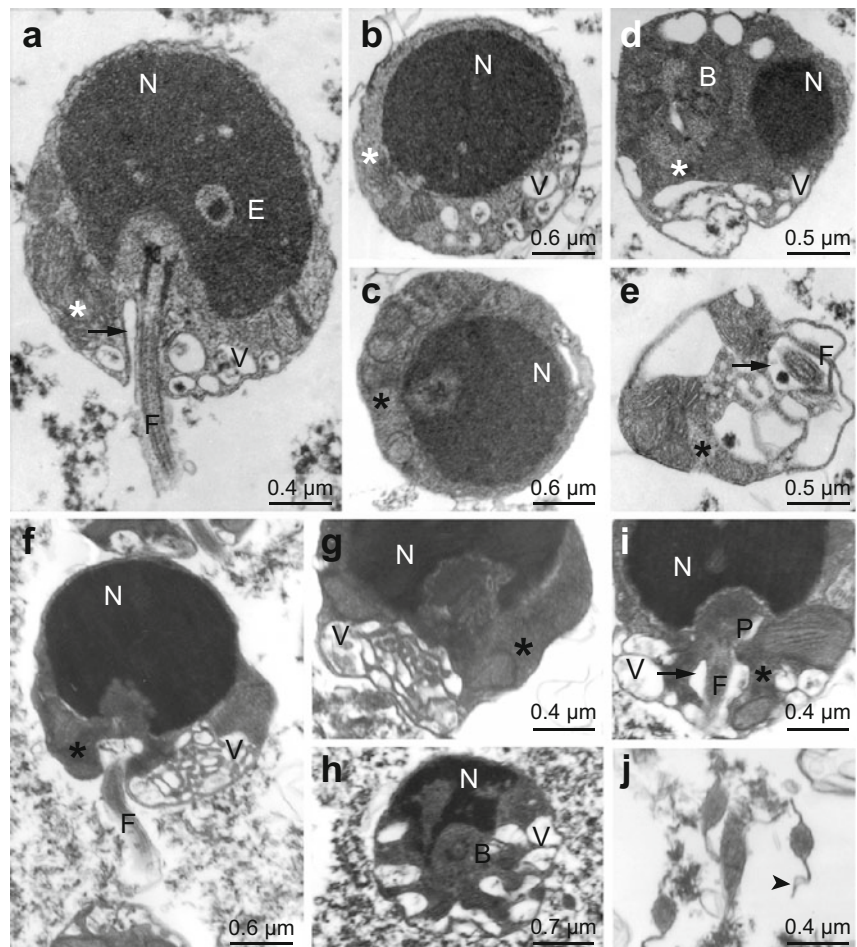
centriolar complex lies medially to the nucleus and is anchored to the plasma membrane (Fig. 7b, c). In the centriolar complex, the proximal centriole is perpendicular to the distal (Fig. 7b). The centriolar complex does not move toward the nucleus and remains associated with the plasma membrane, resulting in a flagellum with a medial position in relation to the nucleus (Fig. 7b, c). The nucleus does not rotate and the nuclear fossa is not formed. The cytoplasm moves toward the initial segment of the flagellum and gives rise to the midpiece and to the cytoplasmic canal (Fig. 7d). In the early spermatids of *F. oxyrryncha* and *L. lata*, the centriolar complex lies laterally to the nucleus and is anchored to the plasma membrane with the proximal centriole perpendicular to the distal centriole (Fig. 7e, i, j). The centriolar complex moves toward the nucleus and brings with it the plasma membrane and the initial segment of the flagellum, originating the cytoplasmic canal (Fig. 7f, g, j). The nuclear rotation occurs in different degrees in these species of Loricariinae, being total in *F. oxyrryncha* and partial in *L. lata*. This results in the medial position of the flagellum in relation to the nucleus in *F. oxyrryncha*, while in *L. lata*, the flagellum is eccentrically positioned (Fig. 7f, j).

The nuclear fossa is formed, and at the end of the process, only the proximal centriole is inserted into it (Fig. 7i, j). In all species, the midpiece contains mitochondria and vesicles (Fig. 7e, k). Among the species analyzed, the formation of lateral projections in the flagella is observed only in *F. oxyrryncha*. All species have a flagellum with a 9 + 2 axoneme (Fig. 7d-inset, h, l).

Spermatozoa

The characteristics of spermatozoa were described based on the analysis of *L. platymetopon* and *L. lata*, since in the testes of *F. oxyrryncha* spermatozoa were not found. The spermatozoa of Loricariinae are observed in the luminal compartment of the testis without any electron-dense secretion around them (Figs. 8a, 9a). They exhibit a spherical nucleus with highly condensed homogeneous chromatin interspersed with electron-lucent areas, and a symmetric midpiece (Figs. 8a–c, 9a–c). The flagellum is medially positioned in relation to the nucleus in *L. platymetopon* and is eccentrically placed in *L. lata* (Figs. 8d, p, 9a, d). In *L. lata*, the single nuclear fossa is eccentrically positioned.

Fig. 7 Spermiogenesis in Loricariinae: **a–d** *Loricariichthys platymetopon*, **e–h** *Farowella oxyrryncha*, and **i–l** *Loricaria lata*. **a** Spermatids cyst, **b**, **e**, **i** early spermatids in longitudinal sections, **c**, **f**, **j** late spermatids (longitudinal sections), **d** flagellum in cross-section (*inset*), **g** centriolar complex arrangement, **d**, **h**, **k** midpiece showing mitochondria and vesicles (cross- and longitudinal sections), **l** flagellum in longitudinal section. *D* distal centriole, *F* flagellum, *N* nucleus, *P* proximal centriole, *S* Sertoli cell, *V* vesicles, *asterisk* mitochondria, *arrow* cytoplasmic canal
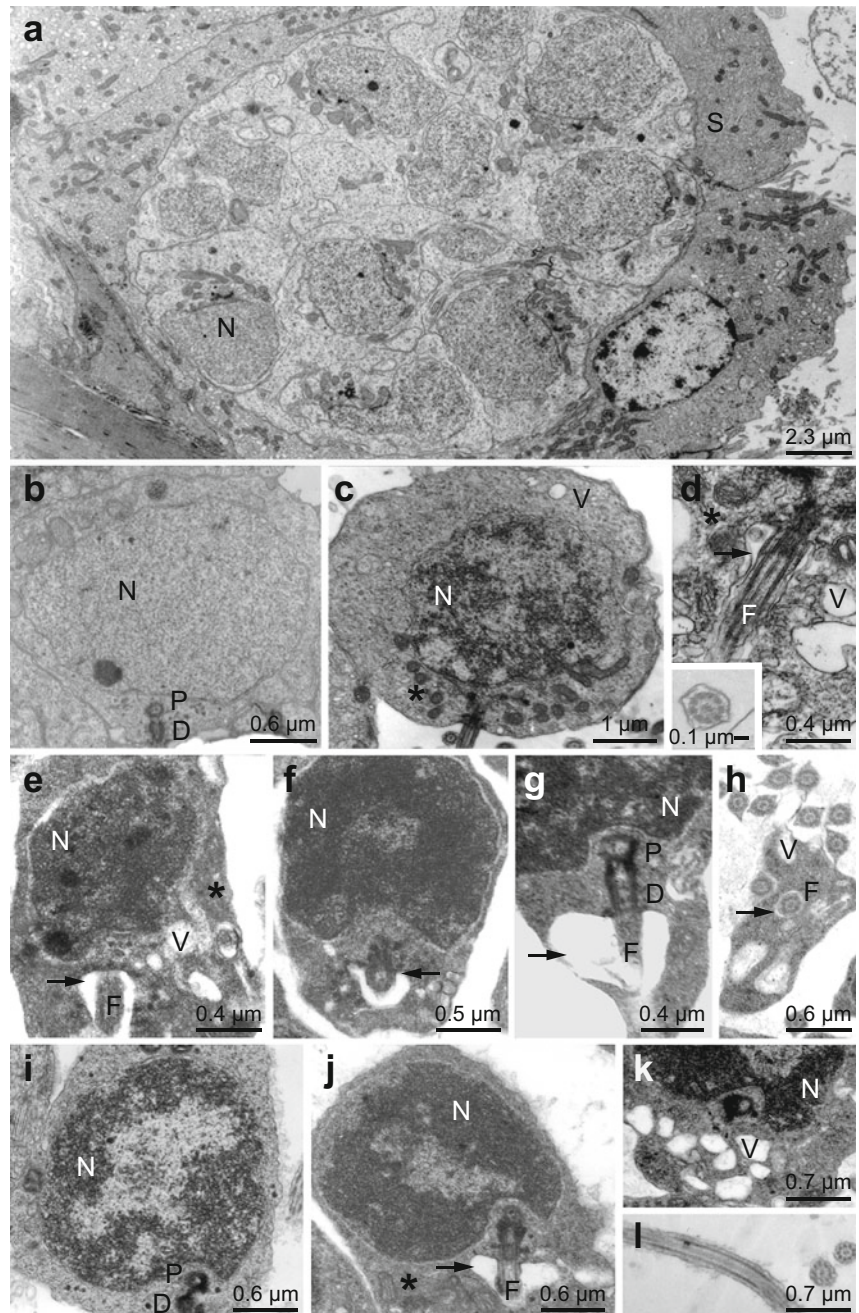
In *L. platymetopon*, the nuclear fossa is absent (Figs. 8a, 9d). In Loricariinae, the centrioles are perpendicular to each other, and in *L. lata*, only the proximal centriole is inserted into the nuclear fossa (Figs. 8d, e, 9d). In *L. platymetopon*, the midpiece has many spherical to elongated mitochondria, generally concentrated in the peripheral region. Thus, the mitochondria are not present around the centriolar complex. In *L. lata*, few mitochondria are randomly scattered throughout the midpiece. In both species, the mitochondria are separated from the flagellum by the cytoplasmic canal. Few isolated vesicles are concentrated in the basal region in

L. platymetopon and are randomly scattered throughout the midpiece in *L. lata* (Figs. 8c, f–o, o-inset, 9e–h).

Hypostominae

Spermatogenesis

In *Hypostomus ancistroides*, spermatogenesis occurs inside the cysts. At the end of the differentiation process, spermatozoa are released into the luminal compartment of the testis (Fig. 10a).

Fig. 8 Spermatozoa in *Loricariichthys platymetopon* (Loricariinae). **a** Longitudinal section of spermatozoon, **b**, **c** head region, **d**, **e** detail of centrioles arrangement, **f–n** midpiece in longitudinal and cross-sections showing mitochondria, vesicles, and cytoplasmic canal, **o**, **p** flagella in cross- (*inset*) and longitudinal sections showing classical axoneme (9 + 2). *B* basal body, *D* distal centriole, *E* electron-lucent area, *F* flagellum, *N* nucleus, *P* proximal centriole, *V* vesicles, *asterisk* mitochondria, *arrow* cytoplasmic canal
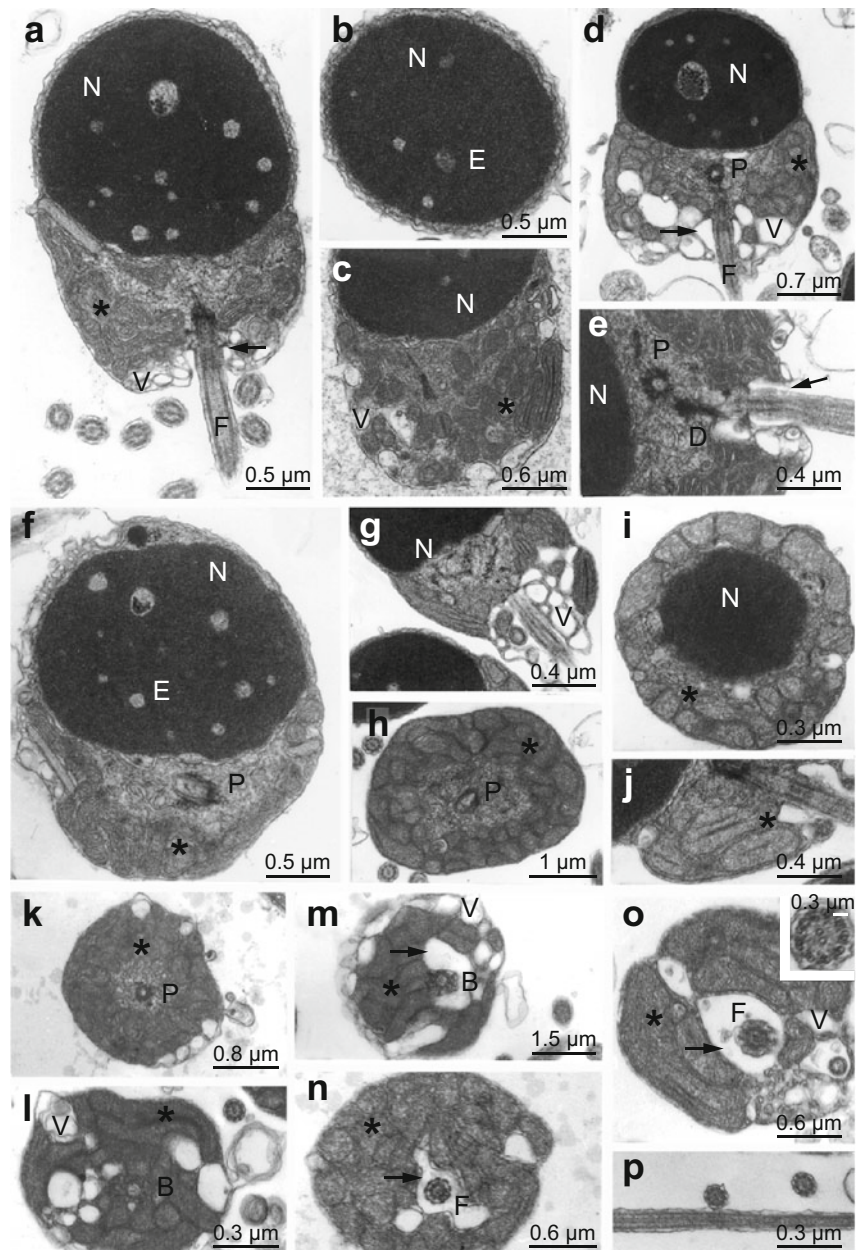
Spermiogenesis

Spermiogenesis was studied in *Hypostomus ancistroides* (Fig. 10a–h). In the early spermatids, the cytoplasm is symmetrically distributed around the nucleus, which contains diffuse homogeneous chromatin. In the spermatids, the centriolar complex, with the proximal centriole perpendicular to the distal, lies medially to the nucleus. The centriolar complex is anchored to the plasma membrane. The flagellum development from the distal centriole takes place medially to the nucleus (Fig. 10b, c). The nuclear rotation does not occur, and the flagellum remains in a medial position to the nucleus. Along with the differentiation process, a narrow nuclear fossa is formed in the

nuclear outline (Fig. 10b–e). The movement of the centriolar complex does not occur and the centrioles remain associated with the plasma membrane (Fig. 10b, d). The cytoplasm moves toward the flagellum and gives rise to the midpiece with a cytoplasmic canal (Fig. 10d). The midpiece has mitochondria and vesicles. The flagellum exhibits a 9 + 2 axoneme and the formation of two lateral projections (Fig. 10h).

Spermatozoa

The spermatozoa of *Hypostomus ancistroides* are found in the luminal compartment of the testis, which is free of secretion (Fig. 11a). Spermatozoa exhibit a spherical head,

Fig. 9 Spermatozoa in *Loricaria lata* (Loricariinae). **a** Longitudinal section, **b**, **c** nucleus in cross-sections, **d** centriolar complex arrangement, **e–g** midpiece in longitudinal and cross-sections showing mitochondria, vesicles, and cytoplasmic canal, **h** flagella in longitudinal and cross-sections. *B* basal body, *D* distal centriole, *E* electron-lucent area, *F* flagellum, *N* nucleus, *P* proximal centriole, *V* vesicles, *asterisk* mitochondria, *arrow* cytoplasmic canal
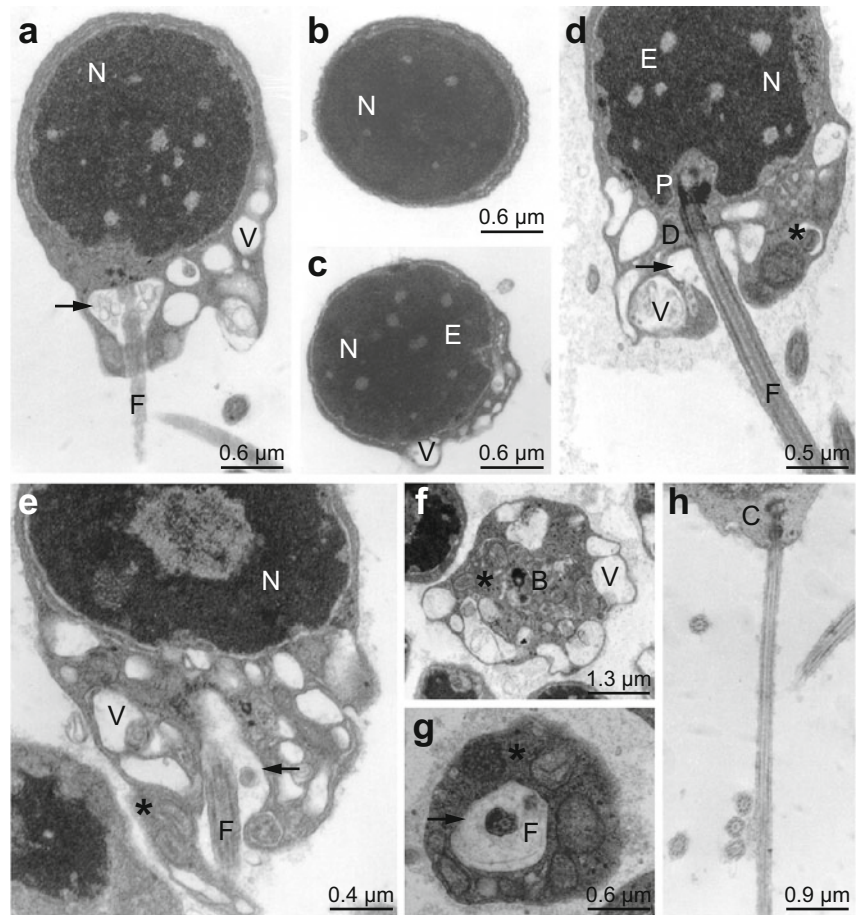
a symmetric midpiece, and one flagellum medially positioned to the nucleus (Fig. 11a, d, f, i, i-inset). The spherical nucleus contains highly condensed homogeneous chromatin interspersed with electron-lucent areas (Fig. 10b, c). The centrioles are perpendicular to each other, and only the proximal centriole is inserted into the single nuclear fossa (Fig. 11d, e). In the midpiece, few rounded mitochondria are centrally distributed, concentrated around the centriolar complex. Many isolated vesicles are found in the periphery of the midpiece (Fig. 11c, f–h).

Discussion

Spermatogenesis and spermiogenesis

In the loricariids analyzed, the differentiation of spermatids into spermatozoa occurs completely within cysts in the germinal epithelium, characterizing the spermatogenesis as the cystic type. Cystic spermatogenesis is present in most Teleostei (Mattei 1993; Quagio-Grassiotto et al. 2005), and it was also described in other groups of Loricarioidei, as in Trichomycteridae (Spadella et al. 2010), in Callichthyinae (family Callichthyidae) (Spadella et al. 2007), and in

Scoloplacidae (Spadella et al. 2006b), representing a plesiomorphic character state. In Astroblepidae (Spadella et al. 2012), the sister group of Loricariidae, the later stages of the spermatids differentiation occur in the lumina of the seminiferous tubules, characterizing a spermatogenesis partially cystic.

According to Mattei (1970), the spermiogenesis can be of type I or II in Teleostei with external fertilization. In early spermatids, the initial development of the flagellum, in general, occurs laterally to the nucleus in both types. If nuclear rotation occurs (type I), the flagellum axis will be perpendicular to the nucleus. If there is no nuclear rotation (type II), the flagellum will be parallel to the nucleus (Mattei 1970). A third type of spermiogenesis has been described in Pimelodidae and Heptapteridae (Quagio-Grassiotto et al. 2005; Quagio-Grassiotto and Oliveira 2008; Burns et al. 2009). Here, the development of the flagellum is medial, the nucleus does not rotate, and both the nuclear fossa and cytoplasmic canal are not formed during spermiogenesis. The spermiogenesis process observed in most species of the Loricariidae is characterized by an initial lateral development of the flagellum, a centriolar complex migration, a cytoplasmic canal formation, a complete or partial nuclear rotation, and the formation of

Fig. 10 Spermiogenesis in
Hypostomus ancistroides
(Hypostominae). **a** Spermatids
cyst, **b** early spermatid in longi-
tudinal section, **c** early sperma-
tid showing centriolar complex
arrangement, **d** late spermatid,
e–g midpiece showing
mitochondria and vesicles
(longitudinal and cross-
sections), **h** flagella in cross-
sections exhibiting lateral
projections. *B* basal body,
D distal centriole, *F* flagellum,
N nucleus, *P* proximal centriole,
S Sertoli cell, *T* spermatids,
V vesicles, *asterisk* mitochon-
dria, *arrow* cytoplasmic canal,
*arrowhead* lateral projections
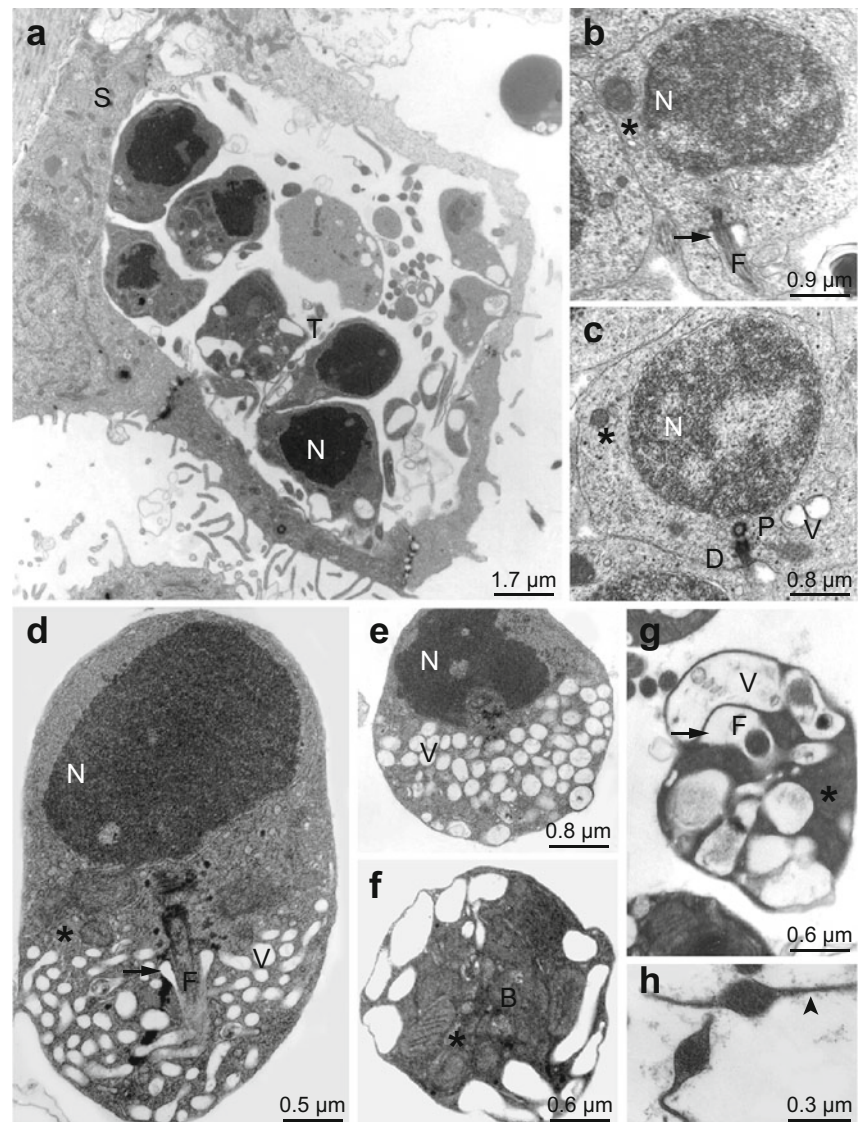
either a medial or an eccentric nuclear fossa. These charac-
teristics resemble to type I spermiogenesis previously
described, which is also found in the species of the Trich-
omycteridae (Spadella et al. 2010), Callichthyinae (Spad-
ella et al. 2007), and Scoloplacidae (Spadella et al. 2006b).
For Astroblepidae, the type of spermiogenesis found in this
group remains unknown (Spadella et al. 2012).

Among the loricariids analyzed, only in *Loricariichthys
platymetopon* and *Hypostomus ancistroides*, the spermio-
genesis process is characterized by a medial development
of the flagellum, the absence of nuclear rotation, the cyto-
plasmic canal formation, and the absence of centriolar com-
plex migration. Moreover, the medial nuclear fossa
formation is observed, but only in *H. ancistroides*. This set
of characteristics is distinct from those previously
described for the other loricariids analyzed in this study,
but is also observed in species of the Nematogenyidae,

except for the nuclear fossa formation (personal observa-
tion). With the exception of the medial development of the
flagellum and medial nuclear fossa formation, this unusual
spermiogenesis process is also found in species of the
Corydoradinae, in which an eccentric formation of flagel-
lum and of the nuclear fossa is observed (Spadella et al.
2007). In the cited groups, it is probable that the existent
cytoplasmic canal results from the accommodation and
interconnection of the vesicles around the flagella, since the
movement of the centriolar complex toward the nucleus
does not occur, as previously reported in species of the
Heptapteridae (Quagio-Grassiotto et al. 2005).

Then, the present data show that all the groups of Lori-
cariidae present three common characteristics of the sper-
matogenesis and spermiogenesis, cystic spermatogenesis,
formation of cytoplasmic channel, and process of chroma-
tin condensation homogeneous. On the other hand, the

Fig. 11 Spermatozoa of *Hypostomus ancistroides* (Hypostominae). **a**, **c**, **d** Spermatozoon in longitudinal sections, **b** nucleus in cross-section, **e** detail of centriolar complex arrangement, **f**–**h** midpiece showing cytoplasmic canal, mitochondria, and vesicles (longitudinal and cross-sections), **i** flagella in longitudinal and cross-sections (*inset*) showing classical axoneme (9 + 2) and lateral projections. *D* distal centriole, *E* electron-lucent area, *F* flagellum, *N* nucleus, *P* proximal centriole, *V* vesicles, *asterisk* mitochondria, *arrow* cytoplasmic canal, *arrowhead* lateral projections
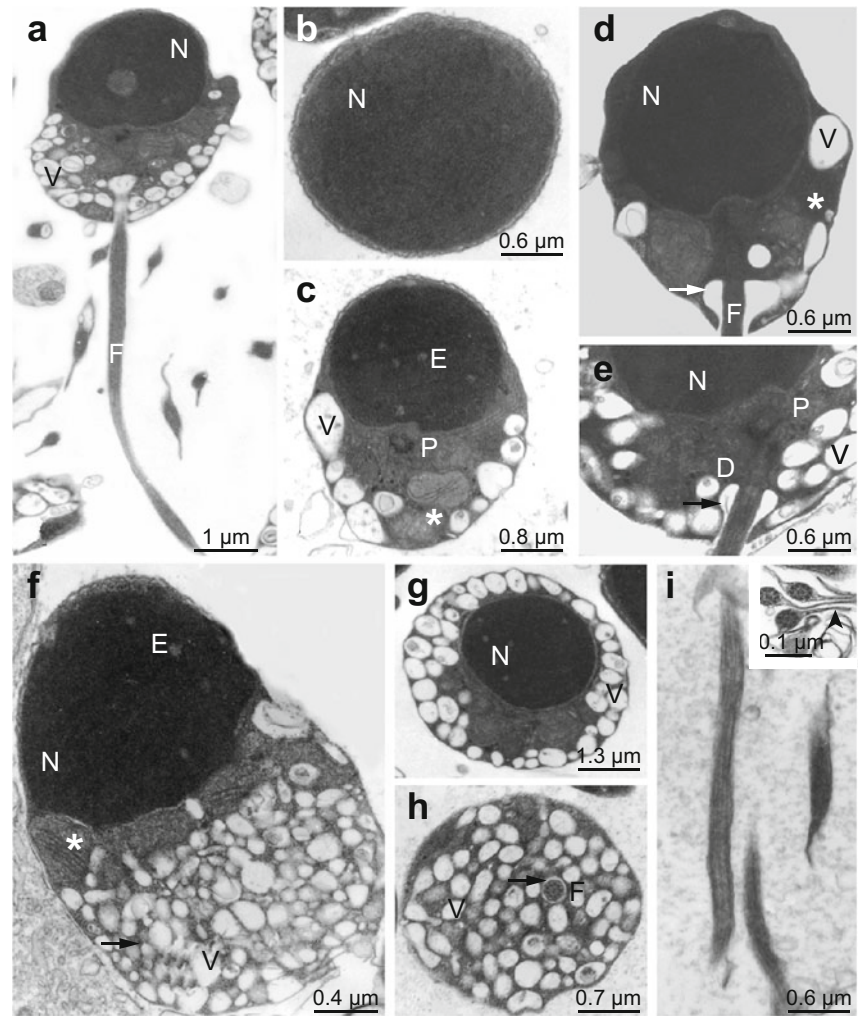
characteristic variables in the group are as follows: the initial position of the flagellum relative to the nucleus in the spermatids that can be lateral or medial, presence or absence of centriolar complex movement, of nuclear rotation, and of nuclear fossa formation. The variation in these characteristics is exclusively observed in Loricariinae and Hypostaminae than in Neoplecostominae, Hypoptopomatinae, and Otothyrinae. These data agree with the hypothesis of phylogenetic relationships among the subfamilies of Loricariidae proposed by Armbruster (2004) and Chiachio et al. (2008). In the phylogenetic analysis of Cramer et al. (2011), Hypoptomatinae and Neoplecostominae form a clade, but this molecular study did not recognize Hypoptopomatinae, Otothyrinae, or Neoplecostominae as monophyletic groups.

Spermatozoa

The comparative analyses of spermatozoa ultrastructure show that Loricariidae species herein studied present the following characteristics in common: one flagellum, presence of vesicles in the midpiece, and midpiece symmetric. Except Nematogenyidae (Spadella et al. 2006a), all the Loricarioidei has one flagellum. Vesicles in the midpiece are not observed in Loricarioidei only in *Trichomycterus reinhardti* (Trichomycteridae) (Spadella et al. 2010) and Astroblepidae (Spadella et al. 2012). In relation to the symmetry of the midpiece, all Loricarioidei has a symmetric midpiece, except Trichomycteridae (Spadella et al. 2010) and some Callichthyidae (Spadella et al. 2007).

All other characters evaluated are polymorphic among loricariids. Thus, the arrangement of the centriolar complex is lateral and in obtuse angle in Neoplecostominae species, while in the other loricariid species, the centrioles are perpendicular to each other. The arrangement of centrioles lateral and at an obtuse angle to each other is also observed in some species of Trichomycteridae (Spadella et al. 2010) and Callichthyidae (Spadella et al. 2007), while the perpendicular arrangement is found in Scoloplacidae (Spadella et al. 2006b) and Astroblepidae (Spadella et al. 2012).

The flagellar membrane specializations are variable, with two lateral projections in the flagellum of the loricari-

ids: Neoplecostominae, Hypoptopomatinae, Otothyrinae, *Farlowella oxyrryncha*, and *Hypostomus ancistroides*, as in the trichomycterids *Trichomycterus areolatus* and *T. reinhardti* (Spadella et al. 2010), Scoloplacidae (Spadella et al. 2006b), and Astroblepidae (Spadella et al. 2012). In *Loricariichthys platymetopon* and *Loricaria lata*, the lateral projections are absent. Lateral flagellar projections are also absent in species of the Nematogenyidae (Spadella et al. 2006a) and Callichthyinae (Spadella et al. 2007).

The nuclear shape is another distinct characteristic among loricariids. It is ovoid in *Corumbataia cuestae* and *Hypoptopoma guentheri*, as observed in some species of Trichomycteridae (Spadella et al. 2010) and Callichthyidae (Spadella et al. 2007). In the other species of Loricariidae, the nucleus is spherical, as found in some trichomycterids (Spadella et al. 2010) and callichthyids (Spadella et al. 2007). In Astroblepidae (Spadella et al. 2012) considered the sister group of Loricariidae, the nucleus shape is conic as observed in Scoloplacidae (Spadella et al. 2006b).

Another variable characteristic among loricariids is the nuclear fossa position, which is medial in the Neoplecostominae, Hypoptopomatinae, Otothyrinae, and Hypostominae. This same position of the nuclear fossa is found in Trichomycteridae (Spadella et al. 2010), in most Callichthyidae (Spadella et al. 2007), in Scoloplacidae (Spadella et al. 2006b), and in Astroblepidae (Spadella et al. 2012). In *Loricaria lata*, an eccentric nuclear fossa is present as in some trichomycterid species (Spadella et al. 2010).

The position of the centrioles in relation to the nuclear fossa is variable, with the centriolar complex totally inserted into the nuclear fossa in Neoplecostominae, Hypoptopomatinae, and Otothyrinae. This characteristic is also present in some trichomycterids (Spadella et al. 2010), in some Callichthyinae (Spadella et al. 2007), in Scoloplacidae (Spadella et al. 2006b), and in Astroblepidae (Spadella et al. 2012). Only the proximal centriole inserted into the nuclear fossa is found in *Loricaria lata* and *Hypostomus ancistroides*. This condition is also described in most of the trichomycterids (Spadella et al. 2010) and *Callichthys callichthys* (Spadella et al. 2007).

The presence of electron-dense structure surrounded by membrane in the midpiece is only observed in the Neoplecostominae, Hypoptopomatinae, and Otothyrinae. In *Neoplecostomus paranensis*, Hypoptopomatinae, and Otothyrinae, the spermatozoa are found in the luminal compartment of the testis surrounded by an electron-dense secretion. Moreover, in light microscope analysis, this secretion presented a positive response to eosin, featuring a protein secretion (personal observation). These characteristics represent exclusive characters of these subfamilies, not observed in any other siluriform.

The position of the flagellum in relation to the nucleus is medial in Neoplecostominae, Hypoptopomatinae, Otothyri-

nae, *Loricariichthys platymetopon*, and Hypostominae as in Callichthyidae (Spadella et al. 2007), in Scoloplacidae (Spadella et al. 2006b), in Astroblepidae (Spadella et al. 2012), in some Trichomycteridae (Spadella et al. 2010), and in Nematogenyidae (Spadella et al. 2006a). In the Loricariidae, *Loricaria lata*, the flagellum is eccentric in relation to the nucleus. This position is also described in some trichomycterid species (Spadella et al. 2010).

Conclusion

Considering the variability of the characteristics of spermatozoa ultrastructure founded in the Loricariidae analyzed, the representing of Loricariinae and Hypostominae present more distinct characteristics than Neoplecostominae, Hypoptopomatinae, and Otothyrinae, as previously was also observed in the analysis of the characteristics of spermatogenesis and spermiogenesis ultrastructure. Prior to any cladistic analysis, the following conclusion highlights the significant contribution of sperm ultrastructure to unraveling the phylogeny. In conclusion, the studies on spermatic characteristics of those groups traditionally classified as families belonging to Loricarioidei reveal that the use of this kind of characters could be very informative in the context of restrict groups, such as families and genera that are more closely related.

The general analysis of the spermatozoa ultrastructure revealed that the spermatozoa from Neoplecostominae have more common characteristics with species of Hypoptopomatinae, Otothyrinae, and Hypostominae, while the spermatozoa of Loricariinae are more similar to those from Hypostominae. Considering that Neoplecostominae, Hypoptopomatinae, and Otothyrinae also have the presence of an electron-dense secretion in the luminal compartment of the testis besides the characters already mentioned, these three groups share more characteristics in common between themselves than among any other loricariids analyzed. In addition, these three groups also have, exclusively, the presence of electron-dense structure surrounded by membrane in the midpiece, which is not observed in any other siluriform described until now. Thus, the ultrastructure of spermatozoa reinforces the characteristics of spermatogenesis and spermiogenesis and strongly indicates that Neoplecostominae, Hypoptopomatinae, and Otothyrinae form a monophyletic subgroup within the Loricariidae. This observation is in concordance with the phylogenic hypothesis presented by Armbruster (2004) and Chiachio et al. (2008).

Considering the available data (Spadella et al. 2006a, b, 2007, 2010, 2012) and of the current paper, Loricariidae has many ultrastructural characteristics of spermatogenesis and spermiogenesis that are also observed in Astroblepidae, its hypothesized sister group (de Pinna 1998; Sullivan et al.

2006) and also in other Loricarioidei. However, most of the characteristics of spermatozoa ultrastructure in Astroblepidae (Spadella et al. 2012) are only found in Scoloplacidae (Spadella et al. 2006b), the sister group of a clade composed of Astroblepidae and Loricariidae (de Pinna 1998; Sullivan et al. 2006). This condition can be due to the fact that these two groups, Astroblepidae and Scoloplacidae, different from all remaining Loricarioidei, are inseminating groups.

References

Armbruster JW (2004) Phylogenetic relationships of the suckermouth armoured catfishes (Loricariidae) with emphasis on the Hypostominae and the Ancistrinae. Zool J Linnean Soc 141:1–80

Burns JR, Quagio-Grassiotto I, Jamieson BGM (2009) Ultrastructure of spermatozoa: Ostariophysi. In: Jamieson BGM (ed) Reproductive biology and phylogeny of fishes (agnathans and bony fishes), part A. Science Publishers, Enfield, pp 287–388

Chiachio MC, Oliveira C, Montoya-Burgos JI (2008) Molecular systematic and historical biogeography of the armored Neotropical catfishes Hypoptopomatinae and Neoplecostominae (Siluriformes: Loricariidae). Mol Phylogenet Evol 49:606–617

Cramer CA, Bonatto SL, Reis RE (2011) Molecular phylogeny of the Neoplecostominae and Hypoptopomatinae (Siluriformes: Loricariidae) using multiple genes. Mol Phylogenet Evol 59:43–52

de Pinna MCC (1998) Phylogenetic relationship of neotropical Siluriformes (Teleostei: Ostariophysi): historical overviews and synthesis of hypotheses. In: Malabarba LR, Reis RE, Vari RP, Lucena ZMS, Lucena CAS (eds) Phylogeny and classification of neotropical fishes. Porto Alegre, Edipucrs, pp 279–330

Mattei X (1970) Spermiogenése comparé des poisson. In: Baccetti B (ed) Comparative spermatology. Academic Press, New York, pp 57–72

Mattei X (1993) Peculiarities in the organization of testis of *Ophidion* sp. (Pisces: Teleostei). Evidence for two types of spermatogenesis in teleost fish. J Fish Biol 43:931–937

Quagio-Grassiotto I, Oliveira C (2008) Sperm ultrastructure and a new type of spermiogenesis in two species of Pimelodidae with a comparative review of sperm ultrastructure in Siluriformes (Teleostei: Ostariophysi). Zool Anzeiger 247:55–66

Quagio-Grassiotto I, Spadella MA, Carvalho M, Oliveira C (2005) Comparative description and discussion of spermiogenesis and spermatozoal ultrastructure in some species of Heptapteridae and Pseudopimelodidae (Telesotei: Siluriformes). Neotropical Ichthyol 3(3):403–412

Reis RE, Pereira EHL, Armbruster JW (2006) Delturinae, a new loricariid catfish subfamily (Teleostei, Siluriformes), with revisions of *Delturus* and *Hemipsilichthys*. Zool J Linnean Soc 147:277–299

Spadella MA, Oliveira C, Quagio-Grassiotto I (2006a) Occurrence of biflagellate spermatozoa in the Siluriformes families Cetopsidae, Aspredinidae, and Nematogenyidae (Teleostei: Ostariophysi). Zoomorphology 125:108–118

Spadella MA, Oliveira C, Quagio-Grassiotto I (2006b) Spermiogenesis and introsperm ultrastructure of *Scoloplax distolothrix* (Ostariophysi: Siluriformes: Scoloplacidae). Acta Zool 87:341–348

Spadella MA, Oliveira C, Quagio-Grassiotto I (2007) Comparative analysis of the spermiogenesis and sperm ultrastructure in Callichthyidae (Teleostei: Ostariophysi: Siluriformes). Neotropical Ichthyol 5(3):337–350

Spadella MA, Oliveira C, Quagio-Grassiotto I (2010) Analysis of the spermiogenesis and spermatozoal ultrastructure in Trichomycteridae (Teleostei: Ostariophysi: Siluriformes). Acta Zool 91(4):373–389

Spadella MA, Oliveira C, Ortega H, Quagio-Grassiotto I, Burns JR (2012) Male and female reproductive morphology in the inseminating genus Astroblepus (Ostariophysi: Siluriformes: Astroblepidae). Zool Anzeiger 251:38–48

Sullivan JP, Lundberg JG, Hardman M (2006) A phylogenetic analysis of the major groups of catfishes (Teleostei: Siluriformes) using rag1 and rag2 nuclear gene sequences. Mol Phylogenet Evol 40:636–662