UNIVERSIDADE ESTADUAL DE CAMPINAS

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ANÁLISE FILOGENÉTICA DA SUPERFAMÍLIA LORICARIOIDEA (TELEOSTEI: SILURIFORMES) COM BASE NA ULTRA-ESTRUTURA DA ESPERMIOGÊNESE E DOS ESPERMATOZÓIDES

Este exemplar corresponde à redação final da tese defendida pelo(a) candidato (a) MARIA ANGÉLICA SPADELLA SANTOS e aprovada pela Comissão Julgadora.

Tese apresentada ao Instituto de Biologia para obtenção do Título de Doutor em Biologia Celular e Estrutural, na área de Biologia Celular.

Orientadora: Profa. Dra. Irani Quagio-Grassiotto

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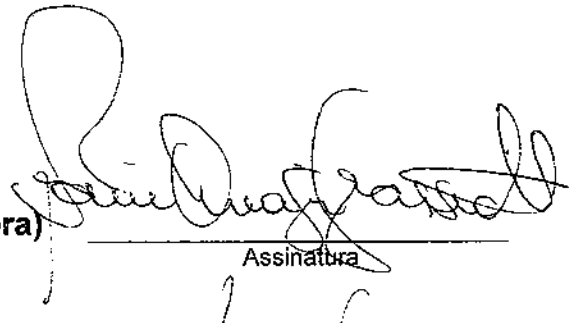
Profa. Dra. Irani Quagio-Grassiotto (Orientadora) 
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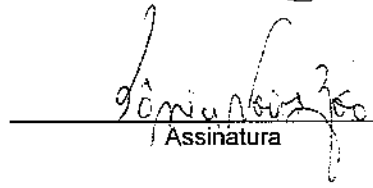
Profa. Dra. Sônia Nair Báo 
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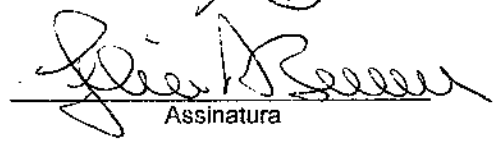
Prof. Dr. Flávio Alicino Bockmann 
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Ninguém pode construir em teu lugar as pontes que precisarás para atravessar o rio da vida. Ninguém exceto tu, só tu. Existem, por certo, atalhos sem número, e pontes, e semideuses que se oferecerão para levar-te além do rio; mas isso te custaria a tua própria pessoa; tu te hipotecarias e te perderias. Existe no mundo um único caminho por onde tu podes passar. Onde leva? Não perguntes, segue-o.
(Nietzsche)

De tudo ficaram três coisas: A certeza de que estamos sempre começando... A certeza de que precisamos continuar... A certeza de que seremos interrompidos antes de terminar... Portanto, devemos: fazer da interrupção um caminho novo... Da queda, um passo de dança... Do medo, um trampolim... Do sonho uma ponte... Da procura um encontro...
(Fernando Pessoa)

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SUMÁRIO

RESUMO

A ordem Siluriformes compreende um grupo diverso e amplamente distribuído dentre os Ostariophysi, apresentando 36 famílias com aproximadamente 480 gêneros e mais de 3.000 espécies. Entre os grupos de Siluriformes neotropicais, reconhecidamente monofiléticos, está a superfamília Loricarioidea. Os relacionamentos filogenéticos entre as famílias de Siluriformes, sugerem que Loricarioidea é grupo irmão de Amphiliidae, uma família africana de Siluriformes. A superfamília Loricarioidea encontra-se atualmente constituída por seis famílias: Nematogenyidae, Trichomycteridae, Callichthyidae, Scoloplacidae, Astroblepidae e Loricariidae. Apesar do conhecimento atual sobre os relacionamentos entre as famílias de Siluriformes vir sendo obtido com base em caracteres osteológicos, outros dados como a ultra-estrutura da espermiogênese e dos espermatozóides parecem ser potencialmente úteis na elucidação dos relacionamentos de grupos. O objetivo deste estudo foi a realização da análise filogenética da superfamília Loricarioidea, com base nas características ultra-estruturais da espermiogênese e dos espermatozóides, visando testar a real habilidade destes dados na resolução dos relacionamentos filogenéticos intra e inter-familiar, bem como na ordem Siluriformes. Foi feita a descrição das características ultra-estruturais das células germinativas masculinas em 27 representantes de diferentes famílias de Loricarioidea. A análise geral dos dados obtidos revelou que quando essa classe de caracteres ultra-estruturais reprodutivos é utilizada em grupos mais restritos como a superfamília Loricarioidea, observa-se que ela pode ser mais informativa e pode corroborar o monofiletismo de alguns grupos. Entretanto, o uso desses caracteres nas análises filogenéticas em nível de ordem não é informativo, uma vez os grupos sugeridos são muito incongruentes com a hipótese de relacionamento disponível para os Siluriformes. Além disso, alguns caracteres que poderiam representar sinapomorfias, tornam-se homoplasias quando se considera a ocorrência da mesma característica em outros grupos não relacionados. Portanto, o emprego desses caracteres ultra-estruturais reprodutivos em análises filogenéticas deverá ser criteriosamente planejado, evitando-se interpretações equivocadas.

ABSTRACT

The order Siluriformes comprises the most diverse and widely distributed ostariophysan groups, presenting thirty-six families with approximately 480 genera and over 3.000 species. Among the neotropical siluriform lineages likely to be monophyletic is the superfamily Loricarioidea. The relationships among catfish families suggest that Loricarioidea is sister group of Amphiliidae, an African siluriform family. The Loricarioidea is constituted by six families: Nematogenyidae, Trichomycteridae, Callichthyidae, Scoloplacidae, Astroblepidae, and Loricariidae. Although the current knowledge of the relationships among siluriform families has been acquired on the basis on osteological characters, other data such as the spermiogenesis and spermatozoal ultrastructure seem be potentially useful in the clarification of the relationships of the groups. The aim of the present study was to develop a phylogenetic analysis of the superfamily Loricarioidea, using the ultrastructural characteristics of both spermiogenesis and spermatozoa as a test to evaluate the ability of this data in resolving the phylogenetic relationships inside the families, among families and in the order Siluriformes. The description of the ultrastructural characteristics of male germinative cells in 27 specimens of different families of Loricarioidea was presented. The general analysis of the data obtained revealed that when this class of reproductive ultrastructural characters is employed in a more restrict group, as the superfamily Loricarioidea, it is really informative and can strongly support the monophyly of some groups. However, the phylogenetics analysis using these characters is not informative at order level as the suggested groups are very incongruent with the available hypotheses for Siluriformes. Moreover, some characters that could represent synapomorphies, change to homoplasies considering the occurrence of the same characters in other unrelated groups. Then, the use these reproductive ultrastructural characters in phylogenetics analysis should be carefully planed to avoid erroneous conclusions.

1. INTRODUÇÃO

1.1. Ictiofauna neotropical de água doce

A fauna neotropical de peixes de água-doce é bastante rica, incluindo cerca de 4.500 espécies reconhecidamente válidas segundo o levantamento da diversidade de peixes de água doce das Américas do Sul e Central realizado por Reis et al. (2003). Além disso, esses autores estimam que possam existir aproximadamente 1.600 espécies ainda não descritas, o que resultaria em uma estimativa total de cerca de 6.000 espécies de peixes neotropicais de água doce. Já Schaefer (1998) calculou, em um levantamento das tendências históricas de descrição de espécies em Characidae e Loricariidae, que possam existir aproximadamente 8.000 espécies de peixes de água doce neotropicais o que corresponderia a 25% de todas as espécies de peixes do mundo. Esse número foi discutido e aceito por Vari e Malabarba (1998) que acrescentaram que toda essa diversidade de peixes neotropicais de água doce ocorre em menos de 0,003% da água do planeta.

De acordo com Reis et al. (2003), a ictiofauna de águas continentais centro e sul-americanas, é dominada, tanto em termos de diversidade taxonômica quanto em biomassa, por peixes da superordem Ostariophysi, alcançando aproximadamente 70% das espécies descritas, divididas primariamente entre as ordens: Siluriformes (15 famílias e aproximadamente 40% das espécies) e Characiformes (14 famílias e aproximadamente 30% das espécies) e, secundariamente, a ordem Gymnotiformes (cinco famílias e 3% das espécies). O restante das espécies divide-se entre a ordem Cyprinodontiformes (aproximadamente 10% das espécies), a família Cichlidae, da ordem Perciformes (aproximadamente 10% das espécies), ambas da superordem Acanthopterygii, e ainda todo um conjunto (7% do total de espécies) formado por vários grupos taxonômicos (Potamotrygonidae, Lepidosirenidae, Osteoglossidae, Engraulidae, Clupeidae, Synbranchidae, Sciaenidae, Gobiidae, Nandidae, Belonidae, Tetraodontidae e outros).

A superordem Ostariophysi encontra-se incluída em Teleostei, Euteleostei (Lauder e Liem, 1983). Os Ostariophysi compreendem duas séries: Anotophysi, constituída pelos Gonorynchiformes e Otophysi constituída pelos Cypriniformes, Characiformes,

Gymnotiformes e Siluriformes (Fink e Fink, 1996). Os Ostariophysi, série Otophysi compreendem um grupo de peixes ósseos diagnosticado pela presença do aparelho de Weber, um complexo formado por um conjunto de ossículos modificados (*tripus*, *intercalarium*, *claustrum* e *scaphium*) que permitem a conexão da bexiga natatória com o ouvido interno (Fink e Fink, 1996). Fink e Fink (1996) propõem a seguinte classificação para os Ostariophysi, Otophysi: Cypriniphysi, que inclui a ordem Cypriniformes (carpas, barbos) e, Characiphysi, incluindo os Characiformes (lambaris, piranhas, pacús) e Siluriphysi, que inclui duas ordens, Gymnotiformes (poraquês, tuviras, morenitas) e Siluriformes (peixes de couro, cascudos). Neste estudo, a análise filogenética das relações entre os grupos de Otophysi, estabelece Cypriniformes como grupo irmão dos demais Otophysi e Characiformes como grupo-irmão do clado Siluriformes mais Gymnotiformes.

O monofiletismo de Gymnotiformes e Siluriformes, considerados grupos-irmãos, baseia-se em 16 características listadas em Fink e Fink (1996), sendo grande parte delas referentes à anatomia neural do sistema eletro-sensorial.

Os estudos sistemáticos da ictiofauna neotropical, com base em dados morfológicos, têm-se expandido consideravelmente nos últimos anos (Malabarba *et al.*, 1998). Apesar dos notáveis progressos, muito ainda resta a ser conhecido sobre a filogenia dos diversos grupos, devido à magnitude da biodiversidade neotropical.

1.2. A ordem Siluriformes

A ordem Siluriformes compreende um grupo de peixes extremamente grande, diverso e amplamente distribuído nas regiões Tropical e Neotropical, principalmente na América do Sul, África e sul e sudeste da Ásia (Burgess, 1989; Teugels, 1996; Ferraris, 1998; de Pinna, 1998; Arratia *et al.*, 2003). Desta forma, sua distribuição parece ser limitada pela temperatura, uma vez que a maioria das espécies habita tanto região Tropical quanto Neotropical e, poucas são as espécies que alcançam o extremo sul da América do Sul ou o extremo norte da América do Norte (Nelson, 2006).

O número de gêneros em Siluriformes é de aproximadamente 480, agrupando as cerca de 3.100 espécies reconhecidas, representando 1/3 do percentual de peixes de água

doce conhecidos (Ferraris, 1998, 2007). Sua enorme diversidade ecológica os faz foco de vários estudos (Fink e Fink, 1981; de Pinna, 1998).

Embora a grande maioria dos peixes da ordem Siluriformes seja encontrada em ambientes de água doce, duas famílias, Ariidae e Plotosidae, têm representação significativa de espécies primariamente marinhas (Burgess, 1989; de Pinna, 1998). Além disso, as famílias Pangasiidae, Aspredinidae e Auchenipteridae incluem algumas espécies estuarinas, que podem ainda, apresentar certa tolerância a ambientes marinhos (de Pinna, 1998).

Os Siluriformes são conhecidos popularmente no Brasil como "bagres", "cascudos", "armados", "mandis", "jaús" ou "pintados". São peixes que possuem formas e tamanhos extremamente variados e caracterizam-se, principalmente, por não possuírem escamas sobre o corpo, por apresentarem barbilhões e, freqüentemente, acúleos fortes e pungentes à frente do primeiro raio das nadadeiras dorsal e peitorais ("tripé defensivo"), capazes de infringir graves ferimentos e em alguns casos, de injetar um veneno produzido por células glandulares localizadas no tecido epidérmico que cobre estes acúleos (Alexander, 1965; Burgess, 1989; Ferraris, 1998). O corpo desprovido de escamas pode ser revestido por uma pele espessa conhecida popularmente como couro ("peixes de couro"), ou então, coberto total ou parcialmente por placas ósseas ("cascudos", "bodós", "caborjas") (Burgess, 1989). Apresentam hábitos predominantemente crepusculares e noturnos, o que os leva a habitar, em geral, locais com águas turvas como o fundo dos rios, e a permanecer entre rochas e vegetação (Ferraris, 1998). Os hábitos alimentares também são variados, havendo espécies, herbívoras, planctófogas, carnívoras e onívoras. Além disso, há espécies com hábitos alimentares extremamente particulares como os representantes de duas subfamílias de Trichomycteridae, os Vandelliinae, que são hematófagos (Machado e Sazima, 1982) e os Stegophilinae, que são lepidófagos ("comedores de escamas") (Nelson, 2006). Provavelmente localizam alimentos no fundo e orientam sua natação por meio dos barbilhões gustativos e tácteis, ou ainda, por meio de receptores de campo elétrico presentes no corpo, como é verificado em representantes de Malapteruridae ("bagres elétricos") (Alexander, 1965; Ferraris, 1998).

Apesar da importância científica e econômica dos Siluriformes, o grupo apresenta ainda inúmeros problemas sistemáticos e taxonômicos devido à sua ampla diversidade. A própria classificação das famílias de Siluriformes ainda não é consensual. Assim, por exemplo, o número de famílias reconhecidas para a ordem é de 29 segundo Ferraris (1998), 33 para Teugels (1996) e Eschmeyer (1998), 34 para Nelson (1994), 35 segundo Ferraris e de Pinna (1999), Britto (2003) e Nelson (2006), e 36 segundo Ferraris (2007).

O número de estudos enfocando as relações entre as várias famílias de Siluriformes, com base em dados morfológicos, tem-se expandido consideravelmente nos últimos anos devido à incorporação de novas técnicas de obtenção e interpretação de dados, entre as quais está o uso da metodologia de análise filogenética proposta inicialmente por Hennig (1966) e implementada por diversos autores.

Embora nos últimos anos notem-se progressos, a grande maioria dos estudos cladísticos sobre a filogenia dos Siluriformes é dedicada aos intra-relacionamentos nas famílias de Siluriformes (Diogo, 2003). Contudo, três importantes trabalhos que atentam para uma análise filogenética mais global entre as famílias de Siluriformes são o de Mo (1991 *apud* de Pinna, 1998), o de de Pinna (1993) e o de Britto (2003), os quais se baseiam em uma grande quantidade de novos caracteres. Entre os tópicos abordados por de Pinna (1998), em seu estudo sobre as relações filogenéticas dos Siluriformes neotropicais, há uma comparação entre os resultados obtidos por Mo (1991) e por de Pinna (1993), concluindo que existe concordância em alguns pontos destes trabalhos, que podem ser vistos como uma evidência de corroboração independente, apresentada em ambos, sobre as relações entre as famílias de Siluriformes.

A recente análise filogenética realizada por Britto (2003), com base em 331 caracteres morfológicos de representantes dos principais grupos da ordem Siluriformes indicou que algumas famílias formam agrupamentos polifiléticos e vários grupos tradicionais tiveram seu monofiletismo corroborado, concordando com as hipóteses previamente propostas por Mo (1991 *apud* de Pinna, 1998) e de Pinna (1993, 1998) (Figura 1). Como exemplo, tem-se a posição basal da família Diplomystidae dentro da ordem e o monofiletismo da superfamília Loricarioidea e a relação entre suas famílias. Dentre as novas hipóteses, não foi confirmada a relação da família Pseudopimelodidae com o grupo

formado por Sisoroidea, Loricarioidea e Amphiliidae, que se mostra agora mais relacionada a Heptapteridae e outros Siluriformes. Entre os grupos apresentados, os que possuem representantes na região neotropical são: Diplomystidae, Cetopsidae, Aspredinidae, Loricarioidea, Pseudopimelodidae, Heptapteridae, Doradoidea, Pimelodidae e Ariidae. O grupo-irmão de cada uma destas linhagens inclui alguns membros com distribuição fora da região neotropical (de Pinna, 1998; Britto, 2003). Possivelmente isto indica que a diversificação dos Siluriformes na região neotropical precede a separação entre a América do Sul e os outros blocos continentais, justificando, por exemplo, os componentes afro-neotropicais presentes em Siluriformes (de Pinna, 1998).

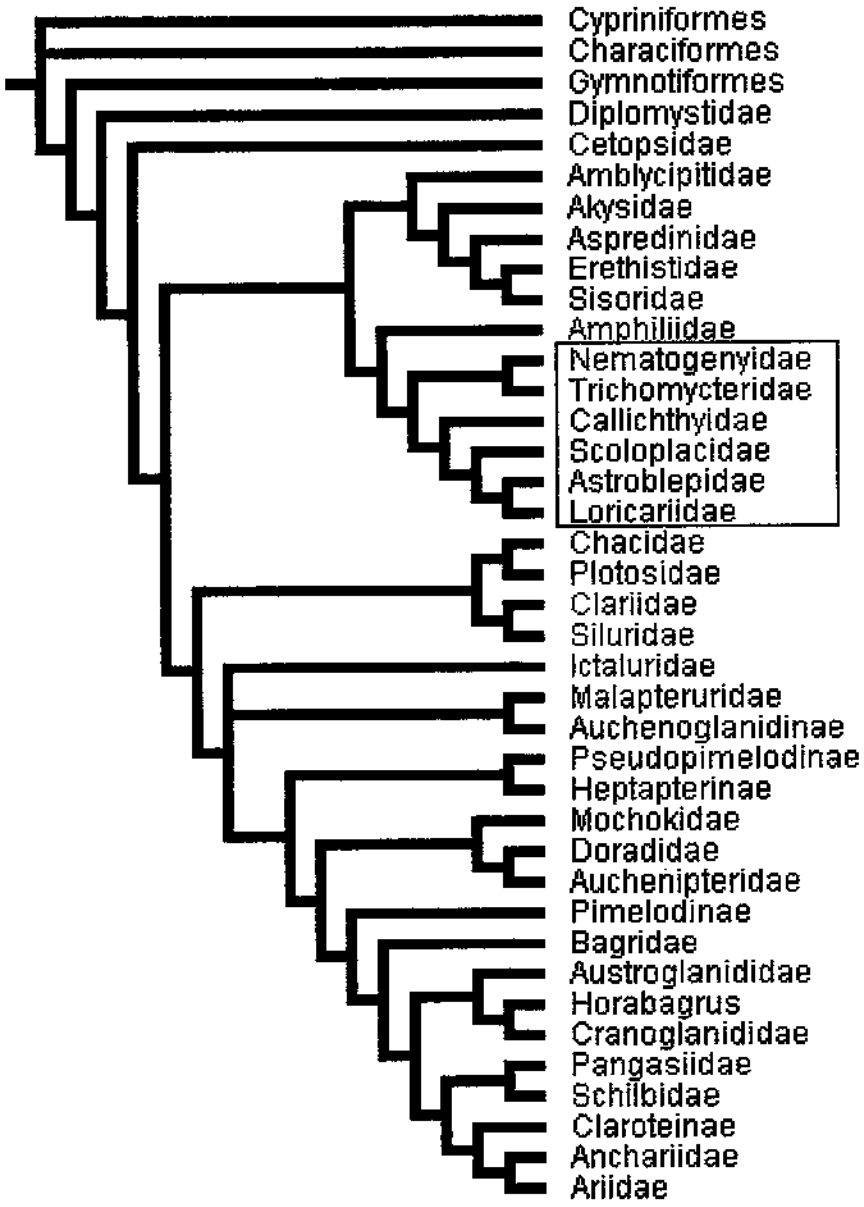

Figura 1: Cladograma proposto por Britto (2003), mostrando as relações filogenéticas entre os grupos da ordem Siluriformes. O retângulo destaca a superfamília Loricarioidea.

1.3. Superfamília Loricarioidea

Atualmente, a superfamília Loricarioidea é composta por seis famílias: Nematogenyidae (1 espécie), Trichomycteridae (171 espécies), Callichthyidae (177 espécies), Scoloplacidae (4 espécies), Astroblepidae (54 espécies) e Loricariidae (673 espécies) (de Pinna, 1998; Reis *et al.*, 2003). Dentro desse grupo estão os três maiores gêneros de Siluriformes: *Hypostomus*, *Corydoras* e *Trichomycterus*, além de um grande número de espécie de interesse econômico, principalmente como espécies ornamentais.

A superfamília Loricarioidea é o maior grupo monofilético de Siluriformes da região neotropical (de Pinna, 1998; Britto, 2003). Essa superfamília foi o primeiro grupo natural de famílias de Siluriformes reconhecido. Essa afinidade foi primeiramente proposta por Peyer (1922), com base na presença de odontódios e na estrutura do primeiro raio da nadadeira peitoral. Além destes caracteres propostos, de Pinna (1998) determinou mais dois caracteres que corroboram o monofiletismo da superfamília, que são: dentes mandibulares com cúspides bífidas e ramos anteriores do basipterígio sem cartilagem nos adultos. De acordo com a recente análise de Britto (2003), a presença de ossículos do aparelho de Weber encapsulados mostrou-se também um caráter exclusivo para Loricarioidea.

A relação da superfamília Loricarioidea com as outras famílias de Siluriformes observada no cladrograma de Britto (2003) sugere sua proximidade com a família Amphiliidae e com a superfamília Sisoroidea, sendo Amphiliidae considerada grupo-irmão de Loricarioidea.

As hipóteses de relacionamento entre as famílias constituintes de Loricarioidea proposta por de Pinna (1998) e Britto (2003) mostram que as famílias Callichthyidae, Scoloplacidae, Astroblepidae e Loricariidae divergem de Nematogenyidae e Trichomycteridae, com a formação de dois grupos, um com Nematogenyidae como grupo-irmão de Trichomycteridae e outro grupo com os demais membros da superfamília, tendo Loricariidae e Astroblepidae como grupos-irmão. Várias sinapomorfias são conhecidas para as diferentes famílias de Loricarioidea, como pode ser observado na Figura 2. Por outro lado, as filogenias apresentadas por de Pinna (1998) revelam que são relativamente poucas as hipóteses de relacionamento dentro das famílias de Loricarioidea.

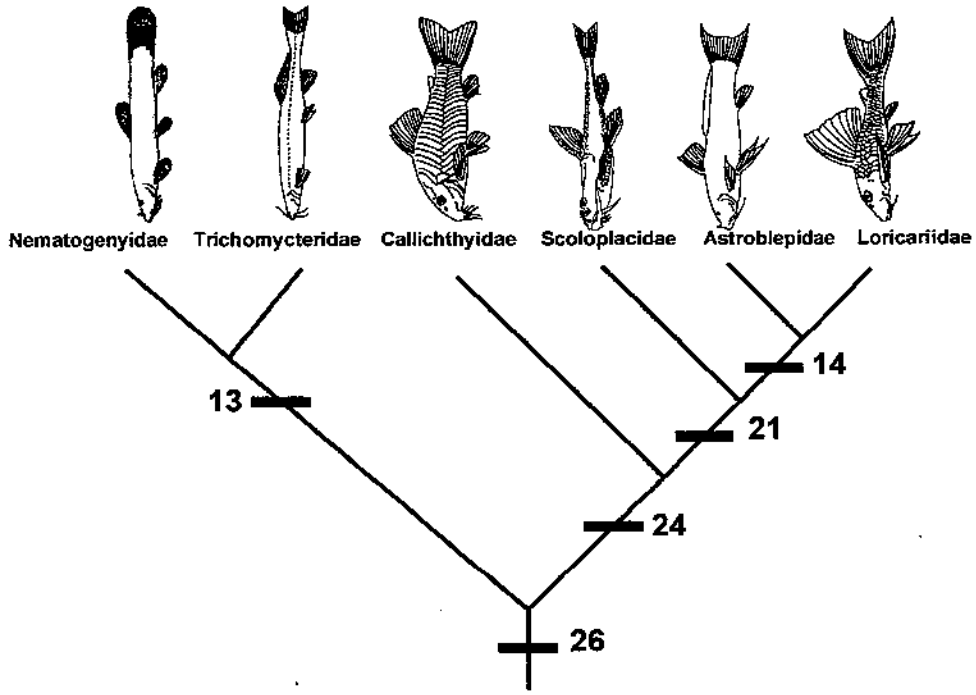

Figura 2. Cladograma mostrando as relações entre as famílias de Loricarioidea. Os retângulos pretos mostram o número de sinapomorfias suportando cada ramo, segundo Britto (2003). Modificado de Britto (2003).

1.4. O aparelho reprodutor masculino em peixes

Nos Teleostei, e particularmente nos Siluriformes, o aparelho reprodutor masculino pode apresentar uma série de diferenciações. Em termos anatômicos, os testículos variam de simples bolsas alongadas que convergem para a papila genital a órgãos compostos por várias franjas com função predominantemente espermatogênica, contendo ou não regiões diferenciadas com função secretora ou armazenadora de espermatozóides (Loir *et al.*, 1989; Meisner *et al.*, 2000; Santos *et al.*, 2001; Quagio-Grassiotto *et al.*, 2005; Spadella *et al.*, 2006a, b). Alguns grupos chegam inclusive a apresentar estruturas similares em função a vesículas seminais, as ampolas (Loir *et al.*, 1989).

Microscopicamente, os testículos encontram-se envolvidos por uma cápsula delgada. A ultra-estrutura desta cápsula revela que ela é constituída por duas camadas: uma mais externa, que corresponde ao mesotélio, o qual é constituído por um epitélio simples pavimentoso sustentado pela lâmina basal e, uma mais interna formada por tecido conjuntivo frouxo e células mióides (Koulish et al., 2002). Os testículos estão conectados à região dorsal da cavidade celomática por meio do mesórquio, uma fina camada de peritônio (Pudney, 1993; Le Gac e Loir, 1999). Ao longo do ciclo reprodutivo anual das espécies são observadas alterações na morfologia dos testículos, bem como na coloração e principalmente no tamanho e peso (Le Gac e Loir, 1999).

A estruturação clássica dos testículos encontrada nos peixes também é compartilhada por outros vertebrados, mostrando ser essa uma característica plesiomórfica (Pudney, 1993, 1995). Nesta organização o testículo encontra-se dividido em dois compartimentos: o germinativo e o intersticial (Pudney, 1993, 1995; Le Gac e Loir, 1999; Grier, 2002; Koulish et al., 2002). O compartimento germinativo é constituído pelo epitélio germinativo, o qual é formado pelas células de Sertoli, pelas células germinativas e pela lâmina basal mais fibras reticulares. O compartimento intersticial é formado pelas células de Leydig, fibroblastos, células mióides, macrófagos, vasos sanguíneos, nervos e fibras colágenas. Ambos os compartimentos encontram-se separados pela lâmina basal (Pudney, 1995; Grier, 2002; Lo Nostro *et al.*, 2003).

Nos Teleostei, a organização do compartimento germinativo no interior dos testículos pode se dar em lóbulos ou em túbulos (Grier, 1993; Parenti e Grier, 2004). Os

conceitos morfológicos contidos nos termos "lóbulo" e "túbulo" têm por base o formato e a maneira como o compartimento germinativo termina na periferia do testículo (Grier, 1993).

Nos testículos lobulares, o compartimento germinativo termina em fundo cego, com formato de dedos voltados para baixo. Pode sofrer anastomoses, porém apenas na região do ducto principal. Esse tipo de organização testicular é encontrado nos Teleostei mais derivados, Percomorpha e Atherinomorpha. Nos testículos tubulares, o compartimento germinativo termina em forma de alças. Nos Teleostei mais basais, os túbulos sofrem anastomoses em diferentes alturas do órgão, principalmente na região do ducto espermático. Esse último tipo de organização testicular é denominado de tubular anastomosado (Grier, 1993).

Os testículos podem ser classificados também conforme a distribuição das espermatogônias, sendo tal classificação válida somente para os testículos lobulares (Grier, 1981, 1992). Esses podem conter espermatogônias confinadas apenas na porção distal dos lóbulos, e serem restritos, ou apresentarem as espermatogônias distribuídas ao longo de todo o lóbulo, e serem irrestritos (Grier, 1992). Os testículos lobulares restritos são típicos dos Atherinomorpha, enquanto que os irrestritos são encontrados nos Percomorpha (Grier, 1993). Parenti e Grier (2004) num levantamento recentemente concluído sobre a estrutura testicular nos Teleostei, contabilizando 136 descrições, confirmam esses dados.

A produção dos gametas masculinos, processo denominado espermatogênese, ocorre no compartimento germinativo (Pudney, 1995; Miura, 1999; Koulish et al., 2002). Mattei (1993) sugere que, em peixes teleósteos, há dois tipos de espermatogênese: a cística e a semi-cística. Na espermatogênese cística, a produção dos espermatozóides se dá completamente no interior de cistos ou espermatocistos presentes no compartimento germinativo; com a abertura desses cistos somente no final do processo, resultando na liberação dos gametas no lúmen. Os cistos são formados por grupos de células germinativas em desenvolvimento mais ou menos sincrônico, sejam as espermatogônias primárias ou secundárias, os espermatócitos primários ou secundários, ou as espermátides, que se encontram envolvidos por prolongamentos citoplasmáticos das células de Sertoli (Grier, 1981, 1993; Miura, 1999). Já na espermatogênese semi-cística, os cistos se abrem antes do final da produção dos espermatozóides, a qual é completada no lúmen do compartimento

germinativo (Mattei, 1993). A abertura do cisto pode ocorrer em variados níveis de diferenciação das células germinativas, levando a uma mescla de diferentes tipos celulares no lúmen. Em geral, encontram-se mesclados as espermátides e os espermatozóides recém-formados. A maioria dos peixes teleósteos apresenta espermatogênese do tipo cística (Mattei, 1993).

A espermatogênese inicia-se com a proliferação mitótica das espermatogônias, prossegue passando pela divisão meiótica e termina com a espermiogênese, na qual a espermátide haplóide diferencia-se em espermatozóide (Miura, 1999). Desta forma, nos cistos as espermatogônias primárias (uma única espermatogônia envolvida pela célula de Sertoli) dividem-se por mitoses e dão origem as espermatogônias secundárias (mais de uma espermatogônia envolvida pela célula de Sertoli). Estas também se multiplicam por mitose e, se diferenciam em espermatócitos primários, os quais entram em meiose e dão origem aos espermatócitos secundários após a primeira divisão meiótica. Os espermatócitos secundários originam as espermátides após a segunda divisão meiótica (Pudney, 1995). Nestas divisões celulares, as citocineses são incompletas e as células germinativas permanecem interligadas por meio de pontes citoplasmáticas (Pudney, 1995; Le Gac e Loir, 1999).

A diferenciação das espermátides ou espermiogênese resulta na formação dos espermatozóides. Durante a espermiogênese ocorrem marcantes mudanças morfológicas nas espermátides que consistem na formação da cabeça do espermatozóide e na compactação da cromatina, na formação da peça intermediária, na perda de citoplasma e no desenvolvimento do flagelo (Pudney, 1995; Miura, 1999). Ao término destes eventos, os processos citoplasmáticos das células de Sertoli se afastam e os espermatozóides são liberados no lúmen do compartimento germinativo (Pudney, 1995; Grier, 1993). Apesar dos espermatozóides terem completado seu desenvolvimento no interior dos testículos após a espermiogênese; na maioria das espécies, estes gametas ditos testiculares ainda não são considerados maduros, uma vez que são imóveis; não sendo capazes de fecundar o gameta feminino. O processo de maturação do espermatozóide ocorre à medida que o gameta percorre o ducto espermático, onde adquire a sua motilidade. Este processo envolve, portanto, mudanças fisiológicas na célula e não morfológicas (Miura, 1999).

Na maioria dos peixes teleósteos a fertilização é externa. Em menor número, algumas espécies apresentam fertilização interna, com os machos possuindo um órgão copulatório (gonopódio ou pseudopênis), com o qual depositam o esperma diretamente no trato reprodutivo da fêmea através da papila urogenital (Nakatani *et al.*, 2001). Burns *et al.* (2002), consideram inadequado o uso do termo fertilização interna e sugerem que ele seja substituído por inseminação, uma vez que o tempo exato entre a introdução do esperma e a fertilização propriamente dita não é conhecido até o momento para nenhuma espécie de Ostariophysi que exibe esse modo de reprodução.

1.5. Classificação da espermiogênese em peixes

Mattei (1970), em uma análise comparativa da espermiogênese em peixes das classes Chondrichthyes e Osteichthyes (em Sarcopterygii e Actinopterygii), propõe dois tipos de espermiogênese, a do tipo I e a II. Em ambos os tipos, o início do desenvolvimento do flagelo, nas espermátides jovens se dá lateralmente ao núcleo, sendo que a ocorrência ou não de rotação nuclear diferencia estes dois tipos. Desta forma, se ao longo da espermiogênese ocorrer rotação nuclear, o eixo flagelar do espermatozóide se posicionará perpendicularmente ao núcleo, caracterizando a espermiogênese do tipo I, porém se não ocorrer rotação nuclear, o eixo flagelar permanecerá paralelamente ao núcleo, caracterizando a espermiogênese do tipo II.

Na espermiogênese do tipo I, as espermátides jovens apresentam núcleo central, mitocôndrias esparsas pelo citoplasma e complexo centriolar lateral ao núcleo e preso à membrana plasmática. O centríolo distal diferencia-se em corpúsculo basal e desenvolve o flagelo. O complexo centriolar movimenta-se em direção ao núcleo, trazendo a membrana, que sofre uma invaginação, e o segmento inicial do flagelo. Forma-se assim o canal citoplasmático, um espaço existente entre as membranas plasmática e flagelar. O flagelo dispõe-se tangencialmente ao núcleo e nessa face do contorno nuclear forma-se uma depressão, denominada fossa nuclear. O núcleo sofre uma rotação de 90° em relação ao eixo flagelar e o complexo centriolar se insere na fossa nuclear. A região da fossa nuclear determina a base do núcleo, região para a qual migram as mitocôndrias.

Na espermiogênese do tipo II, as espermátides jovens são bastante similares àquelas caracterizadas na espermiogênese do tipo I. Como nestas células a rotação nuclear não ocorre durante a sua diferenciação, o flagelo posiciona-se paralelo ao núcleo. Embora ocorra a formação da fossa nuclear, os centríolos permanecem fora dela (Mattei, 1970; Jamieson, 1991). Este tipo de espermiogênese é característico de teleósteos mais derivados, como por exemplo, os Perciformes (Mattei, 1970; Jamieson, 1991).

Variações nos tipos I e II de espermiogênese resultam em um canal citoplasmático pequeno ou inexistente, o mesmo ocorrendo com a fossa nuclear (Jamieson, 1991; Mattei, 1991).

Um outro tipo de espermiogênese, a do tipo III, foi recentemente descrito em Siluriformes, para as famílias Pimelodidae e Heptapteridae (Quagio-Grassiotto e Carvalho, 2000; Quagio-Grassiotto *et al.*, 2005; Quagio-Grassiotto e Oliveira, manuscrito submetido). Nessa, as espermátides jovens apresentam núcleo central, mitocôndrias esparsas pelo citoplasma, complexo centriolar medial ao núcleo e preso à membrana plasmática. O complexo centriolar não se movimenta em direção ao núcleo e o canal citoplasmático e a fossa nuclear não se formam. Não há também rotação nuclear. A peça intermediária é formada pela movimentação da massa citoplasmática em direção a região inicial do flagelo. Apesar de neste tipo de espermiogênese não ocorrer rotação nuclear, o flagelo é perpendicular ao núcleo.

Portanto, a morfologia final encontrada nos espermatozóides em peixes está diretamente relacionada ao tipo de espermiogênese ocorrido na espécie, podendo variar, por exemplo, quanto ao posicionamento do flagelo em relação ao núcleo, à presença ou não de rotação nuclear, à presença ou não da fossa nuclear e do canal citoplasmático e ao número de flagelos, caracterizando tipos intermediários de espermatozóides (Jamieson, 1991; Mattei, 1970, 1991).

Entre os tipos de espermiogênese descritos, a espermiogênese observada no mais basal dos Siluriformes, *Diplomystes mesembrinus*, (Quagio-Grassiotto *et al.*, 2001), corresponde à do tipo I de Mattei (1970).

1.6. Classificação dos espermatozóides em peixes

Franzén (1970), em um estudo sobre os aspectos filogenéticos da morfologia dos espermatozóides em vários filos de invertebrados aquáticos, propõem uma classificação para estes gametas em primitivos ou modificados, considerando a estrutura e organização encontrada, bem como o tipo de fertilização. Entende-se por primitivos, os espermatozóides que ocorrem nos grupos com fertilização externa e que, portanto, são liberados no meio aquático. Estes apresentam numa seqüência ântero-posterior: acrossomo, cabeça arredondada ou cônica, núcleo esférico, dois centríolos (proximal e distal), peça intermediária, com mitocôndrias arredondadas e pouco numerosas e flagelo contendo axonema clássico (9+2). Já os espermatozóides modificados são encontrados em espécies de fertilização interna, sendo liberados diretamente no trato reprodutivo da fêmea. A morfologia destes gametas diverge em variados níveis da encontrada nos espermatozóides do tipo primitivo. Tais diferenças que destoam da condição primitiva correspondem principalmente à morfologia da cabeça, que se mostra mais alongada e à peça intermediária, que também exibe um alongamento, além de uma redistribuição das mitocôndrias. Os espermatozóides do tipo primitivo são encontrados nos grupos mais primitivos dos invertebrados analisados, enquanto que os grupos mais derivados apresentam espermatozóides modificados e que esta divergência está associada à mudança no tipo de fertilização encontrada nestes dois grupos.

A revisão sobre o conhecimento da estrutura dos espermatozóides de diversos grupos de peixes, tanto da superclasse Agnatha quanto da Gnathostomata apresentada por Jamieson (1991), assinala modificações estruturais que ocorreram em cada um desses grupos. Nesta revisão, o autor considera que a classificação dos espermatozóides em primitivos e modificados proposta por Franzén (1970), mostra-se equivocada para os espermatozóides dos peixes. Isto porque os espermatozóides tidos como primitivos estão presentes em grupos de peixes "superiores", enquanto grupos "inferiores" apresentam espermatozóides estruturalmente considerados avançados. Desta forma, Jamieson (1991) sugere uma classificação para os espermatozóides dos peixes em *aquasperm* e *introsperm*, para designar, respectivamente, os espermatozóides dos peixes com fertilização externa,

liberados no meio aquático, e os dos peixes de fertilização interna, liberados diretamente no trato reprodutivo da fêmea.

Jamieson (1991) considera que o espermatozóide encontrado na maioria dos Neopterygii corresponde a um típico espermatozóide aquático uniflagelado (*aquasperm*), que apresenta, em geral, em uma seqüência anterior-posterior: núcleo esférico, sem vesícula acrossomal, presença de fossa nuclear, centríolos proximal e distal freqüentemente arranjados em ângulo reto e inseridos na fossa nuclear; centríolo distal se diferenciando em corpúsculo basal e desenvolvendo o axonema, mitocôndrias arredondadas e pouco numerosas e flagelo contendo o axonema clássico (9+2). A perda do acrossoma neste grupo é um caráter que o distingue dos demais grupos de peixes e vem acompanhada da presença da micrópila nos ovos destes animais. A micrópila é uma abertura no envoltório do ovo que permite a passagem do espermatozóide no momento da fertilização (Amanze e Yvengar, 1990). Para Jamieson (1991), os espermatozóides dos Neopterygii podem ter se desenvolvido secundariamente a partir de espermatozóides mais complexos encontrados nos grupos de peixes mais basais.

Mattei (1991), no estudo sobre a ultra-estrutura dos espermatozóides em peixes pertencentes à superclasse Gnathostomata, incluindo Chondrichthyes e Ostheichthyes, e suas implicações sistemáticas, revela que é imensa a diversidade de formas encontrada nos espermatozóides dos peixes, não sendo possível estabelecer um único modelo para esses gametas, como é o caso, por exemplo, dos mamíferos. Nesse estudo, não há qualquer proposta de classificação para os espermatozóides dos peixes. Com base nas comparações realizadas entre os espermatozóides dos grupos analisados, dois caracteres, considerados novos, foram encontrados nos Neopterygii, a redução no tamanho do núcleo e a perda do acrossomo, sendo esse último também evidenciado por Jamieson (1991). Estas duas características associadas à presença de uma peça intermediária curta, ocorrendo já no Actinopterygii primitivo, produzem um espermatozóide cuja estrutura simplificada é bastante similar a dos espermatozóides dos invertebrados aquáticos com fertilização externa considerados como sendo do tipo primitivo por Franzén (1970).

Mattei (1991) sugere que a estrutura dos espermatozóides encontrada nos Neopterygii, em geral, consiste em um gameta sem acrossomo e uniflagelado. Esta

estrutura corresponde ao espermatozóide aquático (*aquasperm*) descrito por Jamieson (1991). No entanto, há grupos em Neopterygii, em particular nos Teleostei primitivos, em que são encontradas variações na estrutura dos seus espermatozóides. Como exemplos que chamam a atenção, têm-se a ocorrência de espermatozóides aflagelados em Osteoglossomorpha e de espermatozóides com flagelo constituído por 9+0 microtúbulos em Elopomorpha (Jamieson, 1991; Mattei, 1991).

Embora os estudos de Jamieson (1991) e Mattei (1991) forneçam dados sobre ultra-estrutura dos espermatozóides de praticamente todos os grandes grupos de peixes vivos, os espermatozóides dos teleósteos neotropicais de água doce são pouco conhecidos.

1.7. Implicação filogenética dos caracteres reprodutivos masculinos em peixes

O conhecimento atual sobre o padrão de relacionamento entre os Siluriformes tem sido inferido com base em caracteres morfológicos, com ênfase no esqueleto (de Pinna, 1998; Britto, 2003). Contudo, outros caracteres, relacionados aos aspectos reprodutivos das espécies de peixes, parecem ser potencialmente úteis no estudo do grupo, uma vez que várias dessas características podem conter traços filogenéticos (Jamieson, 1991; Mattei, 1991; Spadella *et al.*, 2006a, b).

De acordo com Loir *et al.* (1989), a presença de regiões anatomicamente diferenciadas nos testículos, em especial nos Siluriformes, quanto à função, estruturas secretoras anexas e a presença de porções armazenadoras de espermatozóides, constituem uma outra fonte de informação filogenética e deve ser melhor explorada. Parenti e Grier (2004), em um recente estudo sobre a evolução e filogenia da morfologia da gônada em peixes ósseos, mostram que a organização do compartimento germinativo no interior dos testículos, em lóbulos ou em túbulos, também encerra informações de caráter filogenético, verificando que a estrutura gonadal dos Teleostei mostra diferenças marcantes entre os grupos mais basais e os grupos mais derivados, os Neoteleostei.

Em relação à ultra-estrutura da espermiogênese e dos espermatozóides, é possível obter um elevado número de características, qualitativas e quantitativas, que podem ser extraídas em análises dessa natureza. Dentre estas características, pode-se citar: o tipo de espermiogênese, a forma e dimensão do núcleo, o padrão de compactação da cromatina, a

presença ou não de fossa nuclear, canal citoplasmático e de projeções laterais nos flagelos, forma e distribuição de mitocôndrias e a presença de vesículas na peça intermediária, entre outros. Entende-se por caracteres qualitativos as características descritas por palavras, como, por exemplo, peça intermediária simétrica ou assimétrica, mitocôndrias arredondadas ou alongadas. Já os caracteres quantitativos representam as características descritas por meio de medidas, como comprimento e largura da peça intermediária, altura da fossa nuclear (Thiele, 1993). Em análises filogenéticas, os caracteres quantitativos são geralmente codificados como caracteres discretos, ou seja, de forma qualitativa, com o objetivo de facilitar as comparações entre as variáveis quantitativas e qualitativas.

Dessa forma, os estudos sobre a ultra-estrutura da espermiogênese e dos espermatozóides em peixes, vêm sendo direcionados no sentido da sua utilização em análises filogenéticas, podendo acrescentar dados importantes na elucidação de padrões de relacionamento em diversos grupos de peixes (Jamieson, 1991, Mattei, 1991, Spadella, 2004, Spadella et al., 2006a). Atualmente alguns grupos são suportados com base na estrutura de seus espermatozóides. Como exemplo, tem-se a monofilia da superordem Elopomorpha que é corroborada por cinco sinapomorfias derivadas da estrutura dos espermatozóides (Jamieson, 1991).

O emprego da ultra-estrutura dos espermatozóides em análises filogenéticas de outros grupos de vertebrados também é apresentado em vários trabalhos (Jamieson, 1991; Garda, 2002; Teixeira, 2003). Para diferentes gêneros de lagartos da família Teiidae, Teixeira (2003) realiza uma análise filogenética com base em 24 caracteres da ultra-estrutura dos espermatozóides, mostrando que o uso deste dado é um bom indicador de filogenia da família Teiidae, apresentando poucas regiões conflitantes e de pouco suporte com a topologia com base em outros caracteres morfológicos.

A espermiogênese e a ultra-estrutura dos espermatozóides têm sido estudadas em vários grupos de peixes (Jamieson, 1991; Mattei, 1970; 1991) e seu emprego na identificação do padrão de relacionamento tem sido amplamente reconhecido. Com relação aos Siluriformes, não pertencentes à Loricarioidea, encontram-se disponíveis, atualmente, descrições detalhadas dos espermatozóides das seguintes famílias de Siluriformes: Diplomystidae (Quagio-Grassiotto *et al.*, 2001); Cetopsidae (Spadella *et al.*, 2006a);

Amblycipitidae (Lee e Kim, 1999); Aspredinidae (Mansour e Lahnsteiner, 2003; Spadella, *et al.*, 2006a); Loricariidae (Mansour e Lahnsteiner, 2003); Clariidae (Mansour *et al.*, 2002); Siluridae (Emel'yanova e Makeyeva, 1992; Kwon *et al.*, 1998; Lee e Kim, 2001); Ictaluridae (Poirier e Nicholson, 1982; Emel'yanova e Makeyeva, 1991, 1992); Auchenipteridae (Burns *et al.*, 2002; Burns e Weitzman, 2005); Pimelodidae (Quagio-Grassiotto e Carvalho, 2000; Quagio-Grassiotto e Oliveira, manuscrito submetido); Bagridae (Emel'yanova e Makeyeva, 1992; Lee, 1998; Kim e Lee, 2000; Mansour e Lahnsteiner, 2003); Pseudopimelodidae e Heptapteridae (Quagio-Grassiotto *et al.*, 2005) e Malapteruridae (Shahin, 2006). Informações não detalhadas estão disponíveis sobre os espermatozóides de Heteropneustidae (Nath e Chand, 1998); Mochokidae (desenhos esquemáticos, Mattei, 1991); Doradidae (Quagio-Grassiotto, 2002); Schilbidae (desenhos esquemáticos, Mattei, 1991); Ariidae (desenhos esquemáticos, Mattei, 1991); e *Conorhynchus conirostris* (Lopes *et al.*, 2004). Não existem dados sobre os espermatozóides dos Amphiliidae, Chacidae, Plotosidae, Auchenoglanidinae, Austroglanididae, *Horabagrus*, Cranoglanididae, Pangasiidae, Claroteinae e Anchariidae.

Em especial, às famílias da superfamília Loricarioidea, têm-se disponíveis, até o momento, descrições dos espermatozóides das espécies: *Nematogenys inermis*, da família Nematogenyidae (Spadella et al., 2006a); *Trichomycterus* aff. *iheringi*, *T. areolatus*, *T. reinhardti*, *Trichomycteurs* sp. (Spadella, 2004), da família Trichomycteridae; *Corydoras flaveolus*, da família Callichthyidae (Spadella, 2004); *Scoloplax distolothrix*, da família Scoloplacidae (Spadella *et al.*, 2006b) e *Loricariichthys platymetopon* (Spadella, 2004) e *Ancistrus triradiatus* (Mansour e Lahnsteiner, 2003), da família Loricariidae.

As descrições dos espermatozóides dos Loricarioidea somadas aos dados disponíveis na literatura de outras famílias de Siluriformes representam ainda um número pequeno de descrições em relação ao grande número de espécies e de famílias que compõem essa ordem, tornando, portanto, limitada uma discussão geral sobre a evolução dessas características no grupo.

Assim, a ultra-estrutura da espermiogênese e dos espermatozóides de representantes da superfamília Loricarioidea poderá ser bastante útil para testar hipóteses de relacionamento entre os Loricarioidea e também servirem de base para estudos mais

abrangentes em Siluriformes, procurando avaliar a real extensão da variabilidade deste tipo de caráter e o nível taxonômico em que eles possam ser mais informativos.

2. OBJETIVOS

A presente tese se insere em um programa geral de estudo da superfamília Loricarioidea, intitulado "Filogenia e evolução de Loricarioidea: uma abordagem multidisciplinar", cujo objetivo é ampliar o conhecimento sobre a diversidade e os padrões de relacionamento neste grupo. Este trabalho, em particular, contribuiu com a obtenção de um conjunto de caracteres referentes à ultra-estrutura da espermiogênese e dos espermatozóides de representantes de Loricarioidea, bem como de outras famílias de Siluriformes que servirão como grupos externos. Assim, os objetivos deste trabalho foram:

1. Descrever, por meio de análises à microscopia eletrônica de transmissão, as características ultra-estruturais da espermiogênese e dos espermatozóides de espécies de Loricarioidea e de grupos externos significativos, disponibilizando um conjunto de caracteres para esse clado e procurando avaliar o grau de variabilidade encontrada nesses caracteres reprodutivos em relação à diversidade de espécies do grupo;

2. Propor uma lista de caracteres morfológicos que reflita as características ultra-estruturais da espermiogênese e dos espermatozóides de espécies da superfamília Loricarioidea, bem como de outras espécies da ordem Siluriformes;

3. Realizar estudos comparativos com base na ultra-estrutura da espermiogênese e dos espermatozóides, visando a identificação de caracteres particulares e compartilhados pelas famílias de Loricarioidea e elaborar hipóteses sobre a filogenia dos Loricarioidea e de seus grupos constituintes com base nestes caracteres;

4. Comparar as hipóteses obtidas a partir dos dados de ultra-estrutura com a hipótese de relacionamento proposta por Britto (2003) para a ordem Siluriformes, particularmente para a superfamília Loricarioidea, elaborada com base em outros caracteres morfológicos, principalmente osteológicos e de morfologia externa, procurando verificar se há ou não congruência entre os diferentes conjuntos de caracteres.

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4. CAPÍTULOS

O trabalho desenvolvido na presente tese resultou na produção de nove manuscritos para publicação, referentes aos dados obtidos para cada família de Loricarioidea. A seguir, estes manuscritos serão apresentados, subdivididos em capítulos.

4.1. CAPÍTULO 1

Spadella, M.A., Oliveira, C., Quagio-Grassiotto, I., 2006. Occurrence of biflagellate spermatozoa in Cetopsidae, Aspredinidae, and Nematogenyidae (Teleostei: Ostariophysi: Siluriformes). Zoomorphology 125: 135-145.

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ORIGINAL ARTICLE

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Occurrence of biflagellate spermatozoa in Cetopsidae, Aspredinidae, and Nematogenyidae (Teleostei: Ostariophysi: Siluriformes)

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Abstract In the present study spermiogenesis was investigated in *Cetopsis coecutiens* (Cetopsidae), and *Bunocephalus amazonicus* (Aspredinidae), while spermatozoa ultrastructure was investigated in *C. coecutiens*, *B. amazonicus*, and *Nematogenys inermis* (Nematogenyidae). Aspredinidae and Cetopsidae share a spermatogenesis of the semicystic type, and a particular type of spermiogenesis process not reported in any fish group. In the three species analyzed, spermatozoa are biflagellate with flagella having the classical axoneme formulae (9 + 2). The analysis of thirteen characters showed the presence of eight characters shared by Cetopsidae and Aspredinidae, and six characters shared by Cetopsidae and Nematogenyidae, which may suggest that these three families may be more related than actually hypothesized, comprising a very primitive siluriform lineage originated after Diplomystidae.

Keywords Fish evolution · Catfish · Sperm · Semicystic spermatogenesis · Morphology

Introduction

Recent phylogenetic studies of siluriforms were conducted by Mo (1991), de Pinna (1998) and Britto (2003). A resumed phylogeny is presented in Fig. 1 (redrawn from Britto 2003). Basically, the only difference between this phylogeny and the one presented by de Pinna (1998) is the position of Pseudopimelodidae, which, according to Britto (2003), is a sister group of Heptapteridae (included in "other Siluriformes" in Fig. 1). According to de Pinna (1998) it is the sister group of the clade composed of Sisuroidea, Amphiliidae, and Loricarioidea. There is a general understanding that Diplomystidae is the most primitive siluriform, followed by Cetopsidae. The remaining siluriforms belong to two lines: the first, composed of Sisuroidea (Amblycipitidae, Akysidae, Aspredinidae, Erethystidae, and Sisoridae), Loricarioidea (Nematogenyidae, Trichomycteridae, Callichthyidae, Scoloplacidae, Astroblepidae, and Loricariidae), and Amphiliidae, and the second, composed of the other siluriforms (Britto 2003).

Ultrastructural studies of fish spermatozoa have shown that characteristics of the spermatozoa structure and spermiogenesis process may be phylogenetically analyzed providing important data for the elucidation of relationship patterns in several fish groups (Jamieson 1991; Mattei 1991). Although the studies by Jamieson (1991) and Mattei (1991) present data related to spermatozoa structure of practically all major fish groups, the information about siluriform spermatozoa is still quite incomplete, since there are data lacking for several families and absence of data for several families (Quagio-Grassiotto et al. 2001; Santos et al. 2001). Studies of spermiogenesis and spermatozoal ultrastructure in *Diplomystes mesembrinus*, member of the most primitive siluriform family, have shown that many of the characteristics found in this species are not found in other groups of Siluriformes or Ostariophysi (Quagio-Grassiotto et al. 2001).

In the current study, the characterization of spermiogenesis and spermatozoa in specimens of three neotropical siluriform families: Cetopsidae, Aspredinidae, and Nematogenyidae are presented by the first time. The data were employed to test the hypothesis that these three families are more related among themselves than

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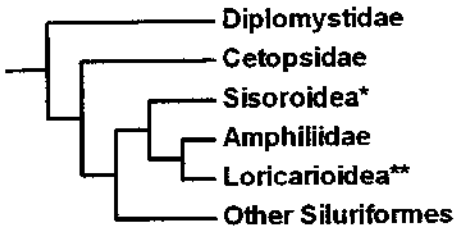

Fig. 1 Simplified cladogram of Siluriformes (redrawn from Britto 2003). *Sisuroidea is composed of Amblycipitidae, Akysidae, Aspredinidae, Erethystidae, and Sisoridae. **Loricarioidea is composed of Nematogenyidae, Trichomycteridae, Callichthyidae, Scoloplacidae, Astroblepidae, and Loricariidae

with other neotropical siluriform families. Additionally, comparisons between the data obtained in the present study and those available for other siluriform families were conducted in order to investigate the similarities and differences of spermatozoa ultrastructure among the families of this order.

Materials and methods

The present study was conducted with two adult males of *Cetopsis coecutiens* (Lichtenstein, 1819) (Cetopsidae) collected from the Araguaia river, Aragarças, Goiás, Brazil (16°00′ S 52°17′ W); three adult males of *Bunocephalus amazonicus* (Mees, 1989) (Aspredinidae) from a commercial aquarium shop; and two adult males of *Nematogenys inermis* (Guichenot, 1848) (Nematogenyidae) collected from the Aguas de la Gloria river, VIII Region, Aguas de la Gloria, Chile (36°50.304′ S 2°55.642′ W). The fishes were identified and kept in the fish collection of Laboratório de Biologia e Genética de Peixes (LBP), Departamento de Morfologia, Instituto de Biociências, UNESP, Botucatu, São Paulo, Brazil.

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 for 2 h in the dark in 1% osmium tetroxide 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.

For comparative purposes, all the available information about siluriform spermatozoa was reviewed. Data were obtained for the following families: Amblycipitidae (Lee and Kim 1999), Auchenipteridae (Burns et al. 2002), Bagridae (Emel'yanova and Makeyeva 1991a; Lee 1998; Kim and Lee 2000; Mansour and Lahnsteiner 2003), Clariidae (Mansour et al. 2002), Diplomystidae (Quagio-Grassiotto et al. 2001), Ictaluridae (Poirier and Nicholson 1982; Emel'yanova and Makeyeva 1991a, b), Loricariidae (Mansour and Lahnsteiner 2003), Pimelodidae (Quagio-Grassiotto and Carvalho 2000; Santos et al. 2001), and Siluridae (Emel'yanova and Makeyeva 1991a; Kwon et al. 1998; Lee and Kim 2001). The available information regarding the families Ariidae and Malapteruridae are restricted to some schematic drawings (Mattei 1991). However, they were included, since both families present species with biflagellate spermatozoa.

Thirteen characters, present in at least one family analyzed in the present study, were employed in the comparative analyses (Table 1). Based on the siluriform

Table 1 General view of the distribution of spermatozoa character states analyzed in the present study

Families	Character																		
	1	2	3	4	5a	5b	5c	6a	6b	7	8	9	10a	10b	11a	11b	12a	12b	13a	13b
Cetopsidae	+	+	+	–	+	–	–	+	–	–	+	+	+	–	+	–	+	–	+	–
Aspredinidae	+	+	+	–	–	+	–	+	–	–	+	+	–	+	–	+	–	+	–	+
Nematogenyidae	+	+	+	–	–	–	+	–	+	+	–	–	–	+	+	–	+	–	–	–
Amblycipitidae	+	+	–	+	+	–	–	–	+	–	–	+	+	–	+	–	–	+	+	–
Ariidae	+	+	?	?	?	?	?	?	?	?	–	?	?	?	?	?	–	+	?	?
Malapteruridae	+	+	?	?	?	?	?	?	?	?	–	?	?	?	?	?	–	+	?	?
Ictaluridae	+	+	+	–	+	–	–	–	+	–	–	–	+	–	+	–	–	+	–	+
Bagridae	?	?	–	+	+	–	–	–	+	–	–	+	+	–	+	–	+	–	+	+
Pimelodidae	–	–	+	–	–	–	–	–	+	+	–	–	+	–	–	–	–	–	–	+
Diplomystidae	–	–	–	+	–	–	–	+	–	–	–	+	+	–	–	–	–	–	–	–
Siluridae	–	–	+	–	–	–	–	–	+	–	–	–	+	–	+	–	+	–	+	+
Clariidae	–	–	–	–	–	–	–	+	–	–	–	+	+	–	–	–	–	–	–	–
Loricariidae	–	–	–	–	–	–	–	+	–	–	–	+	+	–	–	–	+	–	–	+
Auchenipteridae	–	–	–	–	–	+	–	–	+	–	–	–	–	+	–	+	+	–	–	+

+ Present, – absent, ? unavailable or contradictory information

Presence of two flagella, *2* presence of lateral and parallel centrioles, *3* presence of elongated vesicles, *4* presence of lateral fins, *5* shape of the nucleus (*a* semi-ovoid, *b* conic, *c* ovoid with the larger axis in the horizontal position), *6* pattern of chromatin condensation (*a* heterogeneous, *b* homogeneous), *7* absence of nuclear fossa; *8* nuclear fossa as a simple arc in biflagellate spermatozoa, *9* centriole inserted in the nuclear fossa, *10* midpiece size (*a* short, *b* long), *11* cytoplasmic channel size (*a* short, *b* long), *12* number of cytoplasmic channels (*a* one, *b* two), *13* mitochondria shape (*a* rounded, *b* elongated)

spermatozoa described in the literature (Quagio-Grassiotto et al. 2001; Lee and Kim 1999; Mansour et al. 2002; Lee and Kim 2001; Kwon et al. 1998; Poirier and Nicholson 1982; Burns et al. 2002; Quagio-Grassiotto and Carvalho 2000; Santos et al. 2001; Lee 1998; Kim and Lee 2000), the midpiece size (character 10) was considered short when its total length was ≤ 1.7 μm, but considered long with the length > 1.7 μm. The cytoplasmic channel size (character 11) was considered short when its total length was ≤ 1.5 μm, but considered long with the length > 1.5 μm.

Results

Spermiogenesis in *C. coecutiens* and *B. amazonicus*

In the analyzed species, spermatids are found in the lumen of the germinative compartment together with the spermatozoa (Figs. 2a–c, 3a, b). The early spermatids are found in groups in the proximities of the spermatocytes cysts (Figs. 2a, b, 3a). During spermiogenesis, these spermatids move towards the lumen region of the central germinative compartment mixing with the spermatozoa (Figs. 2c, 3e). In the early spermatids, the centrioles lie medial to the nucleus, and anchors to the plasma membrane. The flagella development in the two centrioles occurs medial to the nucleus (Figs. 2d–f, 3c–e). The centrioles do not move towards the nucleus. They remain anchored to the plasma membrane. At the beginning of the spermiogenesis, the centrioles of *C. coecutiens* exhibit an oblique position in relation to their main axis. Along the process, they change their position adopting a lateral and parallel position (Fig. 2d, e). In *B. amazonicus* the centrioles are lateral and parallel to each other (Fig. 3c). During the spermiogenesis, nuclear rotation does not occur, and the flagella remain medial to the nucleus. A depression is formed in the nuclear outline that gives rise to the nuclear fossa. The nuclear fossa is medially positioned. At the time of the fossa formation, the basal region of the nuclei is projected into the direction of the basal bodies. At the end of this process, the centrioles longer than those usually found in fishes remain completely inserted in the nuclear fossa (Figs. 2g, h, 3e, f). The process of centrioles elongation occurs during the spermatogenesis, more precisely during the nuclear fossa formation. This elongation process may have some influence in the nuclear fossa formation. Thus, the final form of the nuclear fossa is possibly a consequence of the depression of the nuclear outline, projection of the basis of the nucleus, and elongation of the basal bodies. The chromatin is heterogeneously condensed in the form of thin filaments juxtaposed in *C. coecutiens* and in the form of condensed clusters in *B. amazonicus* (Figs. 2g, g-inset, h; 3e–g). Chromatin condensation process begins at the basal position of the nucleus, and proceeds until the apical region. Although the centrioles do not move toward the nucleus, the formation of a cytoplasmic channel occurs. In *C. coecutiens*, only one cytoplasmic channel is formed, while two are formed in *B. amazonicus* (Figs. 2g, h, 3d). The cytoplasmic mass moves around the initial segment of the tail, and gives rise to the midpiece of the future spermatozoon. The midpiece has rounded to elongate mitochondria and vesicles (Figs. 2f–h, 3e–g). The flagella have a classical (9 + 2) axoneme without lateral projections or fins (Figs. 2g, h, 3b, g).

Spermatozoa of *C. coecutiens*

Spermatozoa of *C. coecutiens* are found in the lumen of the germinative compartment, either isolated or in groups, with variable number of cells (Fig. 4d). The interaction points among the membranes of different spermatozoa are in general electron-dense (Fig. 4b, d, f). The spermatozoon of this species exhibits a semi-ovoid head, a symmetric midpiece, and two flagella medial to the nucleus (Fig. 2a). They show no acrosomal vesicle. The nuclei with semi-ovoid shape are 1.3 μm in length, and 1.4 μm in width. The chromatin is heterogeneously condensed, and in the form of thin, juxtaposed filaments, interspersed by few electron-lucent areas (Fig. 4a–g). No organelles are seen in the cytoplasmic region, apical to the nucleus (Fig. 4e, f). The nuclear fossa is 0.6 μm in length, and 0.5 μm in width. It is medial to the nucleus, and has an in simple arc shape (Fig. 4f). The midpiece is 0.5 μm in length, and 1.7 μm in width. A few rounded mitochondria are encountered in the apical and medial regions of the midpiece, peripherally distributed in these regions (Fig. 4d–g). Many elongated vesicles are found in all regions of the midpiece, concentrated mainly on the basal region. They might be isolated or interconnected among themselves, forming a membranous compartment (Fig. 4h–j). The mitochondria are separated from the flagella by the cytoplasmic channel (Fig. 4i). The cytoplasmic channel is 0.4 μm in length, and 0.5 μm in width. The centrioles are lateral and parallel to each other, and found completely inserted in the nuclear fossa (Fig. 4c, f). Both centrioles become basal bodies, and give rise to an axoneme that exhibits the classical 9 + 2 microtubular pattern. In all segments of the tail, the axonemes are individualized and separated so as not to partake in the same membrane. No flagellar lateral projections or fins are present (Fig. 4k, l).

Spermatozoa of *B. amazonicus*

Spermatozoa of *B. amazonicus* exhibit a conic head, a symmetric midpiece, and two flagella medial to the nucleus (Fig. 5a, b). They show no acrosomal vesicle. The nuclei with conic shape are 1.9 μm in length, and 1.6 μm in width. The chromatin is heterogeneously condensed, and in the form of chromatin clusters (Fig. 5a–d). The cytoplasmic region around the nucleus is narrow, and

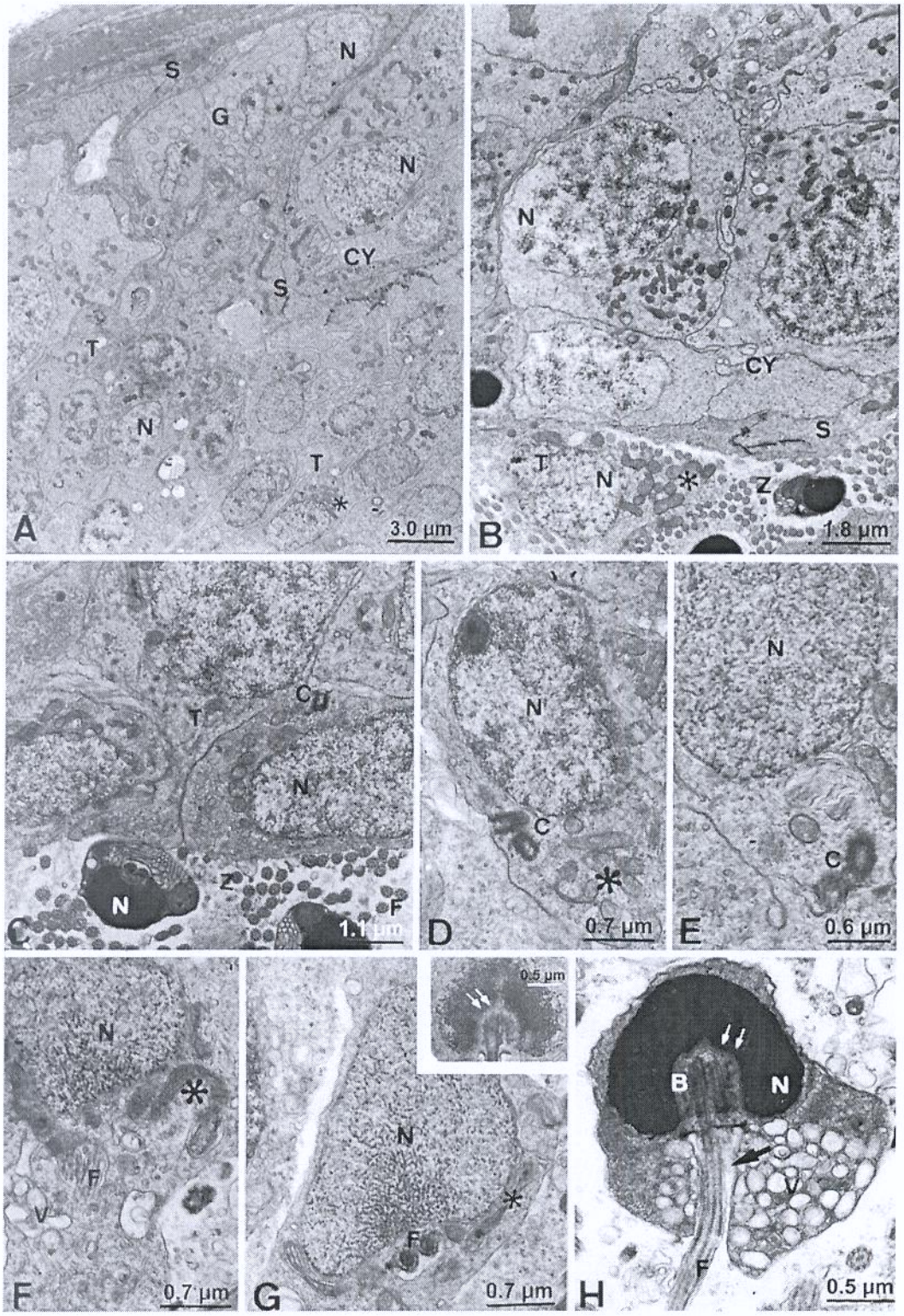

Fig. 2 Spermiogenesis of *Cetopsis coecutiens*: **a** Spermatids group near spermatocytes cysts. **b** Details of the proximity between the spermatids and spermatocytes cysts. **c** Spermatids together with the spermatozoa in the lumen of the germinative compartment. **d**, **e** Details of the centrioles position. **f** Midpiece in formation showing mitochondria and vesicles. **g** and (g-inset) Nucleus showing the chromatin process of condensation. **h** Late spermatid. *B* basal bodies, *C* centrioles, *CY* spermatocytes cysts, *F* flagella, *G* spermatogonia cysts, *N* nucleus, *S* Sertoli cell, *T* spermatids, *V* vesicles, *Z* spermatozoon, *Arrow* cytoplasmic channel, *Double arrow* nuclear fossa, *Asterisk* mitochondria.

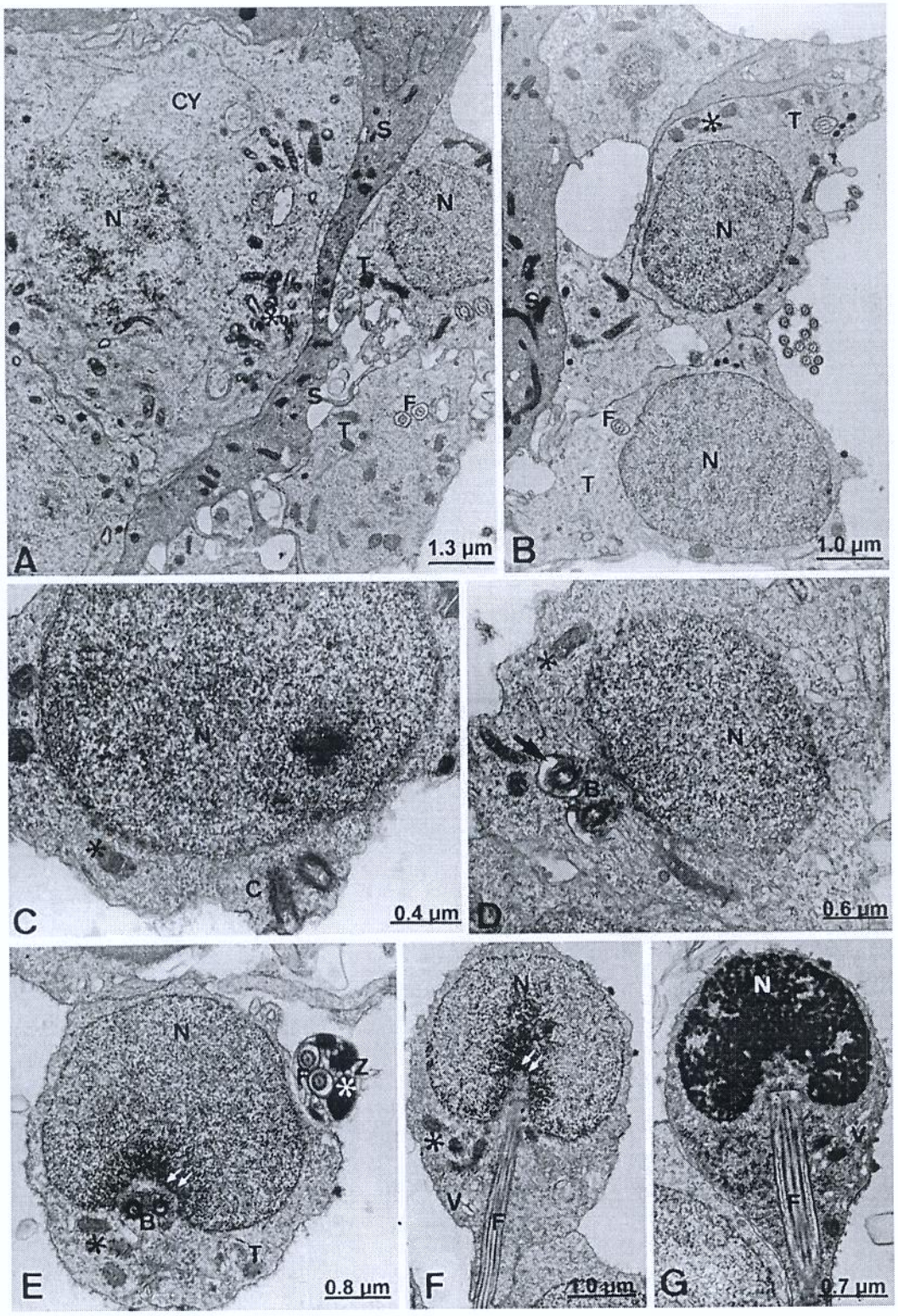

Fig. 3 Spermiogenesis of *Bunocephalus amazonicus*: **a** Spermatids near spermatocytes cyst. **b** Spermatids in the lumen of germinative compartment. **c** Details of the centrioles arrangement. **d** Early spermatids with the midpiece in formation showing mitochondria and cytoplasmic channel. **e**, **f** Early spermatids showing the chromatin condensation process in the nucleus, and mitochondria and vesicles in the midpiece. **g** Late spermatid. *B* basal bodies, *C* centrioles, *CY* spermatocytes cysts, *E* electron-lucent area, *F* flagella, *N* nucleus, *S* Sertoli cell, *T* spermatids, *V* vesicles, *Z* spermatozoon, *Arrow* cytoplasmic channel, *Double arrow* nuclear fossa, *Asterisk* mitochondria

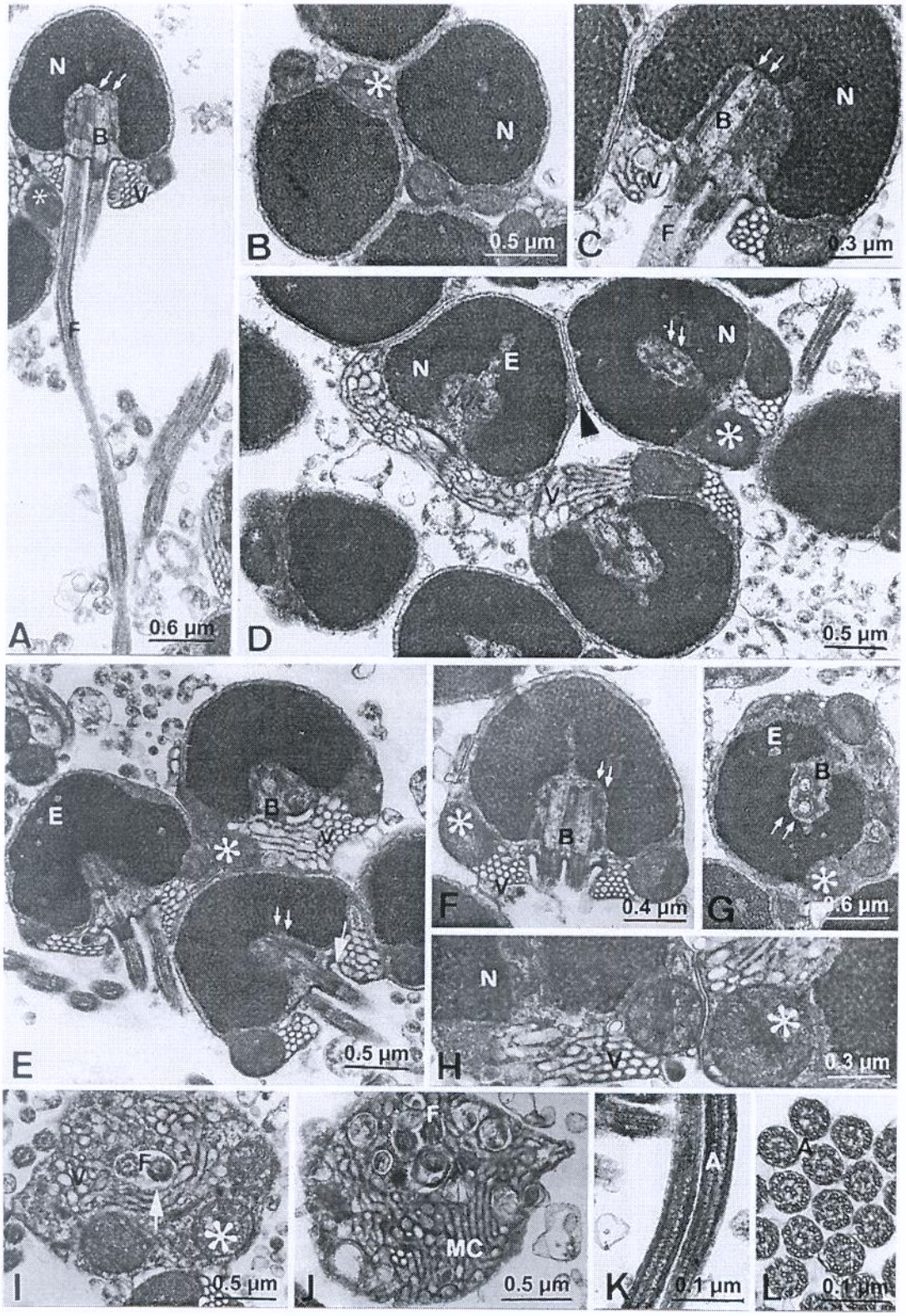

Fig. 4 Spermatozoa of *C. coecutiens*: **a** Spermatozoon longitudinal section. **b** Head region. **c** Centrioles arrangement. **d, e** Spermatozoa groups showing interaction points between plasmic membranes. **f** Spermatozoon longitudinal section showing nuclear fossa in the nucleus, and cytoplasmic channel, mitochondria and vesicles in the midpiece. **g–j** Midpiece sections showing mitochondria and vesicles. **k** Flagella longitudinal section. **l** Flagella cross-section. *A* axoneme, *B* basal bodies, *E* electron-lucent area, *F* flagella, *MC* membranous compartment, *N* nucleus, *V* vesicles, *Arrow* cytoplasmic channel, *Arrowhead* interaction points, *Double arrow* nuclear fossa, *Asterisk* mitochondria

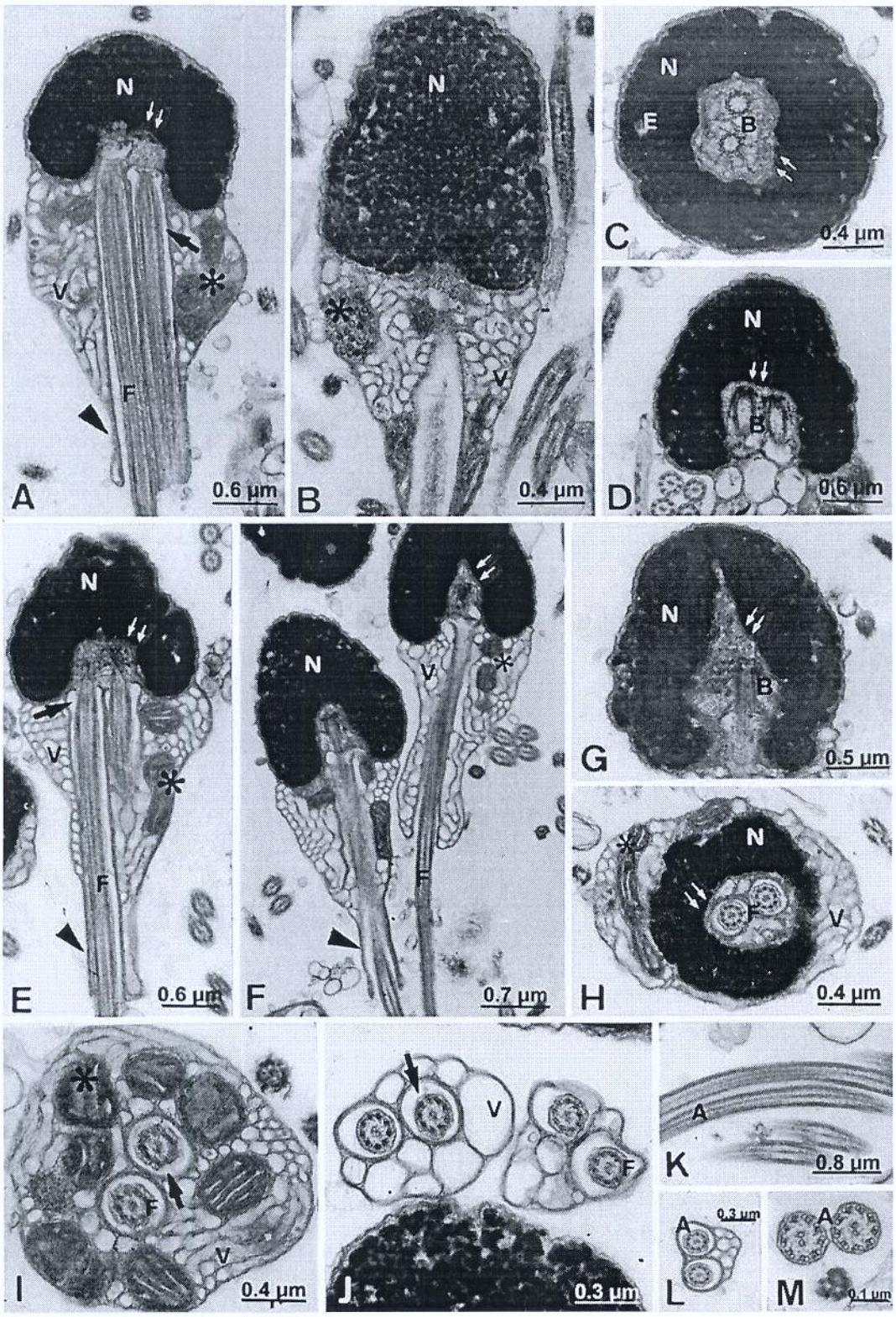

Fig. 5 Spermatozoa of *B. amazonicus*: **a**, **b** Spermatozoa longitudinal sections. **c** Head cross section. **d** Centrioles arrangement. **e**, **f** Spermatozoa longitudinal sections showing mitochondria, vesicles and cytoplasmic sheath of the midpiece. **g** Details of the nuclear fossa. **h–j** Midpiece cross sections. **k** Flagella longitudinal section. **l**, **m** Flagella cross sections. *A* axoneme, *B* basal bodies, *E* electron-lucent area, *F* flagella, *MC* membranous compartment, *N* nucleus, *V* vesicles, *Arrow* cytoplasmic channel, *Arrowhead* cytoplasmic sheath, *Double arrow* nuclear fossa, *Asterisk* mitochondria

organelles are not found in it (Fig. 5e–F). The nuclear fossa is 1.2 μm in length, and 0.7 μm in width. It is medial to the nucleus and lain in simple arc shape (Fig. 5g). The midpiece is 2.6 μm in length, 1.7 μm in width, and exhibits a cytoplasmic sheath (Fig. 5e, f). Few elongated mitochondria are found in the apical and medial regions of the midpiece. They are distributed central and peripherally in these regions (Fig. 5e, f, h, i). Many elongated vesicles, either isolated or interconnected, are found in all regions of the midpiece (Fig. 5e, f, h–j). The mitochondria are separated from the flagella by the cytoplasmic channels (Fig. 5i). The cytoplasmic channels are 2.8 μm in length, and 0.6 μm in width. The centrioles are lateral and parallel to each other, and are found completely inserted in the nuclear fossa (Fig. 5d). Both centrioles are differentiated into basal bodies and give rise to an axoneme exhibiting the classical 9 + 2 microtubular pattern. In all segments of the tail, the axonemes are individualized. No flagellar lateral projections or fins are present (Fig. 5k–m).

Spermatozoa of *N. inermis*

Spermatozoa of *N. inermis* display the head and the midpiece joined in a single structure, with no clear delimitation between them, and two flagella medial to the nucleus (Fig. 6a). They do not show acrosomal vesicle. The ovoid nucleus has the larger axis displayed in the horizontal position and is 1.6 μm in length, and 2.8 μm in width. The nucleus contains highly condensed homogeneous chromatin interspersed with some electron-lucent areas. In the peripheral electron-lucent areas, small chromatin clusters are present (Fig. 6a–c). In the cytoplasmic region, apical to the nucleus, no organelles are seen. The nuclear fossa is absent (Fig. 6a). The midpiece is 2.0 μm in length, and 3.9 μm in width, and shows a radial symmetry with the flagellum axis. Surrounding the basal region of the nucleus and filling the midpiece, a large number of very long, sometimes ramified mitochondria, radially arranged are found (Fig. 6a, d, e). In the basolateral region of the midpiece base, several elongate and interconnected vesicles form a membranous compartment (Fig. 6a, e, f). The cytoplasmic channels are 0.2 μm in length, and 0.9 μm in width. The centrioles are lateral, parallel to one another, and positioned distal to the midpiece region. They are anchored by cytoskeletal components (Fig. 6a, e-inset). Each centriole is differentiated into a basal body, and gives rise to an axoneme exhibiting the classical 9 + 2 microtubular pattern. The axonemes are individualized in the initial segment of the tail, and separated from the elongated distal vesicles of the midpiece by the cytoplasmic channel. The axonemes are not individualized in the other segments of the tail, and share the same flagellar membrane. No flagellar lateral projections or fins are present (Fig. 6g–j).

Discussion

Spermiogenesis

The occurrence of spermatids in the lumen of the germinative compartment in Cetopsidae and Aspredinidae, together with the spermatozoa, suggests that spermatogenesis in these families are of the semicystic type. The semicystic spermatogenesis is uncommon among Teleostei and has been described in very few groups as Opheliidae (Mattei 1993), Scorpaenidae (Muñoz et al. 2002), and Bleniidae (Lahnsteiner and Patzer 1990).

In the spermatozoa, the flagellum axis may be either perpendicular or parallel to the nucleus, depending on whether nuclear rotation during spermiogenesis occurs (type I spermiogenesis) or not (type II spermiogenesis) (Mattei 1970). In the Pimelodidae the flagellum is medial, the nucleus does not rotate, and both the nuclear fossa and the cytoplasmic channel are absent during spermiogenesis, characterizing a third type of spermiogenesis (I. Quagio-Grassiotto and C. Oliveira, submitted). The spermiogenesis process observed in Cetopsidae and Aspredinidae is characterized by a medial development of the flagella, the absence of nuclear rotation, a medial nuclear fossa formation, a cytoplasmic channel formation, and by the absence of centriolar migration. This set of characteristics is different from those previously described. The unusual spermiogenesis found in Cetopsidae and Aspredinidae may represent a synapomorphy evolved in a common stem lineage of both taxa or it independently evolved in each group. It then was an autapomorphy of either taxon.

It is possible that the existing cytoplasmic channel is a result of the accommodation and interconnection of the vesicles around the flagella of Cetopsidae, Aspredinidae and Nematogenyidae, instead of the movement of the centrioles toward the nucleus.

Spermatozoa

The comparative analyses of siluriform spermatozoa ultrastructure showed that some characteristics are only found in Aspredinidae, for example, the presence of cytoplasmic sheath in the midpiece. Some are only found in Nematogenyidae, such as the presence of very long, sometimes ramified mitochondria, centrioles or basal bodies positioned far from the nucleus, and in some segments of the axonemes sharing the same flagellar membrane. There was not any characteristic exclusively found in the spermatozoa of Cetopsidae.

The main characteristic found in the present study was the presence of two flagella in the three species analyzed. In teleosts, the flagellum is usually single (Jamieson 1991; Mattei 1991). In Cetopsidae, Aspredinidae and Nematogenyidae, spermatozoa are biflagellate, with the flagella medial to the nucleus. The same happens in Amblycipitidae (Lee and Kim 1999), Ariidae and Mal-

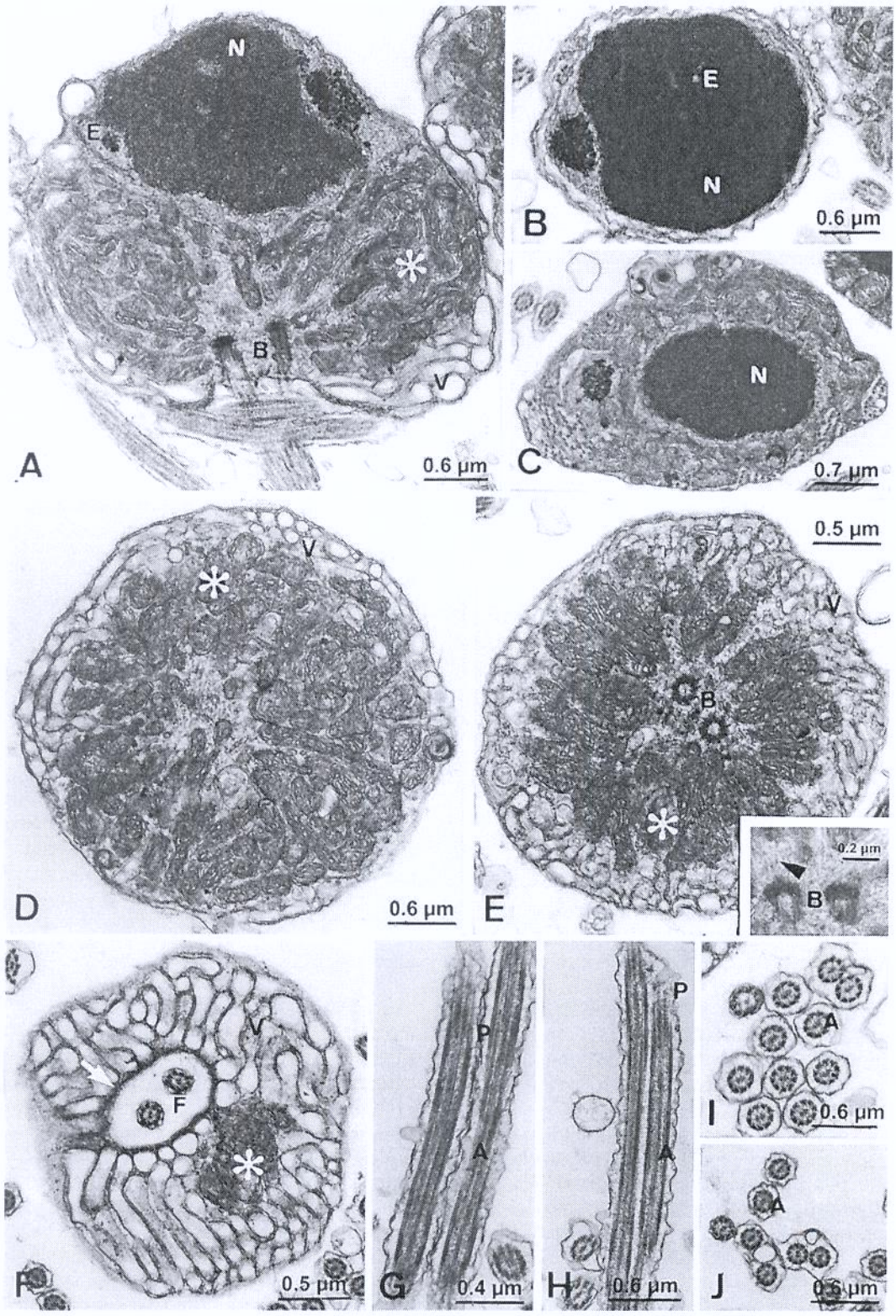

Fig. 6 Spermatozoa of *Nematogenys inermis*: **a** Spermatozoon longitudinal section. **b**, **c** Head cross sections; **d**–**f** Midpiece cross sections. (**e**-inset) Details of centrioles position. **g**, **h** Individualized and associated axonemes (*longitudinal sections*). **i**, **j** Individualized and associated axonemes (*cross sections*). *A* axoneme, *B* basal bodies, *E* electron-lucent area. *F* flagella, *N* nucleus, *P* plasmic membrane, *V* vesicles, *Arrowhead* cytoskeletal components, *Asterisk* mitochondria

apteruridae (Mattei 1991), and Ictaluridae (Poirier and Nicholson 1982; Emel'yanova and Makeyeva 1991a, b).Mansour and Lahnsteiner (2003) described the occurrence of biflagellate spermatozoa in Aspredinidae and Bagridae. However, two previous studies with species of Bagridae described their spermatozoa as uniflagellate (Lee 1998; Kim and Lee 2000). In the species of all other siluriforms studied, such as Diplomystidae, there is only one flagellum (Quagio-Grassiotto et al. 2001; Mansour and Lahnsteiner 2003) (Table 1).

Although the lack of information on spermiogenesis and spermatozoa ultrastructure of siluriforms prevents a more accurate investigation, stalling the use of modern methods of parsimony, the comparative analysis presented in Table 1 point to some interesting features. Among them the 13 spermatozoa characters depicted that the spermatozoa of the Cetopsidae are more similar to those of the Amblycipitidae, Aspredinidae, and Ictaluridae, sharing eight similar characteristics (Table 1). Furthermore, the spermatozoa of the Cetopsidae share seven characteristics with Bagridae and Siluridae and six characteristics with Nematogenyidae (Table 1). The spermatozoa of Nematogenyidae are more similar to those of the Aspredinidae, sharing six characteristics (Table 1).

As discussed above, Table 1 shows that the spermatozoa of the Cetopsidae, Aspredinidae, Amblycipitidae, Ictaluridae, and Nematogenyidae share more similar characteristics among themselves than with any other siluriform. This observation is only partially in accordance with the phylogeny proposed by Britto (2003) (Fig. 1). Besides the characters presented in Table 1, Aspredinidae and Cetopsidae also share a spermatogenesis of the semicystic type, and a particular type of spermiogenesis process not reported in any fish group, as discussed above. Thus, the position of Aspredinidae may be different from that proposed by de Pinna (1998) and Britto (2003). It is important to notice that according to de Pinna (1996; 1998) and Britto (2003), Aspredinidae is the only Sisuroidea found in South America, as all remaining families are found in Africa.

The position of Nematogenyidae is also controversial, because the spermatozoa of this group share only one characteristic with those of Loricariidae, the second representative of Loricarioidea with spermatozoa ultrastructure published (Table 1). Moreover, the main characteristics found in the Nematogenyidae spermatozoa are quite different from those of the Trichomycteridae, Scoloplacidae, and Callichthyidae (our unpublished data). The presence of eight characteristics shared by Cetopsidae and Aspredinidae and six characters shared by Cetopsidae and Nematogenyidae (Table 1) may suggest that these three families (only found in South America) belong to a very primitive siluriform lineage originated after Diplomystidae, the most primitive siluriform (de Pinna 1998; Britto 2003), which nowadays is also found only in South America.

Further studies characterizing the spermiogenesis and the spermatozoa ultrastructure of additional siluriform families will be very useful for a better understanding of the relationship among the Siluriformes families, and the evolutionary transformation of the male germinative cells in fishes.

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4.2. CAPÍTULO 2

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

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Spermiogenesis and introsperm ultrastructure of *Scoloplax distolothrix* (Ostariophysi: Siluriformes: Scoloplacidae)

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Abstract

Spadella, M. A., Oliveira, C. and Quagio-Grassiotto, I. 2006. Spermiogenesis and introsperm ultrastructure of *Scoloplax distolothrix* (Ostariophysi: Siluriformes: Scoloplacidae). — *Acta Zoologica* (Stockholm) 87: 341–348

Spermiogenesis in scoloplacids is characterized by initial lateral development of the flagellum, nuclear rotation, medial nuclear fossa formation, complex centriolar migration, and cytoplasmic channel formation. The scoloplacid spermiogenesis is similar to those found in Diplomystidae, the most primitive siluriform family. The scoloplacid spermatozoa have all the main characteristics of introsperm. They exhibit a conic head, a symmetric midpiece, a medial flagellum, and no acrosome. The conic forward-elongated nuclei contain homogeneous chromatin. The thin extremity of the nuclei is strongly curved and along its internal face there is a well-developed membranous compartment. The centrioles are completely inside the medial nuclear fossa, perpendicular to each other and with an electron-dense material between them. In a cross view of the midpiece, the mitochondria form a ring surrounding internally the cytoplasmic channel, and in a longitudinal view they are organized in a row along it. Several elongated vesicles are distributed peripherally, mainly concentrated in the mid-piece basal region. The flagellum contains the classical axoneme (9 + 2) and has two lateral projections or fins. The spermatozoa of scoloplacids share several characteristics with those of Auchenipteridae. Since these two families are not phylogenetically related this similarity seems to be due to convergence once both families are, until now, the only known siluriform families with introsperm.

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Introduction

Scoloplacidae, with only four species, is one of the most recent Neotropical siluriform family described (de Pinna 1998; Schaefer 2003). Scoloplacids were initially supposed to be representative of the Aspredinidae family (Lundberg and Baskin 1969). Bailey and Baskin (1976) described the first species of scoloplacids and suggested that it could belong to a new subfamily of Loricariidae named Scoloplacinae. Only in 1980 Isbrücker elevated the subfamily to the family status and named it Scoloplacidae. Howes (1983) confirmed that action via a cladistic analysis of higher loricarioid relationships. Recent phylogenetic studies (reviewed in de Pinna 1998) showed that Scoloplacidae belongs to the superfamily Loricarioidea and is the sister group of the clade composed by Loricariidae and Astroblepidae. Scoloplacids are known from the Amazon, Tocantins, and Parana/Paraguay River systems of South America (Schaefer 2003). Fishes of this family are usually referred to as miniature catfishes, since the adults do not exceed the standard length of about 20 mm (Schaefer 2003).

The reproductive biology of scoloplacids is poorly known. In a sample of 874 scoloplacid specimens caught at the end of the rainy season between May and August, Sazima *et al.* (2000) related the presence of six sexually dimorphic males measuring 14.0–14.6 mm SL, and several possible adult

females measuring 12.8–16.4 mm SL, with up to 300 oocytes per ovary. In this study, any female was found with mature oocytes. Burns and Weitzman (2005) describe *Scoloplax dicra* as being an inseminating species, based on the histological demonstration of spermatozoa within the ovaries. Among siluriforms, insemination has only been documented in Auchenipteridae (Loir *et al.* 1989; Meisner *et al.* 2000; Burns *et al.* 2002).

The present study describes the spermiogenesis and spermatozoa in *Scoloplax distolothrix* demonstrating the occurrence of introsperm in this species. The data are compared with those of other siluriforms with the main objective of investigating if the characteristics found in *S. distolothrix* spermatozoa are more influenced by the reproductive mode than by the phylogenetic position of the family.

Materials and methods

The present study was conducted with adult males of *S. distolothrix* Schaefer, Weitzman and Britski, 1989 (Scoloplacidae), collected in a temporary lagoon on the right margin of the Rio Itiquira, Itiquira, Mato Grosso, Brazil (17°28′13″S, 55°14′46.7″W) in May (LBP 1424) and September (LBP 1938) of 2003. The fishes were identified and kept in the fish collection of Laboratório de Biologia e Genética de Peixes (LBP), Departamento de Morfologia, Instituto de Biociências, UNESP, Botucatu, São Paulo, Brazil.

For ultrastructural analysis, the gonad fragments were fixed in 2% glutaraldehyde and 4% paraformaldehyde in 0.1 M Sorensen phosphate buffer, pH 7.4. The material was postfixed for 2 h in the dark in 1% osmium tetroxide 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.

Results

Spermiogenesis in Scoloplax distolothrix

The spermiogenesis occurs in cysts in the germinative epithelium. In these cysts, groups of spermatids at the same development stage are surrounded by cytoplasmic processes of the Sertoli cells (Fig. 1A). In the early spermatids, the cytoplasm is symmetrically distributed around the nucleus, which contains diffuse chromatin and has a circular outline (Fig. 1A–C). The centriolar complex, with the proximal centriole perpendicular to the distal, lies laterally to the nucleus and is anchored to the plasma membrane. Between the centrioles there is a deposit of electron-dense material (Fig. 1K). The flagellum development from the distal centriole takes place laterally to the nucleus (Fig. 1B, 1B inset). The centriolar complex moves towards to the nucleus, bringing with it the plasma membrane and the initial segment of the flagellum (Fig. 1B,C). With the movement of the centrioles towards to the nucleus, the cytoplasmic channel is formed; the nuclear rotation occurs and the flagellum is positioned medially to the nucleus. During the nuclear rotation, a depression is formed in the nuclear outline that gives rise to the nuclear fossa. At the end of this process, the centrioles and the initial segment of the flagellum are found completely inserted in the nuclear fossa, in medial position (Fig. 1B–E,H). During the nuclear elongation, the nuclear fossa initially long in relation to the nucleus becomes short (Fig. 1C–E, 1H,J,K). During the nuclear rotation, the chromatin condensation starts. The process culminates with the formation of thin juxtaposed filaments (Fig. 1B–E, 1H,J,K). The cytoplasmic mass moving towards and around the initial segment of the tail gives rise to the midpiece of the future spermatozoon (Fig. 1D,E,H,K). In the early spermatids, the midpiece has rounded mitochondria randomly distributed. During the spermatid differentiation, the mitochondria elongate. They can fuse to each other and take place along the flagellar initial region (Fig. 1F). In a cross view, the mitochondria form a spiral surround inside the cytoplasmic channel, while in a longitudinal view, they are organized in a row along it (Fig. 1F–K). Few vesicles are found in the spermatids midpiece. The flagellum has the classical (9 + 2) axoneme, with nine peripheral microtubular doublets of microtubules and a single central pair of microtubules, surrounded by the flagellar membrane. Two lateral projections or fins develop from the flagellar membrane (Fig. 1L, 1L inset).

Scoloplax distolothrix *spermatozoa*

Scoloplax distolothrix spermatozoa are found in the light of the germinative compartment, isolated or in groups, with a variable number of cells. In the groups, the spermatozoa can be tightly packed along their entire lengths (Fig. 2B). However, these groups do not constitute a spermatozeugmata. The spermatozoa of these species exhibit a conic head, symmetric midpiece, and one medial flagellum (Fig. 2A,F,I). They do not show acrosomal vesicle. The nuclei have a conic shape, are very elongated forward and have its thin extremity curved (Fig. 2A, 2B inset). The nuclei are 6.0 μm in length and 0.4 μm in width. In a longitudinal view, it forms a long and slight arc, while in a cross view it is oblong (Fig. 2C,D). In the head, along the internal face of the arc, is a well-developed membranous compartment with some electron-dense dots close to the nuclei (Fig. 2A–D). Despite the chromatin being heterogeneously condensed and in the form of thin juxtaposed filaments, it has a homogeneous pattern in the spermatozoon. In the cytoplasmic region, around to the nucleus, no organelles are seen (Fig. 2A,B). The nuclear fossa is 0.8 μm in length and 0.2 μm in width. It is medial to nucleus and in simple arc (Fig. 2E). The midpiece is 4.4 μm in length and 0.8 μm in width. Centrally in the midpiece, but not in the terminal end, in a cross view, the mitochondria form a

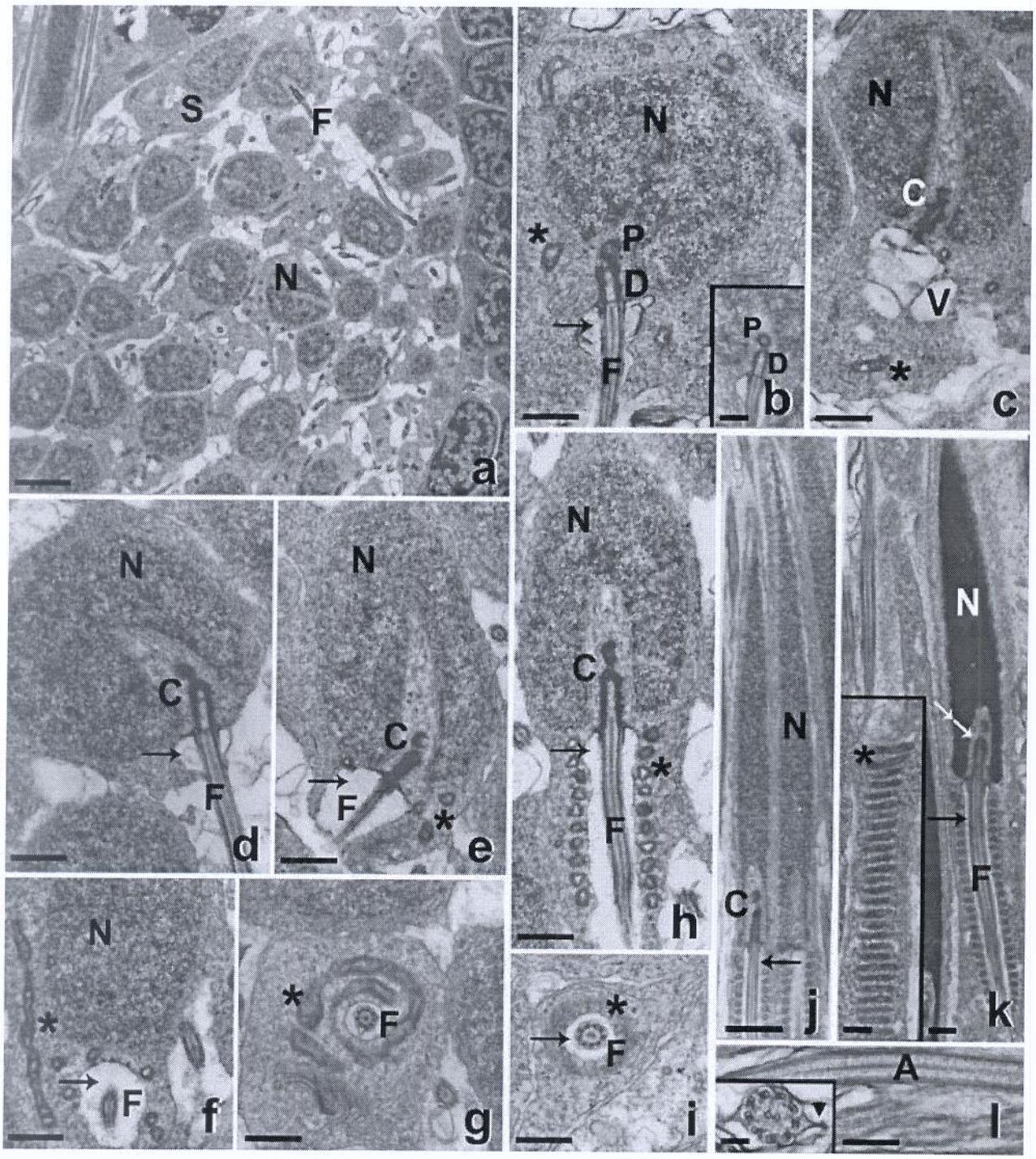

Fig. 1—Spermiogenesis of *Scoloplax distolothrix*. —**A**. Spermatids cyst. CY, cyst; F, flagellum; N, nucleus. X4.850. —**B–E**. Early spermatid (longitudinal sections). C, centriolar complex; D, distal centriole; F, flagellum; P, proximal centriole; V, vesicles; asterisk, mitochondria; arrow, cytoplasmic channel. (B, D) X17.000; (B inset) X10.200; (C, E) X13.250. —**F, G, I**. Midpiece showing mitochondria (longitudinal and cross sections). (F) X13.600; (G) X18.400; (I) X25.200. —**H**. The arrangement between the centrioles and the mitochondria distribution in the midpiece. X17.000. —**J, K**. Late spermatid nucleus showing the heterogeneous condensation process. Double arrow: electron-dense material between centrioles. (J) X9.750; (K) X13.250. (K inset) Mitochondria organized in a row. X13.600. —**L** and L inset. Flagellum (longitudinal and cross sections). A, axoneme; double arrowhead, lateral projections. (L) X31.500; (L inset) X23.000.

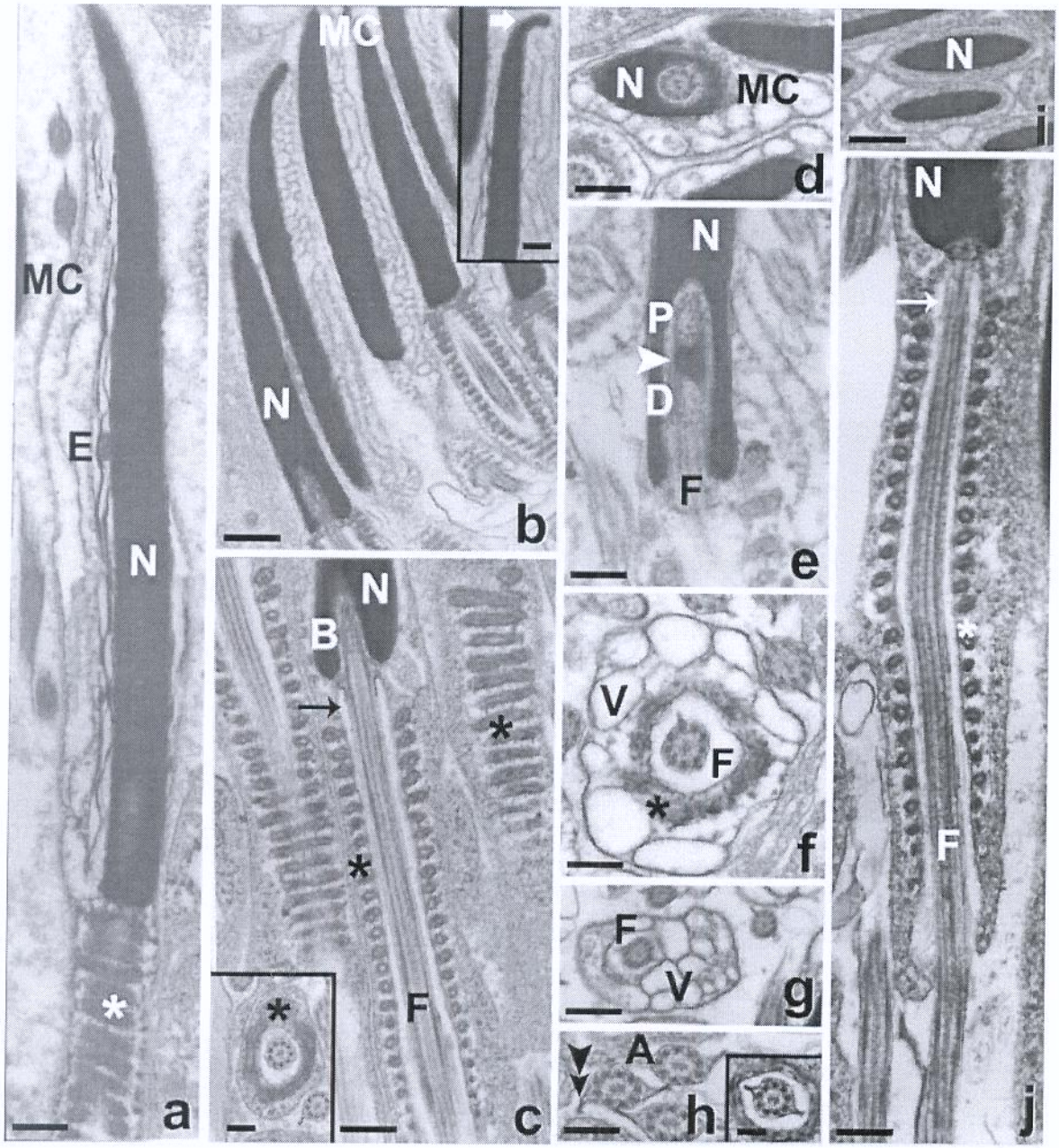

Fig. 2—Spermatozoa of *Scoloplax distolothrix*. —**A**. Spermatozoon longitudinal section. E, electron-dense structure; MC, lateral membranous compartment; N, nucleus; asterisk, mitochondria. X23.000. —**B** and B inset. Nucleus longitudinal sections, showing its extremity curved and lateral membranous compartment. (B) X17.000; (B inset) X13.250. —**C, D**. Head regions (transverse sections). (C) X18.400; (D) X17.000. —**E**. Centrioles arrangement. D, distal centriole; F, flagellum; P, proximal centriole; arrowhead, electron-dense material between centrioles. X42.000. —**F**. Spermatozoon longitudinal section showing nuclear fossa and cytoplasmic channel and mitochondria in the midpiece. B, basal body; arrow, cytoplasmic channel. X23.000. —**F** inset, **G** and **H**. Midpiece transverse sections showing mitochondria and vesicles. V, vesicles. (F inset) X25.200; (G) X42.000; (H) X23.000. —**I**. Midpiece longitudinal section. X31.500. (J and J inset) Flagella transverse sections. A, axoneme; double arrowhead, lateral projections. (J) X42.000; (J inset) X31.500.

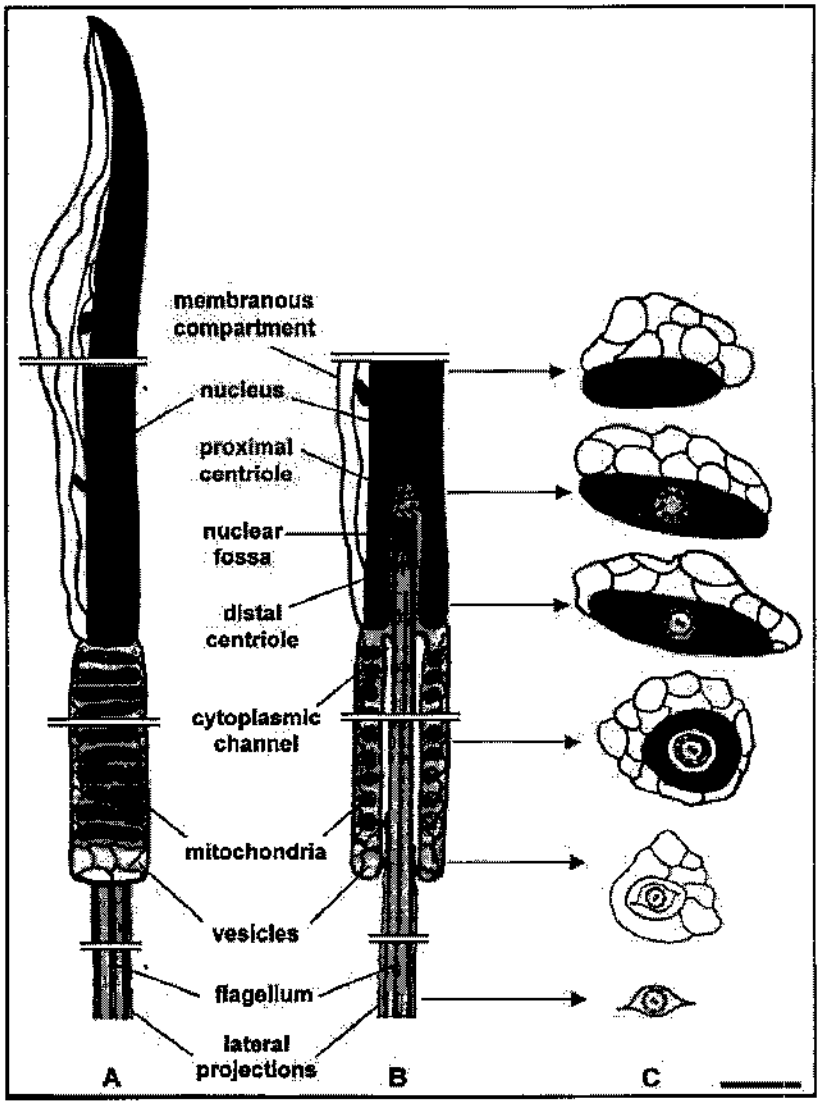

Fig. 3—Diagram of the spermatozoon of *Scoloplax distolothrix* (A) with corresponding longitudinal (B) and transverse (C) sections. Scales of various parts are only approximate. Scale bar = 0.48 μm.

ring internally surrounding the cytoplasmic channel, while in a longitudinal view they are organized in a row along it (Fig. 2F, 2F inset, 2I). Mitochondria are not observed only in the basal extremity of the midpiece (Fig. 2I). Several elongated vesicles are found peripherally distributed in all regions of the midpiece, but mainly concentrated in the basal region (Fig. 2G,H). The mitochondria are separated from the flagellum by the cytoplasmic channel (Fig. 2F,I). The cytoplasmic channel is 4.2 μm in length and 0.3 μm in width. The centrioles are completely inside the nuclear fossa, perpendicular to each other, and show an electron-dense material between them (Fig. 2E). The distal centriole is differentiated in the basal body and gives rise to the axoneme, which exhibits the classical (9 + 2) microtubular pattern. The initial segment of the axonema is also found inserted in the nuclear fossa. The flagellar membrane displays two lateral projections or fins (Fig. 2J, 2J inset). A diagram of the *S. distolothrix* introsperm is presented in Fig. 3.

Discussion

Spermiogenesis

In the spermatozoa, the flagellum axis may be either perpendicular or parallel to the nucleus, depending on whether nuclear rotation during spermiogenesis occurs (type I spermiogenesis) or not (type II spermiogenesis) (Mattei 1970). The spermiogenesis process observed in scoloplacids is characterized by an initial lateral development of the flagellum, presence of nuclear rotation, a medial nuclear fossa formation,

a cytoplasmic channel formation, and the presence of centriolar migration. These characteristics are similar to type I spermiogenesis, which is also observed in Diplomystidae, the most basal siluriform family. However, in Diplomystidae the cytoplasmic channel does not remain in the spermatozoa (Quagio-Grassiotto *et al.* 2001) as observed in scoloplacid.

Spermatozoa

Fish spermatozoa can be divided in two main groups, according to their reproduction mode. In the first group we have the species with external fertilization that have aquasperms. This is the most common condition found among fishes and in this case the spermatozoa have spherical to ovoid nucleus and short midpieces (Jamieson 1991). In siluriforms aquasperms are found in most families, including, for example, Diplomystidae (Quagio-Grassiotto *et al.* 2001), Loricariidae (Mansour and Lahnsteiner 2003), Clariidae (Mansour *et al.* 2002), Siluridae (Kwon *et al.* 1998; Lee and Kim 2001), and Pimelodidae (Quagio-Grassiotto and Carvalho 2000; Santos *et al.* 2001). In the second group, we have the internal inseminated species whose spermatozoa, named introsperm, have very elongated nucleus and midpieces (Jamieson 1991). This condition is relatively rarer among fishes. However, species with introsperm are found in several families of different orders. In Cyprinodontiformes, this reproduction mode was described in Poeciliidae (Grier 1975; Kobayashi and Iwamatsu 2002) and Anablepidae and Jenynsiidae (Grier *et al.* 1981). In Scorpaeniformes, introsperm are observed in several species of Scorpaenidae (Jamieson 1991; Muñoz *et al.* 1999, 2002). In Characiformes, the sister group of Siluriformes and Gymnotiformes (Fink and Fink 1996), some species with introsperm are found in the subfamily Cheirodontinae (Burns *et al.* 1997) and Glandulocaudinae (Burns *et al.* 1995, 1998; Azevedo *et al.* 2000; Burns and Weitzman 2005; Pecio *et al.* 2005). In Siluriformes, Auchenipteridae is the only family with introsperm (Loir *et al.* 1989; Meisner *et al.* 2000; Burns *et al.* 2002).

The presence of very elongated nucleus and midpieces in *S. distolothrix* characterize the occurrence of introsperm in this species. This finding reinforces the hypothesis of Burns and Weitzman (2005) that scoloplacids have insemination. Due to this particular mode of reproduction, the *S. distolothrix* spermatozoa are morphologically more similar to those found in auchenipterids. In a comparative analysis, five characteristics shared between the spermatozoa of *S. distolothrix* and auchenipterids were found: the conic shape of the nuclei, the presence of very long nucleus, and the perpendicular arrangement between the proximal and distal centrioles, the presence of a long midpiece, and the presence of mitochondria along of the midpiece. Since phylogenetic studies demonstrated that Scoloplacidae and Auchenipteridae do not belong to a natural group (de Pinna 1998; Britto 2003), these similar characteristics should be developed by convergence, due to their particular reproductive characteristics.

On the other hand, introsperm of scoloplacids and auchenipterids have several marked differences. In scoloplacids spermatozoa midpiece the mitochondria form a ring internally surrounding the cytoplasmic channel, while in auchenipterids they do not have this arrangement. In scoloplacids the conic nuclei have their extremity strongly curved and a well-developed membranous compartment along its internal face. In auchenipterids, the extremity of the nuclei is lanceolated and any membranous compartment flanking the nucleus is observed (Burns *et al.* 2002). In scoloplacids, spermatozoa midpieces have several vesicles, which they are not found in auchenipterids. In scoloplacids spermatozoa, the flagellum has two lateral fins, not observed in auchenipterids. The organization of the scoloplacid spermatozoa in the germinative compartment is also different from auchenipterids since they are not arranged in well-organized spermatozeugmata, as found in auchenipterids (Meisner *et al.* 2000; Burns *et al.* 2002). These characteristics may be related with the phylogenetic position of scoloplacids.

The family Scoloplacidae belongs to the superfamily Loricarioidea (de Pinna 1998). However, almost all characteristics found in the scoloplacid spermatozoa are quite different from those found in other Loricarioidea families, such as in Nematogenyidae (Spadella *et al.* submitted), Loricariidae (Mansour and Lahnsteiner 2003; our unpublished data), as well as in Trichomycteridae and Callichthyidae (our unpublished data), possibly due its particular mode of reproduction as discussed above. According to Burgess (1989) and our own observations, male astroblepids have an elongate urogenital papilla that apparently functions as an intromittent organ. This suggests the occurrence of insemination in this family, but future analysis with astroblepid spermatozoa should be conducted to test this hypothesis.

Scoloplacidae is the sister group of a clade composed of Astroblepidae and Loricariidae (de Pinna 1998; Britto 2003). The spermatozoa of *S. distolothrix* and those of loricariids share only two morphological characteristics: the perpendicular arrangement between the proximal and distal centrioles and the presence of a symmetric midpiece. The perpendicular arrangement between the proximal and distal centrioles is also found in Diplomystidae (Quagio-Grassiotto *et al.* 2001), Siluridae (Lee and Kim 2001), and Pimelodidae (Quagio-Grassiotto and Carvalho 2000; Santos *et al.* 2001). The presence of a symmetric midpiece is also found in Diplomystidae (Quagio-Grassiotto *et al.* 2001), Amblycipitidae (Lee and Kim 1999), Clariidae (Mansour *et al.* 2002), Siluridae (Kwon *et al.* 1998; Lee and Kim 2001), Pimelodidae (Quagio-Grassiotto and Carvalho 2000; Santos *et al.* 2001), Bagridae (Lee 1998; Kim and Lee 2000), and Ictaluridae (Poirier and Nicholson 1982). Thus, the two characteristics shared between scoloplacids and loricariids cannot be directly related with their phylogenetic position.

Histological studies with male and female gonads of scoloplacids will be very important to test the hypothesis of the occurrence of insemination in scoloplacids. Also, further

studies in Astroblepidae will be fundamental for a better discussion of the spermatozoa differentiation in Loricarioidea.

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4.3. CAPÍTULO 3

Spadella, M.A., Oliveira, C., Quagio-Grassiotto, I. Morphology and histology of male and female reproductive systems in the inseminating species *Scoloplax distolothrix* (Ostariophysi: Siluriformes: Scoloplacidae).

Morphology and histology of male and female reproductive systems in the inseminating species *Scoloplax distolothrix* (Ostariophysi: Siluriformes: Scoloplacidae)

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ABSTRACT

The morphology and histology of male and female reproductive systems were examined in *Scoloplax distolothrix*. Internal insemination was documented in this species by the presence of sperm within the ovaries. The mature males and females have elongated genital papillas, exhibiting a tubular shape in male and a plain heart-shape with two median protuberances in females. The testes are two elongated structures that converge ventrally, under the intestine, towards a genital papilla. They are joined in the caudal end forming an ovoid single chamber to sperm storage. Any secretory regions were observed. In the lumen of the testicular tubules, spermatozoa can be tightly packed along their lengths, but they do not constitute a spermatozeugmata. The lumen of the sperm storage chamber and spermatic duct are filled with free spermatozoa without accompanying secretions. The ovaries are saccular structures, wing-bird shape, and they converge towards ventrally, under the intestine, a genital papilla. They are joined in the caudal end forming a tubular chamber maybe destined to oocytes storage. An oviduct with an irregular outline connects the chamber to the tubular region of the genital papilla. Any distinct sperm storage structure was found in the ovaries. The peculiar male and female genital papillae suggest that these structures are associated with the reproductive mode in scoloplacids, representing other evidence for insemination. The occurrence of free spermatozoa, without accompanying secretions and not arranged in a spermatozeugmata, can be associate with the presence of a tubular male genital papilla for sperm transference to the female genital tract. This reinforce the idea to that sperm packed is not necessary in all inseminating species. The male reproductive system in scoloplacids is very different from auchenipterids; which indicates that the occurrence of insemination is not connected to the internal morphology of reproductive organs.

KEY WORDS: introsperm, copulatory organ, genital papilla, catfish.

INTRODUCTION

Scoloplacidae, with only four species, is one of the most recent Neotropical siluriform families discovered (de Pinna, 1998; Schaefer, 2003). Scoloplacids were initially described by Lundberg and Baskin (1969) as a representative of Aspredinidae. Bailey and Baskin (1976) described the first species of scoloplacid and suggested that it could belong to a new subfamily of Loricariidae, named Scoloplacinae. In 1980 Isbrücker ranked it to family status. Howes (1983) confirmed that action via a cladistic analysis of higher loricarioid relationships. Recent phylogenetic studies (reviewed in de Pinna, 1998; Britto, 2003) showed that Scoloplacidae belongs to the superfamily Loricarioidea and is the sister group of the clade composed by Loricariidae and Astroblepidae. Scoloplacids are endemic of South America been known from the Amazon, Tocantins, and Parana/Paraguay River systems (Schaefer, 2003).

The reproductive biology of scoloplacids is still poorly known. Burns and Weitzman (2005) suggested that *Scoloplax dicra* could be an inseminating species, based on the histological identification of spermatozoa within the ovaries. Insemination is uncommon among siluriforms, only described in Auchenipteridae (Loir et al., 1989; Burns et al., 2000, 2002; Meisner et al., 2000; Burns and Weitzman, 2005). The spermatozoa of *Scoloplax distolothrix* described by Spadella et al. (2006) show many characters that reinforce the hypothesis of Burns and Weitzman (2005) that scoloplacids are inseminating fishes.

Inseminating species have several morphological specializations associated with the process of insemination as the presence of sperm with nuclei and midpiece very elongated, and distinct copulatory organs in males (Burns and Weitzman, 2005). In adult males of auchenipterids, the intromittent organ or gonopodium usually results from modifications of anterior anal-fin rays (Meisner et al., 2000; Burns et al., 2002). The gonopodium is thought to function in the transfer of sperm to the female reproductive tract (Grier et al., 1981; Meisner et al., 2000; Burns et al., 2002). The intromittent organ can be also derived of modifications of pectoral and pelvic fins, as in Phallostethidae, or appears as elongate

genital papillae, as in *Labidesthes* (Atherinidae) and *Hemirhamphodon* (Hemiramphidae) (Meisner et al., 2000).

Schaefer et al. (1989) described a generic diagnosis for sexual dimorphism in *Scoloplax*, based on the presence of large, fleshy globular tissue at ventral opercle margin in larger male specimens. Specifically, in *S. dicra*, *S. empousa*, and *S. distolothrix*, these authors related in males and immature females the presence of small, knob-like genital papilla located just posterior to the anus, often bearing a slender posterior extension. Additionally, in females the authors describe the presence of an expanded, fleshy sack-like genital papilla, T-shaped slit forming a sperm receptacle.

In order to confirm the insemination occurrence in scoloplacids the morphology and histology of reproductive system were studied in mature male and female of *Scoloplax distolothrix*.

MATERIAL AND METHODS

Adult males and females of *Scoloplax distolothrix* Schaefer et al., 1989 (Scoloplacidae) collected in a temporary lagoon in the right margin of the rio Itiquira, Itiquira, Mato Grosso, Brazil (17°28'13"S 55°14'46.7"W) during two collect expeditions conduced in 05/05/2003 (LBP 1424) and 29/09/2003 (LBP 1938). Fishes were identified and kept in the fish collection of Laboratório de Biologia e Genética de Peixes (LBP), Departamento de Morfologia, Instituto de Biociências, UNESP, Botucatu, São Paulo, Brazil.

Histological studies were conduced in specimens and gonads fixed in 10% formalin. Segments of the fish bodies, and gonads were dehydrated in alcohol and embedded in historesin - Technovit 7100. Serial sections from 3 to 5µm thick were stained with Haematoxilin-eosin. Micrographs were obtained using a JVC camera coupled to microscope and microcomputer with the image capture program.

RESULTS

Mature male and female of *S. distolothrix* have developed genital papillas (Figs. 1a and 1b). In males, the papilla is elongated, and exhibits a tubular shape with a ventral opening close to its distal tip (Fig. 1a). The progressive closing of the ventral opening forms a duct that runs toward the animal body. In histological sections, male genital papilla is constituted by dense connective tissue with smooth muscle cells, and blood vessels. The intern duct is lined by a stratified squamous ephitelium (Figs. 3c-k).

In female, the genital papilla has a plain heart-shape with two median protuberances. The female papilla is connected to the animal body by a short tubular region continuous with the median protuberances. The protuberances limit a central conduit in their median and distal regions, it becomes progressively closed, and forms a tortuous canal that runs toward the animal body within the short tubular connecting segment (Fig. 1b). In histological sections, the female genital papilla, as in males, is constituted by dense connective tissue with smooth muscle cells, and blood vessels. The tortuous canal has a very irregular outline with many folds, and is lined by a stratified squamous ephitelium (Figs. 4c-k).

Anatomically, the testes of *S. distolothrix* are two elongated structures situated in the dorsal region of the celomatic cavity. They are enwrapped by mesenteric tissue, and individually suspended, in the two anterior thirds, along the dorsal wall by a mesorchium. Both of the testes converge ventrally, under the intestine, towards the genital papilla (Figs. 2a and 2b). The testes are joined in the caudal end forming an ovoid single chamber to sperm storage. A spermatic duct connects the sperm storage chamber to the tubular region of the genital papilla. In histological sections, testes are enclosed by the albuginea tunic, internally had anastomosing seminiferous tubules lined by the germinal epithelium that containing cysts with germ cells in different stages of the maturation. In the lumen of the testicular tubules of male in final maturation class (sensu Grier, 2002), spermatozoa can be tightly packed along their lengths, but they do not constitute a spermatozeugmata. The luminal compartment of the sperm storage chamber and spermatic duct are filled with free

spermatozoa no accompanying secretions (Figs. 3a-k). Male reproductive system did not present any secretory regions.

Anatomically, the ovaries of *S. distolothrix* are two saccular structures, wing-bird shape, situated in the dorsal region of the celomatic cavity. They are individually suspended, in their anterior halves, along the dorsal wall by a mesovarium (Figs. 2c and 2d). Both of the ovaries converge ventrally, under the intestine, towards the genital papilla. The ovaries are joined in the caudal end forming a single tubular chamber maybe destined to oocytes storage. An oviduct with an irregular outline connects the chamber to the tubular region of the genital papilla. The female gonads are of the cystovarium type. In histological sections, ovaries are enclosed by the tunic albuginea, and internally the ovigerous lamellae project radially out from the tunic to the lumen. In the maturation class, the lamellae contain oocytes in primary and secondary growth stage, and are lined by a simple cuboidal to a squamous ephitelium (Figs. 4a-k).

S. distolothrix introsperms were found in the ovarian lumen of females in initial maturation class. In the histological sections of the ovary of this class, the lamellae contains only previtellogenic oocytes, are lined by a simple cuboidal to collunar ephitelium, and the introsperms are mainly lined up along the lamellae epithelium. Any distinct sperm storage structure was found (Figs. 5a-e).

DISCUSSION

The presence of introsperm within the ovarian lumen, as previously documented for Burns et al. (2000) and Burns and Weitzman (2005), and showed in the present study, support the hypothesis that scoloplacids are inseminating species. According to Burns and Weitzman (2005) the insemination may permit the temporal and spatial separation of mating and spawning, since the ovary can be a region of sperm storage. The occurrence of introsperm within the ovary in *S. distolothrix,* in initial maturation class, suggests its storage until the spawning period.

The presence of genital papillas in males and females of *S. distolothrix*, in spite of their morphological peculiarities, represent another strong evidence for insemination. The

histological constitution of male genital papilla show that it constitutes an intromitent organ with analogous function of the gonopodium found in other inseminating species, as auchenipterids (Burns, 1991; Downing and Burns, 1995; Burns and Weitzman, 2005). Although the presence of distinct copulatory organs had been described in males of several teleost species (Burns and Weitzman, 2005), its occurrence in females of *S. distolothrix* and possible others scoloplacids (Schaefer et al., 1989), is a new found.

The genital papillae morphology suggests that the spermatozoa flow, during the transfer to the female reproductive tract, occurs from sperm storage chamber across spermatic duct to the ventral opening in the distal tip of male papilla. Thus, the spermatozoa deposited in the conduit between the median protuberances, of the female genital papilla, move throughout the oviduct, and oocytes storage chamber, and reach their final destination in the ovarium lumen. Therefore female genital papilla form a sperm receptacle that help the sperm transfer to the female reproductive tract, as previously discussed for Schaefer et al. (1989).

Differently of some Auchenipteridae (Meisner et al., 2000; Burns et al., 2002) and some Glandulocaudinae species (Pecio et al., 2005), scoloplacid spermatozoa are not organized in spermatozeugmata. They are found free in the lumen of the male germinative compartment, and not accompanied by secretions (Spadella et al., 2006). Grier et al. (1981) describe that sperm transference between sexes in an aqueous medium is facilitated if sperm are packaged as spermatozeugmata or spermatofores, in fish families lacking tubular gonopodia. Additionally, the aggregation of spermatozoa can reduce losses of cells to the environment during the passage to the female genital tract (Burns and Weitzman, 2005). Grier et al. (1981) paied assigned that in Goodeidae and Poecillidae showed evidences of a correlation between the mechanisms of sperm packaging and non-tubular gonopodia.

Conversely, those same authors related that in two families studied, Anablepidae and Jenynsiidae, the gonopodia is tubular and the packaging of sperm is not the rule. The free spermatozoa found in *S. distolothrix* support the idea that the packing not necessarily occurs in all inseminating species.

The morphology and histology of the male reproductive systems is known for representatives of Loricarioidea of the families Loricariidae and Callichthyidae (Loir et al.,

1989; Mansour and Lanhsteiner, 2003). In Callichthyidae, the male reproductive system consists of two elongated and lobulated testis which join posteriorly in a segment that converge towards the genital papilla. The caudal part of the testis is surrounded by a mass composed of several packet seminal vesicles (Loir et al., 1989; Mansour and Lanhsteiner, 2003). In Loricariidae, the male reproductive system has a simple organization with two ovoid and narrow testis localized in the anterior part. In relation to the presence of seminal vesicles in the reproductive system, the literature data are controverty. Loir et al. (1989) related, based on analysis of the species *Hypostomus gymnorynchus* (subfamily Hypostominae), *Pseudoancistrus barbatus* (subfamily Hypostominae), and *Harttia surinamensis* (subfamily Loricariinae), the presence of a seminal vesicle, poorly defined, localized in the posterior part where the deferent duct is somewhat enlarged. Conversely, Mansour and Lahnsteiner (2003) did not consider the existence of seminal vesicle in the male reproductive system in *Ancistrus triradiatus* (subfamily Hypostominae). Considering that the study of Loir et al. (1989) present no image showing more detail of male reproductive system in Loricariidae, and these authors also related had not been possible to study accurately the anatomy; we believe that the occurrence of seminal vesicle in Loricariidae should be more investigated.

Considering the cited above for Loricariidae according to Mansour and Lahnsteiner (2003), the characteristics of loricariids male reproductive system, are similar to those observed in Scoloplacidae, as the presence of elongated testes converging towards the genital papilla, and the absence of the seminal vesicles. However, this similarity seems to be due to convergence, since loricariids not are inseminating species.

On the other hand, the morphology and histology of male reproductive system in scoloplacids is very different from auchenipterids, a known inseminating family of Siluriformes. Auchenipteridae male reproductive system has a male reproductive system composed by numerous finger-like spermatogenic lobes, sperm storage region, and secretory and storage regions of the seminal vesicle (Loir et al., 1989; Meisner et al., 2000) while in Scoloplacidae the testis is not lobular, and there is not a seminal vesicle or secretory lobes. Thus, the occurrence of insemination is not related to the internal morphology of reproductive organs.

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FIGURE CAPTION

Figure 1. Ventral views of an adult male (a) and female (b) of *Scoloplax distolothrix*. The arrowheads points to the male and female genital papilla. In (a-inset) and (b-inset) detail of a male and female genital papilla respectively. Scale bar = 5 mm.

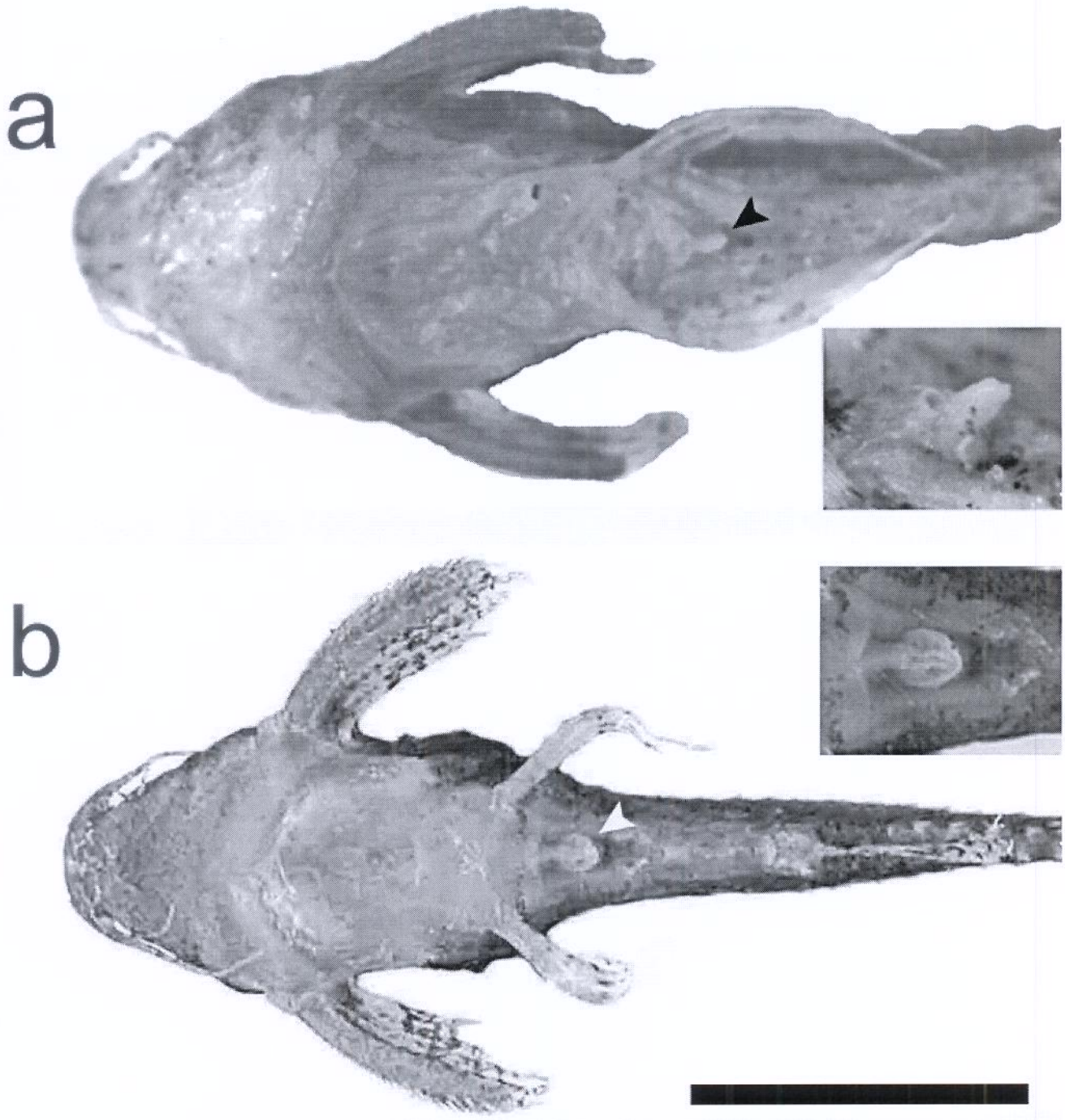
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FIGURE CAPTION

Figure 2. Ventral views of an adult male (a) and female (c) of *Scoloplax distolothrix*. In (b) ventral views of testes. In (d) dorsal views of ovaries. The arrows in (a) and (c) points to the testis and ovary, respectively. Scale bar (a, c) = 0.7 mm. The arrowheads in (b) and (c) details of male and female genital papilla, respectively. Scale bar (b) = 0.8 mm; Scale bar (d) = 1.4 mm.

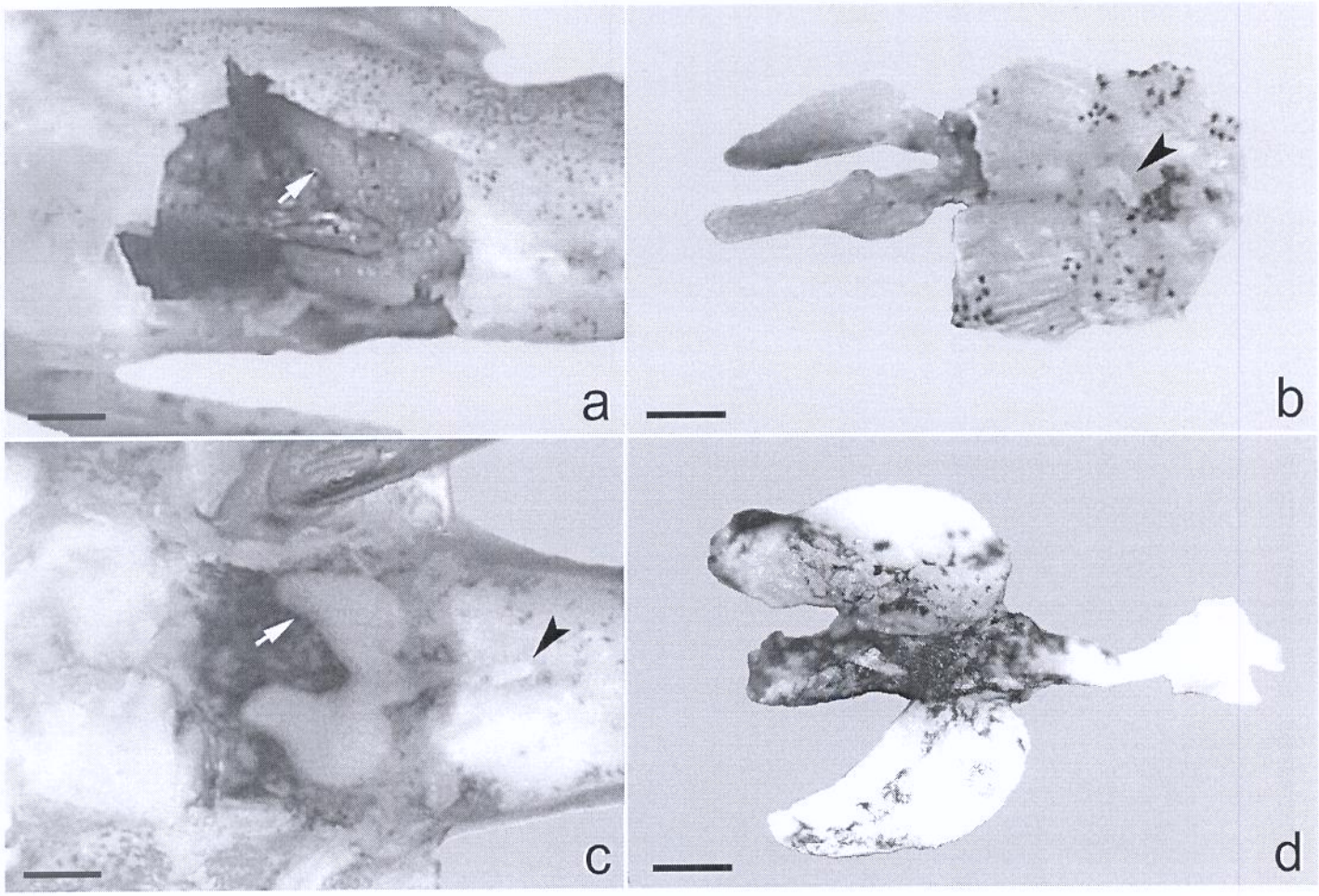
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FIGURE CAPTION

Figure 3. Transversal sections of male adult specimens of *Scoloplax distolothrix* through the genital papilla region. Legends: V: blood vessels; Z: spermatozoa, arrow: genital papilla. Scale bar (a-d, g, h, j) = 10 μm; Scale bar (e, f, i, k) = 5 μm.

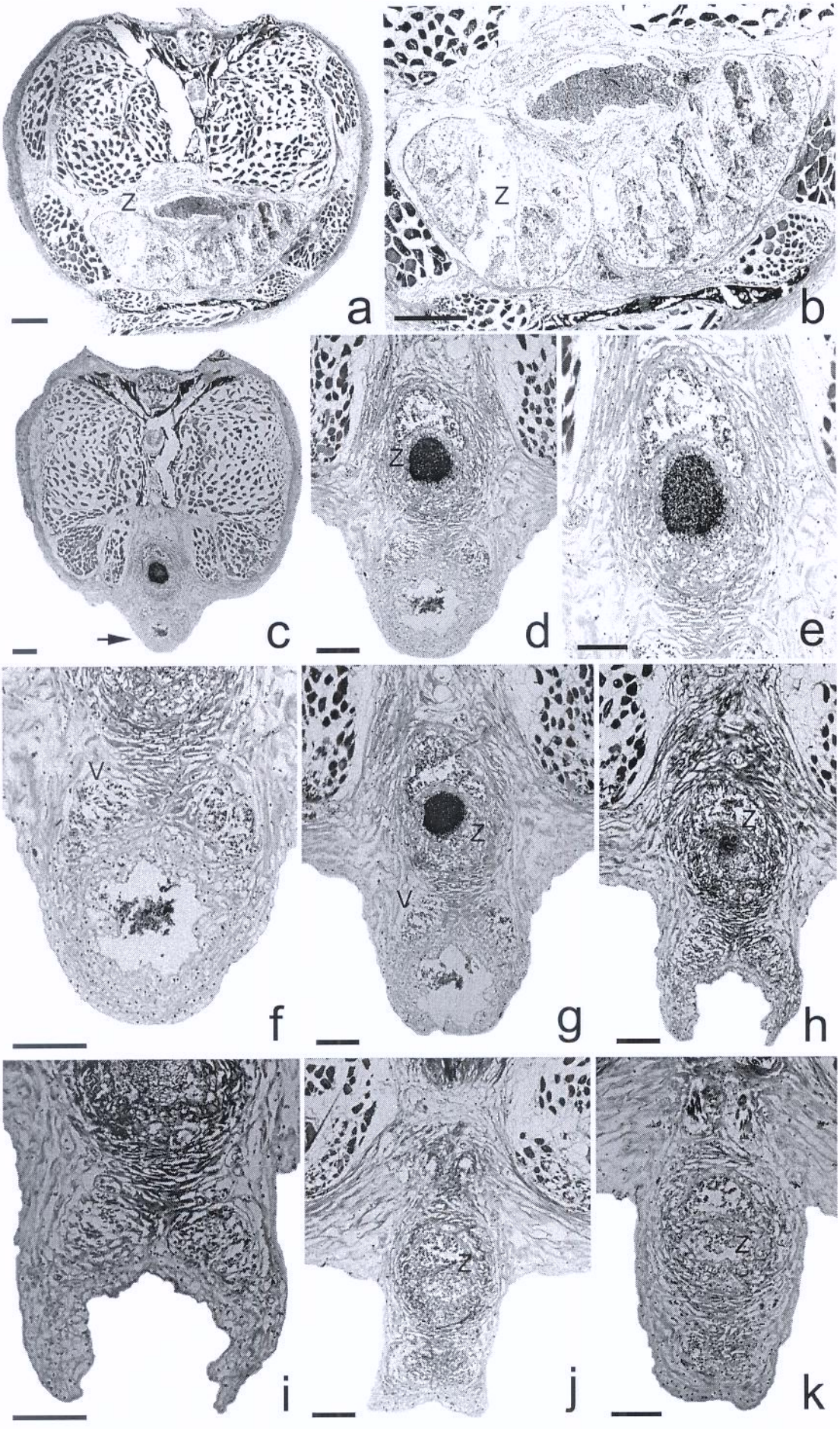
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FIGURE CAPTION

Figure 4. Transversal sections of female adult specimens of *Scoloplax distolothrix* through the genital papilla region. Legends: I: intestine; L: ovarian lumen; O: oocytes; arrow: genital papilla. Scale bar (a, c-g, i, j, k) = 10 μm; Scale bar (b, h) = 5 μm.

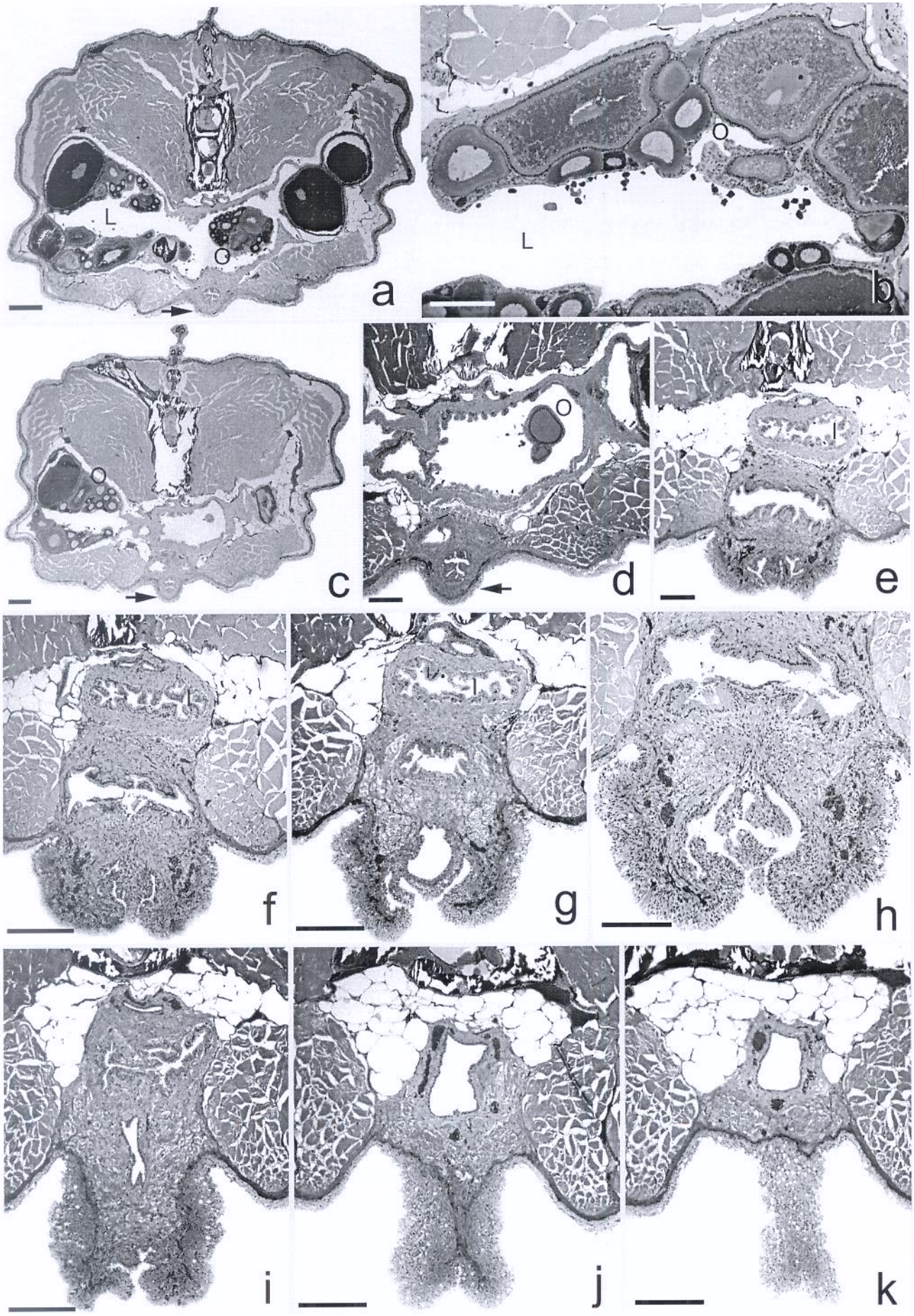
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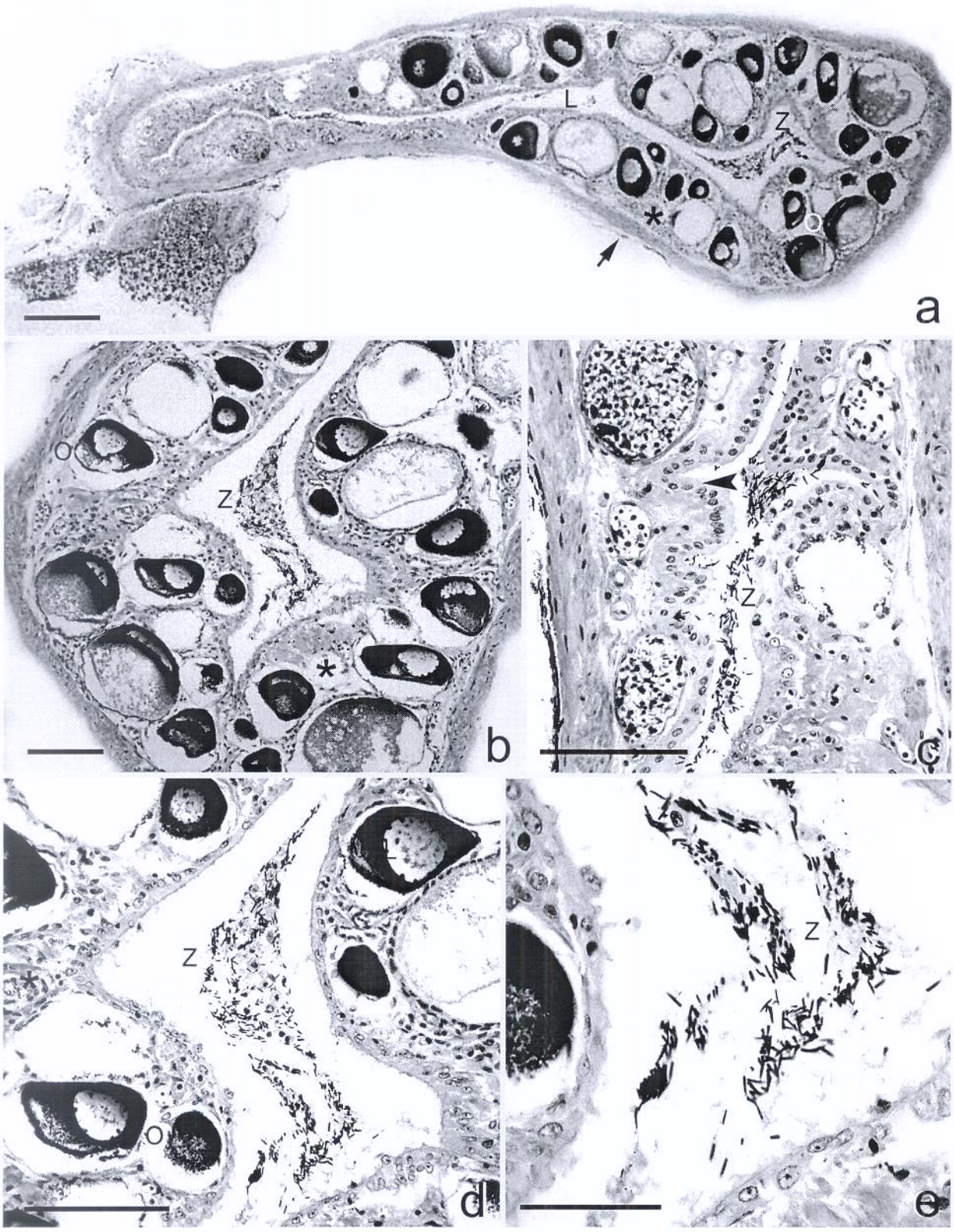
FIGURE CAPTION

Figure 5. Photomicrography of immature ovary showing spermatozoa in the lumen ovarian and oocytes in primary growth stage. Legends: L: ovarian lumen; O: oocytes; Z: spermatozoa, arrow: ovarian bordered, arrowhead: tortuous folds of ephitelium of the ovarian lumen; asterisk: ovigerous lamellae. Scale bar (a) = 20 μm; Scale bar (b-d) = 5 μm; Scale bar (e) = 2 μm.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4.4. CAPÍTULO 4

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Comparative analysis of the spermiogenesis and sperm ultrastructure in Callichthyidae (Teleostei: Ostariophysi: Siluriformes)

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Running headline: Spermiogenesis and spermatozoa ultrastructure in Callichthyidae

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Abstract

In the Corydoradinae, the spermatids occur in the lumen of the germinative compartment, together with the spermatozoa, suggesting that spermatogenesis is of the semicystic type, while in Callichthyinae, the spermatozoa production occurs in cysts in the germinative epithelium, characterizing a cystic spermatogenesis. The spermiogenesis in Callichthyinae is characterized by an initial lateral development of the flagellum, the presence of nuclear rotation in different degrees, an eccentric or medial nuclear fossa formation, a cytoplasmic channel formation, and the presence of centriolar migration, being more similar to type I spermiogenesis. In Corydoradinae, the spermiogenesis is characterized by eccentric development of the flagellum, the absence of nuclear rotation, an eccentric nuclear fossa formation, a cytoplasmic channel formation, and the absence of centriolar migration, differing from the types previously described. The characteristics of the spermatogenesis and spermiogenesis process in Corydoradinae and Callichthyinae revealed clear peculiarities of each of these subfamilies, corroborating the hypotheses that they constitute monophyletic groups. In relation to the spermatozoa ultrastructure, the comparative analysis of the callichthyid species shows that the general characteristics found in the spermatozoa were similar, thus, reinforcing the hypothesis that the family is monophyletic. Any characteristic exclusively found in Callichthyinae spermatozoa was observed.

Key words: catfish, spermatid, spermatozoa, phylogeny, morphology.

Introduction

Siluriformes comprise an extremely large fish group that is widely distributed across the tropical regions of the world (Burgess, 1989; Teugels, 1996; Ferraris, 1998). Among the monophyletic Neotropical Siluriformes lineages, we find the superfamily Loricarioidea that comprises six families: Nematogenyidae, Trichomycteridae, Callichthyidae, Scoloplacidae, Astroblepidae, and Loricariidae (de Pinna, 1998; Britto, 2003a).

Callichthyidae forms a well-corroborated monophyletic group, and it is one of the Loricarioidea families with greater species number, with approximately 180 valid species (Reis, 1998, 2003; Shimabukuro-Dias *et al.*, 2004). Nowadays, callichthyids are divided into two subfamilies: Corydoradinae and Callichthyinae are demonstrably monophyletic based on morphological and molecular characters (Reis, 1998, 2003; Britto, 2003b; Shimabukuro-Dias *et al.*, 2004). The Corydoradinae includes the genera *Aspidoras*, *Scleromystax*, and *Corydoras* and Callichthyinae includes *Dianema*, *Hoplosternum*, *Megalechis*, *Lepthoplosternum*, and *Callichthys*. All genera were found to be monophyletic, with the exception of *Corydoras* and *Hoplosternum* (Reis, 2003; Britto, 2003b).

In the current study, the ultrastructural characterization of both spermiogenesis and spermatozoa in seven specimens of Callichthyidae comprised by four Corydoradinae and three Callichthyinae, are presented. Comparisons between the data obtained in this study and those available for other siluriform families were conducted.

Material and Methods

The present study was conducted with species of the subfamilies Corydoradinae and Callichthyinae. The fish were identified and kept in the fish collection of Laboratório de Biologia e Genética de Peixes (LBP), Departamento de Morfologia, Instituto de Biociências, UNESP, Botucatu, São Paulo, Brazil. For the subfamily Corydoradinae, we utilized two adult males of *Corydoras flaveolus* Ihering, 1911 collected from rio Alambari (22°56'08"S, 48°19'15"W), Botucatu, São Paulo, Brazil (catalog number: LBP 1314), three adult males of *Corydoras aeneus* (Gill, 1858) from rio Araquá (22°47.135'S,

48°28.892'W), Botucatu, São Paulo, Brazil (catalog number: LBP 1406), two adult males of *Scleromystax lacerdai* (Hieronimus, 1995) from rio Ribeira da Terra Firme (15°32'17.9"S, 39°00'28.5"W), Canavieiras, Bahia, Brazil (catalog number: LBP 1966), and three adult males of *Aspidoras poecilus* Nijssen & Isbrücker, 1976 from córrego Voadeira, rio Araguaia (15°52'52.7"S, 52°15'14.3"W), Barra do Garças, Mato Grosso, Brazil (catalog number: LBP 2469). For the subfamily Callichthyinae, we utilized four adult males of *Hoplosternum littorale* (Hancock, 1828) collected from rio Corumbataí (22°15'S, 47°36'W), Corumbataí, São Paulo, Brazil (catalog number: LBP 2015); two adult males of *Megalechis thoracata* (Valenciennes, 1840) from a temporary lagoon, rio Itiquira (17°28'13"S, 55°14'46.7"W), Itiquira, Mato Grosso, Brazil (catalog number: LBP 1930); and two adult males of *Callichthys callichthys* (Linnaeus, 1758) from Corumbá, Mato Grosso do Sul, Brazil (catalog number: LBP 1555).

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 for 2h in the dark in 1% osmium tetroxide in the same buffer, contrasted in block with aqueous solution of 5% uranyl acetate for 2h, dehydrated in acetone, embedded in araldite, and sectioned and stained with a saturated solution of uranyl acetate in 50% alcohol and with lead citrate. Electromicrographs were obtained using a Phillips - CM 100 transmission electron microscope.

Sixteen characters present in Callichthyidae were employed in the comparative analyses with other families of Loricarioidea. Based on the siluriform spermatozoa described in the literature (Porier & Nicholson, 1982; Kwon *et al.*, 1998; Lee, 1998; Lee & Kim, 1999, 2001; Kim & Lee, 2000; Quagio-Grassiotto & Carvalho, 2000, 2001; Quagio-Grassiotto *et al.*, 2001, 2005; Santos *et al.*, 2001; Burns *et al.*, 2002; Mansour *et al.*, 2002; Spadella *et al.*, 2006a, b), the midpiece size (character 10) was considered short when its total length was $\leq$ 1.7 µm, and long when its total length was > 1.7 µm. The cytoplasmic channel size (character 12) was considered short when its total length was $\leq$ 1.5 µm, and long when its total length was > 1.5 µm.

Results

Spermiogenesis in Corydoradinae. In the analyzed species, spermatids are found in the lumen of the germinative compartment together with the spermatozoa (Fig. 1c, 1d, 2a). In the beginning of the process, the early spermatids remain connected to the Sertoli cells surface (Figs. 2b-2d). During spermiogenesis, this connection is lost and differentiated spermatids move towards the lumen region of the central germinative compartment, mixing with the spermatozoa. In the early spermatids, the centriolar complex lies laterally to the nucleus and the distal centriole anchors to the plasma membrane. The flagellum development from the distal centriole occurs lateral to the nucleus (Fig. 2e). The centriolar complex does not move towards the nucleus, remaining anchored to the plasma membrane (Figs. 2f, and 2g). The centrioles are coaxial to each other, and between them an electron-dense structure in transverse position to the centriole axes are observed. In direction of this structure, cytoskeleton elements converge from both centrioles (Figs. 2e, and 2e-inset). Along the spermiogenesis, the nuclear rotation does not occur, and the flagellum takes up an eccentric position in relation to the nucleus. A flat depression is formed in the nuclear outline, giving rise to the nuclear fossa. The nuclear fossa is eccentrically positioned, and the complex centriolar is found completely outside it (Fig. 2e). The chromatin is highly condensed in the form of thin fibers that give an homogeneous aspect to the nucleus (Figs. 2g, and 2i). Although the centrioles do not move toward the nucleus, the formation of a cytoplasmic channel occurs (Fig. 2g). The cytoplasmic mass moves around the initial segment of the tail, and gives rise to the midpiece of the future spermatozoon. The midpiece has elongate mitochondria and vesicles randomly distributed along the midpiece (Figs. 2e-2h). In the midpiece cytoplasm the formation of the electron-dense circular structure is also observed (Fig. 2h). The flagellum has a classical (9+2) axoneme, with nine peripheral microtubular doublets of microtubules and a single central pair of microtubules, with neither lateral projection nor fins, and nor visible membranous compartment (Fig. 2e-inset).

Spermatozoa of Corydoradinae. The measurements of length and width of nucleus, nuclear fossa, midpiece, and cytoplasmic channel of the spermatozoa of Callichthyinae and Corydoradinae species analyzed are presented in the Table 1.

Corydoradinae spermatozoa have a round or ovoid head, asymmetric midpiece, and one flagellum eccentric to the nucleus (Figs. 3a, 4a, 5a, and 6a). They do not show an acrosomal vesicle. In *C. flaveolus* and *C. aeneus*, the nuclei have a round shape, while in *S. lacerdai* and *A. poecilus*, they are ovoid (Figs. 3a-3c, 4a-4c, 5a-5d, and 6a-6d). The chromatin has an homogeneous aspect, interspersed with electron-lucent areas. In the cytoplasmic region around the nucleus no organelles are seen (Figs. 3b-3f, 4c, 4e, 5a-5e, and 6a-6f). The nuclear fossa is eccentric to the nucleus and forms a simple arc (Figs. 3d, 4d, 5d, 5e, and 6d). In the species herein analyzed, there is a large number of elongated mitochondria filling all the regions of the midpiece. The mitochondria are separated from the flagellum by the cytoplasmic channel. Few vesicles are observed in the basal extremity of the midpiece distributed around the flagellum (Figs. 3e-3j, 4e-4j, 5d-5g, and 6e-6i). In *C. flaveolus*, *S. lacerdai*, and *A. poecilus*, the vesicles are small and can be isolated or interconnected to each other, whereas in *C. aeneus* they are interconnected to each other. In the midpiece, few vesicles are observed in the basal extremity of the midpiece distributed around the flagellum (Figs. 3f, 4i, 4j, 5e, 5g, and 6f). In all species analyzed an electron-dense circular structure in the midpiece cytoplasm is observed, which was formed during the spermiogenesis (Figs. 3f, 3g, 4g, 5a, and 6f-6h). The centrioles are completely outside the nuclear fossa, coaxial to each other, and show an electron-dense structure among them (Figs. 3d, 4d, 5a, 5d, 5e, 6a, 6d, and 6f). The distal centriole is differentiated in the basal body and gives rise to the axoneme, which exhibits the classical 9+2 microtubular pattern. Although the flagellum of the spermatids does not present differentiations, the spermatozoon flagellum displays some regions with a membranous compartment, whereas in others this compartment is not observed (Figs. 3k-3n, 4k-4n, 5a, 5h, and 6j-6l).

Spermiogenesis in Callichthyinae. In the analyzed Callichthyinae species, the spermiogenesis occurs in cysts in the germinative epithelium. In these cysts, groups of spermatids at the same development stage are surrounded by cytoplasmic processes of the

Sertoli cells (Figs. 1a, 1b, and 7a). In the cysts, the early spermatids are interconnected by cytoplasmic bridges that result from incomplete cytokinesis during cellular divisions. In the early spermatids, the cytoplasm is symmetrically distributed around the nucleus, which contains diffuse chromatin and has a circular outline (Fig. 7b, 7d, 7i, and 7n). The centriolar complex, with the proximal centriole in obtuse angle to the distal in *M. thoracata* and *C. callichthys*, and lateral and acute angle in *H. littorale*, lies laterally to the nucleus and is anchored to the plasma membrane. The flagellum development from the distal centriole takes place laterally to the nucleus (Figs. 7c, 7d, 7j, 7l, and 7o). The centriolar complex moves towards the nucleus bringing with it the plasma membrane and the initial segment of the flagellum that invaginates (Figs. 7e, 7j, and 7n). With this movement, the cytoplasmic canal, a space between the plasma membranes of the flagellar region, and the main part of the spermatid, is formed. The nuclear rotation occurs in different degrees among the Callichthyinae analyzed, being partial in *H. littorale* and *C. callichthys*, and complete in *M. thoracata*. This results in the eccentric position of the flagellum in relation to the nucleus in *H. littorale* and *C. callichthys*, and medial position in *M. thoracata* (Figs. 7d, 7e, 7i, 7j, 7n, and 7o). During nuclear rotation, a depression is formed in the nuclear outline that gives rise to the nuclear fossa. At the end of this process, the centrioles are found inserted in the nuclear fossa in *H. littorale* and *M. thoracata*, and in *C. callichthys* only the proximal centriole is found inserted in the nuclear fossa. The nuclear fossa is eccentrically positioned in *H. littorale* and *C. callichthys*, and medial in *M. thoracata* (Figs. 7b-7d, 7i, 7j, and 7o). During nuclear rotation, the chromatin condensation commences. The chromatin is progressively and homogeneously condensed, presenting areas of diffuse chromatin among areas of condensing chromatin (Figs. 7e, 7j, and 7o). The cytoplasmic mass moves around the initial segment of the tail, and gives rise to the midpiece of the future spermatozoon. The midpiece has rounded to elongate mitochondria and vesicles randomly distributed (Figs. 7e, 7f, 7j, 7k, 7o, and 7p). The flagellum exhibits the classical (9+2) axoneme surrounded by the flagellar membrane, which does not form lateral projections and membranous compartment in *H. littorale* and *M. thoracata*, whereas in *C. callichthys*, the formation of the membranous compartment from the flagellar membrane occurs in some segments of the tail (Figs. 7g, 7h, 7m, and 7q).

Spermatozoa of Callichthyinae. Callichthyinae spermatozoa are found in the lumen of the germinative compartment. *H. littorale* and *C. callichthys* spermatozoa exhibit an ovoid head with an ovoid nucleus, an asymmetric midpiece, and one flagellum eccentric to the nucleus (Figs. 8a, 8b, 8d, 8e, 10a, and 10e). In the spermatozoa of *M. thoracata* there is a spherical head that contains a spherical nucleus, a symmetric midpiece, and one flagellum medial to the nucleus (Figs. 9a, 9d, and 9e). In this species, the head does not show an acrosomal vesicle. The chromatin has an homogeneous aspect, interspersed by electron-lucent areas (Figs. 8a, 8d, 9a-9d, 10b, 10c, and 10e). No organelles are seen in the cytoplasmic region around the nucleus. The nuclear fossa in *H. littorale* and *C. callichthys* is eccentric in relation to the nucleus, and forms a simple arc (Figs. 8d, 8e, 10a, 10d, and 10e). In *M. thoracata*, the nuclear fossa is medial to the nucleus and is simple arc shaped (Figs. 9a, and 9d). Many large vesicles or cisternae, either interconnected to each other or not, are observed in the midpiece of *H. littorale* and *M. thoracata*, concentrated in the basal region around the flagellum (Figs. 8e, 8f-8i, 9a, 9d, and 9e-9g). In the midpiece of *C. callichthys*, few elongated vesicles are mainly found around the flagellum (Figs. 10d, 10f, and 10g). In Callichthyinae, several mitochondria are randomly distributed in all regions, concentrated on the apical and medial midpiece regions (Figs. 8c-8e, 8g, 8h, 9e-9h, 10a, and 10d-10g). The mitochondria are separated from the flagella by the cytoplasmic canal (Figs. 8h, 8i, 9i, 9j, and 10g). In *M. thoracata* and *C. callichthys*, the proximal centrioles form an obtuse angle in relation to the distal, whereas in *H. littorale* the arrangement of the centriolar complex is lateral and form an acute angle. The centriolar complex lies within the nuclear fossa in *H. littorale* and *M. thoracata*, whereas in *C. callichthys* the proximal centrioles are found only in the nuclear fossa (Figs. 8d, 8e, 9a, 9d, 10d, and 10e). The distal centriole becomes the basal body, and gives rise to an axoneme that exhibits the classical 9+2 microtubular pattern. No flagellar lateral projections or membranous compartment are present in *H. littorale* and *M. thoracata*, while in *C. callichthys*, there is a membranous compartment in some regions of the flagellum (Figs. 8j, 8k, 9k, 9l, 10h, and 10i).

Discussion

Spermatogenesis and Spermiogenesis. The occurrence of spermatids in the lumen of the germinative compartment together with the spermatozoa in the Corydoradinae species, suggests that spermatogenesis in this subfamily is of the semicystic type. This type of spermatogenesis was described in Cetopsidae and Aspredinidae (Spadella *et al.*, 2006a), and also found in Nematogenyidae (our unpublished data). Although uncommon, the semicystic spermatogenesis has been described in other families of Teleostei as Opheliidae (Mattei *et al.*, 1993), Scorpaenidae (Muñoz *et al.*, 2002), and Bleniidae (Lahnsteiner & Patzer, 1990).

In the Callichthyinae, the spermatozoa production occurs completely in cysts in the germinative epithelium, characterizing a spermatogenesis of the cystic type. This type of spermatogenesis is present in most Teleostei (Mattei *et al.*, 1993; Quagio-Grassiotto *et al.*, 2001, 2003, 2005).

In the spermatozoa, the flagellum axis may be either perpendicular or parallel to the nucleus, depending on whether nuclear rotation during spermiogenesis occurs (type I spermiogenesis) or not (type II spermiogenesis) (Mattei, 1970). In Pimelodidae and Heptapteridae, the flagellum is medial, the nucleus does not rotate, and both the nuclear fossa and the cytoplasmic channel are absent during spermiogenesis, characterizing a third type of spermiogenesis (Quagio-Grassiotto *et al.*, 2005; Quagio-Grassiotto and Oliveira, submitted). The spermiogenesis process observed in Corydoradinae is characterized by an eccentric development of the flagellum, the absence of nuclear rotation, an eccentric nuclear fossa formation, a cytoplasmic channel formation, and the absence of centriolar migration. This set of characteristics is different from those previously described. The unusual spermiogenesis process found in Corydoradinae, except for the eccentric development of the flagellum and eccentric nuclear fossa formation, was also observed in Cetopsidae and Aspredinidae (Spadella *et al.*, 2006a).

It is possible that the existing cytoplasmic channel is a result of the accommodation and interconnection of the vesicles around the flagellum of the subfamily Corydoradinae,

instead of the movement of the centrioles toward the nucleus. It is probable that the same occurs in Cetopsidae, Aspredinidae, and Nematogenyidae (Spadella *et al.*, 2006a).

The spermiogenesis process observed in Callichthyinae is characterized by an initial lateral development of the flagellum, the presence of nuclear rotation in different degrees, an eccentric or medial nuclear fossa formation, a cytoplasmic channel formation, and the presence of centriolar migration. This set of characteristics is more similar to type I spermiogenesis, which is also observed in Diplomystidae, the most basal siluriform family (Quagio-Grassiotto *et al.*, 2001), in Scoloplacidae, other family of Loricarioidea (Spadella *et al.*, 2006b), and in Pseudopimelodidae, other siluriform family (Quagio-Grassiotto *et al.*, 2005). However, in Diplomystidae the cytoplasmic channel does not remain in the spermatozoa (Quagio-Grassiotto *et al.*, 2001) as observed in Callichthyinae, Scoloplacidae, and Pseudopimelodidae. Furthermore, the nuclear rotation in Diplomystidae, Scoloplacidae, and Pseudopimelodidae is complete, resulting in a flagellum perpendicular to the nucleus in the spermatozoon, whereas in *H. littorale* and *C. callichthys* it is partial, and in *M. thoracata* it is complete, resulting in the eccentric position of the flagellum in *H. littorale* and *C. callichthys* and medial in *M. thoracata*. Mattei (1970) considered that the occurrence of these intermediate processes of spermiogenesis is responsible for the formation of these intermediate spermatozoa, whose flagellum is eccentric to the nucleus.

The characteristics of the spermatogenesis and spermiogenesis processes in Corydoradinae and Callichthyinae revealed clear peculiarities of each of these subfamilies, corroborating the hypotheses that they constitute monophyletic groups (Reis, 1998; Shimabukuro-Dias *et al.*, 2004).

Spermatozoa. The comparative analyses of callichthyids spermatozoa ultrastructure presented in Table 2 showed that species herein analyzed share the same state of character in eight out of the sixteen characters observed (characters 1, 3, 6, 8, and 10 to 13).

The callichthyids spermatozoa ultrastructure showed that the character 2, arrangement of centriolar complex, is variable among the species analyzed, being co-axial in all Corydoradinae, lateral in acute angle in *H. littorale*, and lateral in obtuse angle in *M. thoracata* and *C. callichthys*. The state "co-axial" is only found in Corydoradinae, which

represent a synapomorphy of this subfamily. The other character states, "arrangement of centriolar complex", found in Callichthyinae are also observed in Trichomycteridae (our unpublished data).

Character 15, "presence of electron-dense circular structure in the midpiece", is also shared by all the Corydoradinae species, representing other exclusive character of this subfamily, not observed in any other siluriform up to the present (Poirier & Nicholson, 1982; Emel'yanova & Makeyeva, 1991a, b; Kwon *et al.*, 1998; Lee, 1998; Lee & Kim, 1999, 2001; Kim & Lee, 2000; Quagio-Grassiotto & Carvalho, 2000; Quagio-Grassiotto *et al.*, 2001, 2005; Santos *et al.*, 2001; Burns *et al.*, 2002; Mansour *et al.*, 2002; Mansour & Lahnsteiner, 2003).

Some characteristics are shared by all Corydoradinae and some species of Callichthyinae. For example, the presence of a membranous compartment in the flagellum and centrioles outside of the nuclear fossa occurs in Corydoradinae and in *C. callichthys* (characters 4 and 9, respectively), and the occurrence of eccentric nuclear fossa (character 7), asymmetric midpiece (character 14) and eccentric flagellum position in relation to the nucleus (character 15) in Corydoradinae and in *H. littorale* and *C. callichthys*. There was not characteristic exclusively found in the spermatozoa of Callichthyinae. Most of the characteristics of spermatozoon of *C. callichthys* (Callichthyinae) were similar with those found among the species of Corydoradinae.

The presence of the membranous compartment in the flagellum (character 4) is found in the majority of the species of Callichthyidae (species of Corydoradinae and *C. callichthys*). A similar characteristic was also observed in Trichomycteridae, other members of Loricarioidea (our unpublished data), and in Characiformes of the family Curimatidae (Quagio-Grassiotto *et al.*, 2003).

The only character not shared by all Corydoradinae is the shape of the nucleus (character 5), which is spherical in *C. flaveolus* and *C. aeneus*, whereas in *S. lacerdai* and *A. poecilus*, the nucleus is ovoid. In a phylogenetic study of the subfamily Corydoradinae conducted by Britto (2003b) with 83 morphological characters the author corroborates the monophyly of the subfamily and showed that *Aspidoras* is more related with *Scleromystax*. The data obtained in the present study corroborated this hypothesis.

Considering the phylogeny of the superfamily Loricarioidea, the family Callichthyidae is the sister group of the clade formed by Scoloplacidae, Astroblepidae, and Loricariidae (de Pinna, 1998; Britto, 2003a). Table 2 shows that one or more species of Callichthyidae share eight characteristics with Nematogenyidae (another family of Loricarioidea), ten characters with Scoloplacidae, and 12 with Loricariidae. As observed above, the Callichthyidae spermatozoa share more similarities with Scoloplacidae and Loricariidae than with Nematogenyidae, corroborating the previous hypotheses of relationships of the superfamily Loricarioidea proposed by de Pinna (1998) and Britto (2003a).

Characteristics shared by Callichthyids and other families of Loricarioidea (Table 2) are also found in other siluriform families: Diplomystidae (Quagio-Grassiotto *et al.*, 2001), Amblycipitidae (Lee & Kim, 1999), Clariidae (Mansour *et al.*, 2002), Siluridae (Emel'yanova & Makeyeva, 1991b; Kwon *et al.*, 1998; Lee & Kim, 2001), Ictaluridae (Poirier & Nicholson, 1982; Emel'yanova & Makeyeva, 1991a), Heptapteridae and Pseudopimelodidae (Quagio-Grassiotto *et al.*, 2005), Auchenipteridae (Burns *et al.*, 2002), Pimelodidae (Quagio-Grassiotto & Carvalho, 2000; Santos *et al.*, 2001), and Bagridae (Emel'yanova & Makeyeva, 1991b; Lee, 1998; Kim & Lee, 2000; Mansour & Lahnsteiner, 2003). The states "co-axial centrioles" (character 5) and "presence of electron-dense circular structure in the midpiece" (character 15) are observed only in Corydoradinae.

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Table 1. Spermatozoa dimensions in callichthyids, in micrometers. The "n" shows the number of structures measured.

Subfamilies	**Corydoradinae**				**Callichthyinae**		
Species	*C. flaveolus*	*C. aeneus*	*S. lacerdai*	*A. poecilus*	*H. littorale*	*M. thoracata*	*C. callichthys*
Structures							
Nucleus							
Length (µm)	1.9 (n = 12)	1.6 (n = 7)	1.5 (n = 7)	1.6 (n = 6)	1.8 (n = 6)	1.8 (n = 9)	1.7 (n = 6)
Width (µm)	2.0 (n = 12)	1.7 (n = 7)	1.6 (n = 7)	1.5 (n = 6)	2.0 (n = 6)	1.8 (n = 8)	1.6 (n = 6)
Nuclear Fossa							
Length (µm)	0.07 (n = 10)	0.07 (n = 8)	0.07 (n = 8)	0.1 (n = 5)	0.2 (n = 6)	0.2 (n = 4)	0.2 (n = 5)
Width (µm)	0.3 (n = 10)	0.3 (n = 8)	0.3 (n = 8)	0.3 (n = 5)	0.6 (n = 6)	0.6 (n = 4)	0.4 (n = 5)
Midpiece							
Length (µm)	1.4 (n = 12)	1.3 (n = 6)	1.5 (n = 5)	1.5 (n = 6)	1.7 (n = 7)	1.6 (n = 6)	1.5 (n = 7)
Width (µm)	2.0 (n = 12)	1.9 (n = 6)	1.7 (n = 5)	1.5 (n = 6)	1.9 (n = 7)	2.0 (n = 7)	1.6 (n = 7)
Cytoplasmic Channel							
Length (µm)	0.3 (n = 12)	0.3 (n = 7)	0.3 (n = 7)	0.6 (n = 5)	1.5 (n = 5)	1.3 (n = 4)	0.8 (n = 5)
Width (µm)	0.4 (n = 12)	0.4 (n = 7)	0.4 (n = 7)	0.3 (n = 5)	0.4 (n = 5)	0.7 (n = 4)	0.4 (n = 5)

Table 2. General view of the distribution of spermatozoa character states in the species analyzed in the present study and, in others Loricarioidea families. (+) present; (-) absent.

Characters*	1		2						3	4			5					6		7			8		9	10		11			12		13					14		15	16	
	a	b	a	b	c	d	e	f		a	b	c	a	b	c	d	e	a	b	a	b	c	a	b		a	b	a	b	c	a	b	a	b	c	d	e	a	b		a	b
Families																																										
Nematogenyidae	-	+	+	-	-	-	-	-	+	+	-	-	-	-	-	+	-	-	+	+	-	-	-	-	-	-	+	-	+	-	+	-	-	-	+	-	-	+	-	-	+	-
C. flaveolus	+	-	-	-	-	-	-	+	+	-	-	+	-	-	-	-	+	-	+	-	-	+	+	-	-	+	-	-	+	-	+	-	-	+	-	-	-	-	+	+	-	+
C. aeneus	+	-	-	-	-	-	-	+	+	-	-	+	-	-	-	-	+	-	+	-	-	+	+	-	-	+	-	-	+	-	+	-	-	+	-	-	-	-	+	+	-	+
S. lacerdai	+	-	-	-	-	-	-	+	+	-	-	+	-	-	+	-	-	-	+	-	-	+	+	-	-	+	-	-	+	-	+	-	-	+	-	-	-	-	+	+	-	+
A. poecilus	+	-	-	-	-	-	-	+	+	-	-	+	-	-	+	-	-	-	+	-	-	+	+	-	-	+	-	-	+	-	+	-	-	+	-	-	-	-	+	+	-	+
H. littorale	+	-	-	+	-	-	-	-	+	+	-	-	-	-	+	-	-	-	+	-	-	+	+	-	+	+	-	-	+	-	+	-	-	+	-	-	-	-	+	-	-	+
M. thoracata	+	-	-	-	+	-	-	-	+	+	-	-	-	-	-	-	+	-	+	-	+	-	+	-	+	+	-	-	+	-	+	-	-	+	-	-	-	-	+	-	+	-
C. callichthys	+	-	-	-	+	-	-	-	+	-	-	+	-	-	+	-	-	-	+	-	-	+	+	-	-	+	-	-	+	-	+	-	-	+	-	-	-	-	+	-	-	+
Scoloplacidae	+	-	-	-	-	+	-	-	+	-	+	-	-	+	-	-	-	-	+	-	+	-	+	-	+	-	+	-	+	-	-	+	-	+	-	-	-	-	+	-	+	-
Loricariidae	+	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	+	+	-	-	+	-	+	-	+	+	-	-	+	-	+	-	-	+	-	-	-	-	+	-	+	-

Legends:

*1- flagella number (a- one; b- two); 2- arrangement of centriolar complex (a- lateral and parallel; b-lateral and acute angle; c- lateral and obtuse angle; d- anterior and perpendicular; e- anterior and obtuse angle; f- co-axial); 3- presence of elongated vesicles; 4- flagellar membrane specializations (a- absent; b- two lateral fins; c- membranous compartment); 5- shape of the nucleus (a- semi-ovoid; b- conic; c- ovoid; d-ovoid with its bigger axis in the horizontal direction; e- spherical); 6- pattern of chromatin condensation (a- heterogeneous; b- homogeneous); 7- nuclear fossa (a-absent; b- medial; c- eccentric); 8- nuclear fossa shape (a- simple arc; b- double arc); 9- centrioles totally inserted in the nuclear fossa; 10- midpiece size (a- short; b- long); 11- cytoplasmic channels (a- absent; b- one; c- two); 12- cytoplasmic channel size (a- short; b- long); 13- mitochondria shape (a- rounded; b- elongated; c- elongated and ramificated; d- C-shape; e- irregular); 14- midpiece symmetry (a- symmetric; b- asymmetric); 15- Presence of electron-dense circular structure in the midpiece; 16- flagellum position in relation to the nucleus (a- medial; b- eccentric).

FIGURE CAPTION

Fig. 1. Photomicrography showing a general view of the cystic spermatogenesis in Callichthyinae, and of the semi-cystic spermatogenesis in Corydoradinae. Scale bar **a** and **c** = 20 μm, **b** and **d** = 10 μm. CI: cysts of primary spermatocytes; G: spermatogonia; T: spermatids in the lumen of germinative compartment; Z spermatozoa, Arrow: spermatids cysts.

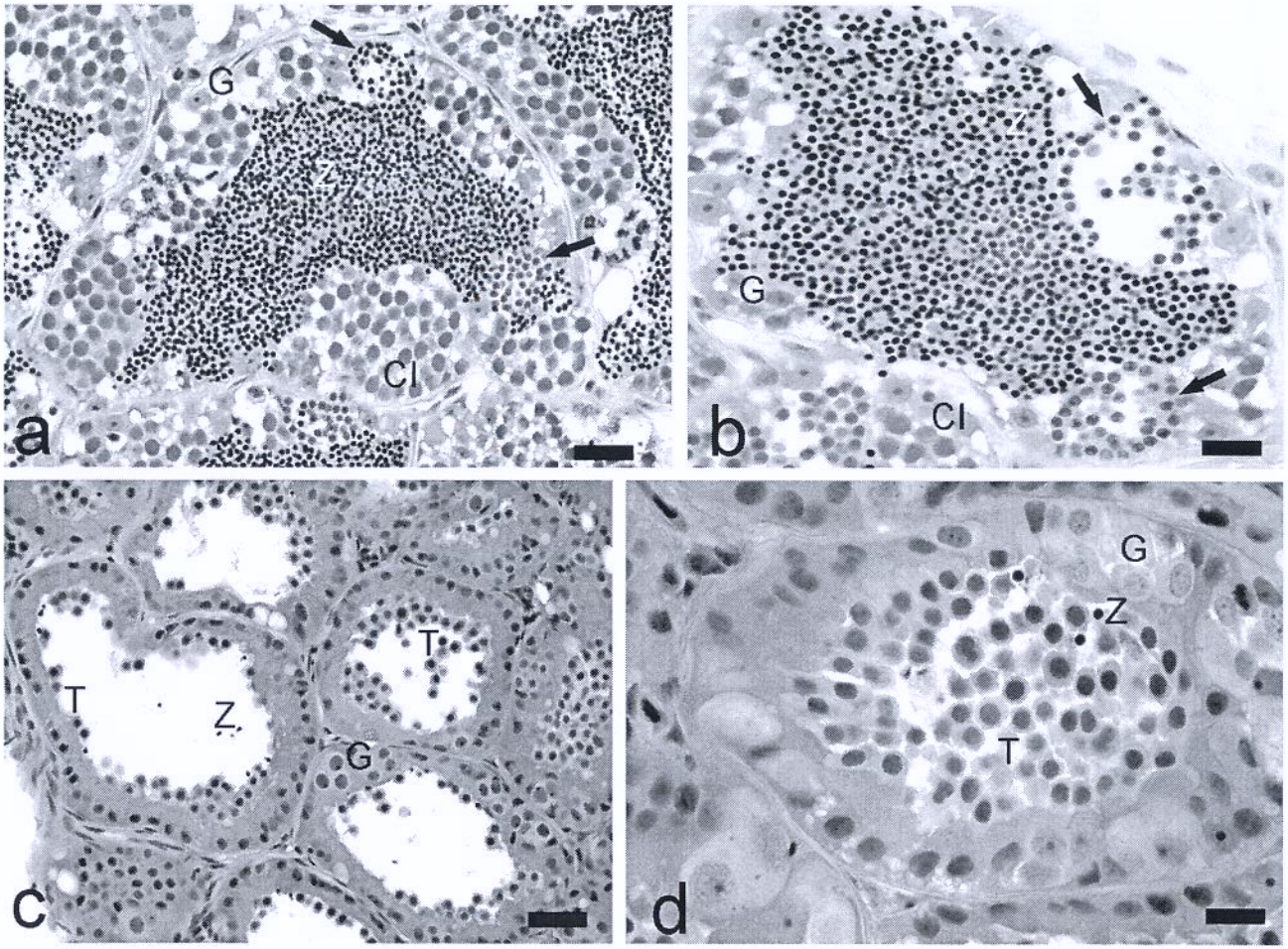
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FIGURE CAPTION

Fig. 2. Spermiogenesis in Corydoradinae. **a**) Spermatids together with the spermatozoa in the lumen of the germinative compartment. **b-d**) Early spermatids connected to the Sertoli cells surface. **e** and **e**-insets) Arrangement of the centriolar complex showing electron-dense material between centrioles, and flagellum with classical (9+2) axoneme. **f** to **h**) Midpiece showing mitochondria, vesicles, and electron-dense circular structure (longitudinal sections). **i**) Late spermatid. (**a**) X 3250; (**b**) X 4875; (**c, d**) X 5750; (**e**) X 13250; (**e**-inset left, **i**) X 17000; (**e**-inset right) X 25850; (**f, g**) X 9750; (**h**) X 7750. C: centriolar complex; D: distal centriole; L: lumen; N: nucleus; P: proximal centriole; S: Sertoli cell; V: vesicles; Z: spermatozoon; Asterisk: mitochondria, Arrow: cytoplasmic channel; Arrowhead: point connection; Double arrowhead: electron-dense material between the centrioles; Lozenge: electron-dense circular structure.

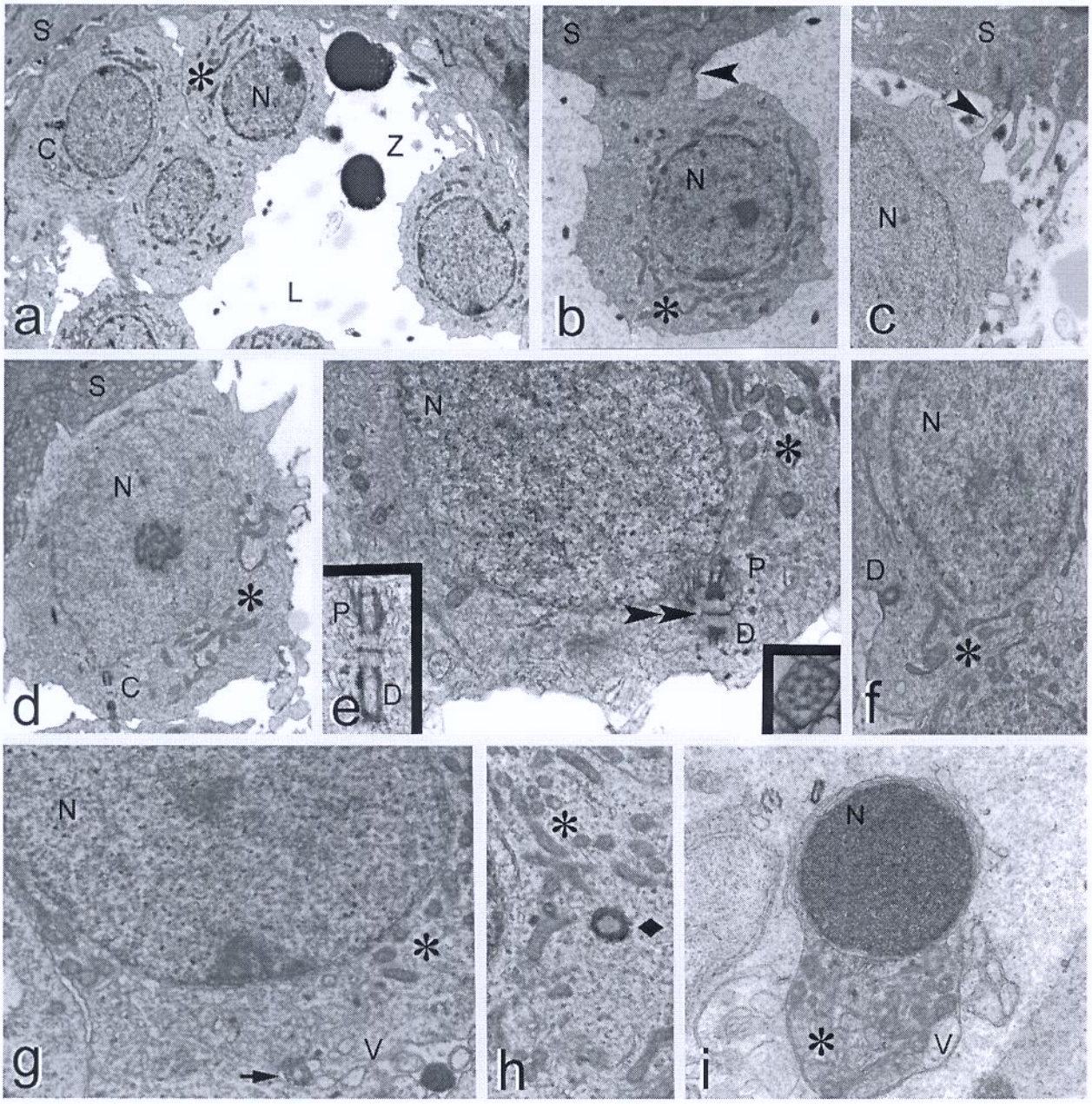
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FIGURE CAPTION

Fig. 3. Spermatozoa of *Corydoras flaveolus*. **a**) Spermatozoon longitudinal section. **b** and **c**) Nucleus in transverse sections. **d**) Detail of centrioles arrangement. **e** to **j**) Midpiece longitudinal and transverse sections showing mitochondria, vesicles, cytoplasmic channel, and electron-dense circular structure. **k** and **l**) Flagellum longitudinal sections. **m** and **n**) Flagellum cross sections. (**a**) X 17000; (**b, c**) X 15750; (**d**) X 28350; (**e, f, h**) X 23000; (**g, m**) X 42000; (**i**) X 18900; (**j**) X 25200; (**k**) X 25300; (**l, n**) X 31500. B: basal body; D: distal centriole; E: electron-dense structure; F: flagellum; N: nucleus; P: proximal centriole; V: vesicles; Asterisk: mitochondria, Arrow: cytoplasmic channel; Arrowhead: lateral membranous compartment; Double arrowhead: electron-dense material between the centrioles; Lozenge: electron-dense circular structure.

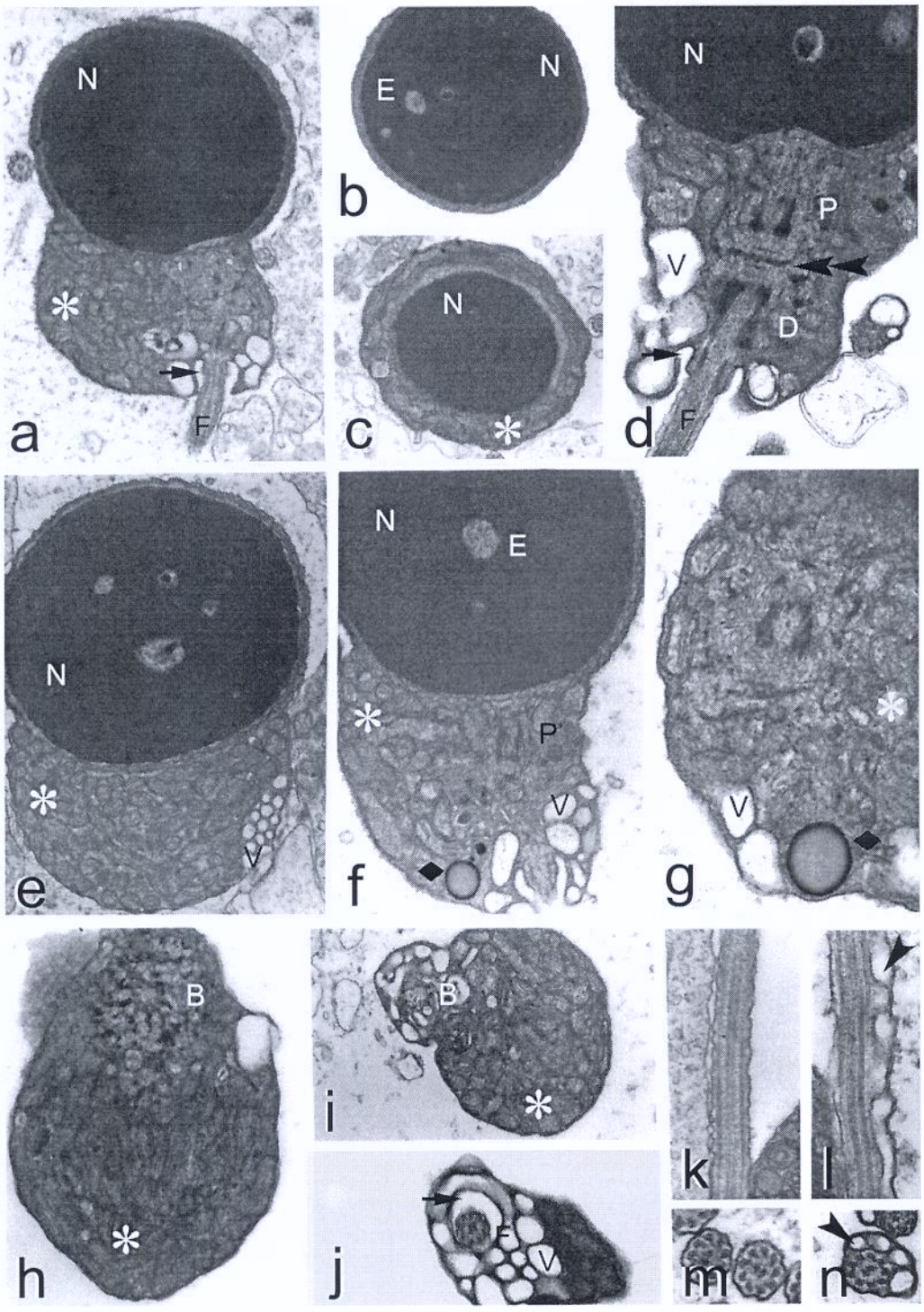
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FIGURE CAPTION

Fig. 4. Spermatozoa of *Corydoras aenèus*. **a)** Longitudinal section. **b** and **c)** Nucleus in cross sections. **d)** Detail of centrioles arrangement. **e** to **j)** Midpiece longitudinal and transverse sections showing mitochondria, vesicles, membranous compartment, and electron-dense circular structure. **k** and **l)** Flagellum in longitudinal sections. **m** and **n)** Flagellum in cross sections. (**a, d**) X 17000; (**b**) X 13800; (**c**) X 15750; (**e, h, k, l**) X 23000; (**f**) X 20400; (**g**) 44100; (**i, j**) X 31500; (**m**) X 47250; (**n**) X 57500. B: basal body; D: distal centriole; E: electron-dense structure; F: flagellum; N: nucleus; P: proximal centriole; V: vesicles; Asterisk: mitochondria, Arrow: cytoplasmic channel; Arrowhead: lateral membranous compartment; Double arrowhead: electron-dense material between the centrioles; Lozenge: electron-dense circular structure.

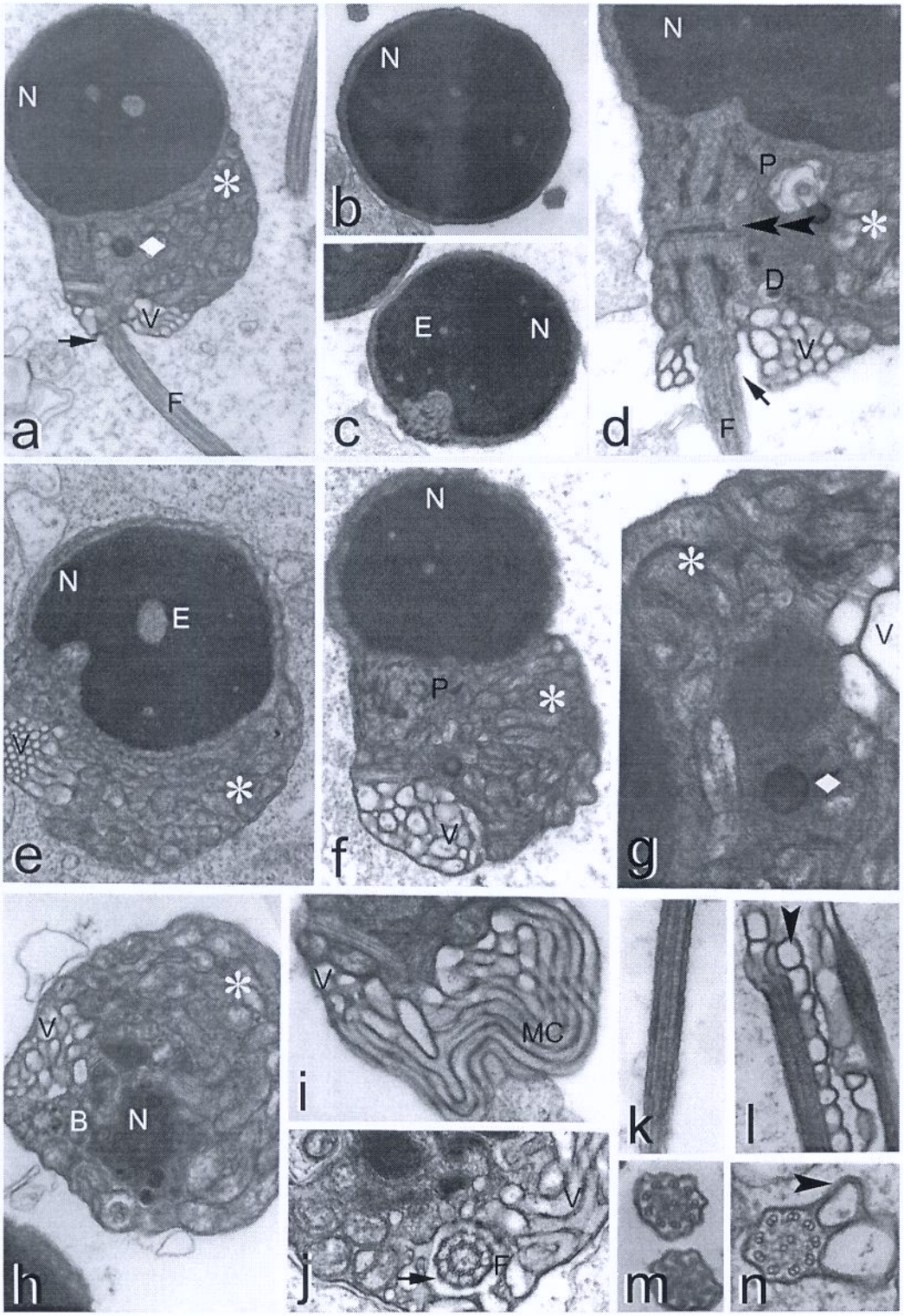
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FIGURE CAPTION

Fig. 5. Spermatozoa of *Scleromystax lacerdai*. **a**) Spermatozoon longitudinal section. **b** and **c**) Nucleus in longitudinal and cross sections. **d**) Detail of centrioles arrangement. **e** to **g**) Midpiece in longitudinal and transverse sections showing mitochondria, and vesicles. **h**) Flagellum in longitudinal section. **h**-inset) Flagellum in cross section. (**a, d, e**) X23000; (**b, c**) X 17000; (**f**) X25200; (**g**) X 20400; (**h**) X 42000; (**h**-inset) X 32200. B: basal body; D: distal centriole; E: electron-dense structure; F: flagellum; N: nucleus; P: proximal centriole; V: vesicles; Asterisk: mitochondria, Arrow: cytoplasmic channel; Arrowhead: lateral membranous compartment; Double arrowhead: electron-dense material between the centrioles; Lozenge: electron-dense circular structure.

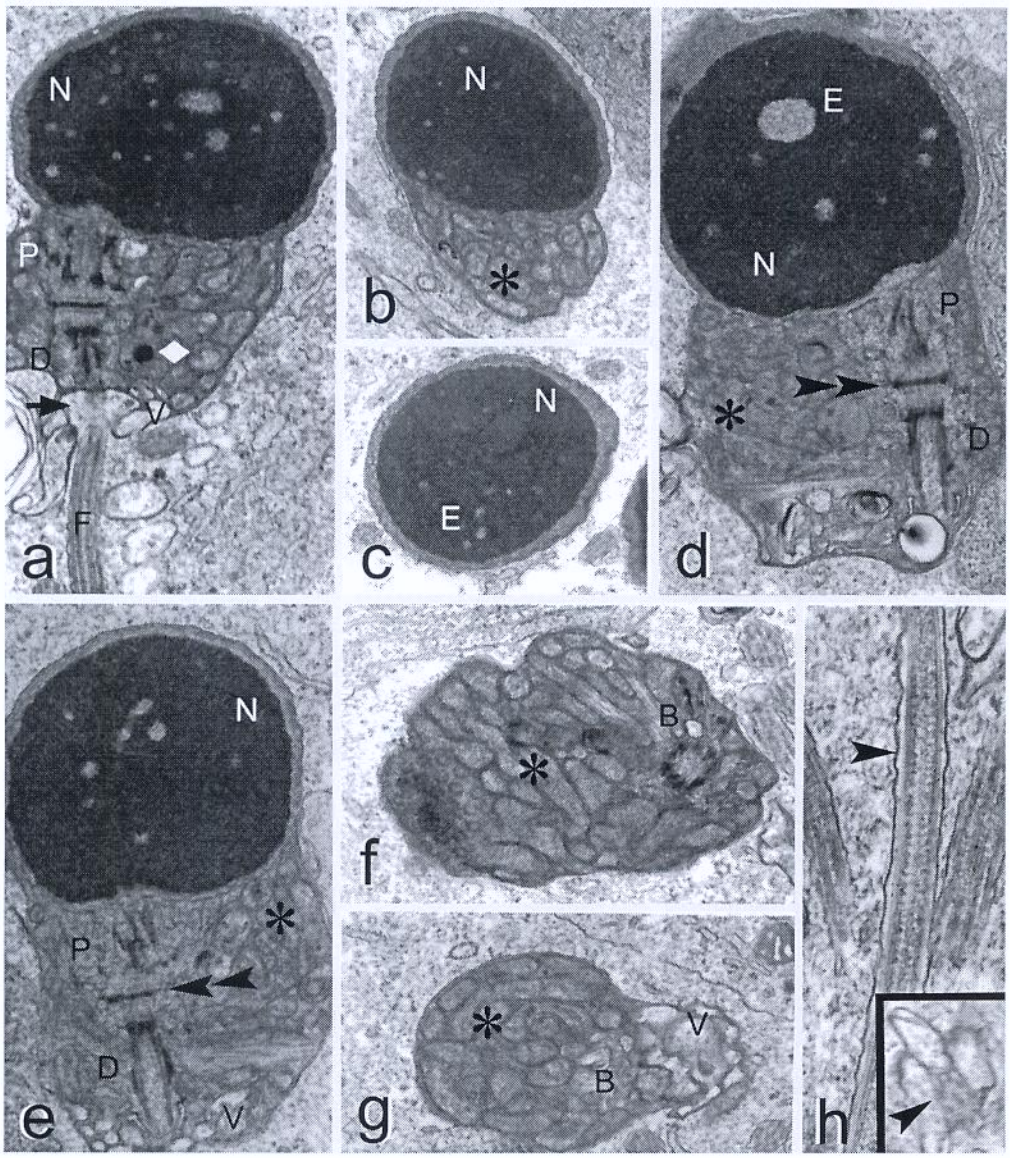
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FIGURE CAPTION

Fig. 6. Spermatozoa of *Aspidoras poecilus*. **a)** Spermatozoon longitudinal section. **b** and **c)** Nucleus in longitudinal and cross sections. **d)** Centrioles arrangement. **e** to **i)** Midpiece in longitudinal and transverse sections showing mitochondria, vesicles, cytoplasmic channel, and electron-dense circular structures. **j** and **k)** Flagellum in longitudinal sections. **j**-inset and **l)** Flagellum in cross sections. **(a, b, f, j, k)** X 23000; **(c, d)** X 17000; **(e)** X 22050; **(g, h)** X 25200; (i) X 18400; (j-inset, **l**) X 57500. B: basal body; D: distal centriole; E: electron-dense structure; F: flagellum; N: nucleus; P: proximal centriole; V: vesicles; Asterisk: mitochondria, Arrow: cytoplasmic channel; Arrowhead: lateral membranous compartment; Double arrowhead: electron-dense material between the centrioles; Lozenge: electron-dense circular structure.

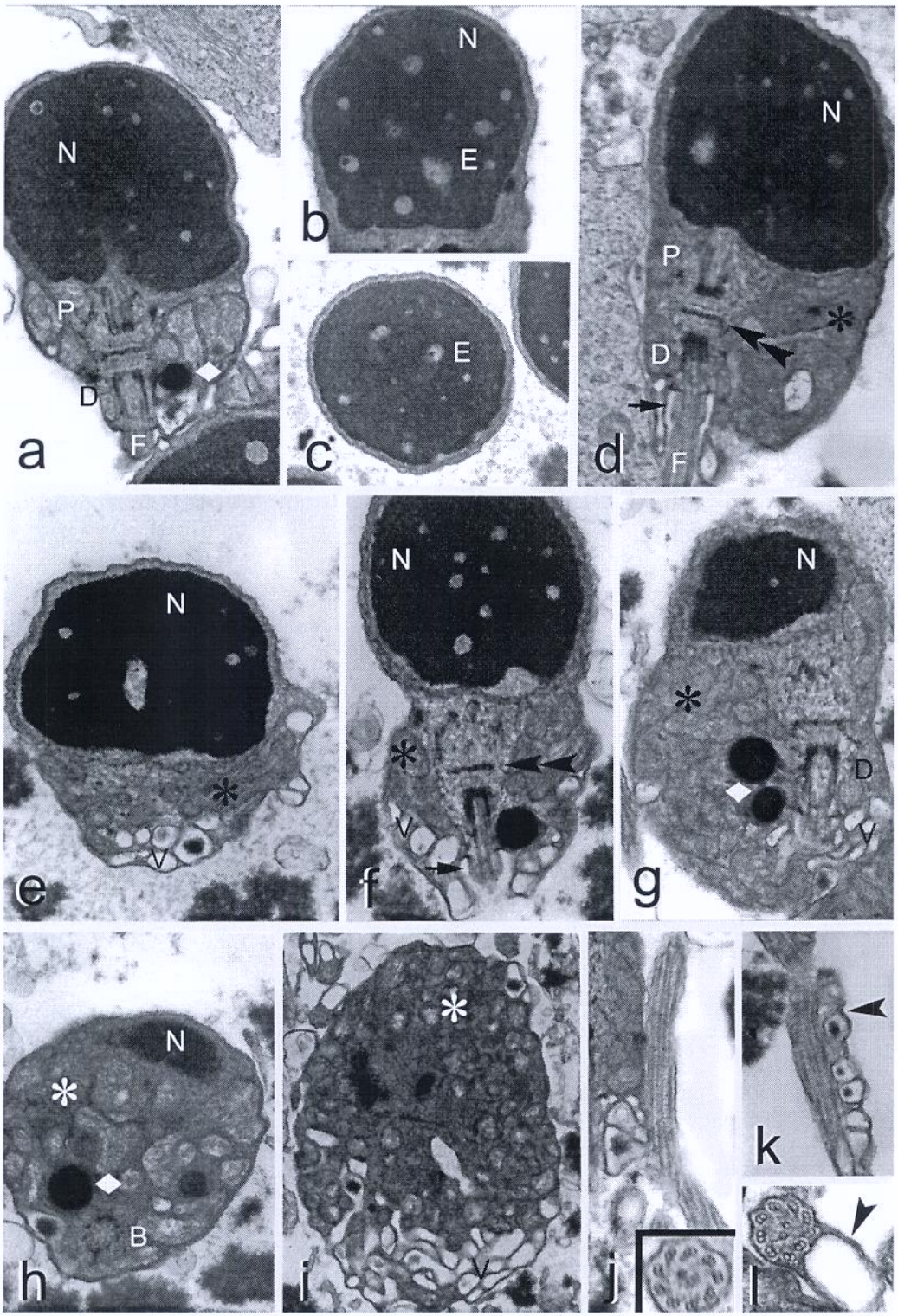
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FIGURE CAPTION

Fig. 7. Spermiogenesis in Callichthyinae. **a** to **h**) *Hoplosternum littorale*. **a**) Spermatids cyst. **b** and **d**) Early spermatids (longitudinal sections). **c**) Centrioles arrangement. **e**) Late spermatid. **f**) Midpiece showing mitochondria, and vesicles (cross section). **g** and **h**) Flagellum (longitudinal and cross sections). **i** to **m**) *Megalechis thoracata*. **i**) Early spermatid in longitudinal section. **j**) Late spermatid. **k**) Midpiece showing mitochondria, cytoplasmic channel, and vesicles (cross section). **l**) Centrioles arrangement. **m**) Flagellum in longitudinal section. **n** to **q**) *Callichtys callichthys*. **n** and **o**) Early spermatids in longitudinal sections. **p**) Midpiece showing mitochondria. **q**) Flagellum in longitudinal section. (**a**) X 7750; (**b, f, i, n, p**) X 13250; (**c, g, k, m**) X 17000; (**d**) X 10200; (**e, j**) X 11900; (**h**) 20400; (**l**) X 23000; (**o**) X 13600; (**q**) X 31500. D: distal centriole; F: flagellum; N: nucleus; P: proximal centriole; V: vesicles; Asterisk: mitochondria, Arrow: cytoplasmic channel.

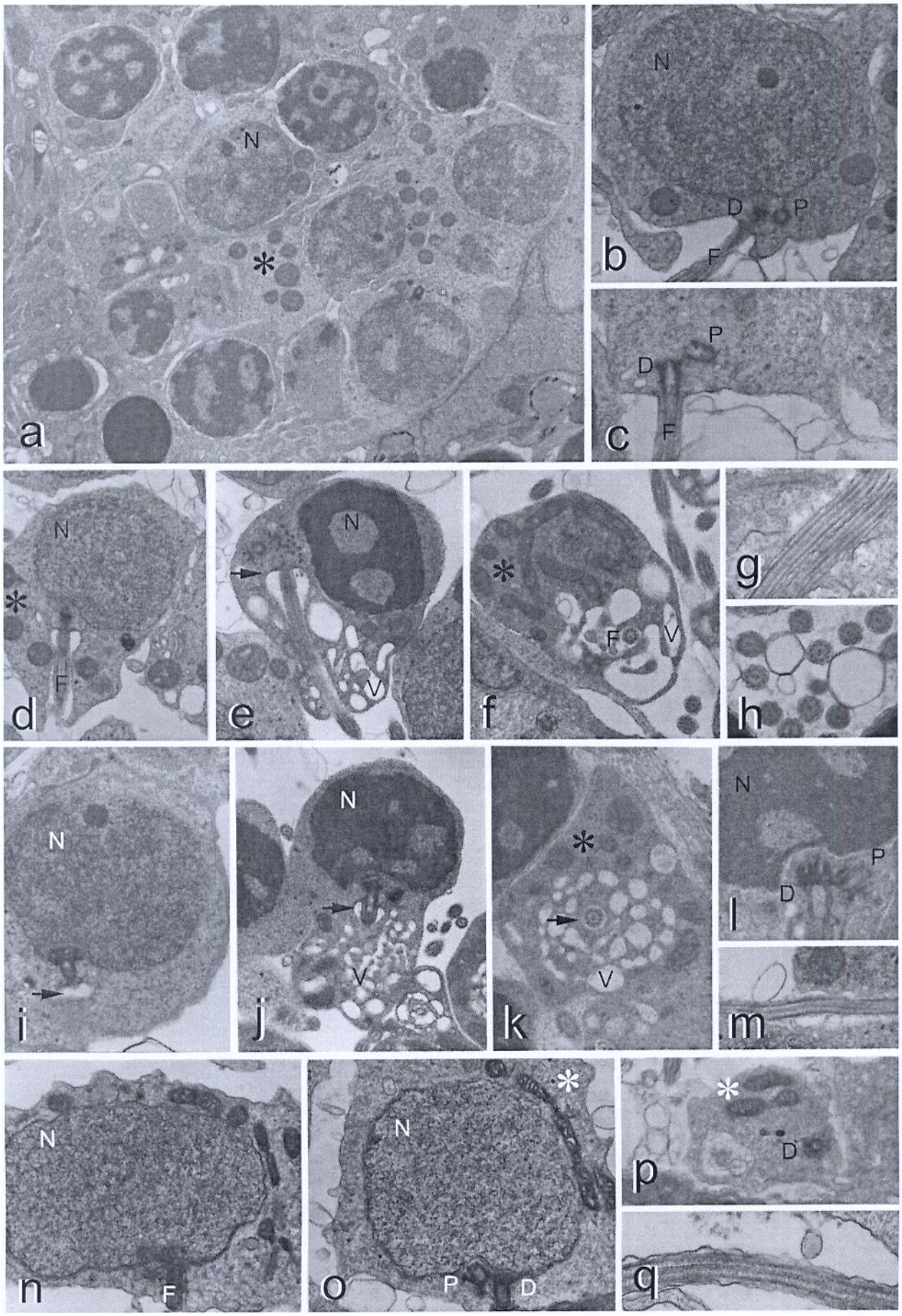
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FIGURE CAPTION

Fig. 8. Spermatozoa of *Hoplosternum littorale*. **a)** Spermatozoon in longitudinal section. **b** and **c)** Nucleus in cross sections. **d)** Detail of centrioles arrangement. **e** to **j)** Midpiece in longitudinal and transverse sections showing mitochondria, elongated vesicles, and cytoplasmic channel. **k)** Flagellum in longitudinal section. j-inset) Flagellum in cross section showing classical (9+2) axoneme. (**a, b, h**) X 17000; (**c**) X 13800; (**d to g**) X 23000; (**i**) X 33600; (**j, k**) X 31500; (**j**-inset) X 57500. D: distal centriole; E: electron-dense lucent structure; F: flagellum; N: nucleus; P: proximal centriole; V: vesicles; Asterisk: mitochondria, Arrow: cytoplasmic channel.

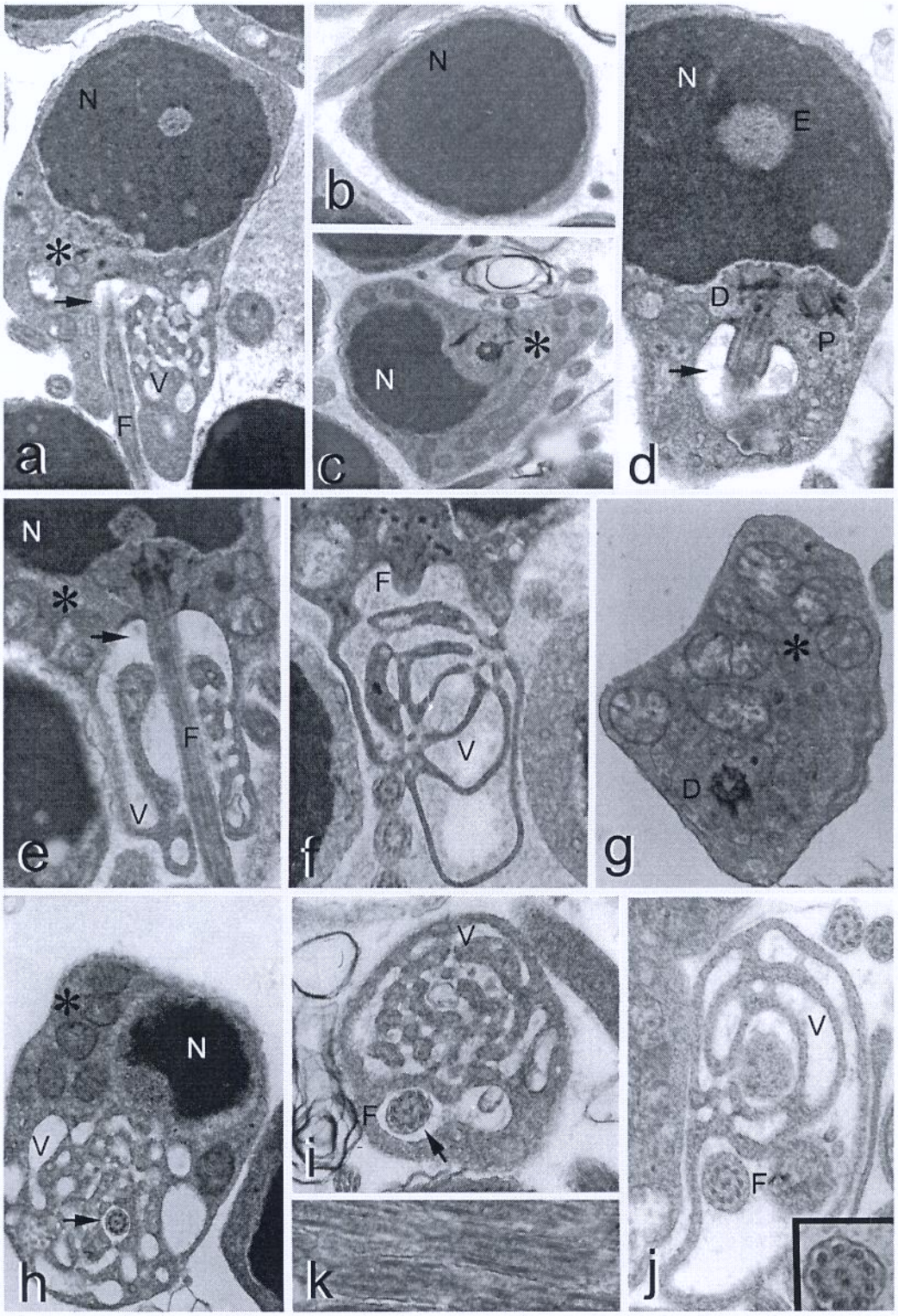
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FIGURE CAPTION

Fig. 9. Spermatozoa of *Megalechis thoràcata*. **a)** Longitudinal section. **b** and **c)** Nucleus in cross sections. **d)** Detail of centrioles arrangement. **e** to **j)** Midpiece in longitudinal and transverse sections showing mitochondria, elongated vesicles, and cytoplasmic channel. **k** and **l)** Flagellum in longitudinal and cross sections. (**a** to **e**, **h**) X 17000; (**f**, **i**, **j**) X 18400; (**g**) X 11925; (**k**) X 23000; (l) X 13950. D: distal centriole; E: electron-dense structure; F: flagellum; N: nucleus; P: proximal centriole; V: vesicles; Asterisk: mitochondria, Arrow: cytoplasmic channel.

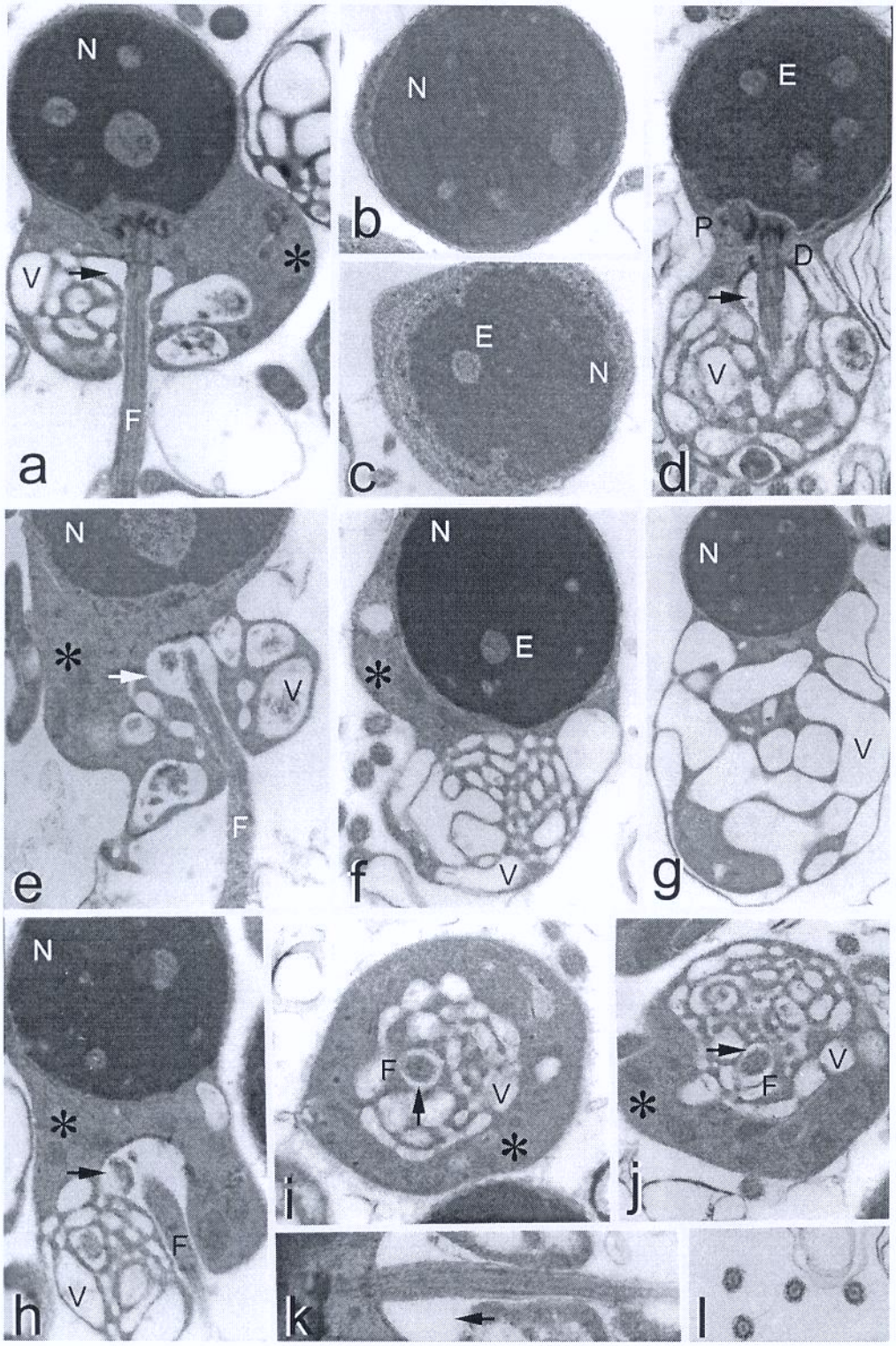
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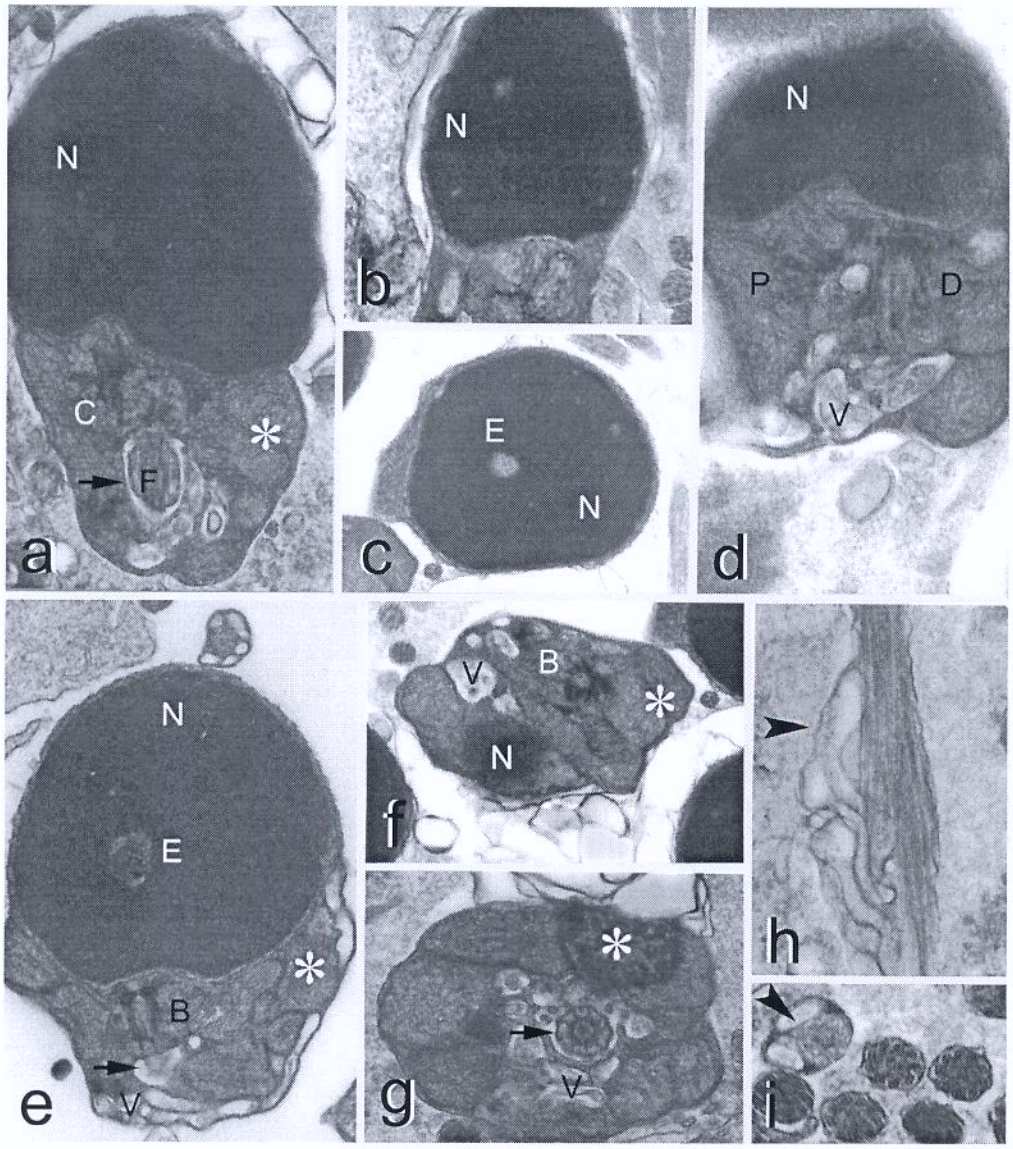
FIGURE CAPTION

Fig. 10. Spermatozoa of *Callichthys callichthys*. **a**) Spermatozoon in longitudinal section. **b** and **c**) Nucleus in longitudinal and cross sections. **d**) Centrioles arrangement. **e** to **g**) Midpiece in longitudinal and transverse sections showing mitochondria, vesicles, and cytoplasmic channel. **h** and **i**) Flagellum in longitudinal and cross sections. (**a, g, h**) X 31500; (**b, e**) X 23000; (**c, f**) X 17000; (**d, i**) X 42000. D: distal centriole; E: electron-dense structure; F: flagellum; N: nucleus; P: proximal centriole; V: vesicles; Asterisk: mitochondria, Arrow: cytoplasmic channel; Arrowhead: lateral membranous compartment.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4.5. CAPÍTULO 5

Spadella, M.A., Oliveira, C., Quagio-Grassiotto, I. Analysis of the spermiogenesis and spermatozoal ultrastructure in Trichomycteridae (Teleostei: Ostariophysi: Siluriformes). Manuscrito a ser submetido à revista Tissue & Cell.

Analysis of the spermiogenesis and spermatozoal ultrastructure in Trichomycteridae (Teleostei: Ostariophysi: Siluriformes)

Maria Angélica Spadella[1,2]
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Running headline: Spermiogenesis and spermatozoa in Trichomycteridae

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ABSTRACT

Siluriformes comprise the most diverse and widely distributed ostariophysan group, a fish assemblage that includes about three quarters of the freshwater fish of the world. In the present study, the ultrastructural characterization of spermiogenesis and spermatozoa in specimens of Copionodontinae (the sister group to all other trichomycterids), Trichomycterinae (a derivate trichomycterid group), and *Ituglanis* (a genus not assigned to any trichomycterid subfamily) are presented. The comparative analyses of the data show that trichomycterid species share six of seven analyzed spermiogenesis characters, reinforcing the hypotheses of group monophyly. Considering the data obtained, the species of Trichomycteridae share more common characteristics of spermatogenesis, spermiogenesis, and spermatozoa with representatives of the families Callichthyidae, Loricariidae, and Scoloplacidae than with Nematogenyidae, its hypothesized sister group. On the other hand, except by the family Nematogenyidae, the similarity observed reinforce the monophyly of the superfamily Loricarioidea.

KEY WORDS: catfish, cysts of spermatids, spermatozoa, structure, phylogeny, morphology.

INTRODUCTION

Siluriformes comprise the most diverse and widely distributed ostariophysan group, a fish assemblage that includes about three quarters of the freshwater fish of the world (Burgess, 1989; Teugels, 1996; Ferraris, 1998; Nelson, 2006). Among the neotropical Siluriformes lineages likely to be monophyletic, we find the superfamily Loricarioidea (de Pinna, 1998; Britto, 2003) that comprises six families: Nematogenyidae, Trichomycteridae, Callichthyidae, Scoloplacidae, Astroblepidae, and Loricariidae. According to the phylogeny of the superfamily Loricarioidea, the family Trichomycteridae is the sister group of the Nematogenyidae (de Pinna, 1998; Britto, 2003).

Trichomycteridae form a well-corroborated monophyletic group, and it is one of the Loricarioidea families with greater species number. Actually, trichomycterids are divided into eight subfamilies: Copionodontinae, Trichogeninae, Trichomycterinae, Vandelliinae, Stegophilinae, Tridentinae, Glanapteryginae, and Sarcoglanidinae (de Pinna and Wosiacki, 2003). All those subfamilies are demonstrably monophyletic, except Trichomycterinae (de Pinna, 1998; de Pinna and Wosiacki, 2003).

In the present study, the ultrastructural characterization of spermiogenesis and spermatozoa in specimens of Copionodontinae (the sister group to all other trichomycterids), Trichomycterinae (a derivate trichomycterids group), and *Ituglanis* (a genus not assigned to any trichomycterid subfamily) are presented. Comparisons between the data obtained in the present study and those available for other siluriform families were conducted in order to investigate the similarities and differences among the families of the order Siluriformes.

MATERIAL AND METHODS

This study was conducted with two adult males of *Copionodon orthiocarinatus* de Pinna, 1992 (Copionodontinae) collected from the Piabas river (12°57'02.2"S, 41°16'37.1"W), Mucugê, Bahia, Brazil (LBP 1964), two adult males of *Ituglanis amazonicus* (Steindachner, 1882) from the Ribeirão dos Veados, Taquari river

(18°25'21.8"S, 54°50'06.3"W), Coxim, Mato Grosso do Sul, Brazil (LBP 1908); and two adult males of each species of subfamily Trichomycterinae: *Trichomycterus* aff. *iheringi* collected from the Capivara river (22°53.963'S, 48°23.204'W), Botucatu, São Paulo, Brazil (LBP 4184); *Trichomycterus areolatus* Valenciennes, 1846, collected from the Caramávida river (37°40.842'S, 73°15.997'W), VIII Region, Antiguala, Cañet, Chile (LBP 997); *Trichomycterus reinhardti* (Eigenmann, 1917) collected from the Sapateiro stream (21°16.432'S, 43°38.613'W), Barbacena, Minas Gerais, Brazil (LBP 10229); *Trichomycterus* sp.1 collected from the Alambari river (22°56'08", 48°19'15"W), Botucatu, São Paulo, Brazil (LBP 1404); and *Trichomycterus* sp.2 collected from the Jacutinga stream (23°09'S, 48°16'W), Bofete, São Paulo, Brazil (LBP 1312). Fishes were identified and kept in the fish collection of Laboratório de Biologia e Genética de Peixes (LBP), Departamento de Morfologia, Instituto de Biociências, UNESP, Botucatu, São Paulo, Brazil.

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 for 2h in the dark in 1% osmium tetroxide in the same buffer, contrasted in block with aqueous solution of 5% uranyl acetate for 2h, 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.

For comparative purposes, the available information about siluriform spermatozoa was reviewed. Data were obtained for the following families: Diplomystidae (Quagio-Grassiotto *et al.*, 2001), Cetopsidae, Aspredinidae, and Nematogenyidae (Spadella *et al.*, 2006a), Amblycipitidae (Lee and Kim, 1999), Ariidae (Mattei, 1991, schematic drawings), Loricariidae (Mansour and Lahnsteiner, 2003), Callichthyidae (Spadella *et al.*, submitted), Scoloplacidae (Spadella *et al.*, 2006b), Malapteruridae (Mattei, 1991, schematic drawings; Shahin, 2006), Ictaluridae (Poirier and Nicholson, 1982; Emel'yanova and Makeyeva, 1991a, b), Bagridae (Emel'yanova and Makeyeva, 1991b; Lee, 1998; Kim and Lee, 2000; Mansour and Lahnsteiner, 2003), Pimelodidae (Quagio-Grassiotto and Carvalho, 2000; Santos *et al.*, 2001), Heptapteridae and Pseudopimelodidae (Quagio-Grassiotto *et al.*,

2005), Siluridae (Emel'yanova and Makeyeva, 1991b; Kwon *et al.*, 1998; Lee and Kim, 2001), Clariidae (Mansour *et al.*, 2002), and Auchenipteridae (Burns *et al.*, 2002).

Twenty four characters of spermatogenesis and spermiogenesis (characters 1 to 7) and spermatozoa (characters 8 to 24), present in at least one species analyzed in the present study, were employed in the comparative analyses with other Loricariodea families. Based on the siluriform spermatozoa described in the literature (Porier and Nicholson, 1982; Kwon *et al.*, 1998; Lee, 1998; Lee and Kim, 1999, 2001; Kim and Lee, 2000; Quagio-Grassiotto and Carvalho, 2000; Quagio-Grassiotto *et al.*, 2001, 2005; Santos *et al.*, 2001; Burns *et al.*, 2002; Mansour *et al.*, 2002), the midpiece size (character 18) was considered short when its total length was ≤1.7 μm and was considered long when its total length was > 1.7 μm. The cytoplasmic channel size (character 20) was considered short when its total length was ≤1.5 μm and was considered long when its total length was > 1.5 μm.

RESULTS

Spermiogenesis in Trichomycteridae

The analysis of spermiogenesis process in Trichomycteridae was based on the observation of spermatids of *C. orthiocarinatus* and *I. amazonicus* (Figs. 1a-k), and *T.* aff. *iheringi*, *T. areolatus*, *T.* sp., and *T.* sp. (Figs. 4a-t).

In the analyzed trichomycterid species, the spermiogenesis occurs in cysts in the germinative epithelium. These cysts contain groups of spermatids at the same development stage, which are surrounded by cytoplasmic processes of the Sertoli cells (Figs. 1a and 4a). In these cysts, the early spermatids are interconnected by cytoplasmic bridges that result from incomplete cytokinesis during cellular divisions (Fig. 1b). In the early spermatids, the cytoplasm is symmetrically distributed around the nucleus, which contains diffuse chromatin and has a circular outline (Figs. 1b, 1g, 4b, 4e, 4k and 4q). The centriolar complex lies laterally to the nucleus and is anchored to the plasma membrane, with the distal centriole forming the flagellum (Figs. 1b, 1c, 1g, 1h, 4b, 4c, 4e, 4f, 4k, 4l, 4q and 4s). In *C. orthiocarinatus*, *T.* aff. *iheringi*, *T. areolatus*, *Trichomycterus* sp.1, and *Trichomycterus* sp.2, the proximal centriole is lateral and obtuse angle in to the distal centriole (Figs. 1b-d, 4b, 4h, 4n, and 4r), while in *I. amazonicus* the centrioles are lateral

and perpendicular to each other (Fig. 1h). The centriolar complex move towards the nucleus, bringing with it the plasma membrane and the initial segment of the flagellum that invaginates (Figs. 1d, 1i, 4c, 4f, 4l, and 4s). With this movement, the cytoplasmic channel, a space between the plasma membranes of the flagellum and of the basal region of midpiece, is formed. The nuclear rotation occurs in different degrees among the trichomycterids analyzed; being partial in *I. amazonicus*, *T.* aff. *iheringi*, and *Trichomycterus* sp.1, resulting in the eccentric position of the flagellum in relation to the nucleus (Figs. 1i, 4c, and 4l). In *C. orthiocarinatus*, *T. areolatus*, and *T.* sp. (Jacutinga), the nuclear rotation is nearly complete, resulting in a flagellum approximately medial to the nucleus (Figs. 1d, 4h, and 4s). During the nuclear rotation, a depression is formed in the nuclear outline that gives rise to the nuclear fossa. At the end of this process, the proximal centriole is only found inserted in the nuclear fossa in *C. orthiocarinatus*, *T.* aff. *iheringi*, *T.* sp., and *T.* sp., while in *I. amazonicus* and *T. areolatus*, the centriolar complex are completely inserted in the nuclear fossa. During the nuclear rotation, the chromatin condensation starts. The chromatin is becomes progressively highly condensed, with areas of diffuse chromatin seen among areas of condensing chromatin (Figs. 1d, 1i, 4c, 4f, 4l, and 4s). Chromatin condensation process begins at the basal position of the nucleus, and proceeds until the apical region. The cytoplasmic mass moves around the initial segment of the tail, and gives rise to the midpiece of the future spermatozoon. The midpiece has rounded to elongate mitochondria and vesicles randomly distributed (Figs. 1e, 1j, 4d, 4g, 4m, 4r, and 4s). The flagellum has the classical (9+2) axoneme, with nine peripheral microtubular doublets of microtubules and a single central pair of microtubules, surrounded by the flagellar membrane. In all trichomycterids analyzed, lateral projections or fins develop from the flagellar membrane (Figs. 1f, 1k, 4d-inset, 4i,4j, 4o, and 4t). In *Trichomycterus* sp.1 and *Trichomycterus* sp.2 is also observed the formation of membranous compartment developed from the flagellar membrane (Fig. 4p).

Spermatozoa of Trichomycteridae

The measurements of length and width of nucleus, nuclear fossa, midpiece, cytoplasmic channel, and of lateral projections length of the spermatozoa of the trichomycterids analyzed are presented in the Table 1.

Trichomycterid spermatozoa are found in the lumen of the germinative compartment. They exhibit an ovoid to spherical head, an asymmetric midpiece, and one flagellum eccentric, medial or lateral positioned in relation to the nucleus. (Figs. 2a, 3a, 5a, 6a, 7a, 8a, and 9a). The head does not show acrosomal vesicle. In *C. orthiocarinatus*, *T. areolatus*, *T. reinhardti*, and *Trichomycterus* sp.2, the nucleus has spherical shape, while in *I. amazonicus*, *T.* aff. *iheringi*, and *Trichomycterus* sp.1, it is ovoid (Figs. 2a, 2d, 3a, 3c, 5a, 6a, 6e, 7a, 7b, 8a, 8b, 9a, and 9b). The chromatin is highly condensed, presenting with a homogeneous aspect. The chromatin can be interspersed by electron-lucent areas (Figs. 2a-e, 3a-d, 5a-d, 6a-d, 7a-c, 8a-c, and 9a-d). No organelles are seen in the cytoplasmic apical and lateral region to the nucleus in *T. areolatus* and *T. reinhardti* (Figs. 6a-d, 7a, and 7b). In *C. orthiocarinatus*, mitochondria and vesicles are observed in the cytoplasmic region lateral of the nucleus, while in *Trichomycterus* sp.2, they also are in the apical region of the nucleus (Figs. 2a, 2c, and 9a-d). In *I. amazonicus*, *T.* aff. *iheringi*, and *Trichomycterus* sp.1, mitochondria are found in the apical region of the nucleus (Figs. 3a, 3b, 3d, and 8a-c). The nuclear fossa is eccentrically positioned in relation to the nucleus in *I. amazonicus*, *T.* aff. *iheringi*, *T. reinhardti*, and *Trichomycterus* sp.1. In *C. orthiocarinatus*, *T. areolatus*, and *Trichomycterus* sp.2, it is nearly medial to the nucleus. In all species analyzed the nuclear fossa has a simple arc shape (Figs. 2d-f, 3d-g, 5e-g, 6a, 6e-g, 7a, 7d, 7f, 8b, 9b, and 9d). In the midpiece, several rounded to elongated mitochondria are randomly distributed in all regions, concentrated in the apical and medial regions. The mitochondria are separated from the flagella by the cytoplasmic channel (Figs. 2f-h, 3f-j, 5g, 5i, 6h, 6j, 7f, 7i, 7j, 8f, 8g, 9g, and 9i). In *C. orthiocarinatus*, *I. amazonicus*, *Trichomycterus* sp.1, and *Trichomycterus* sp.2, many elongated vesicles are found isolated in all regions of the midpiece, mainly concentrated on the basal region (Figs. 2f-k, 3g-j, 8f-k, and 9f-l). However, in *C. orthiocarinatus*, *Trichomycterus* sp.1, and *Trichomycterus* sp.2, they also might be interconnected among themselves, forming a membranous compartment (Fig. 2j, 8i, and

9k). In *T. areolatus* and *T.* aff. *iheringi*, few vesicles are mainly observed in the basal region of the midpiece. Membranous compartment might be also found in the midpiece of *T.* aff. *iheringi* spermatozoon (Figs. 5g-i, 6g, 6h, and 6j). Midpiece of *T. reinhardti* does not contain vesicles (Figs. 7f and 7i). In *C. orthiocarinatus*, *T.* aff. *iheringi*, *T. areolatus*, *Trichomycterus* sp.1, and *Trichomycterus* sp.2, the centrioles are lateral and in obtuse angle to each other, while in *I. amazonicus* and *T. reinhardti*, the proximal centriole is lateral and perpendicular to the distal centriole (Figs. 2d, 2e, 3e, 5f, 6e, 6f, 7e, 8e, 9e, and 9f). The centriolar complex is found completely inserted in the nuclear fossa in *I. amazonicus* and *T. areolatus*. In *C. orthiocarinatus*, *T.* aff. *iheringi*, *T. reinhardti*, *Trichomycterus* sp.1, and *Trichomycterus* sp.2, only the proximal centrioles is inserted in the nuclear fossa (Figs. 2d, 3e, 5f, 6f, 7e, 8e, and 9e). The distal centriole becomes the basal body, and gives rise to an axoneme that exhibits the classical 9+2 microtubular pattern. The flagellum formed is approximately medial to the nucleus in *C. orthiocarinatus*, *T. areolatus*, and *Trichomycterus* sp.2, while in *I. amazonicus*, *T.* aff. *iheringi*, *T. reinhardti*, *Trichomycterus* sp.1, it is eccentric. Two flagellar lateral projections or fins are present in the flagella of *T. areolatus* and *T. reinhardti*. In the others trichomycterids, they occur in variable number. In *Trichomycterus* sp.1 and *Trichomycterus* sp.2, the flagella also have a membranous compartment (Figs. 2l, 2m, 2m-inset, 3k, 3l, 5j-l, 6k, 6l, 7k, 7l, 8l-n, and 9m).

DISCUSSION

Spermatogenesis and Spermiogenesis

In the trichomycterids analyzed, the development of spermatids occurs completely inside the cysts in the germinative epithelium, characterizing a spermatogenesis of the cystic type. This type of spermatogenesis is present in most Teleostei (Mattei, 1993; Quagio-Grassiotto *et al.*, 2001, 2003, 2005), and also observed in other Loricarioidea, as in the subfamily Callichthyinae (family Callichthyidae) (Spadella *et al.*, submitted c) and in Scoloplacidae (Spadella *et al.*, 2006b) (Table 2).

In Teleostei with external fertilization, the spermiogenesis process can be of the types I or II, according to Mattei (1970). In both, the initial development of the flagellum is lateral to the nucleus in the early spermatids, with the occurrence or not of nuclear rotation

the differentiation between these two types. In the type I spermiogenesis, the nuclear rotation occurs, resulting in a spermatozoon with the flagellum axis perpendicular to the nucleus. In the type II spermiogenesis, the nuclear rotation does not occur, determining a position parallel of the flagellum in relation to the nucleus (Mattei, 1970). In Pimelodidae and Heptapteridae, the flagellum is medial, the nucleus does not rotate, the nuclear fossa does not form, and the short pseudo-cytoplasmic channel result of the cytoplasmic mass movement toward the flagellum during spermiogenesis, characterizing a third type of spermiogenesis (Quagio-Grassiotto *et al.*, 2005; Quagio-Grassiotto and Oliveira, submitted). The spermiogenesis process observed in Trichomycteridae is characterized by an initial lateral development of the flagellum, the presence of centriolar migration, a cytoplasmic channel formation, the presence of nuclear rotation in different degrees, and an eccentric to lateral nuclear fossa formation. This set of characteristics is more similar to type I spermiogenesis previously described, which is also observed in Callichthyinae (Spadella *et al.*, submitted) and in Scoloplacidae (Spadella *et al.*, 2006b) (Table 2). This type of spermiogenesis can be found in other siluriform families, as Diplomystidae, the most basal siluriform family (Quagio-Grassiotto *et al.*, 2001), and Pseudopimelodidae (Quagio-Grassiotto *et al.*, 2005). However, in Diplomystidae the cytoplasmic channel does not remain in the spermatozoa (Quagio-Grassiotto *et al.*, 2001) as observed in Trichomycteridae, Callichthyinae, Scoloplacidae and Pseudopimelodidae. Furthermore, the nuclear rotation in Diplomystidae (Quagio-Grassiotto *et al.*, 2001), *Megalechis thoracata* (Spadella *et al.*, submitted), Scoloplacidae (Spadella *et al.*, 2006b), and Pseudopimelodidae (Quagio-Grassiotto *et al.*, 2005) is complete, resulting in a flagellum perpendicular to the nucleus in the spermatozoon, while in *I. amazonicus*, *T.* aff. *iheringi*, and *Trichomycterus* sp.1, it is partial., and in *C. orthiocarinatus*, *T. areolatus*, and *Trichomycterus* sp.2, it is nearly complete, resulting, in both the cases, in the eccentric position of the flagellum. Mattei (1970) considered that the occurrence of these intermediate process of spermiogenesis are responsible by the formation of intermediate spermatozoa, of which flagellum is eccentric to the nucleus.

The comparative analyses of Table 2 show that trichomycterid species share six of seven spermiogenesis characters analyzed (characters 1 to 4, 6 and 7), reinforcing the

hypotheses of group monophyly (de Pinna, 1998; de Pinna and Wosiacki, 2003). The unique spermiogenesis characteristic variable among the species analyzed is the mode of nuclear rotation (character 5).

Considering the Table 2, it is possible to observe that the species of Trichomycteridae share all spermatogenesis and spermiogenesis characteristics with Callichthyinae (subfamily of Callichthyidae), five characters with Scoloplacidae, four with Corydoradinae (other subfamily of Callichthyidae), and two characters with Nematogenyidae. According to above, the Trichomycteridae spermatids share more similar characters with Callichthyidae and Scoloplacidae than with Nematogenyidae, considered sister group of family Trichomycteridae (de Pinna, 1998; Britto, 2003). The type of spermatogenesis and the spermiogenesis characteristics found in Nematogenyidae are present in Cetopsidae and Aspredinidae (Spadella *et al.*, 2006a), suggesting that these families may be more related than actually hypothesized, comprising a primitive siluriform group originated after Diplomystidae.

Spermatozoa

The comparative analyses of siluriform spermatozoa ultrastructure presented in Table 3 showed that Trichomycteridae species herein studied share the same state of character in nine of seventeen characters analyzed (characters 8, 14, 16, and 18 to 23).

Eight characteristics were variable among the species analyzed. Thus, the centrioles are lateral and in obtuse angle to each other in *C. orthiocarinatus*, *T.* aff. *iheringi*, *T. areolatus*, *Trichomycterus* sp.1, and *Trichomycterus* sp.2; and lateral and perpendicular in *I. amazonicus* and *T. reinhardti*. The arrangement of centrioles, lateral and in obtuse angle, is also observed in the species *Megalechis thoracata* and *Callichthys callichthys* of the subfamily Callichthyinae (Spadella *et al.*, submitted c) (character 9). The presence of vesicles in the midpiece occurs in all trichomycterids analyzed, except in *T. reinhardti* (character 10). The absence of vesicles in the midpiece is also found in Diplomystidae (Quagio-Grassiotto *et al.*, 2001), Amblyciptidae (Lee and Kim, 1999), Auchenipteridae (Burns *et al.*, 2002), and Bagridae (Lee, 1998; Kim and Lee, 2000; Mansour and Lahnsteiner, 2003). In all others families of Loricarioidea, vesicles are observed in the

midpiece (Table 3). The occurrence of flagellar membrane specializations (character 11) also varied, thus, two lateral projections in the flagellum were observed in *T. areolatus* and *T. reinhardti* as in Scoloplacidae (Spadela *et al.*, 2006b), Amblycipitidae (Lee and Kim, 1999), Bagridae (Lee, 1998; Kim and Lee, 2000; Mansour and Lahnsteiner, 2003), and Diplomystidae (Quagio-Grassiotto *et al.*, 2001). In *C. orthiocarinatus*, *I. amazonicus*, *T.* aff. *iheringi*, *Trichomycterus* sp.1, and *Trichomycterus* sp.2, more than two lateral fins were observed, a state not described yet in siluriforms. In the flagella of *Trichomycterus* sp.1, and *Trichomycterus* sp.2 spermatozoa are also observed a membranous compartment as in Corydoradinae and *C. callichthys* (Spadella *et al.*, submitted).

Other characteristic that differed among trichomycterids species was the nucleus shape (character 12), which is ovoid in *I. amazonicus*, *T.* aff. *iheringi*, *Trichomycterus* sp.1 being the same observed in some species of Callichthyidae, and Heptapteridae (Quagio-Grassiotto *et al.*, 2005). In *C. orthiocarinatus*, *T. areolatus*, *T. reinhardti*, and *Trichomycterus* sp.2, the nucleus is round as found in some callichthyids (Spadella *et al.*, submitted), Loricariidae (Mansour and Lahnsteiner, 2003), Pimelodidae (Quagio-Grassiotto and Carvalho, 2000; Santos *et al.*, 2001), Pseudopimelodidae (Quagio-Grassiotto *et al.*, 2005), Diplomystidae (Quagio-Grassiotto *et al.*, 2001), Siluridae (Emel'yanova and Makeyeva, 1991b; Kwon *et al.*, 1998; Lee and Kim, 2001), and Clariidae (Mansour *et al.*, 2002). The character 13 is also varied among the trichomycterids, and the cytoplasmic area around the nucleus is narrow in *T. areolatus* and *T. reinhardti*, while in *C. orthiocarinatus*, *I. amazonicus*, *T.* aff. *iheringi*, *Trichomycterus* sp.1, and *Trichomycterus* sp.2, this area is large. The state "narrow cytoplasmic area" is most found in the other families of Loricarioidea (Table 3). Other character variable among trichomycterids analyzed is the nuclear fossa position (character 15), which is medial in *C. orthiocarinatus*, *T. areolatus*, and *Trichomycterus* sp.2, eccentric in *I. amazonicus*, *T.* aff. *iheringi*, *T. reinhardti*, and *Trichomycterus* sp.1. Medial nuclear fossa is also found in most Callichthyidae, Scoloplacidae, Loricariidae (Table 3), and other siluriform families as Diplomystidae (Quagio-Grassiotto *et al.*, 2001), Cetopsidae and Aspredinidae (Spadella *et al.*, 2006a), Amblycipitidae (Lee and Kim, 1999), Ictaluridae (Poirier and Nicholson, 1982), Bagridae (Lee, 1998; Kim and Lee, 2000; Mansour and Lahnsteiner, 2003), Pseudopimelodidae

(Quagio-Grassiotto *et al.*, 2005), Siluridae (Kwon *et al.*, 1998; Lee and Kim, 2001), and Clariidae (Mansour *et al.*, 2002). Eccentric nuclear fossa is also observed in Auchenipteridae (Burns *et al.*, 2002).

The position of centrioles in relation to the nuclear fossa (character 17) is also a variable character, with the centrioles totally inserted in the nuclear fossa in *I. amazonicus* and *T. areolatus* as observed in *Hoplosternum littorale* and *Megalechis thoracata* (subfamily Callichthyinae), Scoloplacidae, Loricariidae (Table 3), Diplomystidae (Quagio-Grassiotto *et al.*, 2001), Cetopsidae and Aspredinidae (Spadella *et al.*, 2006), Amblycipitidae (Lee and Kim, 1999), Bagridae (Lee, 1998; Kim and Lee, 2000; Mansour and Lahnsteiner, 2003), and Clariidae (Mansour *et al.*, 2002); and only the proximal centriole inserted in the nuclear fossa in *C. orthiocarinatus*, *T.* aff. *iheringi*, *T. reinhardti*, *Trichomycterus* sp.1, and *Trichomycterus* sp.2. This state is also described in *C. callichthys* (Table 3). The position of the flagellum in relation to the nucleus is medial in *C. orthiocarinatus*, *T. areolatus*, and *Trichomycterus* sp.2, while in *I. amazonicus*, *T.* aff. *iheringi*, *T. reinhardti*, and *Trichomycterus* sp.1, this position is eccentric. The more common pattern in siluriform is the medial position (Poirier and Nicholson, 1982; Lee, 1998; Lee and Kim, 1999; Kim and Lee, 2000; Quagio-Grassiotto *et al.*, 2001; Spadella *et al.*, 2006a).

These eight characters, as described above, are quite variable among Trichomycteridae and more than one state has been found in other families of Loricarioidea and Siluriformes studied. So, the occurrence of different states of characters in this family permit to suggest that a representative sample of the spermatozoa ultrastructure may be necessary before a generalized conclusion about the spermatozoa morphology of the family.

The analysis of the Table 3 shows that *C. orthiocarinatus* share seventeen spermatozoa characteristics with *Trichomycterus* sp.2, followed of *T.* aff. *iheringi*, *T. areolatus*, and *Trichomycterus* sp.1, sharing fourteen characteristics. Already, the spermatozoa of *I. amazonicus* have fifteen similar characters with *T.* aff. *iheringi* and *Trichomycterus* sp.1. Among the species of *Trichomycterus*, the *T.* aff. *iheringi* spermatozoa share all characteristics with *Trichomycterus* sp.1, while the *T. areolatus* has

the same state of *Trichomycterus* sp.2 in fourteen characters, and *Trichomycterus* sp.1 share also fourteen with *Trichomycterus* sp.2. The characteristics observed in *T. reinhardti* were less shared among the trichomycterids analyzed. This species has one exclusive characteristics as the absence of vesicles in the midpiece (character 10), suggesting autapomorphy.

As presented above, the characteristics of spermatozoa in *C. orthiocarinatus* and *I. amazonicus* share more common characters with species of *Trichomycterus* than among themselves.

The information available on spermatozoa ultrastructure of Loricariodea listed in Table 3 is still incomplete. The lack of these data prevents a more accurate analysis, using accurate methods as parsimony. However, the data showed in the Table 3 points to some interesting points. Then, considering the analyses of seventeen spermatozoa characters depicted that the spermatozoa of the Trichomycteridae species are more similar to those of the Callichthyinae, sharing all characteristics, and Corydoradinae and Loricariidae, sharing fourteen characteristics. Furthermore, the spermatozoa of the Trichomycteridae species share twelve characteristics with the Scoloplacidae, and only seven similar characters with Nematogenyidae.

As discussed above, the Table 3 shows that the spermatozoa of the Trichomycteridae, Callichthyidae, Loricariidae, and Scoloplacidae families share more similar characteristics among themselves than with the family Nematogenyidae.

Considering the phylogeny of the superfamily Loricarioidea, the family Trichomycteridae is the sister group of Nematogenyidae; and this group is a sister group of the clade formed by Callichthyidae, Scoloplacidae, Astroblepidae, and Loricariidae (de Pinna, 1998; Britto, 2003). The spermatozoa of the species of Trichomycteridae and those of Nematogenyidae share only seven morphological characteristics: the presence of elongated vesicles in the midpiece (character 10), narrow area cytoplasmic around the nucleus (character 13), homogeneous aspect of chromatin condensation (character 14), nuclear fossa in simple arc (character 19), the presence of one short cytoplasmic channel (character 20), absence of electron-dense spherical structure in the midpiece (character 23),

and flagellum medial to the nucleus (character 24). This set of characteristics is also found in other Loricarioidea and siluriform families.

Considering the data obtained, the families Callichthyidae, Loricariidae, and Scoloplacidae share more common characteristics of spermatogenesis, spermiogenesis, and spermatozoa with the species of Trichomycteridae than with Nematogenyidae, its hypothesized sister group (de Pinna, 1998; Britto, 2003). On the other hand, except by the family Nematogenyidae, the similarity observed reinforce the monophyly of the superfamily Loricarioidea as proposed by de Pinna (1998) and Britto (2003).

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Table 1. Spermatozoa dimensions observed in the trichomycterids species analyzed. the "n" show the number of structures measured.

Species	*C. orthiocarinatus*	*I. amazonicus*	*Trichomycterus aff. iheringi*	*T. areolatus*	*T. reinhardti*	*Trichomycterus sp. 1*	*Trichomycterus sp. 2*
Structures							
Nucleus							
Length (µm)	1.9 (n = 7)	1.6 (n = 5)	1.5 (n = 15)	1.7 (n = 12)	1.7 (n = 15)	1.7 (n = 12)	1.7 (n = 12)
Width (µm)	1.8 (n = 7)	1.8 (n = 5)	1.3 (n = 15)	1.6 (n = 12)	1.6 (n = 15)	1.5 (n = 12)	1.6 (n = 12)
Nuclear Fossa							
Length (µm)	0.5 (n = 7)	0.3 (n = 6)	0.6 (n = 15)	0.4 (n = 12)	0.3 (n = 6)	0.4 (n = 12)	0.3 (n = 7)
Width (µm)	0.6 (n = 7)	0.6 (n = 6)	0.4 (n = 15)	0.6 (n = 12)	0.2 (n = 6)	0.5 (n = 12)	0.4 (n = 7)
Midpiece							
Length (µm)	1.0 (n = 8)	1.4 (n = 5)	1.4 (n = 12)	1.6 (n = 10)	1.5 (n = 7)	1.6 (n = 7)	1.3 (n = 9)
Width (µm)	1.9 (n = 8)	1.3 (n = 5)	1.6 (n = 12)	1.5 (n = 10)	1.0 (n = 7)	1.6 (n = 7)	1.6 (n = 9)
Cytoplasmic Channel							
Length (µm)	0.7 (n = 6)	0.9 (n = 7)	1.3 (n = 12)	1.2 (n = 10)	1.0 (n = 7)	1.5 (n = 7)	1.2 (n = 8)
Width (µm)	0.4 (n = 6)	0.5 (n = 7)	0.4 (n = 12)	0.4 (n = 10)	0.4 (n = 7)	0.4 (n = 7)	0.5 (n = 8)
Lateral Projections							
Length (µm)	0.2 (n = 7)	0.4 (n = 11)	0.2 (n = 20)	0.2 (n = 20)	0.3 (n = 20)	0.4 (n = 20)	0.3 (n = 20)

Table 2. General view of the distribution of spermatogenesis and spermiogenesis character states in the species analyzed in the present study, and in species of Loricarioidea. (+) present; (-) absent; (?) unavailable.

Characters*	1		2		3		4		5				6		7	
	a	b	a	b	a	b	a	b	a	b	c	d	a	b	a	b
Families																
Nematogenys inermis	-	+	-	+	+	-	-	+	+	-	-	-	-	+	+	-
C.orthiocarinatus	+	-	+	-	-	+	-	+	-	-	+	-	-	+	-	+
I. amazonicus	+	-	+	-	-	+	-	+	-	+	-	-	-	+	-	+
Trichomycterus aff. *iheringi*	+	-	+	-	-	+	-	+	-	+	-	-	-	+	-	+
T. areolatus	+	-	+	-	-	+	-	+	-	-	+	-	-	+	-	+
T. reinhardt	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Trichomycterus sp. 1	+	-	+	-	-	+	-	+	-	+	-	-	-	+	-	+
Trichomycterus sp. 2	+	-	+	-	-	+	-	+	-	-	+	-	-	+	-	+
Corydoras flaveolus	-	+	+	-	+	-	-	+	+	-	-	-	-	+	-	+
Corydoras aeneus	-	+	+	-	+	-	-	+	+	-	-	-	-	+	-	+
Scleromystax lacerdai	-	+	+	-	+	-	-	+	+	-	-	-	-	+	-	+
Aspidoras poecilus	-	+	+	-	+	-	-	+	+	-	-	-	-	+	-	+
Hoplosternum littorale	+	-	+	-	-	+	-	+	-	+	-	-	-	+	-	+
Megalechis thoracata	+	-	+	-	-	+	-	+	-	-	+	-	-	+	-	+
Callichthys callichthys	+	-	+	-	-	+	-	+	-	+	-	-	-	+	-	+
Scoloplax distolothrix	+	-	+	-	-	+	-	+	-	-	-	+	+	-	-	+
Ancistrus triradiatus	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?

Legends:
*1- spermatogenesis type (a-cystic; b- semicystic); 2- initial position of the flagellum in relation to the nucleus (a- lateral; b- medial); 3- centriolar complex movement (a- absent; b- present); 4- cytoplasmic channel formation (a- absent; b- present); 5- nuclear rotation (a- absent; b- partial; c- nearly complete; d- complete); 6- process of chromatin condensation (a- heterogeneous; b- homogeneous); 7- nuclear fossa formation (a-absent; b- present).

Table 3. General view of the distribution of spermatozoa character states in the species analyzed in the present study, and in others species of Loricarioidea. (+) present; (-) absent.

Characters*	8		9						10		11				12					13		14		15			16		17			18		19			20		21					22		23		24	
	a	b	a	b	c	d	e	f	a	b	a	b	c	d	a	b	c	d	e	a	b	a	b	a	b	c	a	b	a	b	c	a	b	a	b	c	a	b	a	b	c	d	e	a	b	a	b	a	b
Families																																																		
Nematogenys inermis	-	+	+	-	-	-	-	-	-	+	+	-	-	-	-	-	-	+	-	+	-	-	+	+	-	-	-	-	-	+	-	+	-	-	+	+	-	-	-	+	-	-	-	+	-	+	-	+	-
C.orthiocarinatus	+	-	-	-	+	-	-	-	-	+	-	-	+	-	-	-	-	-	+	-	+	-	+	+	-	-	+	-	-	+	+	-	+	-	+	-	-	+	-	+	+	-	-	-	+	+	-	+	-
I. amazonicus	+	-	-	-	-	-	+	-	-	+	-	-	+	-	-	-	+	-	-	-	+	-	+	-	-	+	+	-	+	-	-	+	-	-	+	-	+	-	-	+	+	-	-	-	+	+	-	-	+
Trichomycterus aff. *iheringi*	+	-	-	-	+	-	-	-	-	+	-	-	+	-	-	-	+	-	-	-	+	-	+	-	-	+	+	-	-	+	-	+	-	-	+	-	+	-	-	+	+	-	-	-	+	+	-	-	+
T. areolatus	+	-	-	-	+	-	-	-	-	+	-	+	-	-	-	-	-	-	+	+	-	-	+	-	+	-	+	-	+	-	+	+	-	-	+	-	+	-	-	+	+	-	-	-	+	+	-	+	-
T. reinhardt	+	-	-	-	-	-	+	-	+	-	-	+	-	-	-	-	-	-	+	+	-	-	+	-	-	+	+	-	-	+	-	+	-	-	+	-	+	-	-	+	+	-	-	-	+	+	-	-	+
Trichomycterus sp. 1	+	-	-	-	+	-	-	-	-	+	-	-	+	+	-	-	+	-	-	-	+	-	+	-	-	+	+	-	-	+	-	+	-	-	+	-	+	-	-	+	+	-	-	-	+	+	-	-	+
Trichomycterus sp. 2	+	-	-	-	+	-	-	-	-	+	-	-	+	+	-	-	-	-	+	-	+	-	+	-	+	-	+	-	-	+	+	-	+	-	+	-	+	-	-	+	+	-	-	-	+	+	-	+	-
Corydoras flaveolus	+	-	-	-	-	-	-	+	-	+	-	-	-	+	-	-	-	-	+	+	-	-	+	-	-	+	+	-	-	-	-	+	-	-	+	-	+	-	-	+	-	-	+	-	+	-	+	-	+
Corydoras aeneus	+	-	-	-	-	-	-	+	-	+	-	-	-	+	-	-	-	-	+	+	-	-	+	-	-	+	+	-	-	-	-	+	-	-	+	-	+	-	-	+	-	-	+	-	+	-	+	-	+
Scleromystax lacerdai	+	-	-	-	-	-	-	+	-	+	-	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+	+	-	-	-	-	+	-	-	+	-	+	-	-	+	-	-	+	-	+	-	+	-	+
Aspidoras poecilus	+	-	-	-	-	-	-	+	-	+	-	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+	+	-	-	-	-	+	-	-	+	-	+	-	-	+	-	-	+	-	+	-	+	-	+
Hoplosternum littorale	+	-	-	+	-	-	-	-	-	+	+	-	-	-	-	-	+	-	-	+	-	-	+	-	-	+	+	-	+	-	-	+	-	-	+	-	+	-	-	+	-	-	+	-	+	-	+	-	+
Megalechis thoracata	+	-	-	-	+	-	-	-	-	+	+	-	-	-	-	-	-	-	+	+	-	-	+	-	+	-	+	-	+	-	+	-	-	-	+	-	+	-	-	+	-	-	+	-	+	-	+	-	+
Callichthys callichthys	+	-	-	-	+	-	-	-	-	+	-	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+	+	-	-	+	-	+	-	-	+	-	+	-	-	+	-	-	+	-	+	-	+	-	+
Scoloplax distolothrix	+	-	-	-	-	+	-	-	-	+	-	+	-	-	-	+	-	-	-	+	-	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	-	+	-	-	+	-	+	-	+	-	+
Ancistrus triradiatus	+	-	-	-	-	+	-	-	-	+	+	-	-	-	-	-	-	-	+	+	-	+	-	-	+	-	+	-	+	-	+	-	-	-	+	-	+	-	-	+	-	-	+	-	+	-	+	-	+

Legends: *8- flagella number (a- one; b- two); 9- arrangement of centriolar complex (a- lateral and parallel; b- lateral and acute angle; c- lateral and obtuse angle; d- anterior and perpendicular; e- lateral and perpendicular; f- co-axial); 10- vesicles in the midpiece (a- absent; b- present); 11- flagellar membrane specializations (a- absent; b- two lateral fins; c- variable number lateral fins; d- membranous compartment); 12- shape of the nucleus (a- semi-ovoid; b- conic; c- ovoid; d-ovoid with its bigger axis in the horizontal direction; e- round); 13- cytoplasmic area around of the nucleus (a- narrow; b- large); 14- aspect of chromatin condensation (a- heterogeneous; b- homogeneous); 15- nuclear fossa (a-absent; b- medial; c- eccentric); 16- nuclear fossa shape (a- simple arc; b- double arc); 17- Position of centrioles in relation to the nuclear fossa (a- totally inserted in the nuclear fossa; b- only proximal centriole inserted in the nuclear fossa; c- totally outside of the nuclear fossa); 18- midpiece size (a- short; b- long); 19- cytoplasmic channels (a- absent; b- one; c- two); 20- cytoplasmic channel size (a- short; b- long); 21- mitochondria shape (a- rounded; b- elongated; c- elongated and ramificated; d- C-shape; e- irregular); 22- midpiece symmetry (a- symmetric; b- asymmetric); 23- Electron-dense circular structure in the midpiece (a- absent; b- present); 24- flagellum position in relation to the nucleus (a- medial; b- eccentric).

FIGURE CAPTION

Figure 1. Spermiogenesis of Trichomycteridae: *Copionodon orthiocarinatus* (a to f), and *Ituglanis amazonicus* (g to k). a) Spermatids cyst. b, c, g, and h) Early spermatids (longitudinal sections). d and i) Late spermatids (longitudinal sections). e and j) Midpiece showing mitochondria and vesicles (cross sections). f and k) Flagella exhibiting the formation of lateral projections (cross sections). (a) 1.7 μm; (b, c, d, i) 0.7 μm; (e) 0.5 μm; (f) 0.2 μm; (g) 0.8 μm; (h, j) 0.6 μm; (k) 0.1 μm. A: axoneme; C: centriolar complex; D: distal centriole; F: flagellum; N: nucleus; P: proximal centriole; S: Sertoli cell; V: vesicles; Asterisk: mitochondria; Arrow: cytoplasmic channel; Arrowhead: lateral projections; Double arrowhead: cytoplasmic bridges.

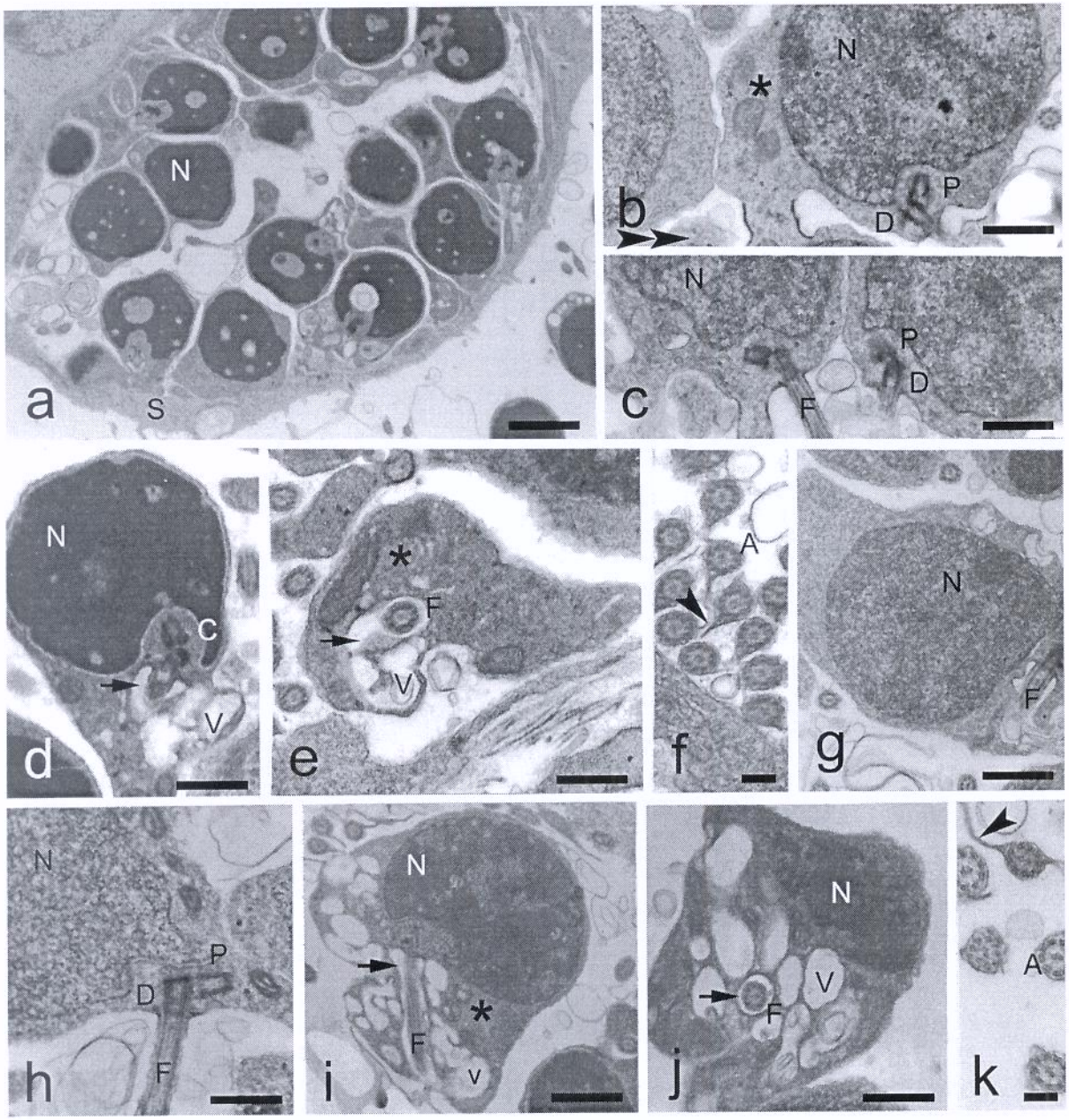
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FIGURE CAPTION

Figure 2. Spermatozoa of *Copionodon orthiocarinatus*. a) Spermatozoon longitudinal section. b and c) Head region. d and e) Centrioles arrangement. f) Spermatozoon longitudinal section showing nuclear fossa, cytoplasmic channel, and mitochondria and vesicles in the midpiece. g to k) Midpiece cross sections showing mitochondria and vesicles. l) Flagellum longitudinal section. m and m-inset) Flagella cross sections. (a) 0.3 μm; (b, c, d, and j) 0.6 μm; (e, f, g, h, i, and l) 0.4 μm; (k) 0.3 μm; (m and m-inset) 0.1 μm. A: axoneme; B: basal body; C: centriolar complex; D: distal centriole; E: electron-lucent area; F: flagellum; MC: membranous compartment; N: nucleus; P: proximal centriole; V: vesicles; Asterisk: mitochondria; Arrow: cytoplasmic channel; Arrowhead: lateral projections.

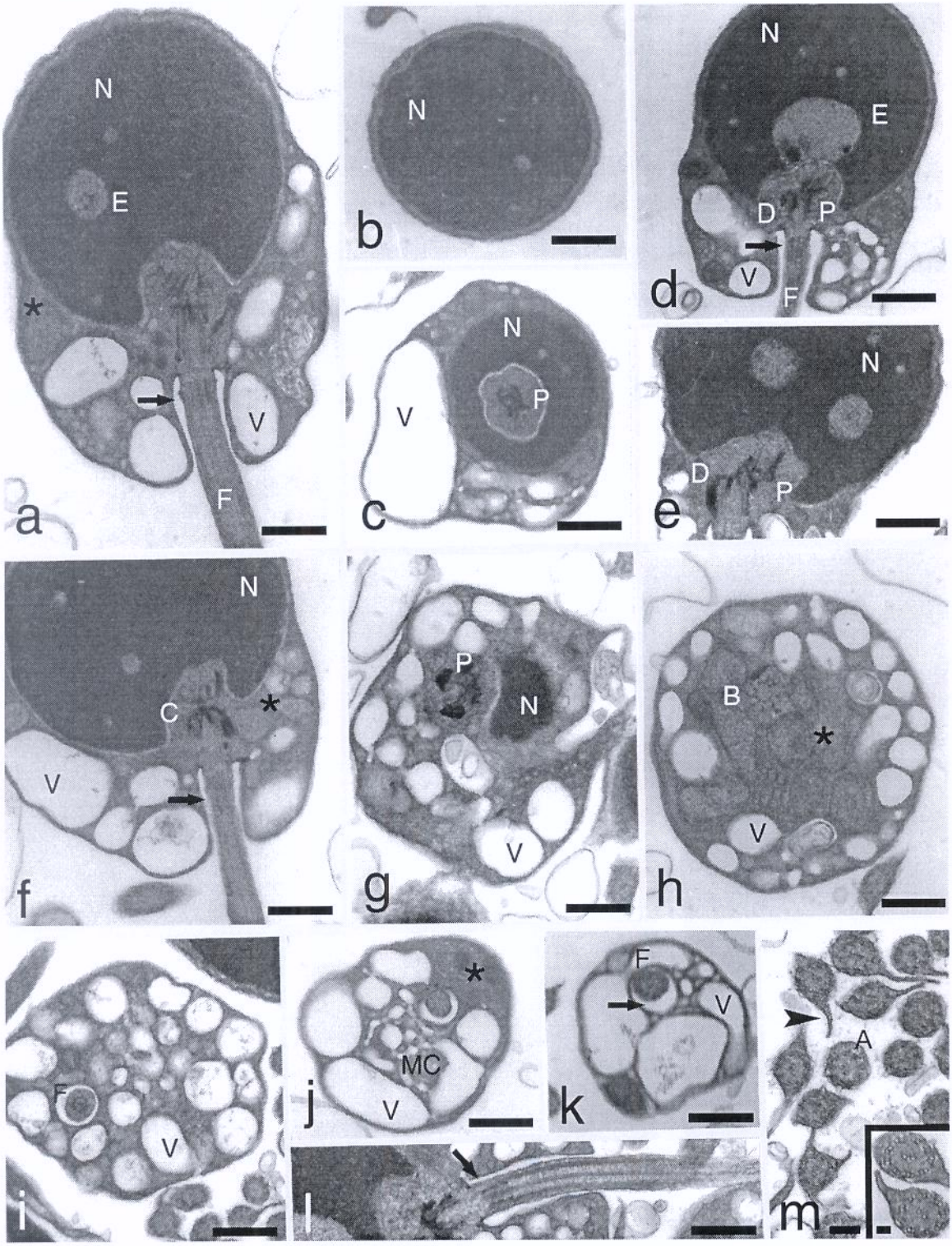
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FIGURE CAPTION

Figure 3. Spermatozoa of *Ituglanis amazonicus*. a and c) Spermatozoon longitudinal sections. b and d) Head cross sections. e) Centriolar complex arrangement. f to h) Spermatozoa longitudinal sections showing mitochondria and vesicles in the midpiece. i and j) Midpiece cross sections. k) Flagellum longitudinal section. l) Flagellum cross section. (a, e, f, and k) 0.3 μm; (b, c) 0.5 μm; (d, g, h, i, and j) = 0.4 μm; (l) 0.1 μm. A: axoneme; D: distal centriole; E: electron-lucent area; F: flagellum; N: nucleus; P: proximal centriole; V: vesicles; Asterisk: mitochondria; Arrow: cytoplasmic channel; Arrowhead: lateral projections.

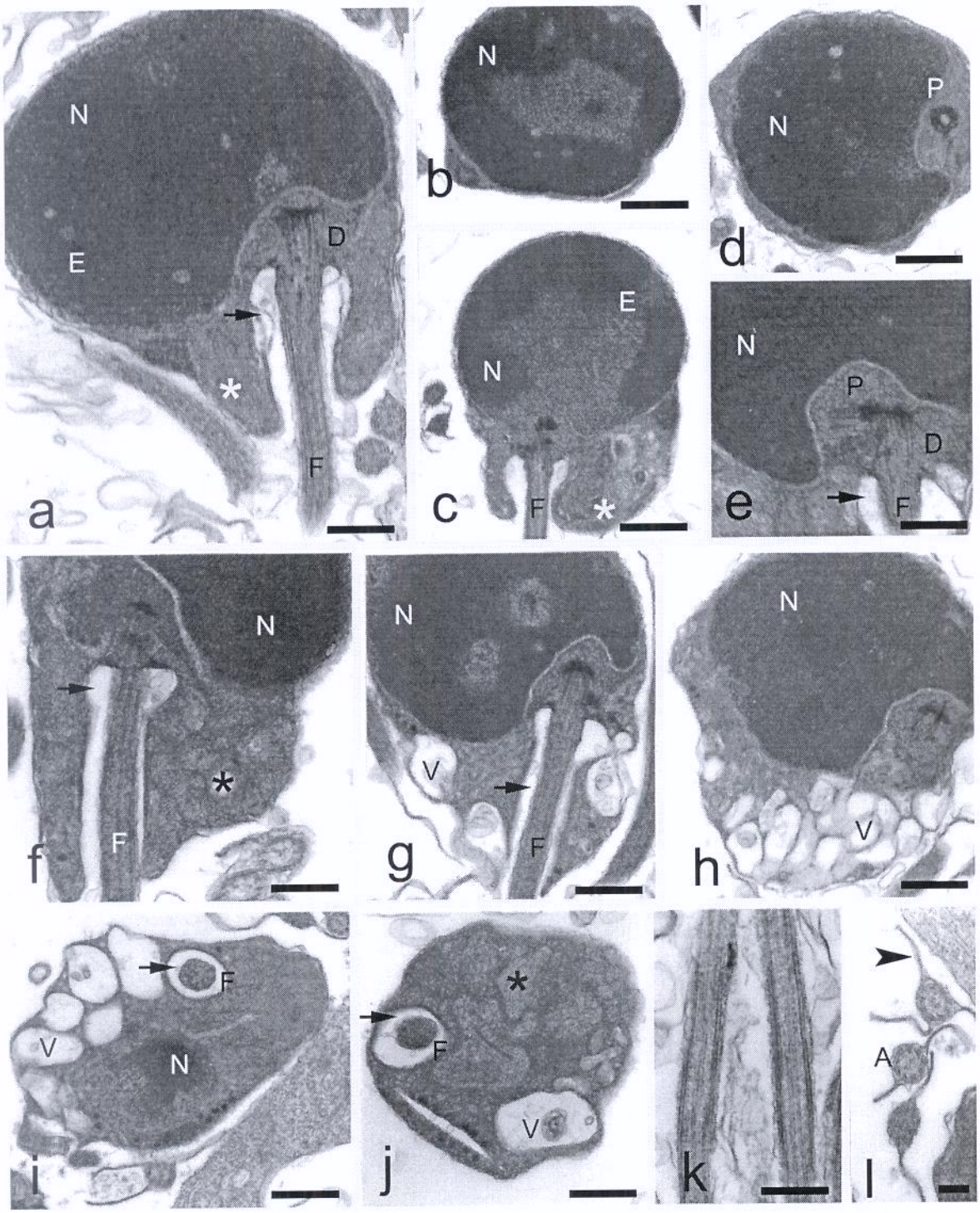
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Figure 4. Spermiogenesis of Trichomycteridae. *Trichomycterus* aff. *iheringi* (a to d), *Trichomycterus areolatus* (e to j), *Trichomycterus* sp. (k to p), and *Trichomycterus* sp. (q to t). a) Spermatids cyst. b, e, k and q) Early spermatids (longitudinal sections). c, f, l and r) Late spermatids. d, g, m and s) Midpiece showing mitochondria and vesicles (cross and longitudinal sections). h and n) Centrioles arrangement. d-inset, i, j, o, p and t) Flagella in longitudinal and cross sections. (a) 1.3 μm; (b) 1.0 μm; (c) 0.8 μm; (d-inset) 0.03 μm; (d, e, k and q) 0.7 μm; (f, g, l and r) 0.6 μm; (h, i, n and o) 0.3 μm; (j, m) 0.4 μm; (p) 0.1 μm; (s) 0.5 μm; (t) 0.2 μm. D: distal centriole; F: flagellum; N: nucleus; P: proximal centriole; S: Sertoli cell; T: spermatids; V: vesicles; Asterisk: mitochondria; Arrow: cytoplasmic channel; Arrowhead: lateral projections; Double arrowhead: membranous compartment.

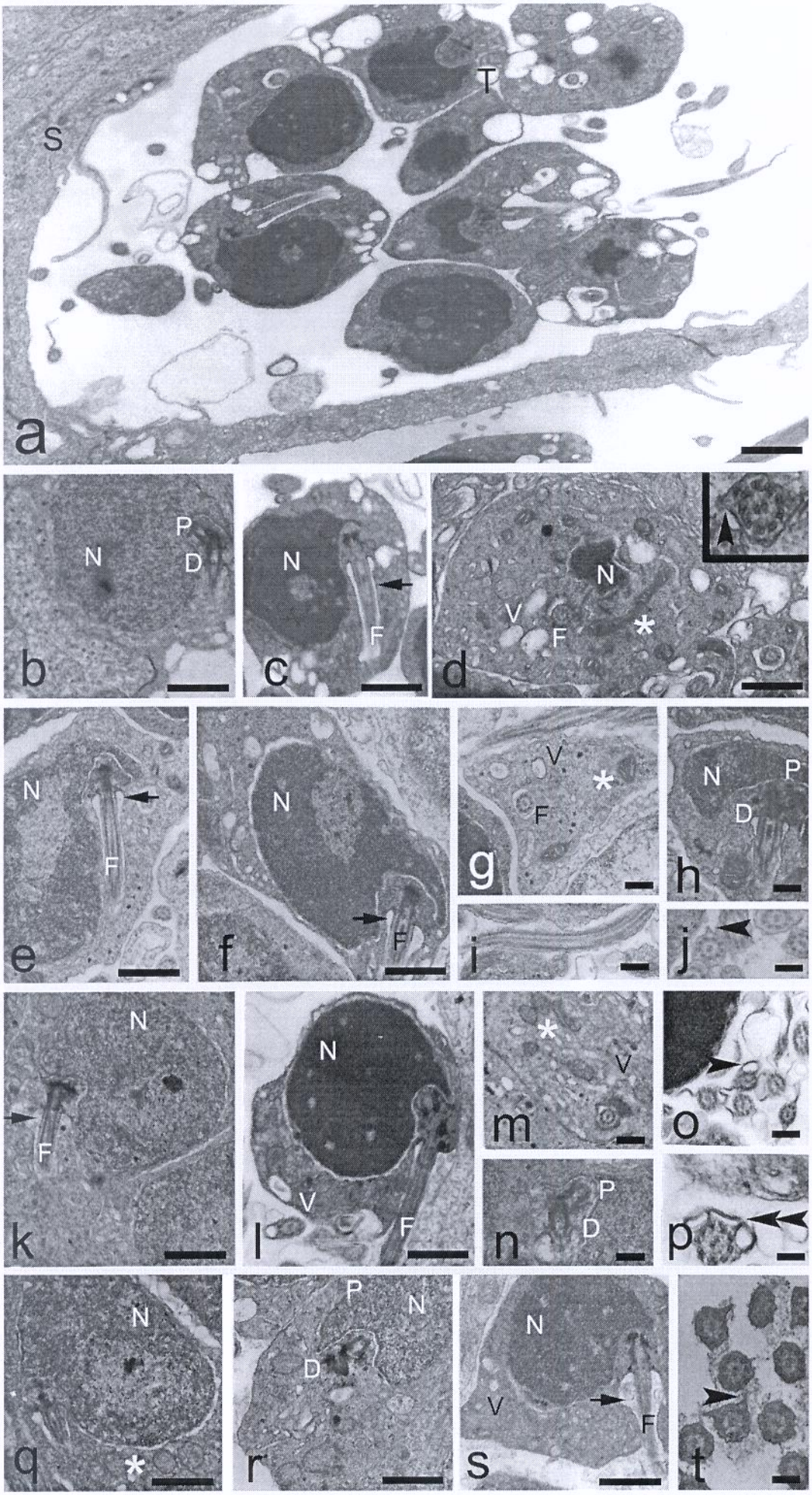
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FIGURE CAPTION

Figure 5. Spermatozoa of *Trichomycterus* aff. *iheringi*. a) Spermatozoon in longitudinal section. b to d) Nucleus in cross sections. e and f) Detail of centrioles arrangement. g to i) Midpiece in longitudinal and cross sections showing mitochondria, elongated vesicles, and cytoplasmic channel. j and k) Flagella in cross sections showing classical (9+2) axoneme. l) Flagellum in longitudinal section. (a, e, g, and h) 0.4 μm; (b, c) 0.7 μm; (d, i) 0.6 μm; (f) 0.2 μm; (j) 0.5 μm; (k) 0.06 μm; (l) 0.1 μm. A: axoneme; B: basal body; C: centriolar complex; D: distal centriole; E: electron-lucent area; F: flagellum; MC: membranous compartment; N: nucleus; P: proximal centriole; V: vesicles; Asterisk: mitochondria; Arrow: cytoplasmic channel; Arrowhead: lateral projections.

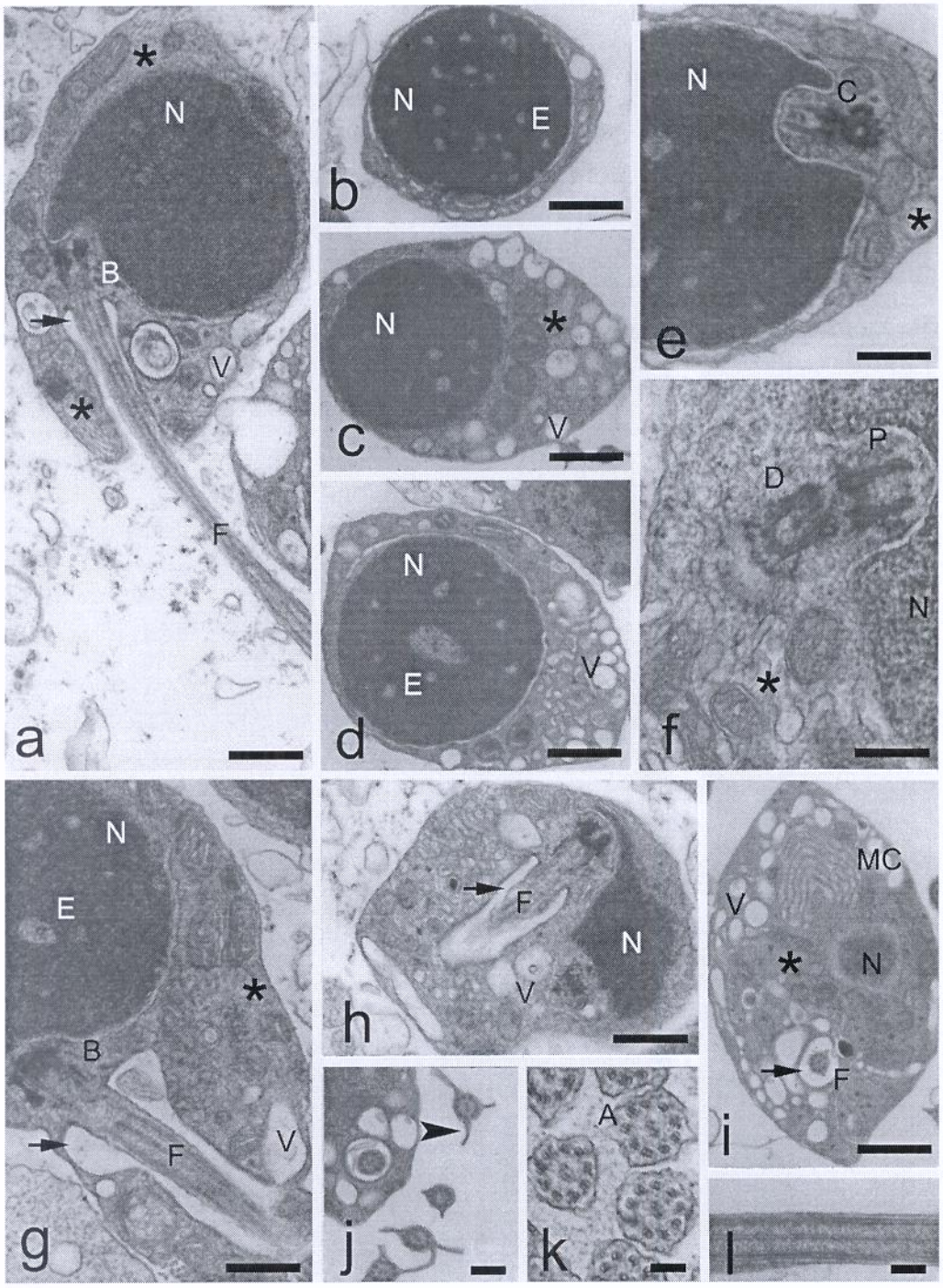
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Figure 6. Spermatozoa of *Trichomycterus areolatus*. a) Longitudinal section. b to d) Head region. e and f) Detail of nuclear fossa and centriolar complex arrangement. g to j) Midpiece region (longitudinal and cross sections) showing mitochondria and vesicles. k and l) Flagella in cross and longitudinal sections. (a) 1.0 μm; (b, c, e, f, j, and k) = 0.4 μm; (d, h) 0.5 μm; (g, i) 0.6 μm; (l) 0.1 μm. A: axoneme; B: basal body; D: distal centriole; E: electron-lucent area; F: flagellum; N: nucleus; P: proximal centriole; V: vesicles; Asterisk: mitochondria; Arrow: cytoplasmic channel; Arrowhead: lateral projections.

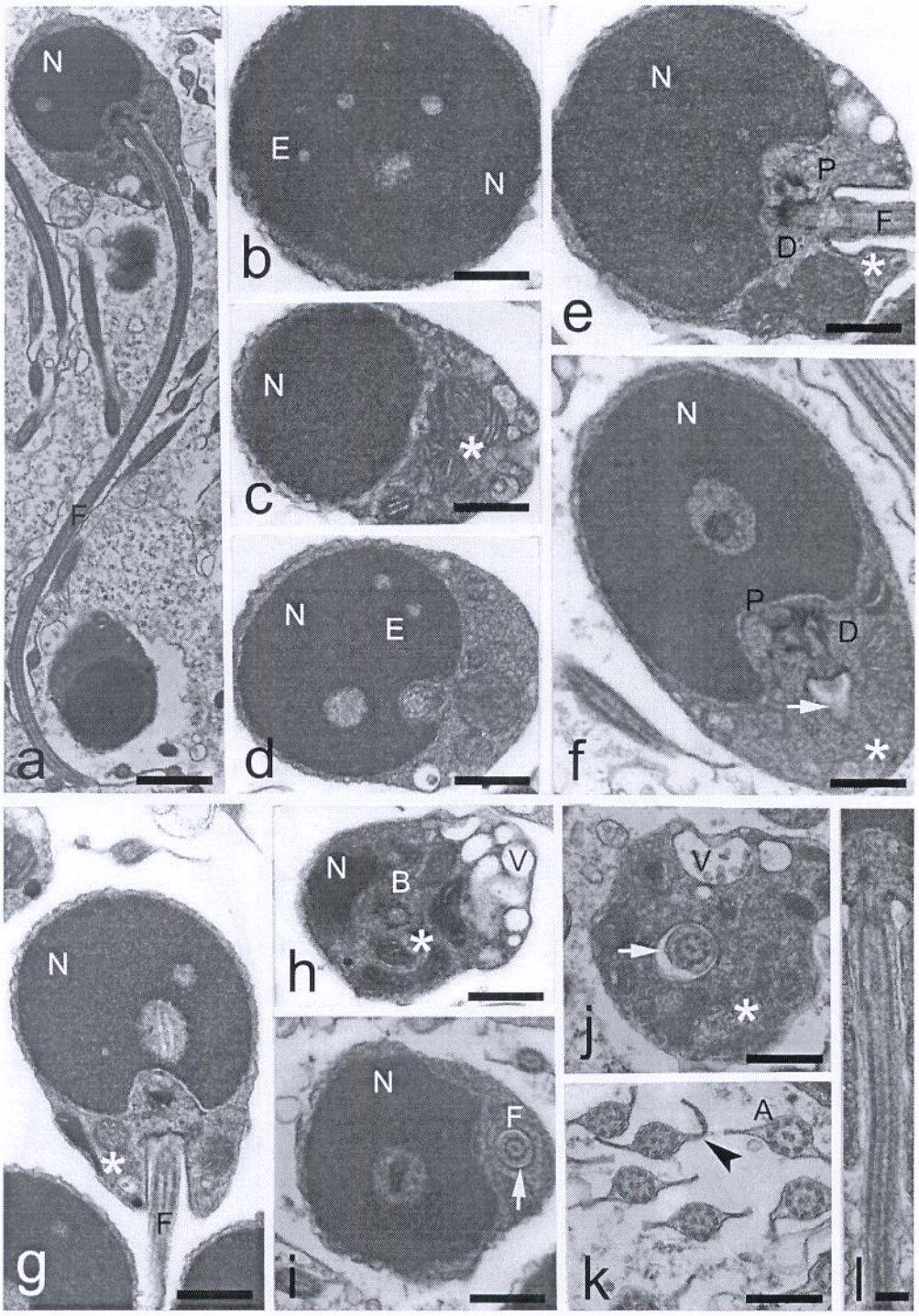
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Figure 7. Spermatozoa of *Trichomycterus reinhardti*. a) Spermatozoon in longitudinal sections. b and c) Head cross sections. d and e) Centriolar complex arrangement. f to j) Spermatozoa longitudinal and cross sections showing mitochondria and vesicles in the midpiece. k) Flagellum longitudinal section. l) Flagellum cross section. (a, f, g and h) 0.4 μm; (b, c) 0.6 μm; (d, e, i, j and l) = 0.2 μm; (k) 0.3 μm. A: axoneme; B: basal body; C: centriolar complex; D: distal centriole; E: electron-lucent area; F: flagellum; N: nucleus; P: proximal centriole; Asterisk: mitochondria; Arrow: cytoplasmic channel; Arrowhead: lateral projections.

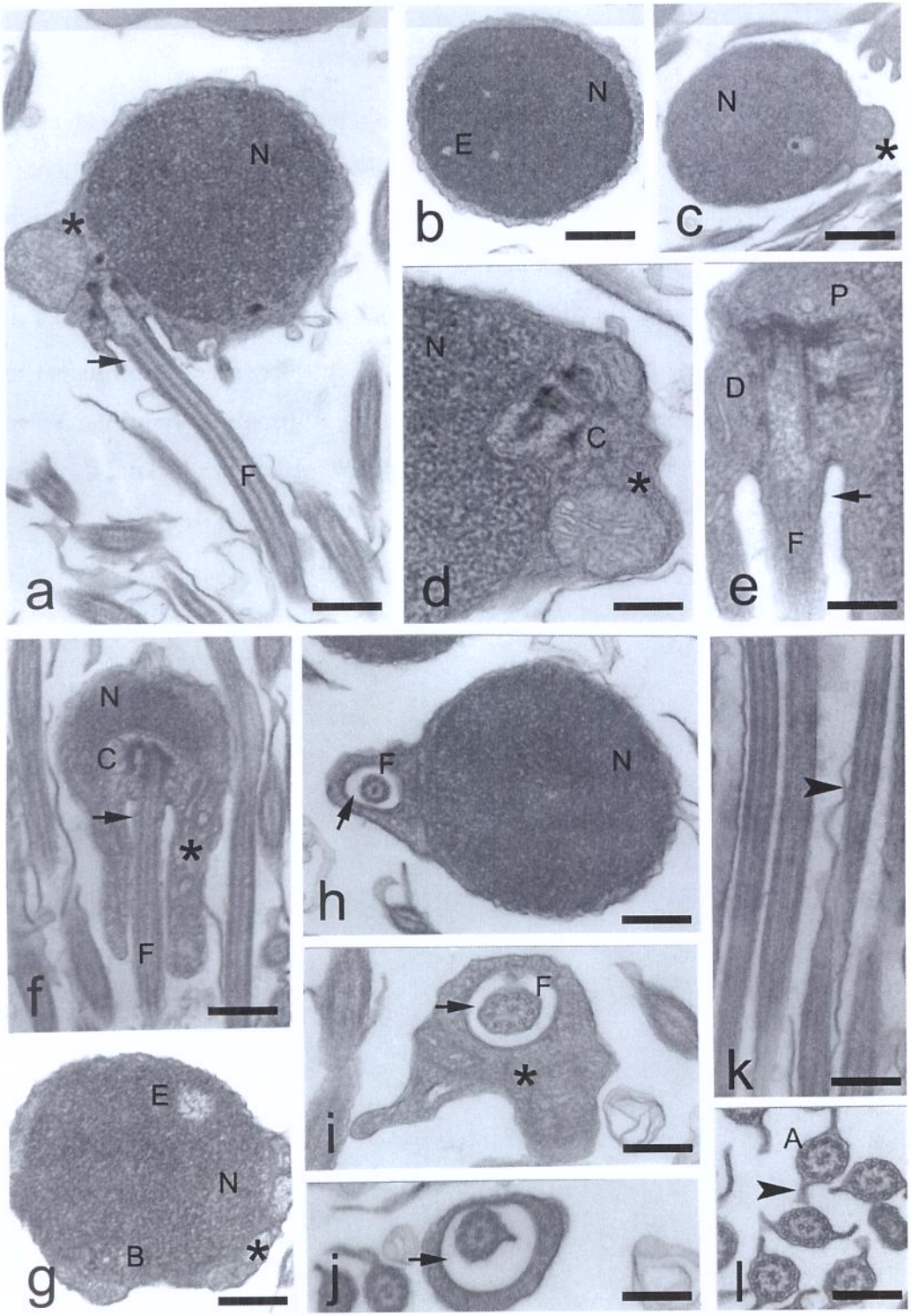
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Figure 8. Spermatozoa of *Trichomycterus* sp.1. a, b and d) Longitudinal sections of spermatozoon. c) Nucleus in cross sections. e) Detail of centrioles arrangement. f to k) Midpiece in longitudinal and cross sections showing mitochondria, elongated vesicles, and cytoplasmic channel. l, m and n) Flagella in cross sections showing classical (9+2) axoneme. (a, j and k) 0.4 μm; (b, d) 0.6 μm; (c, g and h) 0.5 μm; (e, f, i and l) 0.2 μm; (m, n) 0.09 μm. A: axoneme; B: basal body; D: distal centriole; E: electron-lucent area; F: flagellum; MC: membranous compartment; N: nucleus; P: proximal centriole; V: vesicles; Asterisk: mitochondria; Arrow: cytoplasmic channel; Arrowhead: lateral projections; Double arrowhead: membranous compartment.

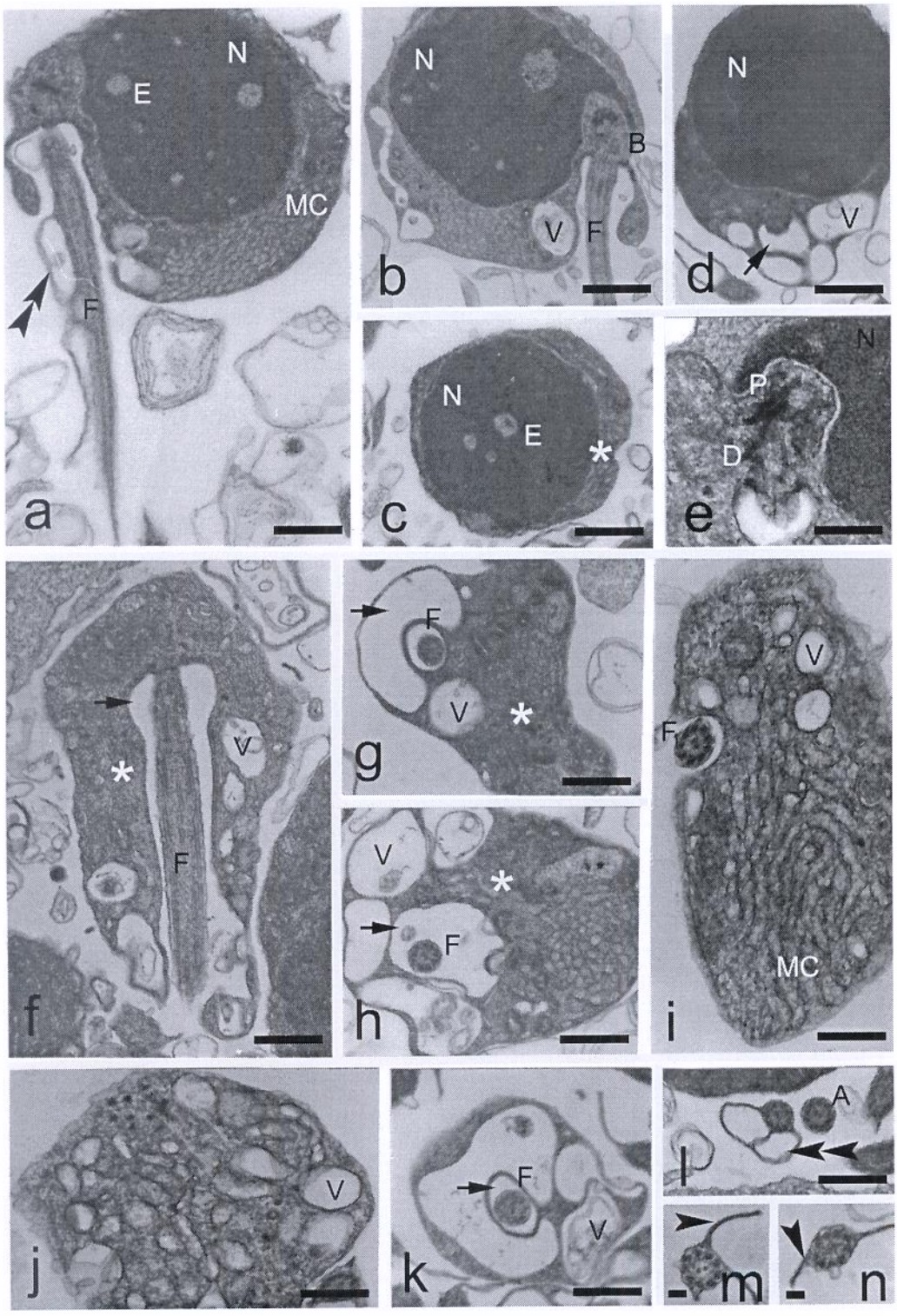
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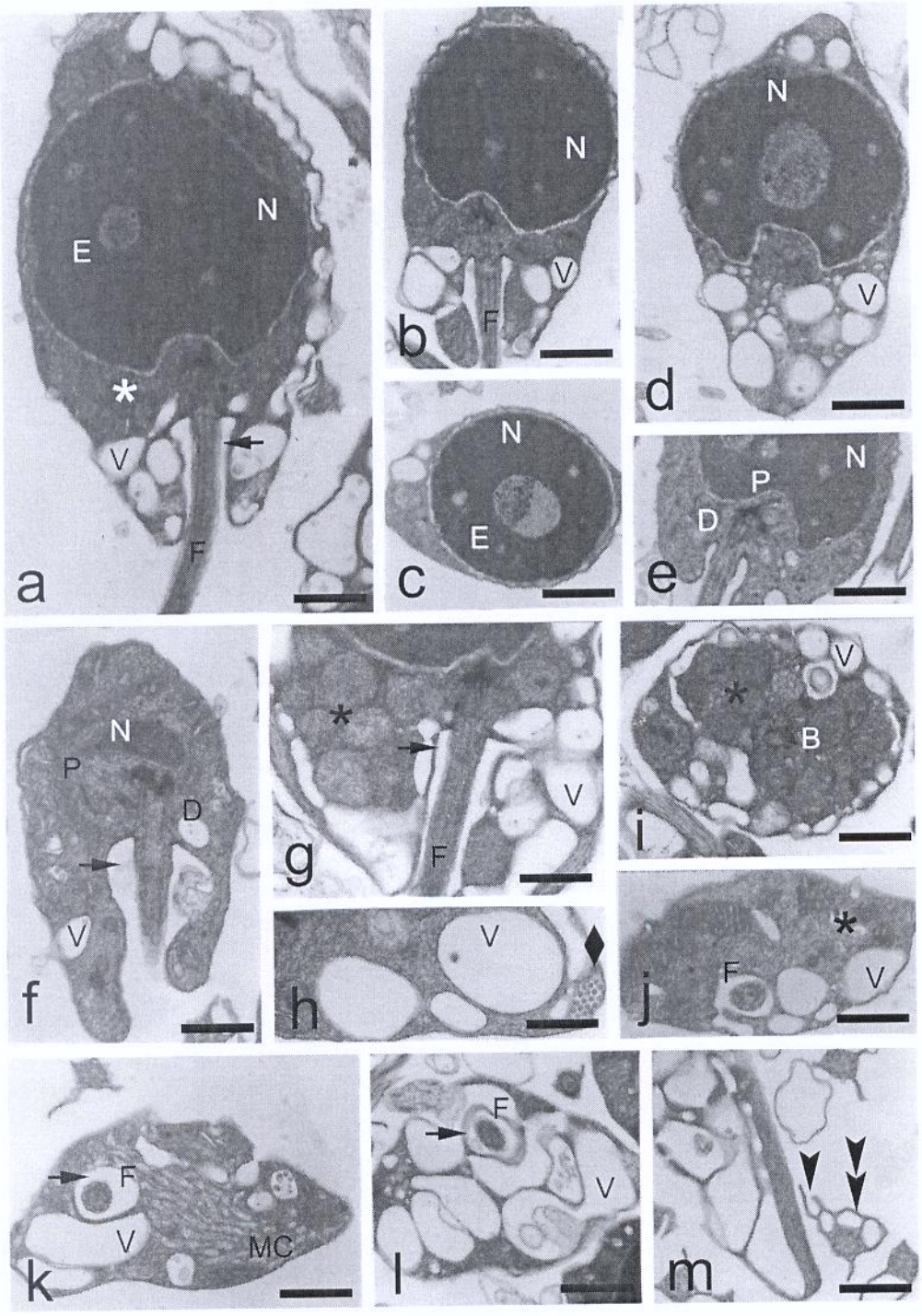
FIGURE CAPTION

Figure 9. Spermatozoa of *Trichomycterus* sp.2. a, b and d) Spermatozoon in longitudinal sections. c) Nucleus in cross sections. e) Centrioles arrangement. f to l) Midpiece in longitudinal and cross sections showing mitochondria, vesicles, and cytoplasmic channel. m) Flagellum in longitudinal and cross sections. (a, f, g, k and m) 0.4 μm; (b, d, i and j) 0.5 μm; (c) 0.7 μm; (e, l) 0.6 μm; (h) 0.2 μm. B: basal body; D: distal centriole; E: electron-lucent area; F: flagellum; MC: membranous compartment; N: nucleus; P: proximal centriole; V: vesicles; Asterisk: mitochondria; Arrow: cytoplasmic channel; Arrowhead: lateral projections; Double arrowhead: membranous compartment; losangue: microtubule.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4.6. CAPÍTULO 6

Spadella, M.A., Oliveira, C., Quagio-Grassiotto, I. Spermiogenesis and spermatozoal ultrastructure in Loricariidae (Teleostei: Ostariophysi: Siluriformes). Manuscrito a ser submetido à revista Tissue & Cell.

Spermiogenesis and spermatozoa ultrastructure in Loricariidae (Teleostei: Ostariophysi: Siluriformes)

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Running headline: Spermiogenesis and spermatozoa in Loricariidae subfamilies.

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ABSTRACT

In the current study, the ultrastructural characteristics of both spermiogenesis and spermatozoa of specimens of Neoplecostominae, Hypoptopomatinae, Loricariinae, and Hypostominae are described. The data show that spermatogenesis and spermiogenesis process, and spermatozoa ultrastructure of Neoplecostominae is more similar to Hypoptopomatinae than Loricariinae and Hypostominae. Furthermore, Loricariinae and Hypostominae share more similar characteristics between them than with any other subfamily of Loricariidae. These data reinforce the phylogenetic hypotheses of relationships among the subfamilies of Loricariidae. Considering all the data obtained, the families Loricariidae and Callichthyidae, share more common ultrastructural characteristics of spermatogenesis, spermiogenesis, and spermatozoa among themselves than any other Loricarioidea suggesting that these families could be more related than actually proposed.

KEY WORDS: catfish, cysts of spermatids, male germ cell, phylogeny, morphology.

INTRODUCTION

Siluriformes comprises the most diverse and widely distributed ostariophysan group (Burgess, 1989; Teugels, 1996; Ferraris, 1998; Nelson, 2006). Among the Neotropical siluriform lineages, the superfamily Loricarioidea is probably a monophyletic group (de Pinna, 1998; Britto, 2003). This superfamily comprises six families: Nematogenyidae, Trichomycteridae, Callichthyidae, Scoloplacidae, Astroblepidae, and Loricariidae. According to the phylogeny of the superfamily Loricarioidea proposed by Britto (2003), the family Loricariidae is sister group of the family Astroblepidae. Loricariidae is the largest family of catfishes, distributed in most of the neotropics (Reis et al., 2006). At present, loricariids are divided into six subfamilies: Lithogeneinae, Delturinae, Neoplecostominae, Hypoptopomatinae, Loricariinae, and Hypostominae (Armbruster, 2004; Reis et al., 2006). The subfamily Hypostominae includes the tribes Corymbophanini, Hypostomini, Pterygoplichthini, Rhinelepini, and Ancistrini (Armbruster, 2004).

In the current study, the ultrastructural characteristics of both spermiogenesis and spermatozoa of specimens of Neoplecostominae, Hypoptopomatinae, Loricariinae, and Hypostominae are described. The data obtained in the present study and those available for other Loricarioidea and siluriform families were compared in order to investigate the occurrence of similarities and differences among families of this clade.

MATERIAL AND METHODS

The present study was conducted with two adult males of each species of subfamily Neoplecostominae: *Kronichthys heylandi* (Boulenger, 1900) collected from the Parati-Mirim river (23°14'25.5"S, W 44°45'47.2"W), Parati, Rio de Janeiro, Brazil (LBP 2122), and *Neoplecostomus paranensis* Langeani, 1990 from the Hortelã stream (22°56'28.3"S, 48°35'03.9"W), Botucatu, São Paulo, Brazil (LBP 3597); two adult males of each species of subfamily Hypoptopomatinae: *Corumbataia cuestae* Britski, 1997 collected from the Alambari river (22°56'08"S, 48°19'15"W), Botucatu, São Paulo, Brazil (LBP 1313, LBP 2001); *Hisonotus* sp. collected from the Araquá river (22°47.135'S, W 48°28.892W),

Botucatu, São Paulo, Brazil (LBP 1292, LBP 1999); *Hypoptopoma guentheri* Boulenger, 1895 collected from the Piraí river (16°25.680'S, 56°25.143'W), Poconé, Mato Grosso, Brazil (LBP 693); and *Schizolecis guntheri* (Miranda-Ribeiro, 1918) collected from the Parati-Mirim river (23°14'25.5"S, W 44°45'47.2"W), Parati, Rio de Janeiro, Brazil (LBP2123); two adult males of each species of subfamily Loricariinae: *Loricariichthys platymetopon* Isbrücker & Nijssen, 1979 collected from the Capivara dam (22°51'52"S, 50°53'56"W), Cândido Mota, São Paulo, Brazil; *Loricaria* sp. collected from the Fundo stream river (15°52'40.4"S, 52°18'15.5"W), Barra do Garças, Mato Grosso, Brazil (LBP 2443); and *Farlowella* sp. collected from the Fundo stream river (15°52'40.4"S, 52°18'15.5"W), Barra do Garças, Mato Grosso, Brazil (LBP 2441); and two adult males of *Hypostomus ancistroides* (Ihering, 1911) of subfamily Hypostominae: collected from the Alambari river (22°56'08", 48°19'15"W), Botucatu, São Paulo, Brazil (LBP 1376). Fishes were identified and kept in the fish collection of Laboratório de Biologia e Genética de Peixes (LBP), Departamento de Morfologia, Instituto de Biociências, UNESP, Botucatu, São Paulo, Brazil.

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 for 2h in the dark in 1% osmium tetroxide in the same buffer, contrasted in block with aqueous solution of 5% uranyl acetate for 2h, 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 available information about siluriform spermatozoa was reviewed for comparative purposes: Diplomystidae (Quagio-Grassiotto *et al.*, 2001), Cetopsidae, Aspredinidae, and Nematogenyidae (Spadella *et al.*, 2006a), Amblycipitidae (Lee and Kim, 1999), Ariidae (Mattei, 1991, schematic drawings), Trichomycteridae (Spadella *et al.*, submitted d), Loricariidae (Mansour and Lahnsteiner, 2003), Callichthyidae (Spadella *et al.*, submitted c), Scoloplacidae (Spadella *et al.*, 2006b), Malapteruridae (Mattei, 1991, schematic drawings; Shahin, 2006), Ictaluridae (Poirier and Nicholson, 1982; Emel'yanova and Makeyeva, 1991a, b), Bagridae (Emel'yanova and Makeyeva, 1991b; Lee, 1998; Kim

and Lee, 2000; Mansour and Lahnsteiner, 2003), Pimelodidae (Quagio-Grassiotto and Carvalho, 2000; Santos *et al.*, 2001), Heptapteridae and Pseudopimelodidae (Quagio-Grassiotto *et al.*, 2005), Siluridae (Emel'yanova and Makeyeva, 1991b; Kwon *et al.*, 1998; Lee and Kim, 2001), Clariidae (Mansour *et al.*, 2002), and Auchenipteridae (Burns *et al.*, 2002).

Twenty four characters of ultrastructure of spermatogenesis and spermiogenesis (characters 1 to 7) and of spermatozoa (characters 8 to 24), present in at least one species analyzed, were employed in the comparative analyses with other Loricariodea families (Tables 2 and 3). Based on the siluriform spermatozoa described in the literature (Porier and Nicholson, 1982; Kwon *et al.*, 1998; Lee, 1998; Lee and Kim, 1999, 2001; Kim and Lee, 2000; Quagio-Grassiotto and Carvalho, 2000; Quagio-Grassiotto *et al.*, 2001, 2005; Santos *et al.*, 2001; Burns *et al.*, 2002; Mansour *et al.*, 2002), the midpiece size (character 18) was considered short when its total length was ≤ 1.7 μm and was considered long when its total length was > 1.7 μm. The cytoplasmic channel size (character 20) was considered short when its total length was ≤ 1.5 μm and was considered long when its total length was > 1.5 μm.

RESULTS

General observations

In the analyzed species, the spermiogenesis occurs in cysts in the germinative epithelium. These cysts contain groups of spermatids in similar developmental stage, which are surrounded by cytoplasmic processes of the Sertoli cells (Figs. 1a, 4a, 7a, and 10a). The early spermatids are interconnected by cytoplasmic bridges resulted from incomplete cytokinesis of cellular divisions. In theses germ cells, the cytoplasm is symmetrically distributed around the nucleus, which contains diffuse homogeneous chromatin and has a circular outline (Figs. 1b, 1c, 1g, 4b, 4f, 7b, 7e, 7i, and 10c). During spermatids differentiation, the chromatin is progressively and highly condensed, with areas of diffuse chromatin interspersed among areas of condensing chromatin (Figs. 1c, 1h, 4d, 4e, 7c, 7f,

7j, 10c, and 10d). Subsequently, the cytoplasmic mass moves toward at the initial segment of the tail, and gives rise to the midpiece of the future spermatozoon. This midpiece in differentiation, has few rounded to elongate mitochondria and vesicles randomly distributed (Figs. 1e, 1f, 1i, 1j, 4d, 4e, 4h, 4i, 7d, 7e, 7h, 7j, 7k, and 10e-10g).

The loricariids spermatozoa have a round to ovoid head without acrossomal vesicle, symmetric midpiece and one flagellum with the classical (9+2) microtubular pattern (Figs. 2a, 3a, 5a, 5e, 6a, 6f, 8a, 9a, and 11a). The nucleus has chromatin highly condensed, of homogeneous aspect, and it is interspersed by electron-lucent areas (Figs. 2d, 2c, 2g, 3b, 3c, 3d, 5f, 6a, 8a, 8b, 8c, 8f, 9b, 9c, 11b, 11c, and 11f). The midpiece has mitochondria and vesicles, being that the mitochondria are separated from the flagellum by the cytoplasmic channel (Figs. 2f-j, 3e-3g, 5b, 5c, 6, d, 6e, 6f-6i, 8m, 8n, 9e-9g, 11f, and 11h). The particular ultrastructural characteristics observed in the spermiogenesis and spermatozoa of each Loricariidae subfamily analyzed, is presented.

Spermiogenesis in Neoplecostominae

The description of spermiogenesis process in Neoplecostominae was based on the observation of spermatids of *K. heylandi* and *N. paranensis* (Figs. 1a-k). In *N. paranensis*, the spermatids have an electron-dense structure surrounded by plasma membrane in the cytoplasm of the midpiece. In the early spermatids of theses species, the centriolar complex lies laterally to the nucleus and is anchored to the plasma membrane, with the flagellum originated from the distal centriole (Figs. 1b and 1g). In the centriolar complex, the proximal centriole is lateral and in obtuse angle in relation to the distal centriole (Figs. 1d and 1h). The centrioles move towards the nucleus, bringing with it the plasma membrane and the initial segment of the flagellum that invaginates (Figs. 1c, 1d, 1g, and 1h). With this movement, the cytoplasmic channel, a space between the plasma membranes of the flagellar region and the midpiece is formed. The nuclear rotation occurs among the Neoplecostominae, resulting in the medial position of the flagellum in relation to the nucleus (Fig. 1d). During the nuclear rotation, a depression is formed in the nuclear outline that gives rise to the nuclear fossa. At the end of this process, the centriolar complex is totally inserted in the nuclear fossa (Figs. 1b-inset, 1d, and 1h). In the Neoplecostominae

analyzed, the formation of lateral projections or fins from the flagellar membrane is observed (Figs. 1k and 1k-inset).

Spermatozoa of Neoplecostominae

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

Neoplecostominae spermatozoa are found in the lumen of the germinative compartment, being surrounded by a secretion only in *N. paranensis* (Fig. 3i). They exhibit a round head, a symmetric midpiece, and one flagellum medially positioned in relation to the nucleus (Figs. 2a, 2f, 2g, 3a, and 3e). The round nucleus contains the nuclear fossa medially positioned, which has a ramified simple arc shape (Figs. 2d, 3d, and 3e). In the midpiece, several rounded to elongated mitochondria are randomly distributed in all regions, concentrated in the apical and medial regions. Few vesicles isolated are observed in the midpiece of *K. heylandi*, while in *N. paranensis*, several isolated vesicles are randomly found in all regions of the midpiece (Figs. 2a, 2g, 2h, 2i, 3a, 3d, and 3g). In Neoplecostominae, the centrioles are lateral and in obtuse angle to each other. The centriolar complex is completely inserted in the nuclear fossa (Figs. 2d, 2e, 2g, and 3d). In *K. heylandi*, the proximal centriole projects a set of microtubules in direction to the nucleus that invaginates (Fig. 2e). The medial flagellum has two flagellar lateral projections or fins (Figs. 2k, 2l, 3h, and 3h-inset).

Spermiogenesis in Hypoptopomatinae

The spermiogenesis process in Hypoptopomatinae was described based on the observation of spermatids of *C. cuestae*, *Hisonotus sp.*, and *H. guentheri* (Figs. 4a-j). For *S. guntheri*, only the characteristics of spermatozoa were described due to the absence of spermatids in the testis.

In the Hypoptopomatinae analyzed, the spermatids have an electron-dense structure surrounded by plasma membrane in the cytoplasm of the midpiece (Figs. 4a, 4b, 4e, 4h, and 4i). In the early spermatids, 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 (Figs. 4b, 4c-inset, and 4f). The centriolar complex moves towards the nucleus bringing with it the plasma membrane and the initial segment of the flagellum, forming the cytoplasmic canal (Figs. 4b-4g). The nuclear rotation occurs, and during this process, the nuclear fossa is formed in the nuclear outline. After nuclear rotation, the flagellum assumed a medial position to the nucleus, and the proximal and distal centrioles are totally inserted in the nuclear fossa (Figs. 4b, 4e, and 4g). The formation of two lateral projections is observed in the flagella (Figs. 4j and 4j-inset).

Spermatozoa in Hypoptopomatinae

Hypoptopomatinae spermatozoa are found in the lumen of the germinative compartment surrounded by an electron-dense secretion. This secretion prevents good plasma membrane preservation in spite of many changes in the fixation procedures. It is possible that this secretion preclude the infiltration of fixative, resulting in this ill-preservation of the plasma membranes of the spermatozoa presented in the Figures 5 and 6. The spermatozoa have ovoid head with an ovoid nucleus in *C. cuestae* and *H. guentheri*, and in *Hisonotus* sp. and *S. guntheri*, the rounded head contains a round nucleus. They also have a symmetric midpiece, and one flagellum medial to the nucleus (Figs. 5a, 5e, 6a, and 6f). In the cytoplasmic region lateral to the nucleus are seen mitochondria in *H. guentheri* and *S. guntheri*, while in *C. cuestae* and *Hisonotus* sp., no organelles are seen (Figs. 5a, 5d, 5e, 6a, and 6f). The nuclear fossa is medial, and forms a simple arc (Figs. 5a, 5b, 5e, 5h, 6a, 6and 6i). In the centriolar complex, the proximal centriole is perpendicular to the distal, and they lies within the nuclear fossa (Figs. 5a, 5g, and 6i). Several vesicles, either interconnected to each other or not, are observed in the midpiece, concentrated in the basal region around the flagellum in *C. cuestae*, *H. guentheri*, and *S. guntheri* (Figs. 5b, 5c, 6d, 6e, 6g, 6h, and 6i). In the midpiece of *Hisonotus* sp., few elongated vesicles are mainly found in the proximity of the nucleus, and around the flagellum (Figs. 5e, 6h, and 6i). Several mitochondria are randomly distributed in all regions, concentrated on the apical and medial midpiece regions. In the midpiece, an electron-dense structure surrounded by

plasma membrane is also observed in *C. cuestae* (Figs. 5b, 5c, 5d, 5f, 5h, 5i, 6c, 6d, 6e, 6h, and 6i). The flagella have two lateral projections or fins (Figs. 5d-inset and 6j).

Spermiogenesis in Loricariinae

The analysis spermiogenesis process in Loricariinae was based on the observation of spermatids of *L. platymetopon*, *Farlowella* sp., and *Loricaria* sp. (Figs. 7a-l). In the species of Loricariinae observed, two types of spermiogenesis are founded. In *L. platymetopon* early spermatids, the centriolar complex lies medially to the nucleus and is anchored to the plasma membrane (Figs. 7b and 7c). The arrangement of centriolar complex consists in the proximal centriole perpendicular to the distal centriole (Fig. 7b). The centriolar complex does not move towards the nucleus, and it remains associated with the plasma membrane, forming the medial flagellum to the nucleus (Figs. 7b and 7c). The nucleus does not rotate and the nuclear fossa is not formed. The cytoplasmic mass moves toward the initial segment of the tail, and gives rise to the midpiece with a cytoplasmic channel (Fig. 7d). In the *Farlowella* sp. and *Loricaria* sp. early spermatids, the centriolar complex lies laterally to the nucleus and is anchored to the plasma membrane, with the proximal centriole perpendicular to the distal centriole (Figs. 7e, 7f, 7i and 7j). The centriolar complex moves towards the nucleus carrying the initial segment of the tail originating the cytoplasmic channel (Figs. 7f, 7g, and 7j). The nuclear rotation occurs in different degrees in these Loricariinae, being total in *Farlowella* sp., and partial in *Loricaria* sp. This result in the medial position of the flagellum in relation to the nucleus in *Farlowella* sp., while in *Loricaria* sp., the flagellum is eccentric positioned (Figs. 7f and 7j). The nuclear fossa is also formed, and at the end of the process, only the proximal centriole is inserted in the nuclear fossa (Figs. 7g, 7i, and 7j). Among the Loricariinae analyzed, the formation of lateral projections or fins is observed only in *Farlowella* sp. (Figs. 7d-inset, 7h, and 7l).

Spermatozoa in Loricariinae

The characteristics of spermatozoa in Loricariinae are described based on analysis of *L. platymetopon* and *Loricaria* sp., since in the testis of *Farlowella* sp. spermatozoa were not found.

Loricariinae spermatozoa are observed in the lumen of the germinative compartment without any secretion around of them (Figs. 8a and 9a). They exhibit a round nucleus, and a symmetric midpiece. The flagellum is medially positioned in *L. platymetopon* and it is eccentric in *Loricaria* sp. (Figs. 8a, 8d, 9a, and 9d). In *Loricaria* sp., the nuclear fossa is eccentrically positioned and has a simple arc shape. In *L. platymetopon*, the nuclear fossa is absent (Figs. 8a, 8d, and 9d). In Loricariinae, the centrioles are perpendicular to each other and in *Loricaria* sp., the proximal centriole is inserted in the nuclear fossa (Figs. 8d, 8e, 9d). In *L. platymetopon*, the midpiece has many rounded to elongated mitochondria, generally concentrated in the periphery. Thus, the mitochondria are not present around the centriolar complex. In *Loricaria* sp., few mitochondria are randomly distributed in all regions of the midpiece. In both species, the mitochondria are separated from the flagellum by the cytoplasmic channel. Few isolated vesicles are observed in the midpiece, concentrated in the basal region in *L. platymetopon*, and randomly distributed in all regions in *Loricaria* sp. (Figs. 8c, 8f-8n, 9e-g). Lateral projections or fins are not observed in the flagella (Figs. 8o, 8o-inset, and 9h).

Spermiogenesis in Hypostominae

The description of spermiogenesis process in Hypostominae was based on the observation of spermatids of *H. ancistroides* (Figs. 10a-h). The early spermatids have the centriolar complex, with the proximal centriole perpendicular to the distal one, lied 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 (Figs. 10b and 10c). The movement of centriolar complex does not occur, remaining associated with the plasma membrane (Figs. 10b and 10d). The nuclear rotation does not also occur, and the flagellum remains in medial position to the nucleus. Along the differentiation, a narrow nuclear fossa is formed in the nuclear outline (Figs. 10b-10e). The cytoplasmic

mass moves toward the tail, and gives rise to the midpiece with a cytoplasmic channel (Fig. 10d). The flagellum exhibits the formation of two lateral projections (Fig. 10h).

Spermatozoa in Hypostominae

Hypostominae spermatozoa are found in the lumen of the germinative compartment free of secretion (Fig. 11a). They exhibit a round head, a symmetric midpiece, and one flagellum medially positioned to the nucleus (Figs. 11a, 11d, and 11f). The medial nuclear fossa has a simple arc shape and contains only the proximal centriole inserted in perpendicular arrangement to the distal one (Figs. 11d and 11e). 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 (Figs. 11c, 11f-11h). The medial flagellum has two flagellar lateral projections or fins (Figs. 11i and 11i-inset).

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 of the cystic type. Cystic spermatogenesis is present in most Teleostei (Mattei, 1993; Quagio-Grassiotto *et al.*, 2001, 2003, 2005), and it was also described in other families of Loricarioidea, as in Trichomycteridae (Spadella *et al.*, submitted d), in the subfamily Callichthyinae (family Callichthyidae) (Spadella *et al.*, submitted c), and in Scoloplacidae (Spadella *et al.*, 2006b) (Table 2).

According to Mattei (1970), the spermiogenesis can be of types I or II in Teleostei with external fertilization. In both types, in the early spermatids the initial development of the flagellum is generally lateral to the nucleus. The occurrence or not of nuclear rotation differentiate these two types. In the type I, the nuclear rotation occurs, resulting in a spermatozoon with the flagellum axis perpendicular to the nucleus. In the type II spermiogenesis, the nuclear rotation does not occurs, determining a parallel position of the

flagellum in relation to the nucleus (Mattei, 1970). In Pimelodidae and Heptapteridae, the development of flagellum is medial, the nucleus does not rotate, and both the nuclear fossa and cytoplasmic channel does not form during spermiogenesis, characterizing a third type of spermiogenesis (Quagio-Grassiotto *et al.*, 2005; Quagio-Grassiotto and Oliveira, submitted). The spermiogenesis process observed in most Loricariidae is characterized by an initial lateral development of the flagellum, the presence of centriolar complex migration, a cytoplasmic channel formation, the presence of complete or partial nuclear rotation, and a medial to eccentric nuclear fossa formation. These characteristics are more similar to type I spermiogenesis previously described, which is also founded in Trichomycteridae (Spadella *et al.*, submitted d), Callichthyinae (Spadella *et al.*, submitted c), and in Scoloplacidae (Spadella *et al.*, 2006b) (Table 2). Type I spermiogenesis can be found in other siluriform families, as Diplomystidae, the most basal siluriform (Quagio-Grassiotto *et al.*, 2001), and Pseudopimelodidae (Quagio-Grassiotto *et al.*, 2005). However, in Diplomystidae the cytoplasmic channel does not remain in the spermatozoa (Quagio-Grassiotto *et al.*, 2001) as observed in other loricariids, Trichomycteridae, Callichthyinae, Scoloplacidae and Pseudopimelodidae.

Of the loricariids analyzed, only in *L. platymetopon* and *Hypostomus* sp., the spermiogenesis process is characterized by a medial development of the flagellum, the absence of nuclear rotation, a cytoplasmic channel formation, the absence of centriolar complex migration, and a medial nuclear fossa formation only in *Hypostomus* sp. (Table 2). This set of characteristics is different from those previously described, also observed in Nematogenyidae, except by nuclear fossa formation (our unpublished data), in Cetopsidae and Aspredinidae (Spadella *et al.*, 2006a), Heptapteridae (Quagio-Grassiotto *et al.*, 2005), and in Pimelodidae (Quagio-Grassiotto and Oliveira, submitted). Except by the medial development of the flagellum and medial nuclear fossa formation, this unusual spermiogenesis process is also found in Corydoradinae in which an eccentric formation of flagellum and of the nuclear fossa is observed (Spadella *et al.*, submitted c). In all cited groups, it is probable that the exiting cytoplasmic channel results of the accommodation and interconnection of the vesicles around the flagella, since the movement of the centriolar complex toward the nucleus does not occurs.

The comparative analyses of Table 2 show that all loricariids species share three of seven spermatogenesis and spermiogenesis characters (characters 1, 4, and 6). The other characteristics are variable among the species analyzed (characters 2, 3, 5, and 7).

The present data shows that spermatogenesis and spermiogenesis of Neoplecostominae is more similar to Hypoptopomatinae than with Loricariinae and Hypostominae, presenting six and four similar characteristics, respectively (Table 2). Furthermore, Loricariinae and Hypostominae share more characteristics between them than with any other subfamily of Loricariidae. These data agree with the new hypothesis of phylogenetic relationships among the subfamilies of Loricariidae (Armbruster, 2004).

The Table 2 shows that at least one species of Loricariidae share with Trichomycteridae and Callichthyidae all spermatogenesis and spermiogenesis characteristics, and six characters with Nematogenyidae and Scoloplacidae. Accordingly, the loricariids species share more similar characteristics with Trichomycteridae and Callichthyidae than with Nematogenyidae and Scoloplacidae. This observation does not agree with the phylogeny actually proposed for Loricarioidea (de Pinna, 1998; Britto, 2003), in which the family Loricariidae is sister group of Astroblepidae, and this clade more related with Scoloplacidae.

Spermatozoa

The comparative analyses of spermatozoa ultrastructure (Table 3) show that Loricariidae species herein studied share the same state of character in nine of seventeen characters analyzed (characters 8, 10, 13, 16, 18, 19 to 22).

Eight characteristics are polymorphic among the loricariids. Thus, the arrangement of centriolar complex is lateral and in obtuse angle in Neoplecostominae, while in the other loricariids, the centrioles are perpendicular to each other (character 9). The arrangement of centrioles, lateral and in obtuse angle, is also observed in some species of Trichomycteridae (Spadella *et al.*, submitted d) and Callichthyidae (Spadella *et al.*, submitted c), and in Clariidae (Mansour *et al.*, 2002); while the perpendicular arrangement is founded in Scoloplacidae (Spadella *et al.*, 2006b) and other siluriform families: Diplomystidae

(Quagio-Grassiotto *et al.*, 2001), Pimelodidae (Quagio-Grassiotto and Carvalho, 2000; Santos *et al.*, 2001), and Auchenipteridae (Burns *et al.*, 2002).

The flagellar membrane specializations (character 11) are also varied, observed two lateral projections in the flagellum of Neoplecostominae, Hypoptopomatinae, *Farlowella* sp., and *H. ancistroides*; as in the trichomycterids *T. areolatus* and *T. reinhardt* (Spadella *et al.*, submitted), Scoloplacidae (Spadela *et al.*, 2006b), Amblycipitidae (Lee and Kim, 1999), Bagridae (Lee, 1998; Kim and Lee, 2000; Mansour and Lahnsteiner, 2003), and Diplomystidae (Quagio-Grassiotto *et al.*, 2001). In *L. platymetopon*, *Loricaria* sp., and in the tribe Ancistrini, the lateral fins are absent. The absence of lateral projections in the flagellum is also pointed in Nematogenyidae, Cetopsidae, and Aspredinidae (Spadella *et al.*, 2006a), in Callichthyinae (Spadella *et al.*, submitted), Pimelodidae (Quagio-Grassiotto and Carvalho, 2000; Santos *et al.*, 2001), Siluridae (Kwon *et al.*, 1998; Lee and Kim, 2001), Clariidae (Mansour *et al.*, 2002), and Auchenipteridae (Burns *et al.*, 2002).

The character 12, the nucleus shape, is other different characteristic among loricariid species, which is ovoid in *C. cuestae* and *H. guentheri*, as observed in some species of Trichomycteridae (Spadella *et al.*, submitted d), Callichthyidae (Spadella *et al.*, submitted c), and Heptapteridae (Quagio-Grassiotto *et al.*, 2005). In the other species of Loricariidae, the nucleus is round as found in some trichomycterids (Spadella *et al.*, submitted d), callichthyids (Spadella *et al.*, submitted c), Pimelodidae (Quagio-Grassiotto and Carvalho, 2000; Santos *et al.*, 2001), Pseudopimelodidae (Quagio-Grassiotto *et al.*, 2005), Diplomystidae (Quagio-Grassiotto *et al.*, 2001), Siluridae (Kwon *et al.*, 1998; Lee and Kim, 2001), and Clariidae (Mansour *et al.*, 2002). Other variable character among loricariids is the nuclear fossa position (character 15), which is medial in Neoplecostominae, Hypoptopomatinae, and Hypostominae. The same is found in Trichomycteridae (Spadella *et al.*, submitted), in most Callichthyidae (Spadella *et al.*, submitted), Scoloplacidae (Spadella *et al.*, 2006b), and in the follow siluriform families: Diplomystidae (Quagio-Grassiotto *et al.*, 2001), Cetopsidae and Aspredinidae (Spadella *et al.*, 2006a), Amblycipitidae (Lee and Kim, 1999), Ictaluridae (Poirier and Nicholson, 1982), Bagridae (Lee, 1998; Kim and Lee, 2000; Mansour and Lahnsteiner, 2003), Pseudopimelodidae (Quagio-Grassiotto *et al.*, 2005), Siluridae (Kwon *et al.*, 1998; Lee and

Kim, 2001), and Clariidae (Mansour *et al.*, 2002). In *Loricaria* sp., an eccentric nuclear fossa is present as in some trichomycterids (Spadella *et al.*, submitted d) and Auchenipteridae (Burns *et al.*, 2002).

The position of centrioles in relation to the nuclear fossa (character 17) varies, the centriolar complex totally inserted in the nuclear fossa in Neoplecostominae, Hypoptopomatinae, and in the tribe Ancistrini. This character is also present in some trichomycterids (Spadella *et al.*, submitted d), in some Callichthyinae (Spadella *et al.*, submitted c), Scoloplacidae (Spadella *et al.*, 2006b), Diplomystidae (Quagio-Grassiotto *et al.*, 2001), Cetopsidae and Aspredinidae (Spadella *et al.*, 2006a), Amblycipitidae (Lee and Kim, 1999), Bagridae (Lee, 1998; Kim and Lee, 2000; Mansour and Lahnsteiner, 2003), and Clariidae (Mansour *et al.*, 2002). Only the proximal centriole inserted in the nuclear fossa is founded in *Loricaria* sp. and *H. ancistroides*. This state is also described in most of the trichomycterids (Spadella *et al.*, submitted) and *Callichthys callichthys* (Spadella *et al.*, submitted).

The character 23, presence of electron-dense structure surrounded by plasma membrane in the midpiece, is only observed in Neoplecostominae and Hypoptopomatinae. In *N. paranensis* and all Hypoptopomatinae analyzed, the spermatozoa are founded in the lumen of the germinative compartment surrounded by a dense secretion. These characteristics represent exclusive characters of these subfamilies, not observed in any other siluriform up to the present.

The position of the flagellum in relation to the nucleus (character 24) is medial in Neoplecostominae, Hypoptopomatinae, *L. platymetopon*, and Hypostominae as in Nematogenyidae (Spadella *et al.*, 2006a), in some Trichomycteridae (Spadella *et al.*, submitted d) and Callichthyidae (Spadella *et al.*, submitted c), and Scoloplacidae (Spadella *et al.*, 2006b). The medial flagellum position is the more common pattern in siluriform (Quagio-Grassiotto *et al.*, 2001; Spadella *et al.*, 2006a; Lee and Kim, 1999; Poirier and Nicholson, 1982; Lee, 1998; Kim and Lee, 2000). In *Loricaria* sp., the flagellum is eccentric in relation to the nucleus. This position is also described in some trichomycterids (Spadella *et al.*, submitted d).

These eight characters, as described above, are more variable among the Loricariinae and Hypostominae than in Neoplecostominae and Hypoptopomatinae. At least one of these states has been found in other families of Loricarioidea and siluriform until now studied, except the character 23.

The Table 3 shows that Neoplecostominae shares sixteen spermatozoa characteristics with Hypoptopomatinae and Hypostominae, followed by Loricariinae, sharing thirteen characteristics. The spermatozoa of Hypoptopomatinae present sixteen similar characters with Hypostominae, and fourteen with Loricariinae. The subfamily Loricariinae shares sixteen characteristics with Hypostominae.

As discussed above, the spermatozoa in Neoplecostominae share more common characters with species of Hypoptopomatinae and Hypostominae, while the spermatozoa of Loricariinae are more similar to the Hypostominae. Considering that the Neoplecostominae and Hypoptopomatinae share the occurrence of spermatozoa involved by secretion in the lumen of germinative compartment, beyond of the characters already mentioned; these subfamilies present more characteristics in common between themselves than with any other loricariids analyzed. Thus, the characters of ultrastructure of spermatozoa reinforce the considerations reported with the characters of spermiogenesis, which shows that the subfamily Neoplecostominae and Hypoptopomatinae are more related. This observation is in concordance with phylogenic hypothesis presented by Armbruster (2004).

Based on the information available the spermatozoa of the Loricariidae species are more similar to those of Callichthyidae species, sharing all characteristics. Furthermore, the spermatozoa of the Loricariidae share sixteen characteristics with Trichomycteridae, fourteen with Scoloplacidae, and ten similar characters with Nematogenyidae. Thus, the spermatozoa of Loricariidae, Callichthyidae, Trichomycteridae, and Scoloplacidae families share more similar characteristics among themselves than with the family Nematogenyidae.

Considering all the data obtained, the families Loricariidae and Callichthyidae, share more common ultrastructure characteristics of spermatogenesis, spermiogenesis, and spermatozoa among themselves than any other Loricarioidea suggesting that these families could be more related than proposed by de Pinna (1998) and Britto (2003).

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Table 1. Spermatozoa dimensions present in the loricariids species analyzed. the "n" show the number of structures measured.

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

Table 2. General view of the distribution of spermatogenesis and spermiogenesis character states in the species analyzed in the present study, and in families and tribe of Loricarioidea. (+) present; (-) absent; (?) unavailable.

Characters*	1		2		3		4		5				6		7	
	a	b	a	b	a	b	a	b	a	b	c	d	a	b	a	b
Families																
Nematogenyidae	-	+	-	+	+	-	-	+	+	-	-	-	-	+	+	-
Trichomycteridae	+	-	+	-	-	+	-	+	-	+	+	-	-	+	-	+
Callichthyidae	+	+	+	-	+	+	-	+	+	+	+	-	-	+	-	+
Scoloplacidae	+	-	+	-	-	+	-	+	-	-	-	+	+	-	-	+
Kronichthys heylandi	+	-	+	-	-	+	-	+	-	-	-	+	-	+	-	+
Neoplecostomus paranensis	+	-	+	-	-	+	-	+	-	-	-	+	-	+	-	+
Corumbataia cuestae	+	-	+	-	-	+	-	+	-	-	-	+	-	+	-	+
Hisonotus sp.	+	-	+	-	-	+	-	+	-	-	-	+	-	+	-	+
Hypoptopoma guentheri	+	-	+	-	-	+	-	+	-	-	-	+	-	+	-	+
Schizolecis guntheri	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Loricariichthys platymetopon	+	-	-	+	+	-	-	+	+	-	-	-	-	+	+	-
Farlowella sp.	+	-	+	-	-	+	-	+	-	+	-	-	-	+	-	+
Loricaria sp.	+	-	+	-	-	+	-	+	-	+	-	-	-	+	-	+
Hypostomus ancistroides	+	-	-	+	+	-	-	+	+	-	-	-	-	+	-	+
Ancistrini	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?

Legends:
*1- spermatogenesis type (a-cystic; b- semicystic); 2- initial position of the flagellum in relation to the nucleus (a- lateral; b- medial); 3- centriolar complex movement (a- absent; b- present); 4- cytoplasmic channel formation (a- absent; b- present); 5- nuclear rotation (a- absent; b- partial; c- nearly complete; d- complete); 6- process of chromatin condensation (a- heterogeneous; b- homogeneous); 7- nuclear fossa formation (a-absent; b- present).

Table 3. General view of the distribution of spermatozoa character states in the loricariids analyzed, and in others families and tribe of Loricarioidea. (+) present; (-) absent; (?) unavailable.

Characters*	8		9						10		11				12					13		14		15				16		17			18		19			20		21					22		23		2
	a	b	a	b	c	d	e	f	a	b	a	b	c	d	a	b	c	d	e	a	b	a	b	a	b	c	d	a	b	a	b	c	a	b	a	b	c	a	b	a	b	c	d	e	a	b	a	b	c	a
Families																																																		
Nematogenyidae	-	+	+	-	-	-	-	-	-	+	+	-	-	-	-	-	-	+	-	+	-	-	+	+	-	-	-	-	-	-	-	-	+	-	+	-	+	-	-	+	-	-	+	-	+	-	-	+
Trichomycteridae	+	-	-	-	+	-	+	-	+	+	-	+	+	+	-	-	+	-	+	+	+	+	+	-	+	+	+	+	-	+	+	-	+	-	+	-	+	-	+	+	+	-	-	-	+	-	-	+
Callichthyidae	+	-	-	+	+	-	-	+	-	+	+	-	-	+	-	-	+	-	+	+	-	-	+	-	+	+	-	+	-	+	+	+	+	-	+	-	+	-	-	+	-	-	+	+	+	-	+
Scoloplacidae	+	-	-	-	-	+	-	-	-	+	-	+	-	-	-	+	-	-	-	+	-	-	+	-	+	-	-	+	-	+	-	-	-	+	-	+	-	-	+	-	-	-	+	-	+	-	-	+
Kronichthys heylandi	+	-	-	-	+	-	-	-	-	+	-	+	-	-	-	-	-	-	+	+	-	-	+	-	+	-	-	+	-	+	-	-	+	-	+	-	+	-	+	+	-	-	+	-	-	-	+	+
Neoplecostomus paranensis	+	-	-	-	+	-	-	-	-	+	-	+	-	-	-	-	-	-	+	+	-	-	+	-	+	-	-	+	-	+	-	-	+	-	+	-	+	-	+	+	-	-	+	-	-	-	+	+
Corumbataia cuestae	+	-	-	-	-	+	-	-	-	+	-	+	-	-	-	-	+	-	-	+	-	-	+	-	+	-	-	+	-	+	-	-	+	-	+	-	+	-	+	+	-	-	+	-	-	-	+	+
Hisonotus sp.	+	-	-	-	-	+	-	-	-	+	-	+	-	-	-	-	-	-	+	+	-	-	+	-	+	-	-	+	-	+	-	-	+	-	+	-	+	-	+	+	-	-	+	-	-	-	+	+
Hypoptopoma guentheri	+	-	-	-	-	+	-	-	-	+	-	+	-	-	-	-	+	-	-	+	-	-	+	-	+	-	-	+	-	+	-	-	+	-	+	-	+	-	+	+	-	-	+	-	-	-	+	+
Schizolecis guntheri	+	-	-	-	-	+	-	-	-	+	-	+	-	-	-	-	-	-	+	+	-	-	+	-	+	-	-	+	-	+	-	-	+	-	+	-	+	-	+	+	-	-	+	-	-	-	+	+
Loricaritchthys platymetopon	+	-	-	-	-	+	-	-	-	+	+	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-	-	+	-	-	-	-	+	-	+	-	+	-	+	+	-	-	+	-	+	-	-	+
Farlowella sp.	+	-	-	-	-	+	-	-	-	+	-	+	-	-	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	-	+	-	?	?	?	?	?	?	?	?	?	?	?	?	?
Loricaria sp.	+	-	-	-	-	+	-	-	-	+	+	-	-	-	-	-	-	-	+	+	-	-	+	-	-	+	-	+	-	-	+	-	+	-	+	-	+	-	+	+	-	-	+	-	+	-	-	-
Hypostomus ancistroides	+	-	-	-	-	+	-	-	-	+	-	+	-	-	-	-	-	-	+	+	-	-	+	-	+	-	-	+	-	-	+	-	+	-	+	-	+	-	+	+	-	-	+	-	+	-	-	+
Ancistrini	+	-	-	-	-	+	-	-	-	+	+	-	-	-	-	-	-	-	+	+	-	+	-	-	+	-	-	+	-	+	-	-	+	-	+	-	+	-	-	+	-	-	+	-	+	-	-	+

Legends: *8- flagella number (a- one; b- two); 9- arrangement of centriolar complex (a- lateral and parallel; b- lateral and acute angle; c- lateral and obtuse angle; d- anterior and perpendicular; e- lateral and perpendicular; f- co-axial); 10- vesicles in the midpiece (a- absent; b- present); 11- flagellar membrane specializations (a- absent; b- two lateral fins; c- variable number lateral fins; d- membranous compartment); 12- shape of the nucleus (a- semi-ovoid; b- conic; c- ovoid; d-ovoid with its bigger axis in the horizontal direction; e- round); 13- cytoplasmic area around of the nucleus (a- narrow; b- large); 14- aspect of chromatin condensation (a- heterogeneous; b- homogeneous); 15- nuclear fossa (a-absent; b- medial; c- eccentric; d- lateral); 16- nuclear fossa shape (a- simple arc; b- double arc); 17- Position of centrioles in relation to the nuclear fossa (a- totally inserted in the nuclear fossa; b- only proximal centriole inserted in the nuclear fossa; c- totally outside of the nuclear fossa); 18- midpiece size (a- short; b- long); 19- cytoplasmic channels (a- absent; b- one; c- two); 20- cytoplasmic channel size (a- short; b- long); 21- mitochondria shape (a- rounded; b- elongated; c- elongated and ramificated; d- C-shape; e- irregular); 22- midpiece symmetry (a- symmetric; b- asymmetric); 23- Electron-dense circular structure in the midpiece (a- absent; b- not surrounded by plasma membrane; c- not surrounded by plasma membrane); 24- flagellum position in relation to the nucleus (a- medial; b- eccentric; c- lateral).

FIGURE CAPTION

Figure 1. Spermiogenesis of Neoplecostominae: *Kronichthys* sp. (a to f) and *Neoplecostomus paranensis* (g to k). a) Spermatids cyst. b, c, and g) Early spermatids in longitudinal sections. d, e, and h) Late spermatids (longitudinal sections). b-inset) Centriolar complex arrangement. f, i, and j) Midpiece showing mitochondria, vesicles, and an electron-dense structure surrounded by plasma membrane (cross sections). k and k-inset) Flagella exhibiting the formation of lateral projections (longitudinal and cross sections). (a) 1.3 μm; (b, d, f, g, and i) 0.6 μm; (c, e, j, and k) 0.7 μm; (b-inset) 0.1 μm; (h) 0.4 μm; (k-inset) 0.2 μm. B: basal body; D: distal centriole; F: flagellum; N: nucleus; P: proximal centriole; S: Sertoli cell; V: vesicles; Asterisk: mitochondria; Arrow: cytoplasmic channel; Arrowhead: lateral projections; Double arrowhead: electron-dense structure surrounded by plasma membrane.

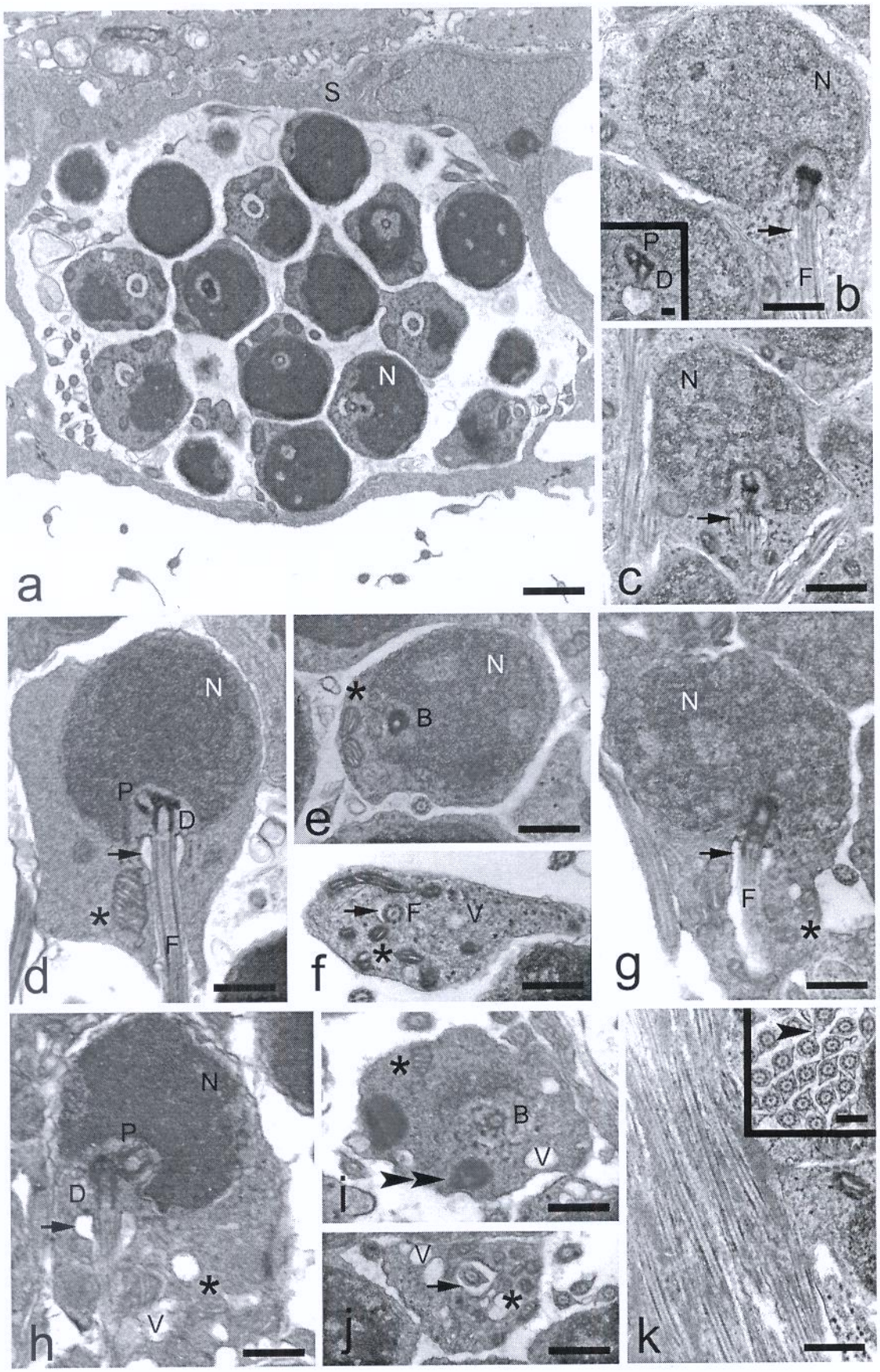

FIGURE CAPTION

Figure 2. Spermatozoa of *Kronichthys heylandi*. a) Longitudinal section. b and c) Head region. d and e) Centriolar complex arrangement. f to h) Spermatozoon longitudinal sections showing nuclear fossa, cytoplasmic channel, mitochondria, and vesicles in the midpiece. i and j) Midpiece cross sections showing mitochondria and vesicles. k and l) Flagella in longitudinal and cross sections. (a, b, and c) 0.5 μm; (d, l) 0.3 μm; (e, g, h, and k) 0.4 μm; (f, i, and j) 0.6 μm. 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 channel; Arrowhead: lateral projections.

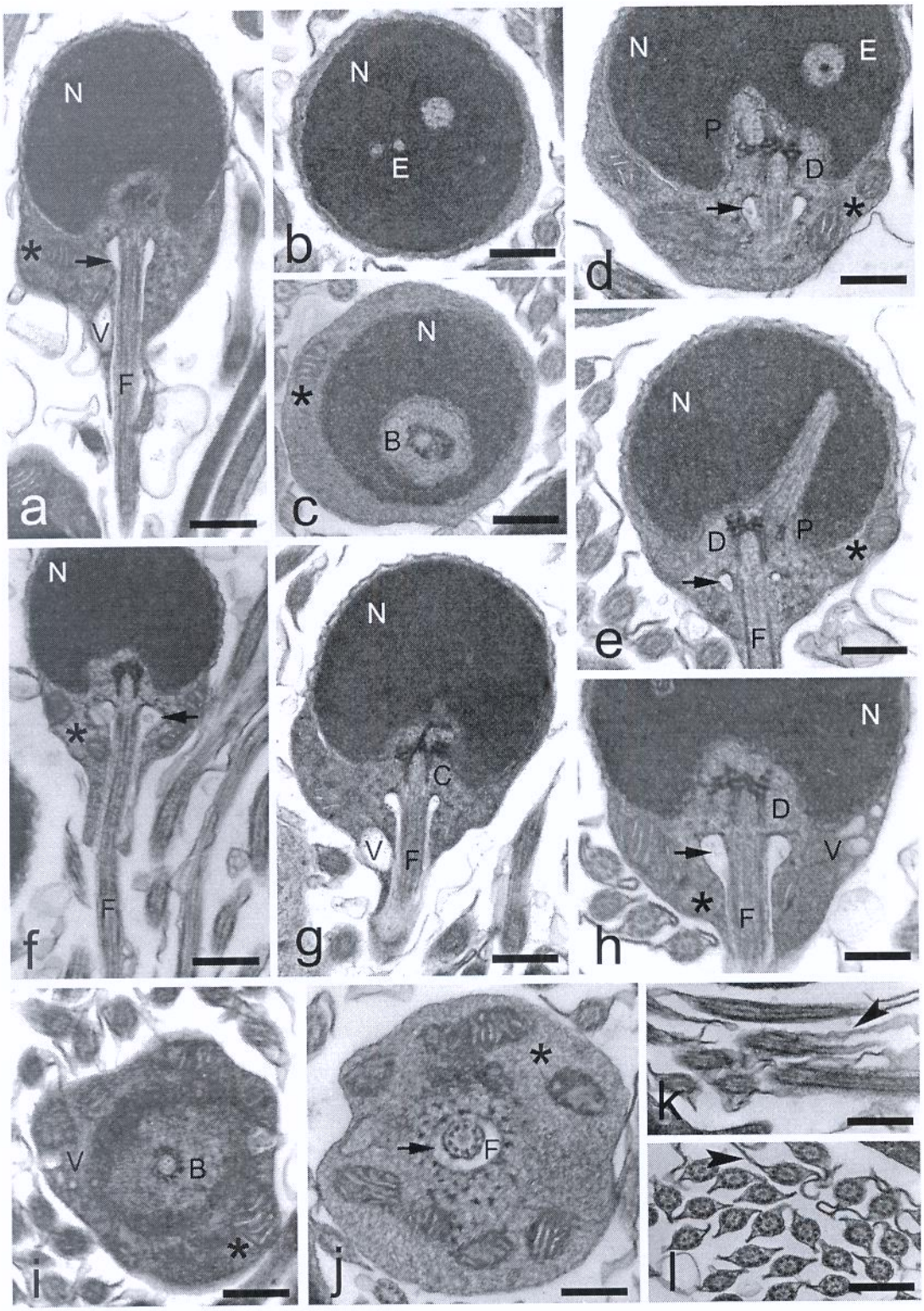
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FIGURE CAPTION

Figure 3. Spermatozoa of *Neoplecostomus paranensis*. a) Spermatozoon in longitudinal section. b and c) Head cross and longitudinal sections. d) Detail of centriolar complex arrangement. e to g) Spermatozoa in longitudinal and cross sections showing mitochondria and vesicles in the midpiece. h and h-inset) Flagella in longitudinal and cross sections. i) Spermatozoa in the lumen of germinative compartment surrounded by secretion. (a and i) 0.4 μm; (b, c, d, and g) 0.6 μm; (e) = 0.5 μm; (f) 0.7 μm; (h) 0.3 μm; (h-inset) 0.1 μm. B: basal body; D: distal centriole; E: electron-lucent area; F: flagellum; N: nucleus; P: proximal centriole; V: vesicles; Asterisk: mitochondria; Arrow: cytoplasmic channel; Arrowhead: lateral projections.

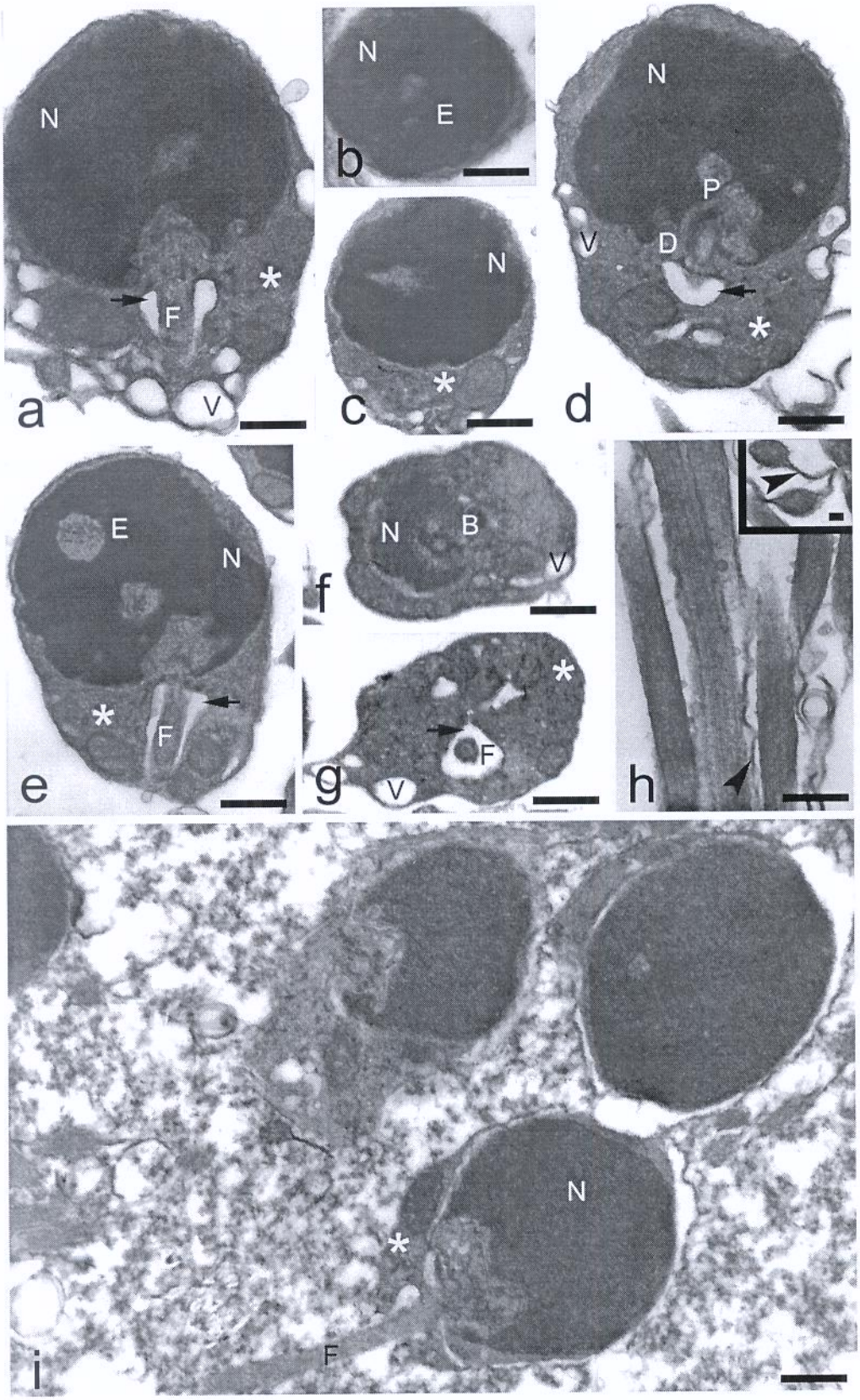
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FIGURE CAPTION

Figure 4. Spermiogenesis of Hypoptopomatinae. *Corumbataia cuestae* (a to c, c-inset, h, and j), *Hisonotus* sp. (d, e, i, and j-inset), and *Hypoptopoma guenteri* (f and g). a) Spermatids cyst. b, f and g) Early spermatids (longitudinal sections). c to e) Late spermatids. c-inset) Detail of centriolar complex arrangement. h and i) Midpiece showing mitochondria and vesicles (cross sections). j and j-inset) Flagella in longitudinal and cross sections. (a) 1.7 μm; (b to e, and i) 0.6 μm; (c-inset) 0.2 μm; (f) 0.5 μm; (g) 0.7 μm; (h, j) 0.4 μm; (j-inset) 0.05 μm. D: distal centriole; F: flagellum; N: nucleus; P: proximal centriole; S: Sertoli cell; V: vesicles; Asterisk: mitochondria; Arrow: cytoplasmic channel; Arrowhead: lateral projections; Double arrowhead: electron-dense structure surrounded by plasma membrane.

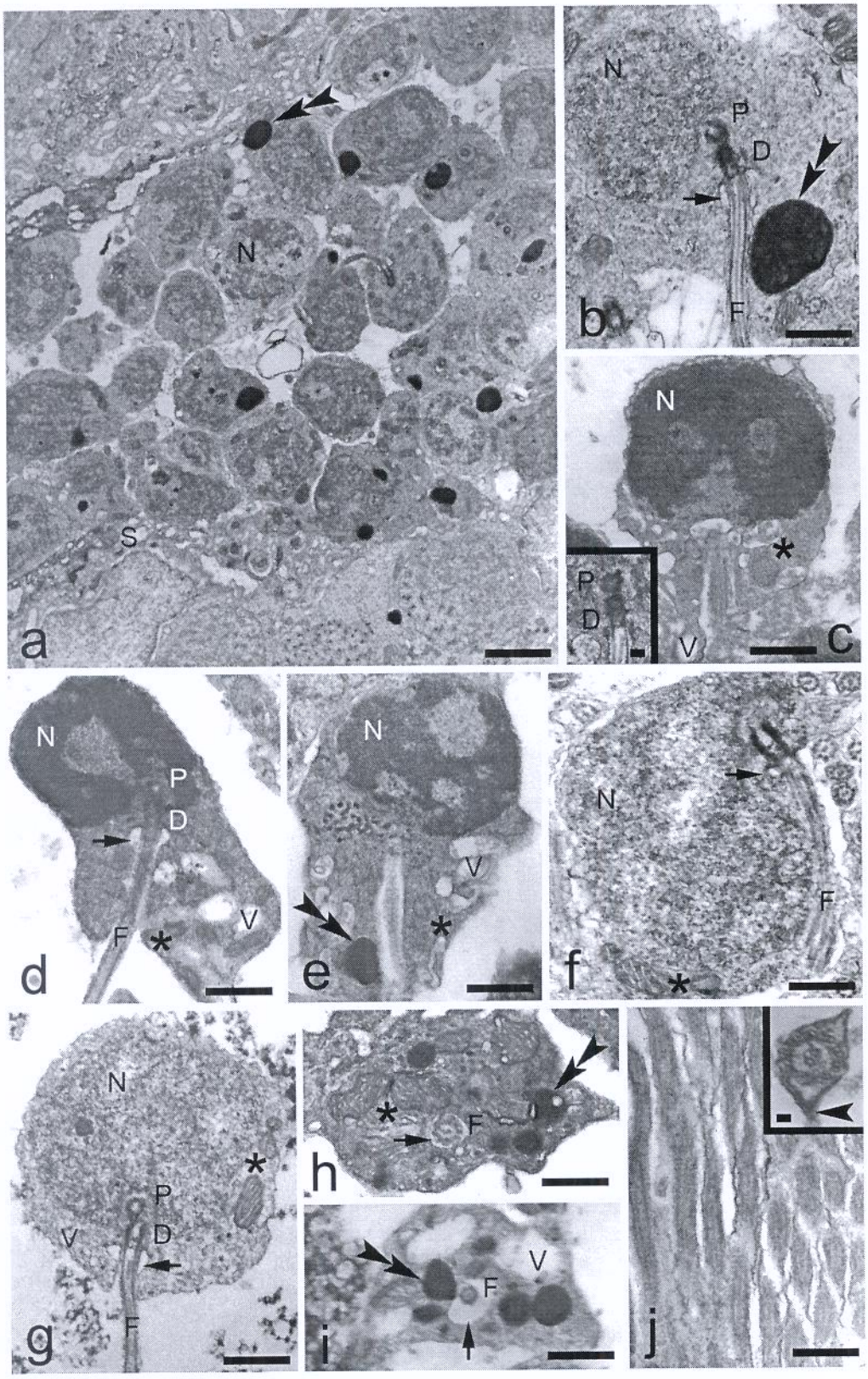
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FIGURE CAPTION

Figure 5. Spermatozoa of *Corumbataia cuestae* (a to d) and *Hisonotus* sp. (e to i). a and g) Spermatozoon in longitudinal sections exhibiting the centriolar complex arrangement. b, c, f, h, and i) Midpiece showing cytoplasmic channel, mitochondria, and vesicles (longitudinal and cross sections). d and e) Spermatozoon in longitudinal sections showing mitochondria and vesicles. d-inset) Flagella in cross sections showing classical (9+2) axoneme and lateral projections. (a, d, f, h, and i) 0.4 μm; (b, c) 0.6 μm; (d-inset) 0.06 μm; (e) 0.5 μm; (g) 0.7 μm. D: distal centriole; F: flagellum; N: nucleus; P: proximal centriole; V: vesicles; Asterisk: mitochondria; Arrow: cytoplasmic channel; Arrowhead: lateral projections; Double arrowhead: electron-dense structure surrounded by plasma membrane.

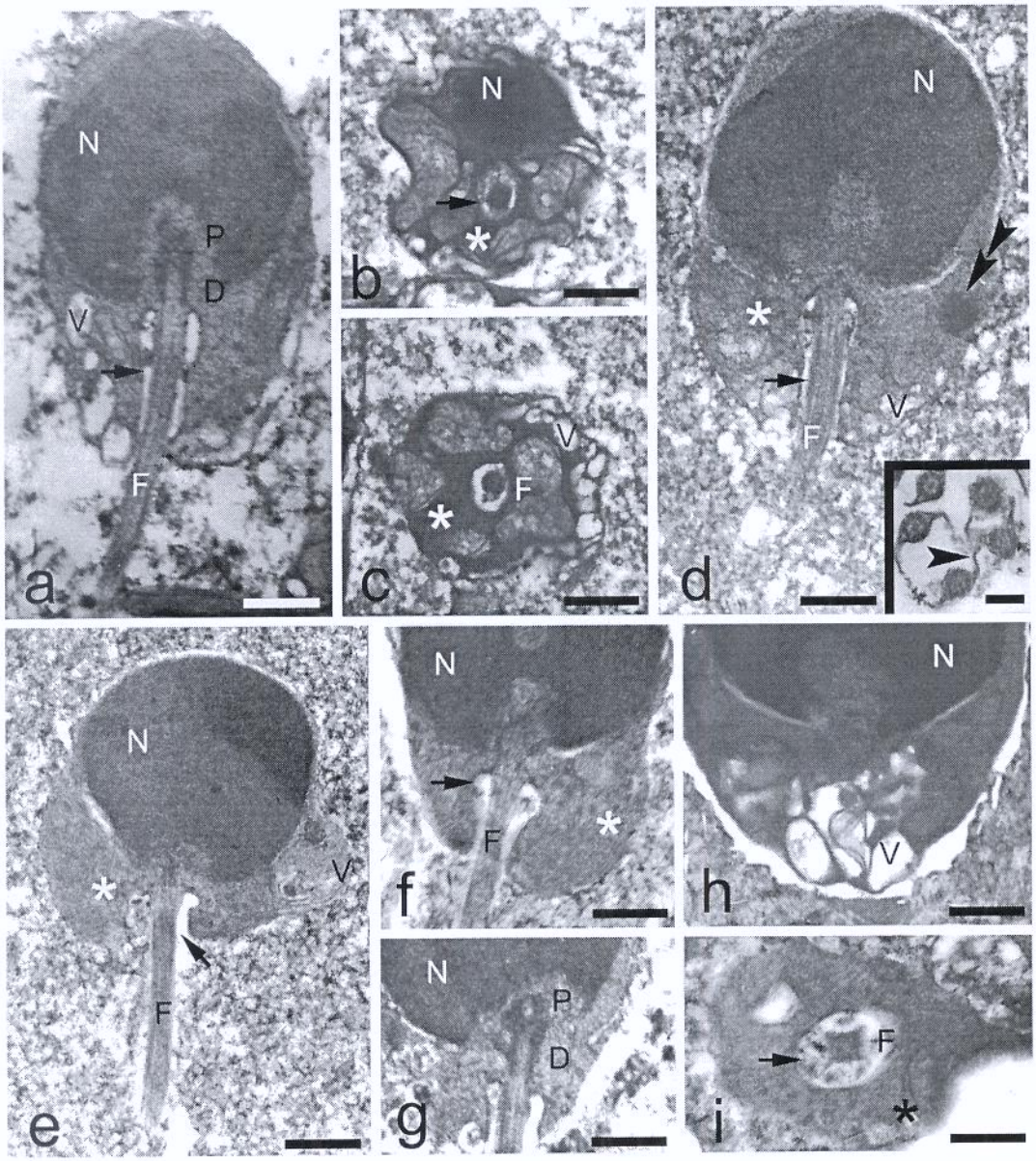
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FIGURE CAPTION

Figure 6. Spermatozoa of *Hypoptopoma guentheri* (a to e) and *Shizolecis guntheri* (f and j). a and f) Longitudinal sections. b and c) Head region. d, e, g, and h) Midpiece (longitudinal and cross sections) showing mitochondria and vesicles. i) Detail of nuclear fossa and centriolar complex arrangement. j) Flagella in cross sections exhibiting lateral projections. (a, g, i, and j) = 0.4 μm; (b, c, and f) 0.6 μm; (d, e) 0.5 μm; (h) 0.7 μm. B: basal body; E: electron-lucent area; F: flagellum; N: nucleus; P: proximal centriole; V: vesicles; Asterisk: mitochondria; Arrow: cytoplasmic channel; Arrowhead: lateral projections.

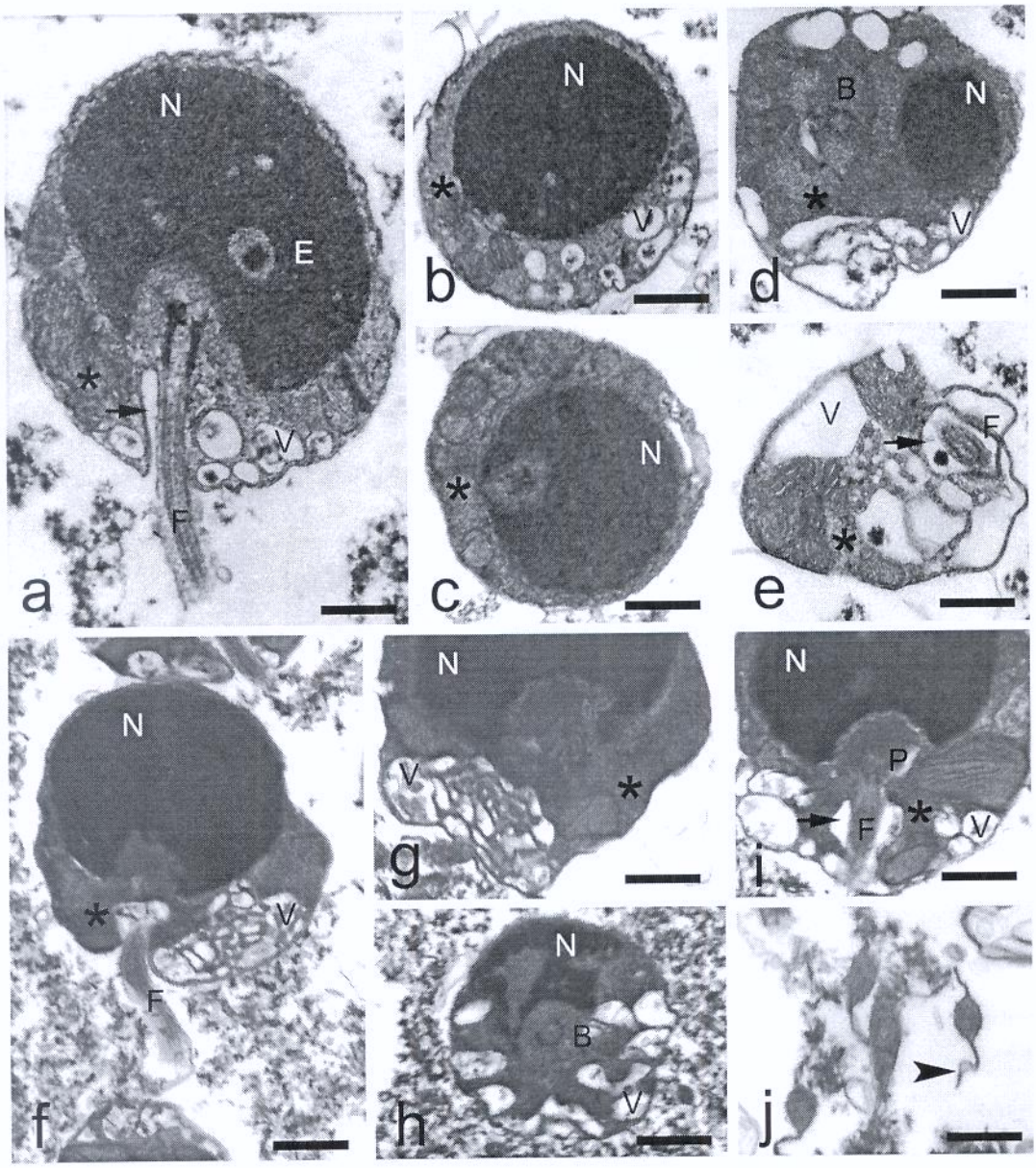
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FIGURE CAPTION

Figure 7. Spermiogenesis of Loricariinae: *Loricariichthys platymetopon* (a to d), *Farowella* sp. (e to h), and *Loricaria* sp. (i to l). a) Spermatids cyst. b, e, and i) Early spermatids in longitudinal sections. c, f, and j) Late spermatids (longitudinal sections). d-inset) Flagellum in cross section. g) Centriolar complex arrangement. d, h, and k) Midpiece showing mitochondria and vesicles (cross and longitudinal sections). l) Flagellum in longitudinal section. (a) 2.3 μm; (b, h, i, and j) 0.6 μm; (c) 1.0 μm; (d, e, and g) 0.4 μm; (d-inset) 0.1 μm; (f) 0.5 μm; (k, l) 0.7 μm. D: distal centriole; F: flagellum; N: nucleus; P: proximal centriole; S: Sertoli cell; V: vesicles; Asterisk: mitochondria; Arrow: cytoplasmic channel; Arrowhead: lateral projections.

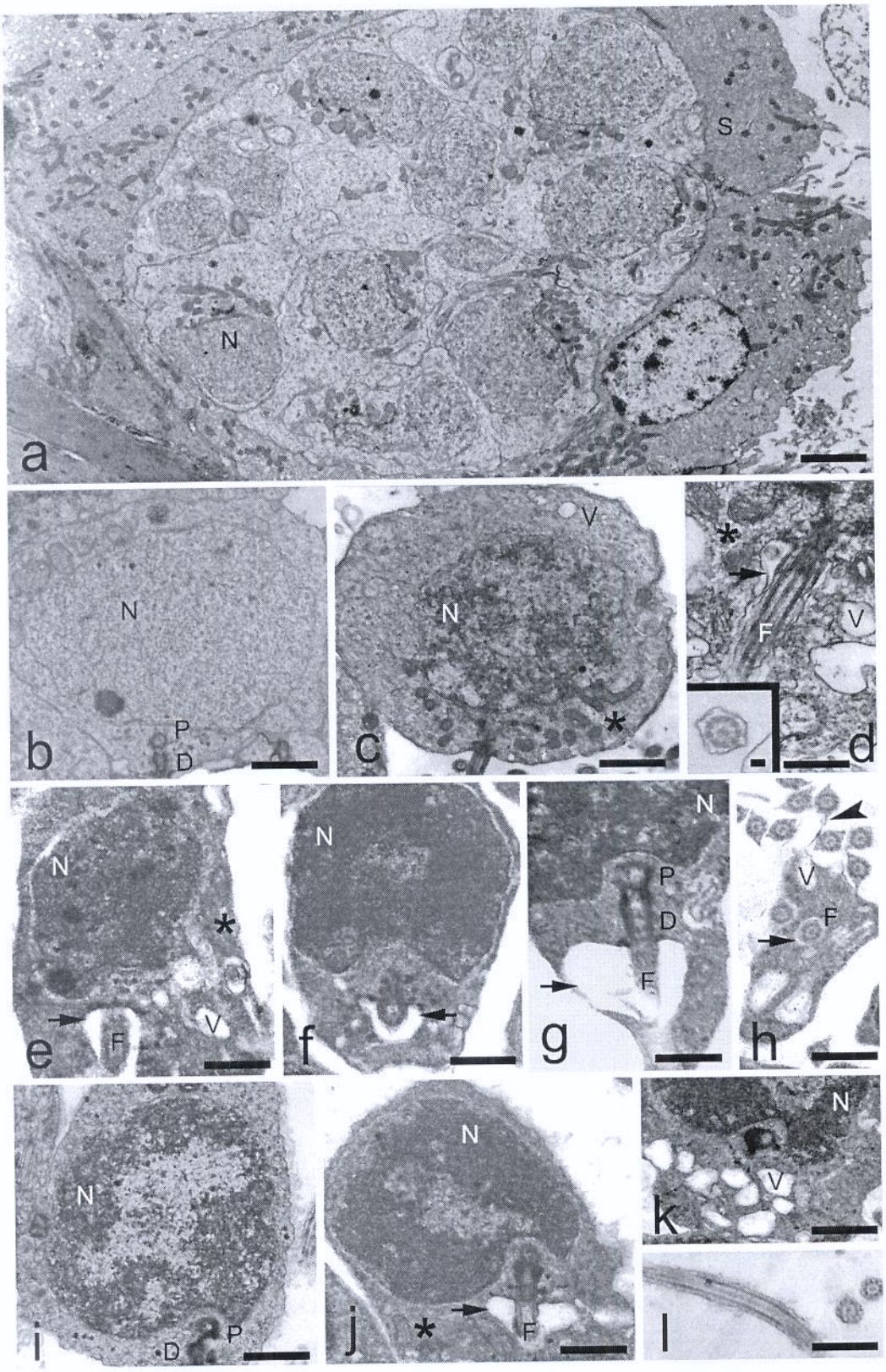
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FIGURE CAPTION

Figure 8. Spermatozoa of *Loricariichthys platymetopon*. a) Longitudinal section of spermatozoon. b and c) Head region. d and e) Detail of centrioles arrangement. f to n) Midpiece in longitudinal and cross sections showing mitochondria, vesicles, and cytoplasmic channel. o, o-inset, and p) Flagella in cross and longitudinal sections showing classical (9+2) axoneme. (a, b, and f) 0.5 μm; (c, n, and o) 0.6 μm; (d) 0.7 μm; (e, g, and j) 0.4 μm; (h) 1.0 μm; (i, l, o-inset, and p) 0.3 μm; (k) 0.8 μm; (m) 1.5 μm. B: basal body; D: distal centriole; E: electron-lucent area; F: flagellum; N: nucleus; P: proximal centriole; V: vesicles; Asterisk: mitochondria; Arrow: cytoplasmic channel.

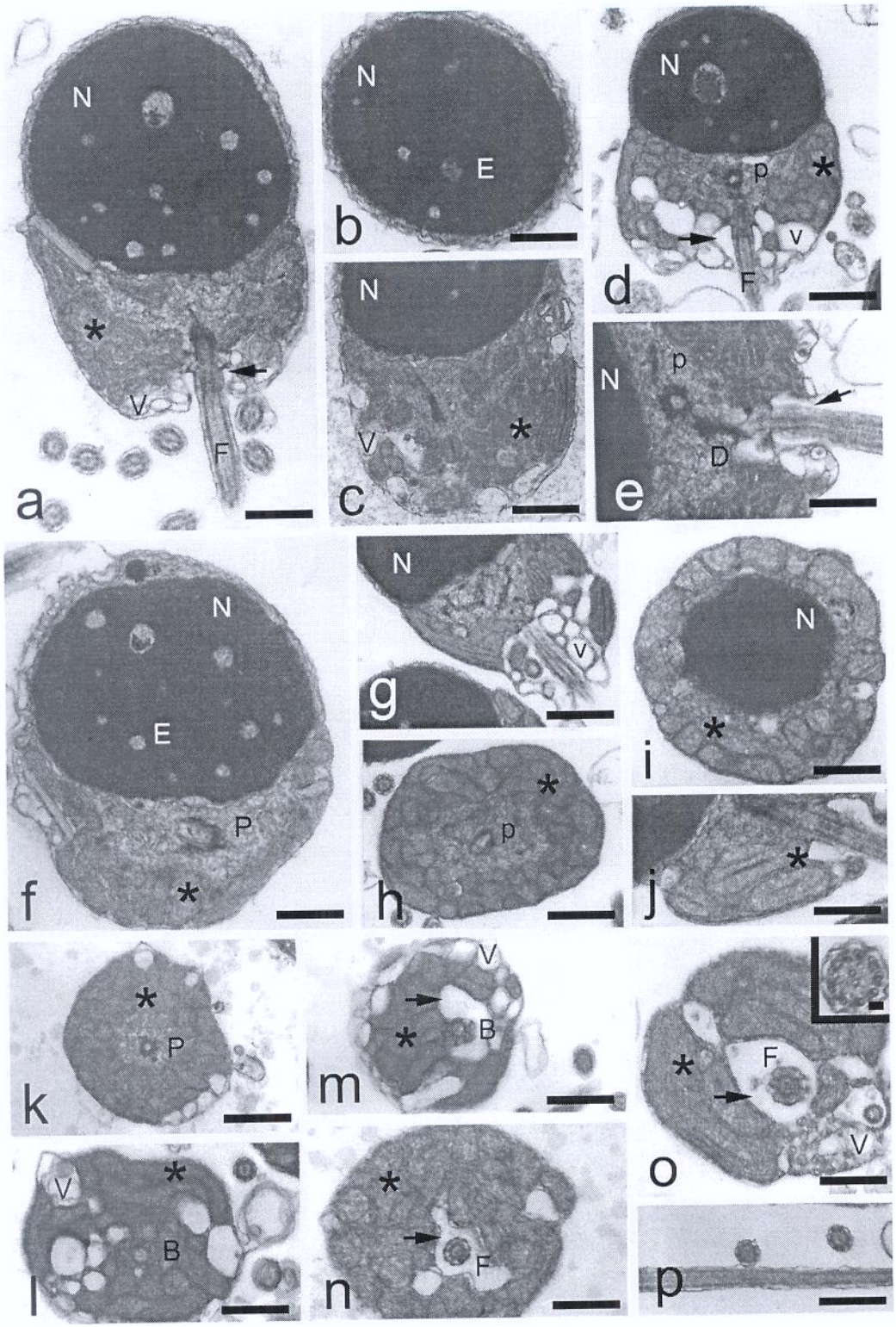
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FIGURE CAPTION

Figure 9. Spermatozoa of *Loricaria* sp.. a) Longitudinal section. b and c) Nucleus in cross sections. d) Centriolar complex arrangement. e to g) Midpiece in longitudinal and cross sections showing mitochondria, vesicles, and cytoplasmic channel. h) Flagella in longitudinal and cross sections. (a to c, and g) 0.6 μm; (d) 0.5 μm; (e) 0.4 μm; (f) 1.3 μm; (h) 0.9 μm. B: basal body; D: distal centriole; E: electron-lucent area; F: flagellum; N: nucleus; P: proximal centriole; V: vesicles; Asterisk: mitochondria; Arrow: cytoplasmic channel.

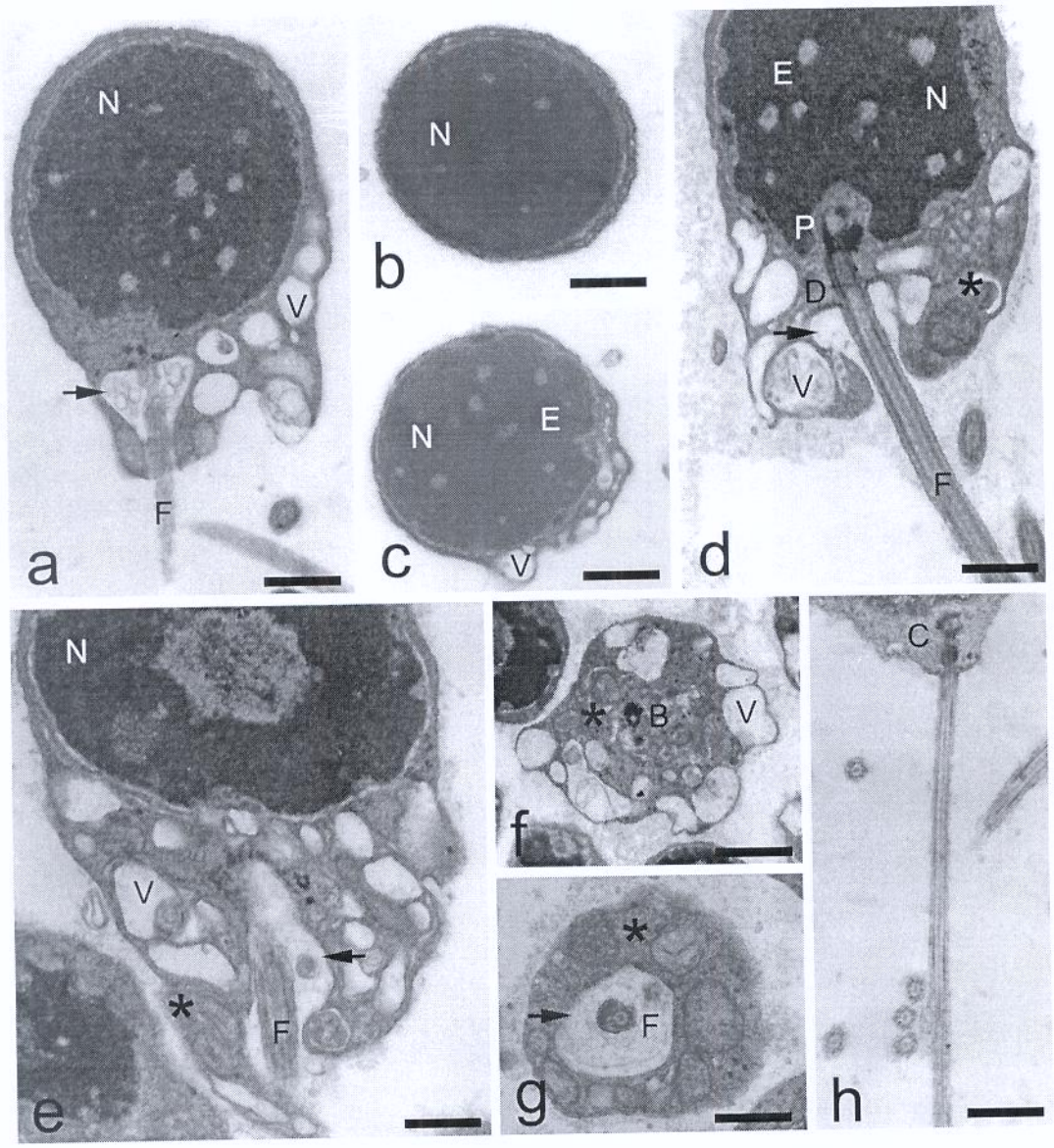
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FIGURE CAPTION

Figure 10. Spermiogenesis of Hypostominae. a) Spermatids cyst. b) Early spermatid in longitudinal section. c) Early spermatid showing centriolar complex arrangement. d) Late spermatid. e to g) Midpiece showing mitochondria and vesicles (longitudinal and cross sections). h) Flagella in cross sections exhibiting lateral projections. (a) 1.7 μm; (b) 0.9 μm; (c, e) 0.8 μm; (d) 0.5 μm; (f, g) 0.6 μm; (h) 0.3 μm. B: basal body; D: distal centriole; F: flagellum; N: nucleus; P: proximal centriole; S: Sertoli cell; T: spermatids; V: vesicles; Asterisk: mitochondria; Arrow: cytoplasmic channel; Arrowhead: lateral projections.

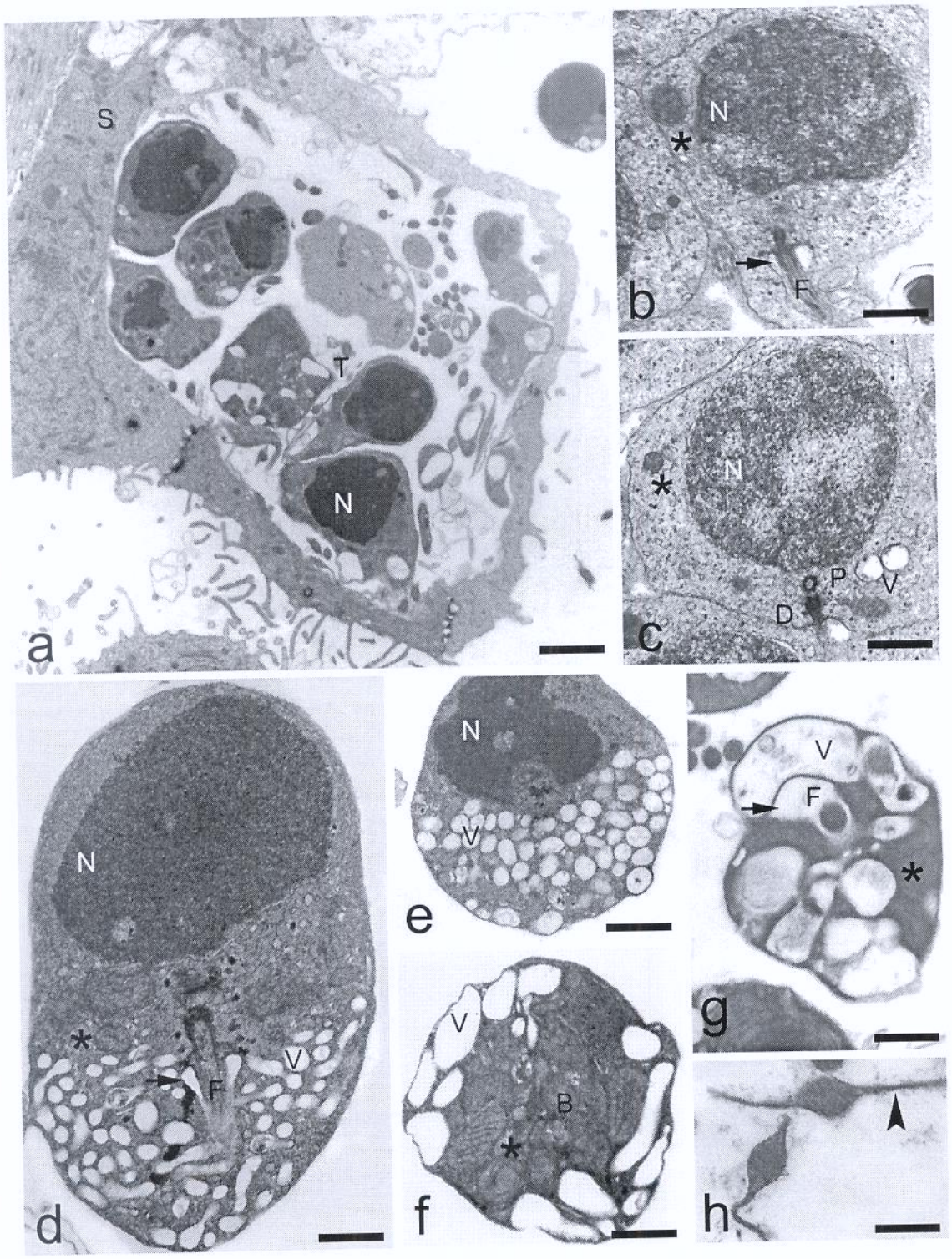
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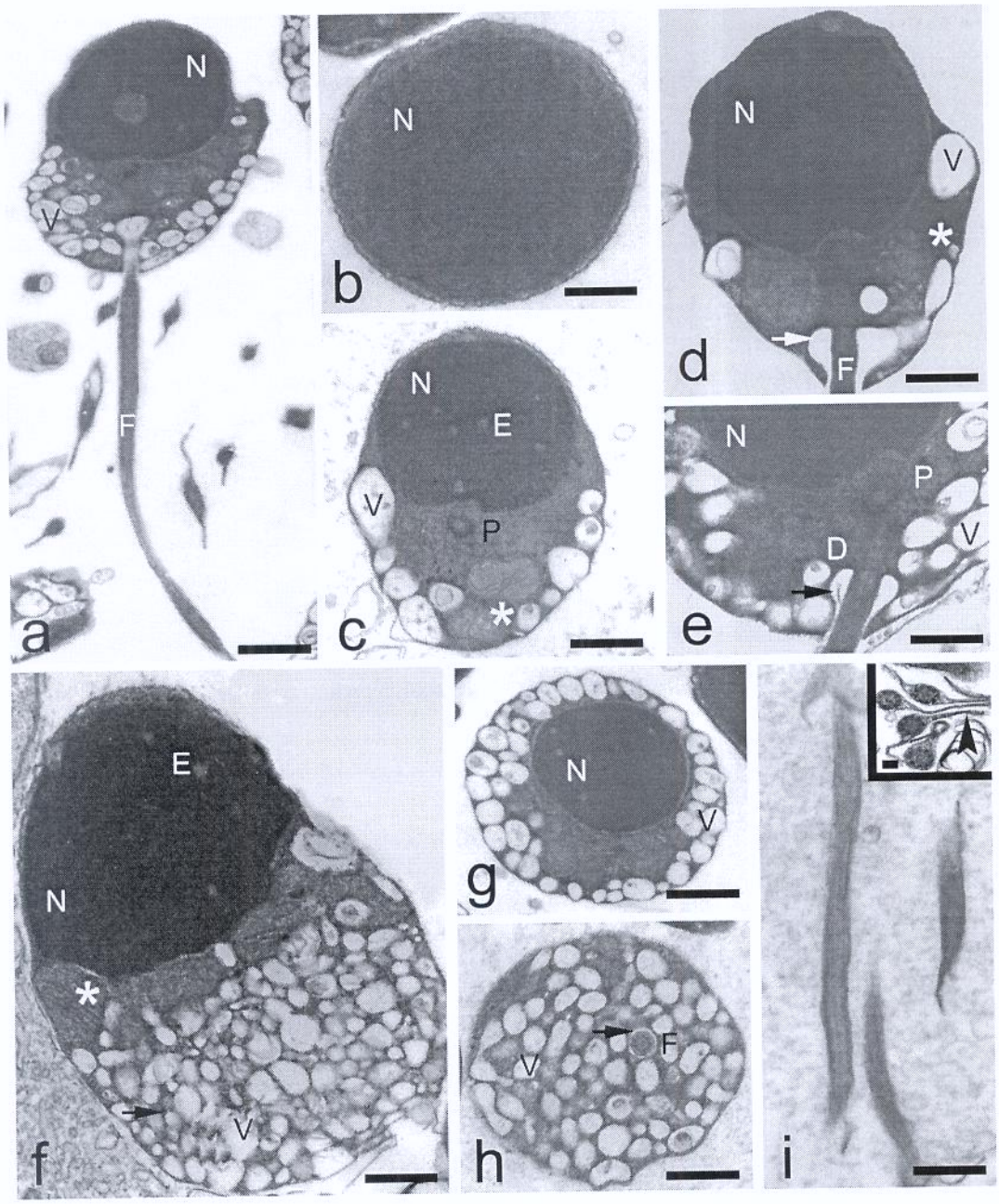
FIGURE CAPTION

Figure 11. Spermatozoa of *Hypostomus ancistroides*. a, c, and d) Spermatozoon in longitudinal sections. b) Nucleus in cross section. e) Detail of centriolar complex arrangement. f to h) Midpiece showing cytoplasmic channel, mitochondria, and vesicles (longitudinal and cross sections). i and i-inset) Flagella in longitudinal and cross sections showing classical (9+2) axoneme and lateral projections. (a) 1.0 μm; (b, d, e, and i) 0.6 μm; (c) 0.8 μm; (f) 0.4 μm; (g) 1.3 μm; (h) 0.7 μm; (i-inset) 0.03 μm. D: distal centriole; E: electron-lucent area; F: flagellum; N: nucleus; P: proximal centriole; V: vesicles; Asterisk: mitochondria, Arrow: cytoplasmic channel; Arrowhead: lateral projections.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Ultrastructure of *Astroblepus* cf. *mancoi* introsperms (Ostariophysi: Siluriformes: Astroblepidae)

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Running headline: Introsperms in Astroblepidae

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4.7. CAPÍTULO 7

Spadella, M.A., Oliveira, C., Ortega, H., Quagio-Grassiotto, I. Ultrastructure of *Astroblepus* cf. *mancoi* introsperms (Ostariophysi: Siluriformes: Astroblepidae). Manuscrito a ser submetido à revista Journal of Morphology.

ABSTRACT

The *Astroblepus* cf. *mancoi* spermatozoa have the main characteristics of introsperms, suggesting the occurrence of internal insemination in this species. These introsperms exhibit a conic head, a symmetric midpiece, a medial flagellum, and no acrosome. The conic forward elongated nuclei have its thin extremity curved. The chromatin exhibits homogeneous aspect. The centrioles are completely inside the medial nuclear fossa, perpendicular to each other. In the midpiece, many fused mitochondria form a ring surrounding internally the cytoplasmic channel. In the midpiece, vesicles are not observed. The flagellum presents the classical axoneme formulae (9+2) and has two lateral projections with extremities dilated and filled by electron-dense material. The introsperms of astroblepids share several characteristics with those of Scoloplacidae and Auchenipteridae, two other siluriform families with internal fecundation.

KEY WORDS: male germinative cells, spermatozoon, morphology, catfish, fish evolution.

INTRODUCTION

The family Astroblepidae has a single genus, *Astroblepus*, with 54 valid species (Schaefer, 2003). Recent phylogenetic studies showed that astroblepids belongs to the superfamily Loricarioidea, and the least understood family of this group (de Pinna, 1998). The monophyly of the family was corroborated by Schaefer (1990), as is its phylogenetic position as the sister group of the family Loricariidae (de Pinna, 1998; Britto, 2003).

The reproductive aspects of astroblepids are poorly known. Burgess (1989) reported that the male astroblepids have an elongate urogenital papilla that apparently functions as an intromittent organ, suggesting the occurrence of insemination in this family. Román-Valencia (2001), in a study of the reproductive ecology in *Astroblepus cyclopus*, shows that in this species ovaries mature between December and May, and that the fecundity is low and the eggs are small.

The ultrastructure of spermatozoa in *Astroblepus* cf. *mancoi* is here described for the first time, demonstrating the occurrence of introsperms in this species.

MATERIAL AND METHODS

The current study was conducted with adult males of *Astroblepus* cf. *mancoi* collected from the Chorobamba river (10°27'58.9''S, 075°29'01.4''W), Ucayali river basin, Huancabamba, Pasco, Peru (LBP 3284). The fishes were identified and kept in the fish collection of Laboratório de Biologia e Genética de Peixes (LBP), Departamento de Morfologia, Instituto de Biociências, UNESP, Botucatu, São Paulo, Brazil.

For ultrastructural analysis, the 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 for 2h in the dark in 1% osmium tetroxide in the same buffer, contrasted in block with aqueous solution of 5% uranyl acetate for 2h, 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.

Seventeen characters of ultrastructure of spermatozoa, present in the species analyzed, were employed in the comparative analyses with the other Loricariodea families, based on available information about spermatozoa ultrastructure of the families: Nematogenyidae (Spadella et al., 2006a), Trichomycteridae (Spadella et al., submitted c), Callichthyidae (Spadella et al., submitted c), Scoloplacidae (Spadella et al., 2006b), and Loricariidae (Mansour and Lahnsteiner, 2003; Spadella et al., submitted e). The midpiece size (character 11) was considered short when its total length was ≤ 1.7 μm and was considered long when its total length was > 1.7 μm. The cytoplasmic channel size (character 13) was considered short when its total length was ≤ 1.5 μm and was considered long when its total length was > 1.5 μm, according to measures of siluriform spermatozoa described in the literature (Lee, 1998; Porter and Nicholson, 1982; Kwon et al.,1998; Lee and Kim, 1999, 2001; Kim and Lee, 2000; Quagio-Grassiotto and Carvalho, 2000; Santos et al., 2001; Quagio-Grassiotto et al., 2001, 2005; Burns et al., 2002; Mansour et al., 2002).

RESULTS

A. cf. *mancoi* spermatozoa are found in the lumen of the germinative compartment, and they are not tightly packed along their entire lengths. These spermatozoa do not constitute a spermatozeugmata due to the absence of a compactness and regularity arrangement (Figs. 1a and 1h). The introsperms of this species exhibit a conic head without acrosomal vesicle, symmetric midpiece, and one medial flagellum (Figs. 1a and 1k). The nuclei have a conic shape, are very elongated forward and have its thin extremity curved (Fig. 1i). The nuclei are 6.0 μm in length and 0.6 μm in width. In its basal extremity, the nucleus has two basal expansions around the midpiece. In the cytoplasmic region, around to the nucleus, no organelles are seen (Figs. 1a, 1i, and 1j). The chromatin present in the nucleus is highly condensed and has a homogeneous aspect, being interspersed by small electron-lucent areas (Figs. 1a, 1b, 1i, and 1j). The nucleus contains a medial nuclear fossa, which is 0.7 μm in length and 0.3 μm in width. The shape of the nuclear fossa is in simple arc, which is irregularly delimited. The proximal and distal centrioles, perpendicular to each other, are completely inside the nuclear fossa. The initial segment of the tail is also

found inserted in the nuclear fossa (Figs. 1a, 1c, 1d and 1j). The midpiece is 5.4 μm in length and 0.6 μm in width. Along of the midpiece, but not in the terminal end, a large number of the mitochondria apparently fused, form a ring surrounding the cytoplasmic channel (Figs. 1e, 1f, 1j, and 1k). The basal extremity of the midpiece has a cytoplasmic shealth, and in this region there is no mitochondria. In the midpiece, vesicles are not found. The cytoplasmic channel is 4.8 μm in length and 0.4 μm in width (Figs. 1a and 1k). The distal centriole is differentiated in the basal body and gives rise to the axoneme which exhibits the classical 9+2 microtubular pattern. The flagellar membrane exhibit two lateral projections or fins, which has dilated extremity and filled with electron-dense material (Figs. 1l and 1l-inset).

DISCUSSION

The presence of very elongated nucleus and midpiece in the spermatozoa of *A.* cf. *mancoi* characterize the occurrence of introsperms in this species. The presence of an elongate urogenital papilla in this species as in other astroblepids species (Burgess, 1989; our personal observations), permit to suggest that Astroblepidae is other internal inseminating siluriform family. This condition is rare among teleosts. Among siluriforms species with introsperms are only reported to occur in Scoloplacidae (Spadella et al, 2006b) and in Auchenipteridae (Loir et al., 1989; Meisner et al., 2000; Burns et al., 2002). Other examples among Teleostei, are found in Cyprinodontiformes, in Poeciliidae (Grier, 1975; Kobayashi and Iwamatsu, 2002), Anablepidae and Jenynsiidae (Grier et al., 1981); in Scorpaeniformes, in several species of Scorpaenidae (Jamieson, 1991; Muñoz et al., 1999, 2002), and in Characiformes, the sister group of Siluriformes and Gymnotiformes (Fink and Fink, 1996), some species with introsperms are found in the Characidae subfamilies Cheirodontinae (Burns et al., 1997), Glandulocaudinae and Stevardiinae (Burns et al., 1995, 1998; Azevedo, et al., 2000; Pecio et al., 2005; Burns and Weitzman, 2005), and in the incertae sedis in Characidae, *Brittanichthys axelrodi* (Javonillo et al., 2007).

The comparative analyses of spermatozoa ultrastructure presented in Table 1 show that the spermatozoa of *A.* cf. ***mancoi*** share sixteen of seventeen characters analyzed with

members of Scoloplacidae. Additionally, Astroblepidae and Scoloplacidae present other characteristics in common not related in the Table 1, as the presence of the mitochondria in the midpiece forming an internal ring surrounding the cytoplasmic channel, the conic nuclei presenting their extremity curved, the presence of two lateral fins in the flagellum, and the organization of the spermatozoa in the germinative compartment not arranged in well organized spermatozeugmata.

According to Table 1, the only state not shared by Astroblepidae and Scoloplacidae is the third character, vesicles in the midpiece, which are absent in Astroblepidae and present in Scoloplacidae. The absence of vesicles in the midpiece is also found in *Trichomycterus reinhardti*, of the family Trichomycteridae (Spadella et al., submitted c), Diplomystidae (Quagio-Grassiotto et al., 2001), Amblyciptidae (Lee and Kim, 1999), Auchenipteridae (Burns et al., 2002), and Bagridae (Lee, 1998; Kim and Lee, 2000; Mansour and Lahnsteiner, 2003). An exclusive character found in Astroblepidae is the presence of expansions in each lateral projections of the flagellum filled by electron-dense material. This character is described here for the first time for siluriforms.

The analysis of Table 1 shows that Astroblepidae share thirteen characteristics with Loricariidae, twelve with Trichomycteridae, eleven with Callichthyidae, and seven similar state of character with Nematogenyidae. Considering all to describe above, *A.* cf. *mancoi* share more similar characteristics with Scoloplacidae than with any other Loricarioidea. In the phylogenies at present proposed for the superfamily Loricarioidea, the family Astroblepidae is a sister group of Loricariidae (de Pinna, 1998; Britto, 2003). However, in the current study, Astroblepidae seems to be more related with Scoloplacidae than with Loricariidae, not corroborating the hypotheses previously proposed. Although this finding can be related with a different phylogenetic position of theses families it can also represent a convergence, due to their exclusive characteristics of reproduction.

In a comparative analysis with others siluriform families, the *A.* cf. *mancoi* spermatozoa are also morphologically more similar to those found in auchenipterids, sharing some characteristics, for example, the conic shape of the nuclei, the presence of very long nucleus, the perpendicular arrangement between the centrioles, the presence of a long midpiece, the absence of vesicles in the midpiece, and the presence of mitochondria

along of the midpiece. It is possible that these similar characteristics should be originated by convergence, due to their particular mode of reproduction, since phylogenetic hypothesis demonstrated that Astroblepidae and Auchenipteridae do not belong to a natural group (de Pinna, 1998; Britto, 2003).

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Spadella MA, Oliveira C, Quagio-Grassiotto I. submitted e. Spermiogenesis and spermatozoal ultrastructure in Loricariidae (Teleostei: Ostariophysi: Siluriformes). Tissue & Cell.

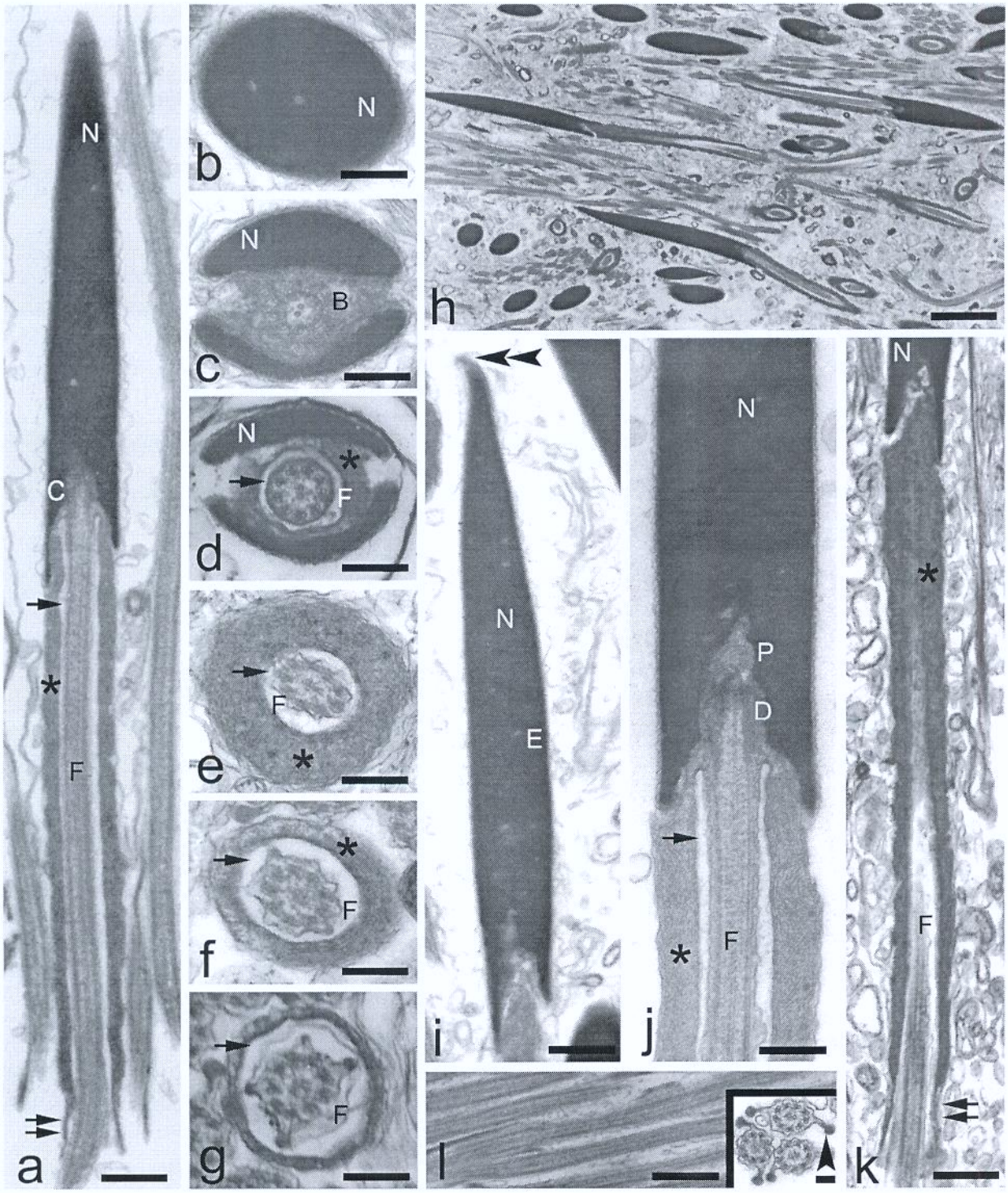
Table 1. General view of spermatozoa character states in the species analyzed and in the others families of Loricarioidea. (+) present; (-) absent.

Characters*	1		2						3		4				5					6		7		8				9		10			11		12			13		14					15		16			1
Families	a	b	a	b	c	d	e	f	a	b	a	b	c	d	a	b	c	d	e	a	b	a	b	a	b	c	d	a	b	a	b	c	a	b	a	b	c	a	b	a	b	c	d	e	a	b	a	b	c	a
Nematogenyidae	-	+	+	-	-	-	-	-	-	+	+	-	-	-	-	-	-	+	-	+	-	-	+	+	-	-	-	-	-	-	-	-	-	+	-	+	-	+	-	-	-	+	-	-	+	-	+	-	-	+
Trichomycteridae	+	-	-	-	+	-	+	-	+	+	-	+	+	+	-	-	+	-	+	+	+	+	+	-	+	+	+	+	-	+	+	-	+	-	-	+	-	+	-	+	+	-	-	-	+	-	+	-	-	+
Callichthyidae	+	-	-	+	+	-	-	+	-	+	+	-	-	+	-	-	+	-	+	+	-	-	+	-	+	+	-	+	-	+	+	+	+	-	-	+	-	+	-	-	+	-	-	-	+	+	+	+	-	+
Scoloplacidae	+	-	-	-	-	+	-	-	-	+	-	+	-	-	-	+	-	-	-	+	-	-	+	-	+	-	-	+	-	+	-	-	-	+	-	+	-	-	+	-	+	-	-	-	+	-	+	-	-	+
Loricariidae	+	-	-	-	+	+	-	-	-	+	+	+	-	-	-	-	+	-	+	+	-	+	+	+	+	+	-	+	-	+	+	-	+	-	-	+	-	+	-	+	+	-	-	-	+	-	+	-	+	+
Astroblepus cf. *mancoi*	+	-	-	-	-	+	-	-	+	-	-	+	-	-	-	+	-	-	-	+	-	-	+	-	+	-	-	+	-	+	-	-	-	+	-	+	-	-	+	-	+	-	-	-	+	-	+	-	-	+

Legends: *1- flagella number (a- one; b- two); 2- arrangement of centriolar complex (a- lateral and parallel; b- lateral and acute angle; c- lateral and obtuse angle; d- anterior and perpendicular; e- lateral and perpendicular; f- co-axial); 3- vesicles in the midpiece (a- absent; b- present); 4- flagellar membrane specializations (a- absent; b- two lateral fins; c- variable number lateral fins; d- membranous compartment); 5- shape of the nucleus (a- semi-ovoid; b- conic; c- ovoid; d-ovoid with its bigger axis in the horizontal direction; e- round); 6- cytoplasmic area around of the nucleus (a- narrow; b- large); 7- aspect of chromatin condensation (a- heterogeneous; b- homogeneous); 8- nuclear fossa (a-absent; b- medial; c- eccentric; d- lateral); 9- nuclear fossa shape (a- simple arc; b- double arc); 10- Position of centrioles in relation to the nuclear fossa (a- totally inserted in the nuclear fossa; b- only proximal centriole inserted in the nuclear fossa; c- totally outside of the nuclear fossa); 11- midpiece size (a- short; b- long); 12- cytoplasmic channels (a- absent; b- one; c- two); 13- cytoplasmic channel size (a- short; b- long); 14- mitochondria shape (a- rounded; b- elongated; c- elongated and ramificated; d- C-shape; e- irregular); 15- midpiece symmetry (a- symmetric; b- asymmetric); 16- Electron-dense circular structure in the midpiece (a- absent; b- not surrounded by plasma membrane; c- not surrounded by plasma membrane); 17- flagellum position in relation to the nucleus (a- medial; b- eccentric; c- lateral).

FIGURE CAPTION

Figure 1. Spermatozoa of *Astroblepus* cf. *mancoi*. a) Spermatozoon longitudinal section. b to d) Nucleus in cross sections. e to g) Midpiece in cross sections exhibits mitochondria and cytoplasmic channel. h) Spermatozoa in the lumen of germinative compartment not arrangement in spermatozeugmata. i) Nucleus in longitudinal section showing its extremity curved. j) Centrioles arrangement. k) Detail of midpiece and cytoplasmic channel. l and l-inset) Flagella in longitudinal and transverse sections. (a) 0.9 µm, (b, c, e, f, j) 0.2 µm, (d, g) 0.3 µm, (h) 1.7 µm, (i) 0.7 µm, (k) 0.5 µm, (l) 0.4 µm, (l-inset) 0.05 µm. B: basal body, C: centriolar complex, D: distal centriole, E: electron-lucent area, F: flagellum, N: nucleus, P: proximal centriole, Asterisk: mitochondria, Arrow: cytoplasmic channel, Arrowhead: lateral projections, Double Arrowhead: curved nucleus extremity, Double Arrow: cytoplasmic sheath.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4.8. CAPÍTULO 8

Spadella, M.A., Oliveira, C., Quagio-Grassiotto, I. Semicystic spermatogenesis and spermiogenesis ultrastructure in *Nematogenys inermis* (Ostariophysi: Siluriformes: Nematogenyidae). Manuscrito a ser submetido à revista Zoomorphology.

Semicystic spermatogenesis and spermiogenesis ultrastructure in *Nematogenys inermis* (Ostariophysi: Siluriformes: Nematogenyidae)

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Running headline: Semicystic spermatogenesis and Spermiogenesis in Nematogenyidae.

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ABSTRACT

In Nematogenyidae, the spermatids are found in the lumen of the germinative compartment, together with the spermatozoa, suggesting that spermatogenesis is semicystic. Spermiogenesis in nematogenyid is characterized by initial medial development of the flagella, absence of nuclear rotation, of nuclear fossa formation, and of migration of centrioles, and cytoplasmic channel formation. The nematogenyid spermatogenesis and spermiogenesis is more similar to those found in Cetopsidae, Aspredinidae, in Corydoradinae and in some Loricariidae than that found in Trichomycteridae suggesting that the phylogenetic position of this family should be revised.

KEY WORDS: catfish, male germinative cells, spermatids, morphology, evolution.

INTRODUCTION

Nematogenyidae, with a single genus and species, *Nematogenys inermis*, is endemic to central Chile (de Pinna, 2003). This family belongs to the superfamily Loricarioidea, the largest monophyletic group of catfishes in the neotropics (de Pinna, 1998; Britto, 2003). According to de Pinna (1998) and Britto (2003), this family is the sister group of the Trichomycteridae. In a recent molecular study conduced in the order Siluriformes the authors were not able to demonstrate the relationship among Nematogenyidae and Trichomycteridae (Sullivan et al., 2006).

The reproductive biology of nematogenyid is poorly known. Spadella et al. (2006) describe the spermatozoa ultrastructure of *N. inermis*, showing that these cells exhibit the head and the midpiece joined in a single structure, and two flagella medial to the nucleus. In the same paper, the occurrence of biflagellate spermatozoa was also described in Cetopsidae and Aspredinidae, other Neotropical siluriforms. The present study describes the occurrence of semicystic spermatogenesis and the ultrastructure of spermiogenesis in *N. inermis*.

MATERIAL AND METHODS

The study was conducted with adult males of *Nematogenys inermis* (Guichenot, 1848) collected from the Aguas de la Gloria river, VIII Region, Aguas de la Gloria, Chile (36°50.304' S 2°55.642' W) (LBP 3105). The fishes were identified and kept in the fish collection of Laboratório de Biologia e Genética de Peixes (LBP), Departamento de Morfologia, Instituto de Biociências, UNESP, Botucatu, São Paulo, Brazil.

The 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 for 2h in the dark in 1% osmium tetroxide in the same buffer, contrasted in block with aqueous solution of 5% uranyl acetate for 2h, dehydrated in acetone, embedded in araldite, and sectioned and stained with a saturated solution of uranyl acetate in 50% alcohol, and lead citrate. The

ultrastructural analysis was realized using a Phillips - CM 100 transmission electron microscope.

RESULTS

In *N. inermis*, spermatids are found in the lumen of the germinative compartment, isolated or in groups, together with the spermatozoa (Figs. 1a and 1b). At the beginning of the spermiogenesis, the early spermatids are connected to the Sertoli cells surface (Figs. 1a and 1d). During the differentiation, this connection is lost and the more differentiated spermatids move towards the lumen region of the central germinative compartment, mixing with the spermatozoa. In the early spermatids, the cytoplasm is symmetrically distributed around the nucleus, which contains diffuse chromatin and has a circular outline (Figs. 1b, 1c, and 1e). The centrioles, lateral and parallel to each other, lie medially to the nucleus and are anchored to the plasma membrane (Figs. 1c, 1d and 1d-inset). The flagella development occurs medially to the nucleus from both centrioles. The centrioles do not move towards to the nucleus, remaining associated with the plasma membrane (Figs. 1c, 1d, and 1d-inset). Although the migration of centrioles does not occur, the formation of one short cytoplasmic channel is observed (Fig. 1c-inset). The nuclear rotation does not occur and the nuclear fossa is not formed (Figs. 1a, 1c and 1e). With the absence of nuclear rotation, the flagella remain medial to the nucleus. In the nucleus, the chromatin condensation begins with areas of progressive and homogeneous condensed chromatin among areas of more diffuse chromatin. Small chromatin clusters are present in the periphery of the nucleus (Fig. 1e-inset). The cytoplasmic mass moving toward and around the initial segment of the tail, gives rise to the midpiece of the future spermatozoon (Figs. 1c and 1e). During the spermatid differentiation, a large number of long mitochondria move from the apical cytoplasmic region of the nucleus to the basal region. After this migration, they surround the basal region of the nucleus and fill the midpiece in formation. In the midpiece few vesicles randomly distributed are also observed (Figs. 1c, 1d, 1e, and 1f). The flagella have the classical (9+2) microtubular pattern, surrounded by the flagellar membrane. In some segments of the tail, the axonemes are individualized, while in others they are not

individualized, sharing the same flagellar membrane. No flagellar lateral projections are observed (Figs. 1g and 1h).

DISCUSSION

The presence of spermatids in the lumen of germinative compartment together with spermatozoa in *N. inermis*, suggesting that the spermatogenesis in this species is of the semicystic type. This same type of spermatogenesis was described in the subfamily Corydoradinae of the family Callichthyidae (Spadella et al., submitted), and in the siluriform families Cetopsidae and Aspredinidae (Spadella et al., 2006). Although uncommon, the semicystic spermatogenesis has been described in other Teleostei families as Opheliidae (Mattei et al., 1993), Scorpaenidae (Muñoz et al., 2002), and Bleniidae (Lahnsteiner and Patzer, 1990). In Trichomycteridae, the sister group of the family Nematogenyidae (de Pinna, 1998; Britto, 2003), spermatogenesis is of the cystic type (Spadella et al., submitted).

In the spermatozoa, the flagellum may develops perpendicular or parallel to the nucleus, depending on whether nuclear rotation during spermiogenesis occurs (type I spermiogenesis) or not (type II spermiogenesis) during spermiogenesis (Mattei, 1970). In Pimelodidae and Heptapteridae, the flagellum is medial, the nucleus does not rotate, and both the nuclear fossa and the cytoplasmic channel are absent, characterizing a third type of spermiogenesis (Quagio-Grassiotto et al., 2005; Quagio-Grassiotto and Oliveira, submitted). The spermiogenesis process observed in *N. inermis* is characterized by an initial medial development of the flagella, a cytoplasmic channel formation, absence of nuclear rotation, absence of nuclear fossa formation, and absence of centriolar migration. These characteristics are more similar to type III spermiogenesis previously described, which was also observed in *Loricariichthys platymetopon* of the family Loricariidae (Spadella et al., submitted), and, except by absence of nuclear fossa formation, this spermiogenesis type is also observed in Cetopsidae and Aspredinidae (Spadella et al., 2006), and *Hypostomus ancistroides* of the family Loricariidae (Spadella et al., submitted). The same also occur in Corydoradinae, except by the medial development of the flagellum and also by absence of

nuclear fossa formation (Spadella et al., submitted). In theses groups, it is possible that the cytoplasmic channel results of the accommodation and interconnection of the vesicles around the flagella, instead of the movement of the centrioles or centriolar complex toward the nucleus. In Trichomycteridae the spermiogenesis is more similar to type I (Spadella et al., submitted).

According to described above Nematogenyidae share more characteristics with Corydoradinae (Spadella et al., submitted), some loricariids (Spadella et al., submitted), Cetopsidae and Aspredinidae (Spadella et al., 2006) than with Trichomycteridae. These observations, based on comparative analysis of ultrastructure of spermatogenesis and spermiogenesis, permit to suggest that the relationship between Nematogenyidae and Trichomycteridae should be revised.

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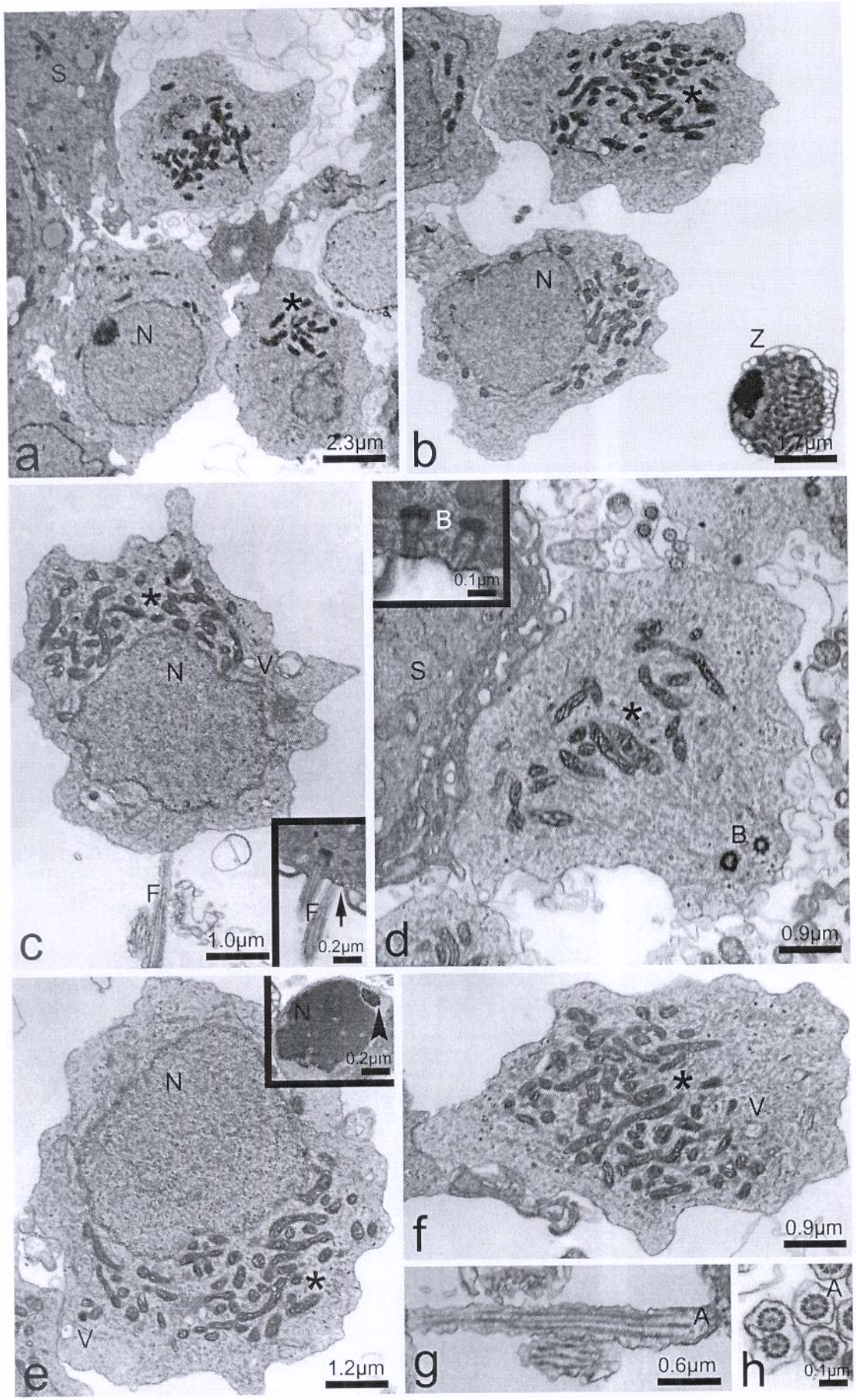
FIGURE CAPTION

Figure 1. Spermiogenesis of *Nematogenys inermis*. a and b Spermatids in the lumen of germinative compartment together with spermatozoa. c Early spermatid (longitudinal section). c-inset Detail of short cytoplasmic channel. d and f Midpiece showing mitochondria and vesicles. d-inset The arrangement of the centrioles. e Late spermatid. e-inset Nucleus showing the chromatin condensation process. g Flagella with axonemes individualized (longitudinal section). h Flagella with axonemes individualized and associated (cross sections). (a) 1.3 μm; (b) 1.0 μm; (c) 0.8 μm; (d-inset) 0.03 μm; (d, e, k and q) 0.7 μm; (f, g, l and r) 0.6 μm; (h, i, n and o) 0.3 μm; (j, m) 0.4 μm; (p) 0.1 μm; (s) 0.5 μm; (t) 0.2 μm. *A* axoneme, *B* basal body, *F* flagellum, *N* nucleus, *S* Sertoli cell, *V* vesicles, *Z* spermatozoon, *Asterisk* mitochondria, *Arrow* cytoplasmic channel, *Arrowhead* chromatin clusters.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4.9. CAPÍTULO 9

Spadella, M.A., Oliveira, C., Quagio-Grassiotto, I. The use of spermiogenesis and spermatozoa ultrastructural characters in the investigation of the relationships among families of Loricarioidea (Teleostei: Ostariophysi: Siluriformes).

The use of spermiogenesis and spermatozoa ultrastructural characters in the investigation of the relationships among families of Loricarioidea (Teleostei: Ostariophysi: Siluriformes)

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Running headline: Phylogeny of Loricarioidea

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ABSTRACT

In the current study, a phylogenetic analysis of the superfamily Loricarioidea, using the spermiogenesis and spermatozoa ultrastructural characteristics is conducted for the first time as a test to evaluate the ability of this data in resolving the phylogenetic inter and intra-relationships of familial level in the order Siluriformes. In general, the data obtained revealed that when these characters are employed in a more restrict group (as the superfamily Loricarioidea), they are really informative and can strongly support the monophyly of some groups. However, the phylogenetic analysis using only this type of reproductive ultrastructural characters is not informative at order level as the suggested groups are very incongruent with the relationship hypotheses available for Siluriformes. Thus, this type of reproductive ultrastructural character should be carefully employed in phylogenetic analysis to avoid misinterpretation.

KEY WORDS: phylogeny, fish, morphology, reproductive ultrastrutural characters.

INTRODUCTION

The order Siluriformes comprises the most diverse and widely distributed ostariophysan groups (Teugels, 1996; Ferraris, 1998; Nelson, 2006; Ferraris, 2007), presenting thirty-six families with approximately 480 genera and over 3.000 species (Ferraris, 2007). Among the Neotropical siluriform lineages probably monophyletic is the superfamily Loricarioidea (de Pinna, 1998; Britto, 2003). This superfamily was one of the oldest natural groups recognized in siluriform systematics (de Pinna, 1998). The relationships among catfish families suggest that Loricarioidea is sister group of African family Amphiliidae (Britto, 2003). The Loricarioidea is currently constituted by six families: Nematogenyidae, Trichomycteridae, Callichthyidae, Scoloplacidae, Astroblepidae, and Loricariidae (de Pinna, 1998; Britto, 2003). According to the phylogeny of the superfamily Loricarioidea proposed by Britto (2003), the family Nematogenyidae is sister group of the Trichomycteridae, and this clade the sister group of all other families of Loricarioidea.

In the present study, a phylogenetic analysis of the superfamily Loricarioidea, using the ultrastructural characteristics of both spermiogenesis and spermatozoa is conducted for the first time as a test to evaluate the ability of this data in resolving the phylogenetic inter and intra-relationships of familial level in the order Siluriformes.

MATERIAL AND METHODS

The material examined of Loricarioidea, and other catfish families is listed in Appendix 1. The analysis of the ultrastrutural characters of spermiogenesis and spermatozoa of the siluriform specimens available in the literature was realized based on observation of the electron-micrographies presented in the cited papers, and in the description of the authors. With these data, a list of ultrastrutuctural characters for the order Siluriformes was elaborated. For the quantitative characters were done mensures of length and width of the following spermatozoa strutuctures: nucleus, midpiece, and when present,

of the nuclear fossa, cytoplasmic channel, and lateral projections. The structures were measured in the bigger axis. The measures were obtained in centimeter and converted for micrometer. Thus, grafics of dispersion were constructed for establish the interval referent to the each quantitative character-state. All characters obtained were coded in a data matrix.

For the hypotheses of relationships was employed the cladistic methodology using the Phylogenetic Analysis Using Parsimony software (PAUP*, version 4.0b10), following the suggestions of Swofford et al. (1996), Swofford (2002), Nei and Kumar (2000), and Schneider (2003). The framework recent phylogenetic hypotheses for Loricarioidea proposed by Britto (2003) were considered. For outgroup comparison were employed a basal group in Siluformes (family Diplomystidae), or a basal group for Loricarioidea (family Aspredinidae). The characters were tested as "ordered" and "unordered" in different analysis. The critery employed in the analysis was the Maximum parsimony, and the parsimonious trees were obtained using heuristic seach performed 1000 random taxon addition replicates and TBR branch swapping. The characters were ACCTRAN optimized, where reversals are chosen over convergences. The support for the internal nodes of the trees obtained was estimated using *bootstrap* (Felsenstein, 1985). Bootstrap values were estimated from 1000 replicates.

RESULTS

Character description

The description of 32 ultrastrutural characters of the order Siluriformes with 49 taxa used in phylogenetic analysis are presented. For each character-state is provided a relation of terminal taxa with the respective condition. The character-state was coded as "0", "1", ..., "n". Character numbers and character-state codes are the same as presented in the data matrix (Table 1). For each character a summary of its characteristics is provided. The characters of 1 to 7 are the ultrastructural charcteres of spermatogenesis and spermiogenesis, while the characters 8 to 32 are spermatozoa characters.

1. Spermatogenesis type

In Teleostei, the spermatogenesis can be cystic or semicystic. In the cystic spermatogenesis (Fig. 1a), the development of the spermatozoa occurs totally within of spermatocysts presents in the germinative compartment. In the semicystic spermatogenesis (Fig. 1b), the spermatocysts open before the final development of the spermatozoa, which is completed in the lumen of the germinative compartment. The spermatogenesis can be: **(0) Cystic**. *Diplomystes mesembrinus*, *Copionodon orthiocarinatus*, *Ituglanis amazonicus*, *Trichomycterus* aff. *iheringi*, *T. areolatus*, *T.* sp. 1, *T.* sp. 2, *Hoplosternum littorale*, *Megalechis thoracata*, *Callichthys callichthys*, *Scoloplax distolothrix*, *Kronichthys heylandi*, *Neoplecostomus paranensis*, *Corumbataia cuestae*, *Hisonotus* sp., *Hypoptopoma guentheri*, *Loricariichthys platymetopon*, *Farlowella* sp., *Loricaria* sp., *Hypostomus ancistroides*, *Microglanis* aff. *parahybae*, *Rhamdia quelen*, *Pimelodella gracilis*, *Pimelodus maculatus*, *Pseudoplatystoma fasciatum*, *Sorubim lima*, *Iheringichthys labrosus*. **(1) Semicystic**. *Cetopsis coecutiens*, *Bunocephalus amazonicus*, *Nematogenys inermis*, *Corydoras flaveolus*, *Corydoras aeneus*, *Scleromystax lacerdai*, *Aspidoras poecilus*, *Malapterurus electricus*.

2. Flagellar initial development in relation to the nucleus

In the early spermatids, the distal centriole differentiates into the basal body and forms the flagellum. This initial development of the flagellum can be medial (Fig. 2a) or lateral to the nucleus (Fig. 2b). **(0) medial to the nucleus**. *Cetopsis coecutiens*, *Bunocephalus amazonicus*, *Nematogenys inermis*, *Loricariichthys platymetopon*, *Hypostomus ancistroides*, *Malapterurus electricus*, *Rhamdia quelen*, *Pimelodella gracilis*, *Pimelodus maculatus*, *Pseudoplatystoma fasciatum*, *Sorubim lima*. **(1) lateral to the nucleus**. *Diplomystes mesembrinus*, *Copionodon orthiocarinatus*, *Ituglanis amazonicus*, *Trichomycterus* aff. *iheringi*, *T. areolatus*, *T.* sp. 1, *T.* sp. 2, *Corydoras flaveolus*, *Corydoras aeneus*, *Scleromystax lacerdai*, *Aspidoras poecilus*, *Hoplosternum littorale*, *Megalechis thoracata*, *Callichthys callichthys*, *Scoloplax distolothrix*, *Kronichthys heylandi*, *Neoplecostomus paranensis*, *Corumbataia cuestae*, *Hisonotus* sp., *Hypoptopoma guentheri*, *Farlowella* sp., *Loricaria* sp., *Microglanis* aff. *parahybae*.

3. Centriolar complex movement

During the spermiogenesis process, the centriolar complex, formed by proximal and distal centrioles, move or not towards the nucleus. This movement can be: **(0) absent**. *Cetopsis coecutiens*, *Bunocephalus amazonicus*, *Nematogenys inermis*, *Corydoras flaveolus*, *Corydoras aeneus*, *Scleromystax lacerdai*, *Aspidoras poecilus*, *Loricariichthys platymetopon*, *Hypostomus ancistroides*, *Malapterurus electricus*, *Rhamdia quelen*, *Pimelodella gracilis*, *Pimelodus maculatus*, *Pseudoplatystoma fasciatum*, *Sorubim lima*. **(1) present**. *Diplomystes mesembrinus*, *Copionodon orthiocarinatus*, *Ituglanis amazonicus*, *Trichomycterus* aff. *iheringi*, *T. areolatus*, *T.* sp. 1, *T.* sp. 2, *Hoplosternum littorale*, *Megalechis thoracata*, *Callichthys callichthys*, *Scoloplax distolothrix*, *Kronichthys heylandi*, *Neoplecostomus paranensis*, *Corumbataia cuestae*, *Hisonotus* sp., *Hypoptopoma guentheri*, *Farlowella* sp., *Loricaria* sp., *Microglanis* aff. *parahybae*.

4. Cytoplasmic channel formation

When present, the cytoplasmic channel consists of a space between the plasma membrane of the basal region of the midpiece and the flagellum. The cytoplasmic channel formation can be absent (Fig. 3a), occurs by movement of centriolar complex towards the nucleus (Figs. 3b) or by projection of the midpiece towards to the initial segment of flagellum, associated to the interconnection of vesicles in this same region (Fig. 3c). Thus, can be: **(0) absent**. *Pimelodus maculatus*, *Pseudoplatystoma fasciatum*, *Sorubim lima*. **(1) originated by centriolar complex movement**. *Diplomystes mesembrinus*, *Copionodon orthiocarinatus*, *Ituglanis amazonicus*, *Trichomycterus* aff. *iheringi*, *T. areolatus*, *T.* sp. 1, *T.* sp. 2, *Hoplosternum littorale*, *Megalechis thoracata*, *Callichthys callichthys*, *Scoloplax distolothrix*, *Kronichthys heylandi*, *Neoplecostomus paranensis*, *Corumbataia cuestae*, *Hisonotus* sp., *Hypoptopoma guentheri*, *Farlowella* sp., *Loricaria* sp., *Microglanis* aff. *parahybae*. **(2) originated by projection of the midpiece towards the initial segment of the flagellum and by vesicullar interconnection**. *Cetopsis coecutiens*, *Bunocephalus amazonicus*, *Nematogenys inermis*, *Corydoras flaveolus*, *Corydoras aeneus*, *Scleromystax lacerdai*, *Aspidoras poecilus*, *Loricariichthys platymetopon*, *Hypostomus ancistroides*, *Malapterurus electricus*, *Rhamdia quelen*, *Pimelodella gracilis*.

5. Nuclear rotation

Along the spermiogenesis, the nucleus of the spermatids can rotate or not in relation to the flagellar axis, establishing the flagellar position in relation to the nucleus. This rotation can be: **(0) absent**. *Cetopsis coecutiens*, *Bunocephalus amazonicus*, *Nematogenys inermis*, *Corydoras flaveolus*, *Corydoras aeneus*, *Scleromystax lacerdai*, *Aspidoras poecilus*, *Loricariichthys platymetopon*, *Hypostomus ancistroides*, *Malapterurus electricus*, *Rhamdia quelen*, *Pimelodella gracilis*, *Pimelodus maculatus*, *Pseudoplatystoma fasciatum*, *Sorubim lima*. **(1) partial**. *Copionodon orthiocarinatus*, *Ituglanis amazonicus*, *Trichomycterus* aff. *iheringi*, *T. areolatus*, *T.* sp. (1), *T.* sp. 2, *Hoplosternum littorale*, *Megalechis thoracata*, *Callichthys callichthys*, *Farlowella* sp., *Loricaria* sp.. **(2) complete**. *Diplomystes mesembrinus*, *Scoloplax distolothrix*, *Kronichthys heylandi*, *Neoplecostomus paranensis*, *Corumbataia cuestae*, *Hisonotus* sp., *Hypoptopoma guentheri*, *Microglanis* aff. *parahybae*.

6. Chromatin condensation process

The chromatin condensation can be heterogeneous (Fig.4a), in the form the thin filaments juxtaposed of granular aspect or condensed clusters. The thin filaments chromatin can also highly condense, resulting in homogeneous apparence (Fig. 4b). Then, this process can be: **(0) heterogeneous**. *Diplomystes mesembrinus*, *Cetopsis coecutiens*, *Bunocephalus amazonicus*, *Scoloplax distolothrix*, *Malapterurus electricus*. **(1) homogeneous**. *Nematogenys inermis*, *Copionodon orthiocarinatus*, *Ituglanis amazonicus*, *Trichomycterus* aff. *iheringi*, *T. areolatus*, *T.* sp. 1, *T.* sp. 2, *Corydoras flaveolus*, *Corydoras aeneus*, *Scleromystax lacerdai*, *Aspidoras poecilus*, *Hoplosternum littorale*, *Megalechis thoracata*, *Callichthys callichthys*, *Kronichthys heylandi*, *Neoplecostomus paranensis*, *Corumbataia cuestae*, *Hisonotus* sp., *Hypoptopoma guentheri*, *Loricariichthys platymetopon*, *Farlowella* sp., *Loricaria* sp., *Hypostomus ancistroides*, *Microglanis* aff. *parahybae*, *Rhamdia quelen*, *Pimelodella gracilis*, *Pimelodus maculatus*, *Pseudoplatystoma fasciatum*, *Sorubim lima*.

7. Nuclear fossa formation

The nuclear fossa consists in a depression in the nuclear outline, which can be absent (Fig. 5a) or present (Fig. 5b). This depression can be: **(0) absent**. *Nematogenys inermis*, *Loricariichthys platymetopon*, *Rhamdia quelen*, *Pimelodella gracilis*, *Pimelodus maculatus*, *Pseudoplatystoma fasciatum*, *Sorubim lima*. **(1) present**. *Diplomystes mesembrinus*, *Cetopsis coecutiens*, *Bunocephalus amazonicus*, *Copionodon orthiocarinatus*, *Ituglanis amazonicus*, *Trichomycterus* aff. *iheringi*, *T. areolatus*, *T.* sp. 1, *T.* sp. 2, *Corydoras flaveolus*, *Corydoras aeneus*, *Scleromystax lacerdai*, *Aspidoras poecilus*, *Hoplosternum littorale*, *Megalechis thoracata*, *Callichthys callichthys*, *Scoloplax distolothrix*, *Kronichthys heylandi*, *Neoplecostomus paranensis*, *Corumbataia cuestae*, *Hisonotus* sp., *Hypoptopoma guentheri*, *Farlowella* sp., *Loricaria* sp., *Hypostomus ancistroides*, *Malapterurus electricus*, *Microglanis* aff. *Parahybae*.

8. Nucleus shape

The nucleus presents different shapes, as rounded (Fig. 6a), semi-rounded (Fig.3, *Liobagrus mediadiposalis*, in Lee and Kim, 1999), semi-ovoid (Fig. 6b), conic (Fig. 6c), ovoid with the large axis in the vertical position (Fig. 6d), or ovoid with the large axis in the horizontal position (Fig. 6e). **(0) rounded**. *Diplomystes mesembrinus*, *Copionodon orthiocarinatus*, *Trichomycterus areolatus*, *T. reinhardti*, *T.* sp. 2, *Corydoras flaveolus*, *Corydoras aeneus*, *Megalechis thoracata*, *Kronichthys heylandi*, *Neoplecostomus paranensis*, *Hisonotus* sp., *Schizolecis guntheri*, *Loricariichthys platymetopon*, *Loricaria* sp., *Hypostomus ancistroides*, *Ancistrus triradiatus*, *Clarias gariepinus*, *Silurus microdorsalis*, *Silurus asotus*, *Microglanis* aff. *parahybae*, *Pimelodella gracilis*, *Sorubim lima*, *Iheringichthys labrosus*. **(1) semi-rounded**. *Liobagrus mediadiposalis*, *Malapterurus electricus*. **(2) semi-ovoid**. *Cetopsis coecutiens*, *Bunocephalus coracoideus*, *Ictalurus punctatus*, *Mystus armatus*, *Pseudobagrus fulvidraco*, *Leiocassis ussuriensis*. **(3) conic**. *Bunocephalus amazonicus*, *Scoloplax distolothrix*, *Astroblepus* cf. *mancoi*, *Trachelyopterus lucenai*. **(4) ovoid with the large axis in the vertical position**. *Ituglanis amazonicus*, *Trichomycterus* aff. *iheringi*, *T.* sp. 1, *Scleromystax lacerdai*, *Aspidoras poecilus*, *Hoplosternum littorale*, *Callichthys callichthys*, *Corumbataia cuestae*, *Hypoptopoma*

guentheri, *Rhamdia quelen*, *Pimelodus maculatus*, *Pseudoplatystoma fasciatum*. **(5) ovoid with the large axis in the horizontal position**. *Nematogenys inermis*.

9. Nuclear length

The length was mensured in longitudinal sections of the nucleus presents in the photomicrografies, from base to its apical region. The Figure 25a shows the interval referent to each character-state of this character (vertical line). The nuclear lenght can be: **(0) nuclear lenght ≤ 3.0 µm: short**. *Diplomystes mesembrinus*, *Cetopsis coecutiens*, *Bunocephalus amazonicus*, *Bunocephalus coracoideus*, *Liobagrus mediadiposalis*, *Nematogenys inermis*, *Copionodon orthiocarinatus*, *Ituglanis amazonicus*, *Trichomycterus* aff. *iheringi*, *T. areolatus*, *T. reinhardti*, *T.* sp. 1, *T.* sp. 2, *Corydoras flaveolus*, *Corydoras aeneus*, *Scleromystax lacerdai*, *Aspidoras poecilus*, *Hoplosternum littorale*, *Megalechis thoracata*, *Callichthys callichthys*, *Kronichthys heylandi*, *Neoplecostomus paranensis*, *Corumbataia cuestae*, *Hisonotus* sp., *Hypoptopoma guentheri*, *Schizolecis guntheri*, *Loricariichthys platymetopon*, *Loricaria* sp., *Hypostomus ancistroides*, *Ancistrus triradiatus*, *Clarias gariepinus*, *Silurus microdorsalis*, *Silurus asotus*, *Ictalurus punctatus*, *Malapterurus electricus*, *Microglanis* aff. *parahybae*, *Rhamdia quelen*, *Pimelodella gracilis*, *Pimelodus maculatus*, *Pseudoplatystoma fasciatum*, *Sorubim lima*, *Iheringichthys labrosus*, *Mystus armatus*, *Pseudobagrus fulvidraco*, *Leiocassis ussuriensis*. **(1) nuclear lenght > 3.0 µm: long**. *Scoloplax distolothrix*, *Astroblepus* cf. *mancoi*, *Trachelyopterus lucenai*.

10. Nucleus width

The width was measured in longitudinal sections of the nucleus presents in the photomicrografies, in the opposite direction to the axis of the nuclear length. The Figure 25a shows the value interval referent to the each character-state of this character (horizontal lines). The width of the nucleus can be: **(0) nucleus width ≤ 1.0 µm: narrow**. *Scoloplax distolothrix*, *Astroblepus* cf. *mancoi*, *Trachelyopterus lucenai*. **(1) 1.0 µm < nucleus width ≤ 2.5 µm: medium**. *Diplomystes mesembrinus*, *Cetopsis coecutiens*, *Liobagrus mediadiposalis*, *Bunocephalus amazonicus*, *Bunocephalus coracoideus*, *Copionodon*

orthiocarinatus, *Ituglanis amazonicus*, *Trichomycterus* aff. *iheringi*, *T. areolatus*, *T. reinhardti*, *T.* sp. 1, *T.* sp. 2, *Corydoras flaveolus*, *Corydoras aeneus*, *Scleromystax lacerdai*, *Aspidoras poecilus*, *Hoplosternum littorale*, *Megalechis thoracata*, *Callichthys callichthys*, *Kronichthys heylandi*, *Neoplecostomus paranensis*, *Corumbataia cuestae*, *Hisonotus* sp., *Hypoptopoma guentheri*, *Schizolecis guntheri*, *Loricariichthys platymetopon*, *Loricaria* sp., *Hypostomus ancistroides*, *Ancistrus triradiatus*, *Clarias gariepinus*, *Silurus microdorsalis*, *Silurus asotus*, *Ictalurus punctatus*, *Malapterurus electricus*, *Microglanis* aff. *parahybae*, *Rhamdia quelen*, *Pimelodella gracilis*, *Pimelodus maculatus*, *Pseudoplatystoma fasciatum*, *Sorubim lima*, *Iheringichthys labrosus*, *Mystus armatus*, *Pseudobagrus fulvidraco*, *Leiocassis ussuriensis*. **(2) nucleus width > 2.5 µm: large**. *Nematogenys inermis*.

11. Final aspect of the condensed chromatin

After the condensation process, the chromatin can present different aspects within the nucleus as heterogeneous forming condensed clusters or in granular aspect (Figs. 7a and 7b); and homogeneous with or without electron-lucent areas (Fig. 7c, and Fig. 2, *Leiocassis ussuriensis*, in Kim and Lee, 2000). Thus, the final aspect of the chromatin can be: **(0) heterogeneous forming condensed clusters or in thin filaments juxtaposed with electron-lucent areas of granular aspect**. *Diplomystes mesembrinus*, *Cetopsis coecutiens*, *Bunocephalus amazonicus*, *Bunocephalus coracoideus*, *Clarias gariepinus*, *Malapterurus electricus*, *Mystus armatus*. **(1) homogeneous with or without electron-lucent areas**. *Liobagrus mediadiposalis*, *Nematogenys inermis*, *Copionodon orthiocarinatus*, *Ituglanis amazonicus*, *Trichomycterus* aff. *iheringi*, *T. areolatus*, *T. reinhardti*, *T.* sp. 1, *T.* sp. 2, *Corydoras flaveolus*, *Corydoras aeneus*, *Scleromystax lacerdai*, *Aspidoras poecilus*, *Hoplosternum littorale*, *Megalechis thoracata*, *Callichthys callichthys*, *Scoloplax distolothrix*, *Astroblepus* cf. *mancoi*, *Kronichthys heylandi*, *Neoplecostomus paranensis*, *Corumbataia cuestae*, *Hisonotus* sp., *Hypoptopoma guentheri*, *Schizolecis guntheri*, *Loricariichthys platymetopon*, *Loricaria* sp., *Hypostomus ancistroides*, *Silurus microdorsalis*, *Silurus asotus*, *Ictalurus punctatus*, *Microglanis* aff. *parahybae*, *Rhamdia quelen*, *Pimelodella gracilis*, *Trachelyopterus lucenai*, *Pimelodus maculatus*,

Pseudoplatystoma fasciatum, *Sorubim lima*, *Iheringichthys labrosus*, *Pseudobagrus fulvidraco*, *Leiocassis ussuriensis*.

12. Cytoplasmic area around the nucleus

The cytoplasmic area that encircles the nucleus can be of two types, narrow (Fig. 8a) or large (Fig. 8b). **(0) narrow**. *Diplomystes mesembrinus*, *Cetopsis coecutiens*, *Liobagrus mediadiposalis*, *Bunocephalus amazonicus*, *Bunocephalus coracoideus*, *Nematogenys inermis*, *Trichomycterus areolatus*, *T. reinhardti*, *Corydoras flaveolus*, *Corydoras aeneus*, *Scleromystax lacerdai*, *Aspidoras poecilus*, *Hoplosternum littorale*, *Megalechis thoracata*, *Callichthys callichthys*, *Scoloplax distolothrix*, *Astroblepus* cf. *mancoi*, *Kronichthys heylandi*, *Neoplecostomus paranensis*, *Corumbataia cuestae*, *Hisonotus* sp., *Hypoptopoma guentheri*, *Schizolecis guntheri*, *Loricariichthys platymetopon*, *Loricaria* sp., *Hypostomus ancistroides*, *Ancistrus triradiatus*, *Clarias gariepinus*, *Silurus microdorsalis*, *Silurus asotus*, *Malapterurus electricus*, *Microglanis* aff. *parahybae*, *Rhamdia quelen*, *Pimelodella gracilis*, *Trachelyopterus lucenai*, *Pimelodus maculatus*, *Pseudoplatystoma fasciatum*, *Sorubim lima*, *Iheringichthys labrosus*, *Mystus armatus*, *Pseudobagrus fulvidraco*, *Leiocassis ussuriensis*. **(1) large**. *Copionodon orthiocarinatus*, *Ituglanis amazonicus*, *Trichomycterus* aff. *iheringi*, *T.* sp. 1, *T.* sp. 2, *Ictalurus punctatus*.

13. Flagellar position in relation to the nucleus

The flagellar axis can be positioned medially (Fig. 9a) or eccentric (Fig. 9b) in relation to the nucleus. **(0) medial**. *Diplomystes mesembrinus*, *Cetopsis coecutiens*, *Liobagrus mediadiposalis*, *Bunocephalus amazonicus*, *Bunocephalus coracoideus*, *Nematogenys inermis*, *Copionodon orthiocarinatus*, *T. areolatus*, *T.* sp. 2, *Megalechis thoracata*, *Scoloplax distolothrix*, *Astroblepus* cf. *mancoi*, *Kronichthys heylandi*, *Neoplecostomus paranensis*, *Corumbataia cuestae*, *Hisonotus* sp., *Hypoptopoma guentheri*, *Schizolecis guntheri*, *Loricariichthys platymetopon*, *Hypostomus ancistroides*, *Ancistrus triradiatus*, *Clarias gariepinus*, *Silurus microdorsalis*, *Silurus asotus*, *Ictalurus punctatus*, *Malapterurus electricus*, *Microglanis* aff. *parahybae*, *Rhamdia quelen*, *Pimelodella*

gracilis, *Trachelyopterus lucenai*, *Pimelodus maculatus*, *Pseudoplatystoma fasciatum*, *Sorubim lima*, *Iheringichthys labrosus*, *Mystus armatus*, *Pseudobagrus fulvidraco*, *Leiocassis ussuriensis*. **(1) eccentric**. *Ituglanis amazonicus*, *Trichomycterus* aff. *iheringi*, *T. reinhardti*, *T.* sp. 1, *Corydoras flaveolus*, *Corydoras aeneus*, *Scleromystax lacerdai*, *Aspidoras poecilus*, *Hoplosternum littorale*, *Callichthys callichthys*, *Loricaria* sp..

14. Nuclear fossa

In the nuclear outline, the nuclear fossa can be absent (Fig. 10a) or present (Figs. 10b and 10c). **(0) absent**. *Nematogenys inermis*, *Loricariichthys platymetopon*, *Rhamdia quelen*, *Pimelodella gracilis*, *Pimelodus maculatus*, *Pseudoplatystoma fasciatum*, *Sorubim lima*. **(1) present**. *Diplomystes mesembrinus*, *Cetopsis coecutiens*, *Liobagrus mediadiposalis*, *Bunocephalus amazonicus*, *Bunocephalus coracoideus*, *Copionodon orthiocarinatus*, *Ituglanis amazonicus*, *Trichomycterus* aff. *iheringi*, *T. areolatus*, *T. reinhardti*, *T.* sp. 1, *T.* sp. 2, *Corydoras flaveolus*, *Corydoras aeneus*, *Scleromystax lacerdai*, *Aspidoras poecilus*, *Megalechis thoracata*, *Hoplostermum littorale*, *Callichthys callichthys*, *Scoloplax distolothrix*, *Astroblepus* cf. *mancoi*, *Kronichthys heylandi*, *Neoplecostomus paranensis*, *Corumbataia cuestae*, *Hisonotus* sp., *Hypoptopoma guentheri*, *Schizolecis guntheri*, *Loricaria* sp., *Hypostomus ancistroides*, *Ancistrus triradiatus*, *Clarias gariepinus*, *Silurus microdorsalis*, *Silurus asotus*, *Ictalurus punctatus*, *Malapterurus electricus*, *Microglanis* aff. *parahybae*, *Trachelyopterus lucenai*, *Iheringichthys labrosus*, *Mystus armatus*, *Pseudobagrus fulvidraco*, *Leiocassis ussuriensis*.

15. Nuclear fossa shape

The nuclear fossa presents different shapes as in simple arc (Figs. 11a and 11b) or in double arc (Fig. 6, *Liobagrus mediadiposalis*, in Lee and Kim, 1999). **(0) simple arc**. *Diplomystes mesembrinus*, *Cetopsis coecutiens*, *Bunocephalus amazonicus*, *Bunocephalus coracoideus*, *Copionodon orthiocarinatus*, *Ituglanis amazonicus*, *Trichomycterus* aff. *iheringi*, *T. areolatus*, *T. reinhardti*, *T.* sp. 1, *T.* sp. 2, *Corydoras flaveolus*, *Corydoras aeneus*, *Scleromystax lacerdai*, *Aspidoras poecilus*, *Hoplosternum littorale*, *Megalechis thoracata*, *Callichthys callichthys*, *Scoloplax distolothrix*, *Astroblepus* cf. *mancoi*,

Kronichthys heylandi, *Neoplecostomus paranensis*, *Corumbataia cuestae*, *Hisonotus* sp., *Hypoptopoma guentheri*, *Schizolecis guntheri*, *Loricaria* sp., *Hypostomus ancistroides*, *Ancistrus triradiatus*, *Clarias gariepinus*, *Silurus microdorsalis*, *Silurus asotus*, *Microglanis* aff. *parahybae*, *Trachelyopterus lucenai*, *Iheringichthys labrosus*, *Pseudobagrus fulvidraco*, *Leiocassis ussuriensis*. **(1) double arc**. *Liobagrus mediadiposalis*, *Ictalurus punctatus*, *Malapterurus electricus*, *Mystus armatus*.

16. Nuclear fossa depth

The depth was mensured in longitudinal sections of the nuclear fossa in the direction of the nucleus length. The Figure 25b shows the interval referent to each character-state of this character (vertical lines). The depth of the nuclear fossa can be: **(0) nuclear fossa depth ≤ 0.25 µm: shallow**. *Corydoras flaveolus*, *Corydoras aeneus*, *Scleromystax lacerdai*, *Aspidoras poecilus*, *Hoplosternum littorale*, *Megalechis thoracata*, *Callichthys callichthys*, *Hypostomus ancistroides*, *Malapterurus electricus*, *Iheringichthys labrosus*. **(1) 0.25 µm < nuclear fossa depth ≤ 0.75 µm: moderate**. *Cetopsis coecutiens*, *Liobagrus mediadiposalis*, *Copionodon orthiocarinatus*, *Ituglanis amazonicus*, *Trichomycterus* aff. *iheringi*, *T. areolatus*, *T. reinhardti*, *T.* sp. 1, *T.* sp. 2, *Kronichthys heylandi*, *Neoplecostomus paranensis*, *Corumbataia cuestae*, *Hisonotus* sp., *Hypoptopoma guentheri*, *Schizolecis guntheri*, *Loricaria* sp., *Ancistrus triradiatus*, *Clarias gariepinus*, *Silurus asotus*, *Ictalurus punctatus*, *Microglanis* aff. *parahybae*, *Trachelyopterus lucenai*, *Mystus armatus*. **(2) nuclear fossa depth > 0.75 µm: deep**. *Diplomystes mesembrinus*, *Bunocephalus amazonicus*, *Bunocephalus coracoideus*, *Scoloplax distolothrix*, *Astroblepus* cf. *mancoi*, *Silurus microdorsalis*, *Pseudobagrus fulvidraco*, *Leiocassis ussuriensis*.

17. Centriolar complex (cc) position in relation to the nuclear fossa

The centriolar complex, formed by proximal and distal centrioles, can be in variable positions in relation to the nuclear fossa. Then, these positions can be: **(0) CC or basal bodies totally inserted in the nuclear fossa** (Fig. 12a). *Diplomystes mesembrinus*, *Cetopsis coecutiens*, *Liobagrus mediadiposalis*, *Bunocephalus amazonicus*, *Bunocephalus coracoideus*, *Ituglanis amazonicus*, *Trichomycterus areolatus*, *Hoplosternum littorale*,

Megalechis thoracata, *Scoloplax distolothrix*, *Astroblepus* cf. *mancoi*, *Kronichthys heylandi*, *Neoplecostomus paranensis*, *Corumbataia cuestae*, *Hisonotus* sp., *Hypoptopoma guentheri*, *Schizolecis guntheri*, *Ancistrus triradiatus*, *Clarias gariepinus*, *Mystus armatus*, *Pseudobagrus fulvidraco*, *Leiocassis ussuriensis*. (1) proximal centriole inserted and basal body partially inserted in the nuclear fossa (Fig. 12b). *Silurus microdorsalis*, *Silurus asotus*, *Microglanis* aff. *parahybae*, *Trachelyopterus lucenai*. **(2) only proximal centriole inserted in the nuclear fossa** (Fig. 12c). *Copionodon orthiocarinatus*, *Trichomycterus* aff. *iheringi*, *T. reinhardti*, *T.* sp. 1, *T.* sp. 2, *Callichthys callichthys*, *Loricaria* sp., *Hypostomus ancistroides*. **(3) basal bodies partially inserted in the nuclear fossa** (Fig. 2, *Ictalurus punctatus*, in Poirier and Nicholson, 1982). *Ictalurus punctatus*, *Malapterurus electricus*. **(4) proximal centriole and basal body totally outside of the nuclear fossa** (Fig. 12d). *Corydoras flaveolus*, *Corydoras aeneus*, *Scleromystax lacerdai*, *Aspidoras poecilus*, *Iheringichthys labrosus*.

18. Arrangement of the proximal and distal centrioles

The proximal and distal centrioles can be arranged in differents positions to each other as: **(0) anterior and perpendicular** (Fig. 13a). *Diplomystes mesembrinus*, *Scoloplax distolothrix*, *Astroblepus* cf. *mancoi*, *Corumbataia cuestae*, *Hisonotus* sp., *Hypoptopoma guentheri*, *Schizolecis guntheri*, *Loricariichthys platymetopon*, *Loricaria* sp., *Hypostomus ancistroides*, *Ancistrus triradiatus*, *Silurus microdorsalis*, *Trachelyopterus lucenai*, *Pimelodus maculatus*, *Pseudoplatystoma fasciatum*, *Sorubim lima*, *Iheringichthys labrosus*. **(1) co-axial** (Fig. 13b). *Corydoras flaveolus*, *Corydoras aeneus*, *Scleromystax lacerdai*, *Aspidoras poecilus*, *Pseudobagrus fulvidraco*, *Leiocassis ussuriensis*. **(2) in obtuse angle** (Fig. 13c). *Copionodon orthiocarinatus*, *Trichomycterus* aff. *iheringi*, *T. areolatus*, *T.* sp. 1, *T.* sp. 2, *Megalechis thoracata*, *Callichthys callichthys*, *Kronichthys heylandi*, *Neoplecostomus paranensis*, *Silurus asotus*, *Microglanis* aff. *parahybae*. **(3) lateral and perpendicular** (Fig. 13d). *Ituglanis amazonicus*, *Trichomycterus reinhardti*, *Rhamdia quelen*, *Pimelodella gracilis*. **(4) in acute angle** (Fig. 13e). *Hoplosternum littorale*, *Clarias gariepinus*. **(5) parallel** (Fig. 13f). *Cetopsis coecutiens*, *Liobagrus mediadiposalis*,

Bunocephalus amazonicus, *Bunocephalus coracoideus*, *Nematogenys inermis*, *Ictalurus punctatus*, *Malapterurus electricus*, *Mystus armatus*.

19. Midpiece length

The length was measured in longitudinal sections of the midpiece, from basis of the nucleus to the terminal end of the midpiece. The Figure 25c shows the interval referent to each character-state of this character (vertical line). The length of the midpiece can be: **(0) midpiece length ≤ 2.0 µm: short**. *Diplomystes mesembrinus*, *Cetopsis coecutiens*, *Liobagrus mediadiposalis*, *Bunocephalus coracoideus*, *Copionodon orthiocarinatus*, *Ituglanis amazonicus*, *Trichomycterus* aff. *iheringi*, *T. areolatus*, *T. reinhardti*, *T.* sp. 1, *T.* sp. 2, *Corydoras flaveolus*, *Corydoras aeneus*, *Scleromystax lacerdai*, *Aspidoras poecilus*, *Hoplosternum littorale*, *Megalechis thoracata*, *Callichthys callichthys*, *Kronichthys heylandi*, *Neoplecostomus paranensis*, *Corumbataia cuestae*, *Hisonotus* sp., *Hypoptopoma guentheri*, *Schizolecis guntheri*, *Loricariichthys platymetopon*, *Loricaria* sp., *Hypostomus ancistroides*, *Ancistrus triradiatus*, *Clarias gariepinus*, *Silurus microdorsalis*, *Silurus asotus*, *Ictalurus punctatus*, *Malapterurus electricus*, *Microglanis* aff. *parahybae*, *Rhamdia quelen*, *Pimelodella gracilis*, *Pimelodus maculatus*,*Pseudoplatystoma fasciatum*, *Sorubim lima*, *Iheringichthys labrosus*, *Mystus armatus*, *Pseudobagrus fulvidraco Leiocassis ussuriensis*. **(1) midpiece length > 2.0 µm: long**. *Bunocephalus amazonicus*, *Nematogenys inermis*, *Scoloplax distolothrix*, *Astroblepus* cf. *mancoi*, *Trachelyopterus lucenai*.

20. Midpiece symmetry in relation to the flagellum

The midpiece can be symmetric (Fig. 14a) or asymmetric (Fig. 14b) in relation to the flagellum. **(0) symmetric**. *Diplomystes mesembrinus*, *Cetopsis coecutiens*, *Liobagrus mediadiposalis*, *Bunocephalus amazonicus*, *Bunocephalus coracoideus*, *Nematogenys inermis*, *Scoloplax distolothrix*, *Astroblepus* cf. *mancoi*, *Kronichthys heylandi*, *Neoplecostomus paranensis*, *Corumbataia cuestae*, *Hisonotus* sp., *Hypoptopoma guentheri*, *Schizolecis guntheri*, *Loricariichthys platymetopon*, *Loricaria* sp., *Hypostomus ancistroides*, *Ancistrus triradiatus*, *Clarias gariepinus*, *Silurus microdorsalis*, *Silurus asotus*, *Ictalurus punctatus*, *Malapterurus electricus*, *Microglanis* aff. *parahybae*, *Rhamdia*

quelen, *Pimelodella gracilis*, *Pimelodus maculatus*, *Pseudoplatystoma fasciatum*, *Sorubim lima*, *Iheringichthys labrosus*, *Mystus armatus*, *Pseudobagrus fulvidraco*, *Leiocassis ussuriensis*. **(1) asymmetric**. *Copionodon orthiocarinatus*, *Ituglanis amazonicus*, *Trichomycterus* aff. *iheringi*, *T. areolatus*, *T. reinhardti*, *T.* sp. 1, *T.* sp. 2, *Corydoras flaveolus*, *Corydoras aeneus*, *Scleromystax lacerdai*, *Aspidoras poecilus*, *Hoplosternum littorale*, *Megalechis thoracata*, *Callichthys callichthys*, *Microglanis* aff. *parahybae*, *Trachelyopterus lucenai*.

21. Cytoplasmic channel

The cytoplasmic channel in the spermatozoon can be absent (Fig. 15a), single (Fig. 15b) or double (Fig. 15c). **(0) absent**. *Diplomystes mesembrinus*, *Clarias gariepinus*, *Pimelodella gracilis*, *Pimelodus maculatus*, *Pseudoplatystoma fasciatum*, *Sorubim lima*, *Iheringichthys labrosus*. **(1) single**. *Cetopsis coecutiens*, *Nematogenys inermis*, *Copionodon orthiocarinatus*, *Ituglanis amazonicus*, *Trichomycterus* aff. *iheringi*, *T. areolatus*, *T. reinhardti*, *T.* sp. 1, *T.* sp. 2, *Corydoras flaveolus*, *Corydoras aeneus*, *Scleromystax lacerdai*, *Aspidoras poecilus*, *Hoplosternum littorale*, *Megalechis thoracata*, *Callichthys callichthys*, *Scoloplax distolothrix*, *Astroblepus* cf. *mancoi*, *Kronichthys heylandi*, *Neoplecostomus paranensis*, *Corumbataia cuestae*, *Hisonotus* sp., *Hypoptopoma guentheri*, *Schizolecis guntheri*, *Loricariichthys platymetopon*, *Loricaria* sp., *Hypostomus ancistroides*, *Ancistrus triradiatus*, *Silurus microdorsalis*, *Silurus asotus*, *Microglanis* aff. *parahybae*, *Rhamdia quelen*, *Trachelyopterus lucenai*, *Mystus armatus*, *Pseudobagrus fulvidraco*, *Leiocassis ussuriensis*. **(2) double**. *Liobagrus mediadiposalis*, *Bunocephalus amazonicus*, *Bunocephalus coracoideus*, *Ictalurus punctatus*, *Malapterurus electricus*.

22. Cytoplasmic channel length

The length was measured in longitudinal sections of the cytoplasmic channel, from of the initial of the flagellum to the terminal end of the midpiece. The Figure 25d shows the interval referent to each character-state of this character (vertical line). The length of the cytoplasmic channel can be: **(0) cytoplasmic channel length ≤ 1.5 µm: short**. *Cetopsis coecutiens*, *Liobagrus mediadiposalis*, *Nematogenys inermis*, *Copionodon orthiocarinatus*,

Ituglanis amazonicus, *Trichomycterus* aff. *iheringi*, *T. areolatus*, *T. reinhardti*, *T.* sp. 1, *T.* sp. 2, *Corydoras flaveolus*, *Corydoras aeneus*, *Scleromystax lacerdai*, *Aspidoras poecilus*, *Hoplosternum littorale*, *Megalechis thoracata*, *Callichthys callichthys*, *Kronichthys heylandi*, *Neoplecostomus paranensis*, *Corumbataia cuestae*, *Hisonotus* sp., *Hypoptopoma guentheri*, *Schizolecis guntheri*, *Loricariichthys platymetopon*, *Loricaria* sp., *Hypostomus ancistroides*, *Ancistrus triradiatus*, *Silurus microdorsalis*, *Silurus asotus*, *Ictalurus punctatus*, *Malapterurus electricus*, *Microglanis* aff. *parahybae*, *Rhamdia quelen*, *Mystus armatus*, *Pseudobagrus fulvidraco*, *Leiocassis ussuriensis*. **(1) cytoplasmic channel length > 1.5 µm: long**. *Bunocephalus amazonicus*, *Bunocephalus coracoideus*, *Scoloplax distolothrix*, *Astroblepus* cf. *mancoi*, *Trachelyopterus lucenai*.

23. Mitochondrial localization in the spermatozoon

The mitochondria can be found in the spermatozoa: **(0) in different regions of the midpiece** (Figs. 16a-c). *Diplomystes mesembrinus*, *Cetopsis coecutiens*, *Liobagrus mediadiposalis*, *Bunocephalus amazonicus*, *Bunocephalus coracoideus*, *Nematogenys inermis*, *Trichomycterus areolatus*, *T. reinhardti*, *Corydoras flaveolus*, *Corydoras aeneus*, *Scleromystax lacerdai*, *Aspidoras poecilus*, *Hoplosternum littorale*, *Megalechis thoracata*, *Callichthys callichthys*, *Scoloplax distolothrix*, *Astroblepus* cf. *mancoi*, *Kronichthys heylandi*, *Neoplecostomus paranensis*, *Corumbataia cuestae*, *Hisonotus* sp., *Loricariichthys platymetopon*, *Loricaria* sp., *Hypostomus ancistroides*, *Ancistrus triradiatus*, *Clarias gariepinus*, *Silurus microdorsalis*, *Silurus asotus*, *Ictalurus punctatus*, *Malapterurus electricus*, *Microglanis* aff. *parahybae*, *Rhamdia quelen*, *Pimelodella gracilis*, *Trachelyopterus lucenai*, *Pimelodus maculatus*, *Pseudoplatystoma fasciatum*, *Sorubim lima*, *Iheringichthys labrosus*, *Mystus armatus*, *Pseudobagrus fulvidraco*, *Leiocassis ussuriensis*. **(1) in all midpiece and around the nucleus** (Fig. 16d). *Copionodon orthiocarinatus*, *Ituglanis amazonicus*, *Trichomycterus* aff. *iheringi*, *T.* sp. 1, *T.* sp. 2, *Hypoptopoma guentheri*, *Schizolecis guntheri*.

24. Mitochondrial amount in the midpiece

The amount of mitochondria in the midpiece can be: **(0) single** (Fig. 17a). *Diplomystes mesembrinus*. **(1) few (contable, usually less than 20)** (Fig. 17b). *Cetopsis coecutiens*, *Liobagrus mediadiposalis*, *Bunocephalus amazonicus*, *Bunocephalus coracoideus*, *Copionodon orthiocarinatus*, *Ituglanis amazonicus*, *Trichomycterus* aff. *iheringi*, *T. areolatus*, *T. reinhardti*, *T.* sp. 1, *T.* sp. 2, *Hoplosternum littorale*, *Megalechis thoracata*, *Callichthys callichthys*, *Kronichthys heylandi*, *Neoplecostomus paranensis*, *Corumbataia cuestae*, *Hisonotus* sp., *Hypoptopoma guentheri*, *Schizolecis guntheri*, *Loricaria* sp., *Hypostomus ancistroides*, *Ancistrus triradiatus*, *Clarias gariepinus*, *Silurus microdorsalis*, *Silurus asotus*, *Ictalurus punctatus*, *Malapterurus electricus*, *Microglanis* aff. *parahybae*, *Rhamdia quelen*, *Pimelodella gracilis*, *Trachelyopterus lucenai*, *Pimelodus maculatus*, *Pseudoplatystoma fasciatum*, *Sorubim lima*, *Iheringichthys labrosus*, *Mystus armatus*, *Pseudobagrus fulvidraco*, *Leiocassis ussuriensis*. **(2) many (uncontable)** (Fig. 17c). *Nematogenys inermis*, *Corydoras flaveolus*, *Corydoras aeneus*, *Scleromystax lacerdai*, *Aspidoras poecilus*, *Scoloplax distolothrix*, *Astroblepus* cf. *mancoi*, *Loricariichthys platymetopon*.

25. Mitochondrial shape

The shape of the mitochondria can be: **(0) rounded** (Fig. 18a). *Cetopsis coecutiens*, *Liobagrus mediadiposalis*, *Silurus microdorsalis*, *Malapterurus electricus*, *Pseudobagrus fulvidraco*. **(1) elongated** (Fig. 18b). *Bunocephalus amazonicus*, *Bunocephalus coracoideus*, *Copionodon orthiocarinatus*, *Ituglanis amazonicus*, *Trichomycterus* aff. *iheringi*, *T. areolatus*, *T. reinhardti*, *T.* sp. 1, *T.* sp. 2, *Corydoras flaveolus*, *Corydoras aeneus*, *Scleromystax lacerdai*, *Aspidoras poecilus*, *Hoplosternum littorale*, *Megalechis thoracata*, *Callichthys callichthys*, *Scoloplax distolothrix*, *Astroblepus* cf. *mancoi*, *Kronichthys heylandi*, *Neoplecostomus paranensis*, *Corumbataia cuestae*, *Hisonotus* sp., *Hypoptopoma guentheri*, *Schizolecis guntheri*, *Loricariichthys platymetopon*, *Loricaria* sp., *Hypostomus ancistroides*, *Ancistrus triradiatus*, *Clarias gariepinus*, *Silurus asotus*, *Ictalurus punctatus*, *Microglanis* aff. *parahybae*, *Rhamdia quelen*, *Pimelodella gracilis*, *Trachelyopterus lucenai*, *Pimelodus maculatus*, *Pseudoplatystoma fasciatum*, *Sorubim*

lima, *Iheringichthys labrosus*, *Mystus armatus*, *Leiocassis ussuriensis*. **(2) C-shape** (Fig. 16, *Diplomystes mesembrinus*, in Quagio-Grassiotto et al., 2001). *Diplomystes mesembrinus*. **(3) elongated and ramified** (Fig. 18c). *Nematogenys inermis*.

26. Electron-dense spherical struture in the midpiece

The electron-dense spherical structure can be: **(0) absent** (Fig. 19a). *Diplomystes mesembrinus*, *Cetopsis coecutiens*, *Liobagrus mediadiposalis*, *Bunocephalus amazonicus*, *Bunocephalus coracoideus*, *Nematogenys inermis*, *Copionodon orthiocarinatus*, *Ituglanis amazonicus*, *Trichomycterus* aff. *iheringi*, *T. areolatus*, *T. reinhardti*, *T.* sp. 1, *T.* sp. 2, *Hoplosternum littorale*, *Megalechis thoracata*, *Callichthys callichthys*, *Scoloplax distolothrix*, *Astroblepus* cf. *mancoi*, *Loricariichthys platymetopon*, *Loricaria* sp., *Hypostomus ancistroides*, *Ancistrus triradiatus*, *Clarias gariepinus*, *Silurus microdorsalis*, *Silurus asotus*, *Ictalurus punctatus*, *Malapterurus electricus*, *Microglanis* aff. *parahybae*, *Rhamdia quelen*, *Pimelodella gracilis*, *Trachelyopterus lucenai*, *Pimelodus maculatus*, *Pseudoplatystoma fasciatum*, *Sorubim lima*, *Iheringichthys labrosus*, *Mystus armatus*, *Pseudobagrus fulvidraco*, *Leiocassis ussuriensis*. **(1) present** (Figs. 19b and 19c). *Corydoras flaveolus*, *Corydoras aeneus*, *Scleromystax lacerdai*, *Aspidoras poecilus*, *Kronichthys heylandi*, *Neoplecostomus paranensis*, *Corumbataia cuestae*, *Hisonotus* sp., *Hypoptopoma guentheri*, *Schizolecis guntheri*.

27. Vesicles in the midpiece

The vesicles in the midpiece of the spermatozoon can be: **(0) absent** (Fig. 20a). *Diplomystes mesembrinus*, *Liobagrus mediadiposalis*, *Trichomycterus reinhardti*, *Astroblepus* cf. *mancoi*, *Ancistrus triradiatus*, *Trachelyopterus lucenai*, *Mystus armatus*, *Pseudobagrus fulvidraco*, *Leiocassis ussuriensis*. **(1) only in the basal region of the midpiece** (Fig. 20b). *Nematogenys inermis*, *Trichomycterus* aff. *iheringi*, *T. areolatus*, *Corydoras flaveolus*, *Corydoras aeneus*, *Scleromystax lacerdai*, *Aspidoras poecilus*, *Hoplosternum littorale*, *Megalechis thoracata*, *Callichthys callichthys*, *Corumbataia cuestae*, *Hisonotus* sp., *Hypoptopoma guentheri*, *Schizolecis guntheri*, *Hypostomus ancistroides*. **(2) in the medial and basal regions of the midpiece** (Fig. 20c).

Loricariichthys platymetopon, *Silurus asotus*, *Rhamdia quelen*, *Pimelodella gracilis*, *Pseudoplatystoma fasciatum*. **(3) in all midpiece** (Fig. 20d). *Cetopsis coecutiens*, *Bunocephalus amazonicus*, *Bunocephalus coracoideus*, *Copionodon orthiocarinatus*, *Ituglanis amazonicus*, *Trichomycterus* sp. 1, *T.* sp. 2, *Scoloplax distolothrix*, *Kronichthys heylandi*, *Neoplecostomus paranensis*, *Loricaria* sp., *Clarias gariepinus*, *Silurus microdorsalis*, *Ictalurus punctatus*, *Malapterurus electricus*, *Microglanis* aff. *parahybae*, *Pimelodus maculatus*, *Sorubim lima*, *Iheringichthys labrosus*.

28. Vesicles amount in the midpiece

The vesicles amount in the midpiece can be: **(0) few (contable, usually less than 20)** (Fig. 21a). *Bunocephalus coracoideus*, *Trichomycterus* aff. *iheringi*, *T. areolatus*, *Corydoras flaveolus*, *Corydoras aeneus*, *Scleromystax lacerdai*, *Aspidoras poecilus*, *Callichthys callichthys*, *Kronichthys heylandi*, *Neoplecostomus paranensis*, *Corumbataia cuestae*, *Hisonotus* sp., *Hypoptopoma guentheri*, *Schizolecis guntheri*, *Loricariichthys platymetopon*, *Loricaria* sp., *Clarias gariepinus*, *Silurus microdorsalis*, *Silurus asotus*, *Ictalurus punctatus*, *Malapterurus electricus*, *Rhamdia quelen*, *Pimelodella gracilis*, *Pimelodus maculatus*, *Sorubim lima*, *Pseudoplatystoma fasciatum*, *Iheringichthys labrosus*. **(1) many (uncontable)** (Fig. 21b). *Cetopsis coecutiens*, *Bunocephalus amazonicus*, *Nematogenys inermis*, *Copionodon orthiocarinatus*, *Ituglanis amazonicus*, *T.* sp. 1, *T.* sp. 2, *Hoplosternum littorale*, *Megalechis thoracata*, *Scoloplax distolothrix*, *Hypostomus ancistroides*, *Microglanis* aff. *parahybae*.

29. Characteristics of the vesicles and tubules presents in the midpiece

The vesicles and tubules observed in the midpiece show varied characteristics as: **(0) small vesicles and tubules interconnected or not** (Figs. 22a and 22b). *Bunocephalus amazonicus*, *Bunocephalus coracoideus*, *Copionodon orthiocarinatus*, *Ituglanis amazonicus*, *Trichomycterus* aff. *iheringi*, *T. areolatus*, *T.* sp. 1, *T.* sp. 2, *Corydoras flaveolus*, *Corydoras aeneus*, *Scleromystax lacerdai*, *Aspidoras poecilus*, *Hoplosternum littorale*, *Megalechis thoracata*, *Callichthys callichthys*, *Scoloplax distolothrix*, *Kronichthys heylandi*, *Neoplecostomus paranensis*, *Corumbataia cuestae*, *Hisonotus* sp., *Hypoptopoma*

guentheri, *Schizolecis guntheri*, *Loricariichthys platymetopon*, *Loricaria* sp., *Hypostomus ancistroides*, *Clarias gariepinus*, *Silurus microdorsalis*, *Malapterurus electricus*. **(1) only tubules interconnected to each other** (Fig. 22c). *Cetopsis coecutiens*, *Nematogenys inermis*, *Silurus asotus*, *Rhamdia quelen*, *Pimelodella gracilis*. **(2) large and irregular vesicles interconnected or not among themselves and to plasma membrane** (Fig. 22d). *Ictalurus punctatus*, *Microglanis* aff. *parahybae*, *Pimelodus maculatus*, *Pseudoplatystoma fasciatum*, *Sorubim lima*, *Iheringichthys labrosus*.

30. Flagellar number

The siluriform spermatozoa presents one (Fig. 23a) or two flagella (Fig. 23b). **(0) one**. *Diplomystes mesembrinus*, *Copionodon orthiocarinatus*, *Ituglanis amazonicus*, *Trichomycterus* aff. *iheringi*, *T. areolatus*, *T. reinhardti*, *T.* sp. 1, *T.* sp. 2, *Corydoras flaveolus*, *Corydoras aeneus*, *Scleromystax lacerdai*, *Aspidoras poecilus*, *Hoplosternum littorale*, *Megalechis thoracata*, *Callichthys callichthys*, *Scoloplax distolothrix*, *Astroblepus* cf. *mancoi*, *Kronichthys heylandi*, *Neoplecostomus paranensis*, *Corumbataia cuestae*, *Hisonotus* sp., *Hypoptopoma guentheri*, *Schizolecis guntheri*, *Loricariichthys platymetopon*, *Loricaria* sp., *Hypostomus ancistroides*, *Ancistrus triradiatus*, *Clarias gariepinus*, *Silurus microdorsalis*, *Silurus asotus*, *Microglanis* aff. *parahybae*, *Rhamdia quelen*, *Pimelodella gracilis*, *Trachelyopterus lucenai*, *Pimelodus maculatus*, *Pseudoplatystoma fasciatum*, *Sorubim lima*, *Iheringichthys labrosus*, *Pseudobagrus fulvidraco*, *Leiocassis ussuriensis*. **(1) two**. *Cetopsis coecutiens*, *Liobagrus mediadiposalis*, *Bunocephalus amazonicus*, *Bunocephalus coracoideus*, *Nematogenys inermis*, *Ictalurus punctatus*, *Malapterurus electricus*, *Mystus armatus*.

31. Flagellar membrane specializations

The flagella exihibits specializations developep from the flagellar membrane. These specializations can be lateral projections or fins which consist in expansions of flagellar membrane or a membranous compartment, which is set of vesicles interspersed by narrow cytoplasmic line. Theses structures can be: **(0) absent** (Fig. 24a). *Cetopsis coecutiens*, *Bunocephalus amazonicus*, *Bunocephalus coracoideus*, *Nematogenys inermis*,

Hoplosternum littorale, *Megalechis thoracata*, *Loricariichthys platymetopon*, *Loricaria* sp., *Ancistrus triradiatus*, *Clarias gariepinus*, *Silurus microdorsalis*, *Silurus asotus*, *Ictalurus punctatus*, *Malapterurus electricus*, *Microglanis* aff. *parahybae*, *Rhamdia quelen*, *Pimelodella gracilis*, *Trachelyopterus lucenai*, *Pimelodus maculatus*, *Pseudoplatystoma fasciatum*, *Sorubim lima*, *Iheringichthys labrosus*. **(1) present as two lateral projections or fins** (Fig. 24b). *Diplomystes mesembrinus*, *Liobagrus mediadiposalis*, *Trichomycterus areolatus*, *T. reinhardti*, *Scoloplax distolothrix*, *Astroblepus* cf. *mancoi*, *Kronichthys heylandi*, *Neoplecostomus paranensis*, *Corumbataia cuestae*, *Hisonotus* sp., *Hypoptopoma guentheri*, *Schizolecis guntheri*, *Hypostomus ancistroides*, *Mystus armatus*, *Pseudobagrus fulvidraco*, *Leiocassis ussuriensis*. **(2) present as variable number lateral projections or fins** (Fig. 24c). *Copionodon orthiocarinatus*, *Ituglanis amazonicus*, *Trichomycterus* aff. *iheringi*. **(3) present as a membranous compartment** (Fig. 24d). *Corydoras flaveolus*, *Corydoras aeneus*, *Scleromystax lacerdai*, *Aspidoras poecilus*, *Callichthys callichthys*. **(4) present as variable number lateral projections or fins and membranous compartment** (Fig. 24e). *Trichomycterus* sp. 1, *T.* sp. 2.

32. Lateral projections length

The length was mensured in cross sections of the flagellum. The Figure 25e shows the value interval referent to the each character-state of this character (vertical line). The length of the lateral projections can be: **(0) lateral projection length ≤ 0.35 µm: short**. *Diplomystes mesembrinus*, *Liobagrus mediadiposalis*, *Copionodon orthiocarinatus*, *Trichomycterus* aff. *iheringi*, *T. areolatus*, *T. reinhardti*, *T.* sp. 2, *Scoloplax distolothrix*, *Astroblepus* cf. *mancoi*, *Kronichthys heylandi*, *Hisonotus* sp., *Hypoptopoma guentheri*, *Schizolecis guntheri*, *Pseudobagrus fulvidraco*. **(1) lateral projection length > 0.35 µm: long**. *Ituglanis amazonicus*, *Trichomycterus* sp. 1, *Neoplecostomus paranensis*, *Corumbataia cuestae*, *Hypostomus ancistroides*, *Mystus armatus*, *Leiocassis ussuriensis*.

Cladograms

The cladograms obtained in the phylogenetic analyses are presented in the Figures 26 to 28. In the Figure 26, the majority-rule (50%) consensus tree shows the hypotheses of relationshiops among Loricarioidea families and the other siluriform families. The phylogenetics analysis involving all Loricarioidea species is presented in the majority-rule (50%) consensus tree of the Figure 27, while in the Figure 28, some species of Loricarioidea were excluded of the analysis; thus, the topology presented show the pattern of relationships among the remaining Loricarioidea taxa.

DISCUSSION

The analysis of the consensus cladogram presented in the Figure 26 show that the hypotheses of interrelationships of siluriform families, excluding Diplomystidae, obtained is very different of that proposed by Britto (2003) and, in general, exhibit a very low statistical support. In this cladogram is observed a dicotomy formed by the clade composed by Scoloplacidae, Astroblepidae and Auchenipteridae, and by the remaining siluriforms. This result is due to the occurrence only in these families of introsperms. Among the Loricarioidea, only the monophyly of the family Callichthyidae is corroborated but with low support. Considering the other families of Loricarioidea, the cladogram shows that *Nematogenys inermis* appear as more related to species of the families Heptapteridae and Pimelodidae and with *Loricariichthys platymetopon* (Loricariidae). Examples of the spermiogenesis characters shared by the spermatids of these families are the absence the nuclear fossa formation and centrioles migration. However, this is an unexpect result since Nematogenyidae have spermatozoa with two flagella and could appear more related to the species of the clade composed by Aspredinidae, Cetopsidae, Ictaluridae and Malapteruridae, all with biflagellate spermatozoa. The families Trichomycteridae and Callichthyidae also appeared as sister group with low statistical support. Curiously some species of Trichomycteridae and Loricariidae are scattered in the phylogeny.

In the cladogram of Loricarioidea presented in the Figures 27 is possible to note that the phylogenetic hypothesis obtained for this superfamily is different of the present

available for the group. Except for phylogenetic position of *Loricariichthys platymetopon*, *Nematogenys inermis* was found as sister group of all the other Loricarioidea, with high bootstrap value. The clade formed by *L. platymetopon* and *N. inermis* is supported by some characteristics of the spermiogenesis and spermatozoa as absence of nuclear fossa, medial origin of the flagellum, cytoplasmic channel formation by migration and interconnection of vesicles around of the initial segment of the flagellum (Table 1). The families Loricariidae, Callichthyidae and Trichomycteridae appear as polyphyletics.

In relation to the Trichomycteridae, the monophyly of this family is corroborated, except by the position of *Trichomycterus reinhardti* (Figure 27). The monophyletic condition is also confirmed for the family Callichthyidae. In both cladograms presented in the Figures 27 and 28, the families Trichomycteridae and Callichthyidae are related to some species of Loricariidae. In the Figure 27, Trichomycteridae was found related to *Farlowella* sp. and *Ancistrus triradiatus*, while Callichthyidae to *Loricaria* sp., but these groups have almost no statistical support. Some of the ultrastructural characteristics shared by Trichomycteridae and Loricariidae are the spermatogenesis type, movement of the centriolar complex towards to the nucleus, characteristics of vesicles and tubules in the midpiece. For Callichthyidae and Loricariidae, some examples of characters shared are the initial development of the flagellum lateral to the nucleus and short nuclear length.

In the cladogram presented in the Figure 28, some species and characters were excluded of the analysis. Basically all species with unresolved position in the phylogeny of the order Siluriformes were removed. In the case of the species *Loricaria* sp., *Farlowella* sp., and *Ancistrus triradiatus*, the exclusion was due to the lack of more data about the ultrastructure of spermiogenesis or spermatozoa. In relation to the characters, some were excluded to test if they could be responsible for the bad resolution of the final tree. After a wide series of tests the better phylogeny was obtained when the characteres numbers 2, 3, 4, 6, 7, 13, 14, 17, 18, 22, 28 were removed of the anaylsis. Characters number 2, 3, 4, 6, and 7 were derived of the spermiogenesis process and they were too similar to other characteres already availabe in the spermatozoa list, thus, they were removed to avoid character duplication. Characteres 13, 14, 17, 18, 22, and 28 were too variable and without a precise identification criteria.

The result of the final analysis support the division of the superfamily Loricarioidea in two clades: one composed by the family Nematogenyidae and the second composed by all remaining Loricarioidea. In this second clade Astroblepidae and Scoloplacidae appear as sister groups with high statistical support. This result was expected since these families are the only ones among Loricarioidea to present introsperm. Among the remaining species we found a polyphyletic Loricariidae as sister group of the clade composed by Callichthyidae and Trichomycteridae (both monophyletics). This result is very different from those already availabe since in previous studies Nematogenyidae is the sister group of Trichomycteridae (de Pinna, 1998; Britto, 2003), and this group is a sister group of the clade composed by Callichthyidae, Scoloplacidae, Astroblepidae, and Loricariidae (de Pinna, 1998; Britto, 2003). This is the first time that the hypothesis of relationship between Scoloplacidae and Astroblepidae is presented.

When the intra-familial relationships are analyzed, it is possible to observe that the monophyly of the subfamilies of the Loricariidae is confirmed (Figure 28), as the group composed by Neoplecostominae and Hypoptopomatinae (subfamilies of Loricariidae) as proposed by Armbruster (2004). The family Callichthyidae appears as monophyletic as well as its subfamilies. The family Trichomycteridae also appears as monophyletic but the species considered belonging to the most primitive group, *Copionodon orthiocarinatus* (de Pinna, 1998) appeared as the most derived in our cladogram. In this case *Trichomycterus reinhardti* appeared as the sister group of all trichomycterides.

In general, the data obtained revealed that the phylogenetics analysis using only these class of reproductive ultrastructural characters is not informative at order level and the suggested groups are very incongruent with the relationship hypotheses available for Siluriformes (de Pinna, 1998; Britto, 2003; Diogo, 2005; Sullivan et al., 2006). This result is not so unexpected because the low number of characters. Moreover, some characters that could represent synapomorphies, as the occurrence of introsperms in Scoloplacidae and Astroblepidae, change to homoplasies considering the occurrence of the same characters in other unrelated groups, as the introsperms of Auchenipteridae. When this information is employed in the context of a more restrict group (as the superfamily Loricarioidea) it is really informative and can strongly support the monophyly of some groups. Thus, these

type reproductive ultrastructural characters in phylogenetic analysis should be carefully employed to avoid misinterpretation.

ACKNOWLEDGMENTS

We wish to thank the E.M. Laboratory of IBB-UNESP, for allowing the use of their facilities. This research was supported by the Brazilian agencies FAPESP (Fundação de Apoio à Pesquisa do Estado de São Paulo), and CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico).

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FIGURE CAPTIONS

Figure 1: Spermatogenesis type. a) *Kronichthys heylandi*, state (0) cystic; b) *Corydoras aeneus*, state (1) semicystic. (a) X 5.812; (b) X 5.270. S: Sertoli cell, T: spermatids, Z: spermatozoon.

Figure 2: Early spermatids showing the flagellar initial development in relation to the nucleus. a) *Loricariichthys platymetopon*, state (0) medial to the nucleus; b) *Diplomystes mesembrinus*, state (1) lateral to the nucleus. (a) X 15.640; (b) X 11.760. F: flagellum, N: nucleus.

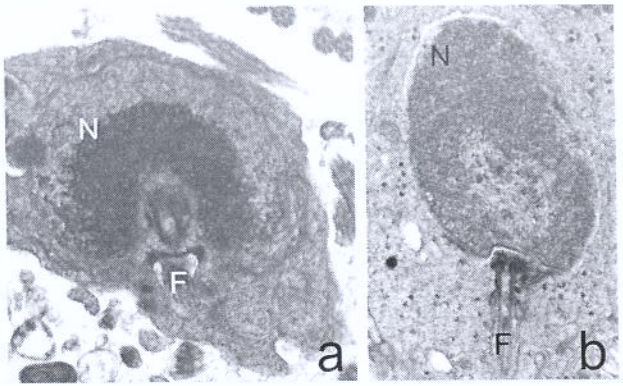
Figure 3: Spermatids exhibiting the cytoplasmic channel formation. a) *Pimelodus maculatus*, state (0) absent; b) *Trichomycterus* sp. 2, state (1) originated by centriolar complex movement; c) *Pimelodella gracilis*, state (2) originated by projection of the midpiece towards to the initial segment of the flagellum and by vesicullar interconnection. (a) X 13.250; (b) X 14.575; (c) X 18.270. F: flagellum, N: nucleus, Arrow: cytoplasmic channel.

FIGURE CAPTIONS

Figure 4: Chromatin condensation process. a) *Cetopsis coecutiens*, state (0) heterogeneous; b) *Trichomycterus* sp. 1, state (1) homogeneous. (a) X 17.000; (b) X 12.580. F: flagellum, N: nucleus.

Figure 5: Spermatids showing the nuclear fossa formation. a) *Pimelodella gracilis*, state (0) absent; b) *Bunocephalus amazonicus*, state (1) present. (a) X 15.120; (b) X 10.805. F: flagellum, N: nucleus, Arrowhead: nuclear fossa.

Figure 6: Nucleus shape of the spermatozoa. a) *Corydoras flaveolus*, state (0) rounded; b) *Cetopsis coecutiens*, state (2) semi-ovoid; c) *Astroblepus* cf. *mancoi*, state (3) conic; d) *Pseudoplatystoma fasciatum*, state (4) ovoid with the large axis in the vertical position; e) *Nematogenys inermis*, state (5) ovoid with the large axis in the horizontal position. (a) X 13.065; (b) X 20.100; (c) X 12.580; (d) X 16.430; (e) X 16.185. N: nucleus.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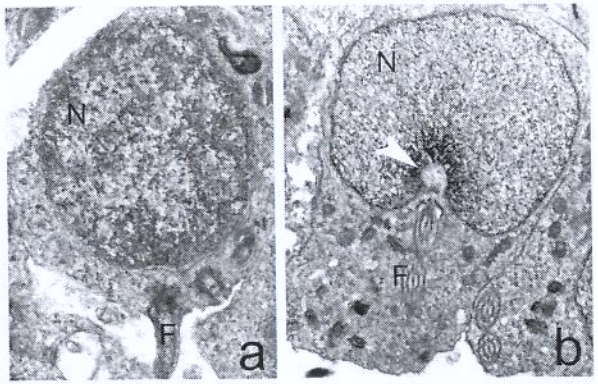
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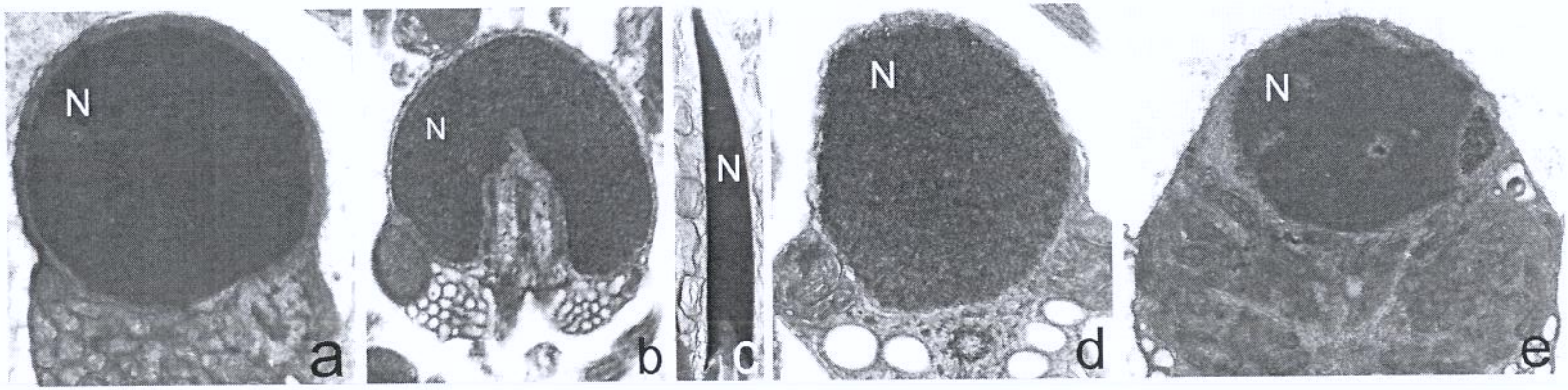
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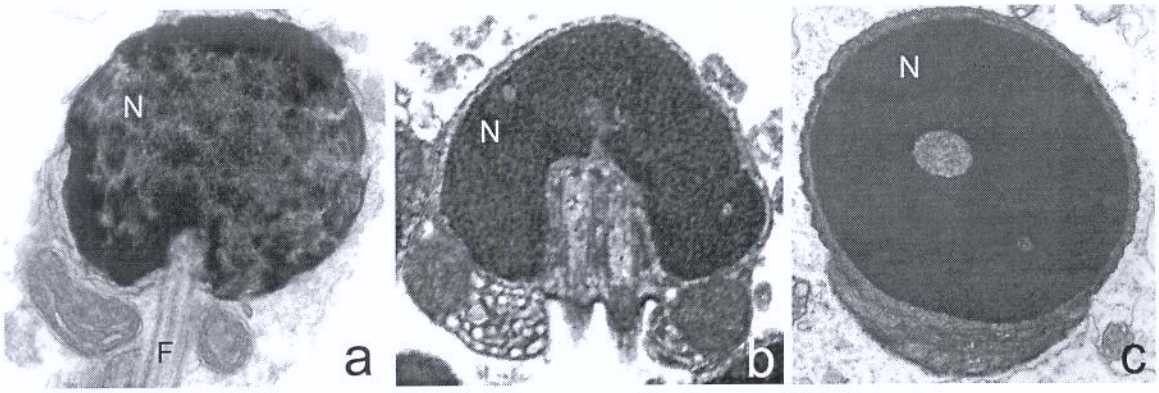
FIGURE CAPTIONS

Figure 7: End aspect of the condensed chromatin in the nucleus. a) *Diplomystes mesembrinus* and b) *Cetopsis coecutiens,* state (0) heterogeneous forming condensed clusters or in thin filaments juxtaposed with electron-lucent areas of granular aspect; c) *Corydoras flaveolus*, state (1) homogeneous with or without electron-lucent areas. (a) X 29.400; (b) X 28.560; (c) X 17.580. N: nucleus.

Figure 8: Cytoplasmic area around of the nucleus. a) *Corydoras aeneus*, state (0) narrow; b) *Trichomycterus* sp. 2, state (1) large. (a) X 16.100; (b) X 17.020. F: flagellum, N: nucleus.

Figure 9: Flagellar position in relation to the nucleus. a) *Kronichthys heylandi*, state (0) medial; b) *Corydoras aeneus*, state (1) eccentric. (a) X 15.640; (b) X 16.560. F: flagellum, N: nucleus.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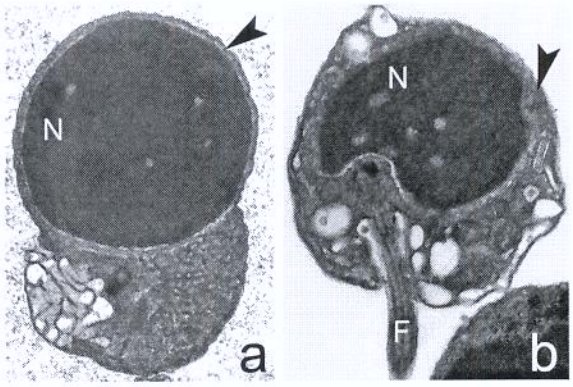
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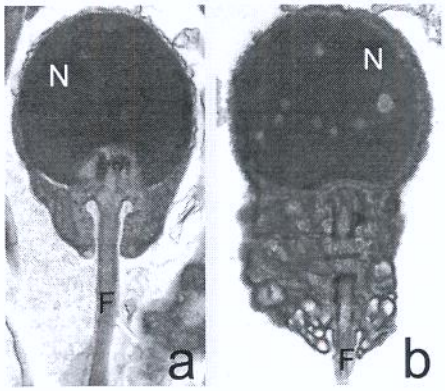
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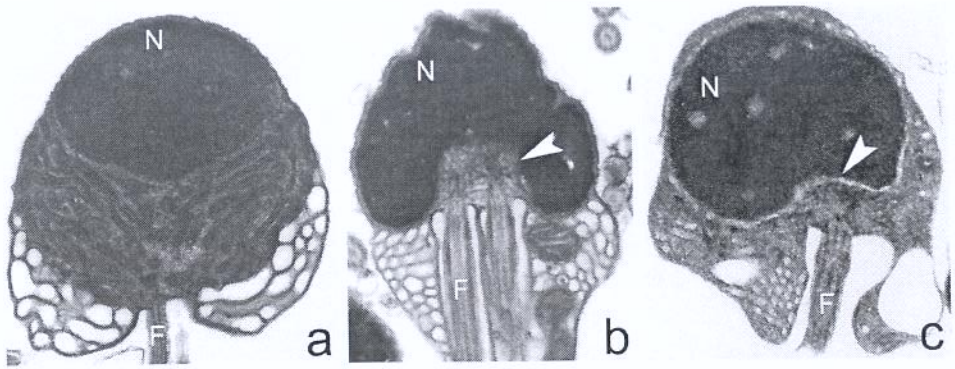
FIGURE CAPTIONS

Figure 10: Nuclear fossa. a) *Nematogenys inermis*, state (0) absent; b) *Bunocephalus amazonicus* and c) *Trichomycterus* sp. 1, state (1) present (medial and eccentric nuclear fossa). (a) X 12.580; (b) X 20.400; (c) X 20.400. F: flagellum, N: nucleus, Arrowhead: nuclear fossa.

Figure 11: Nuclear fossa shape. a) *Diplomystes mesembrinus* and b) *Neoplecostomus paranensis*, state (0) simple arc. (a) X 28.560; (b) X 27.600. F: flagellum, N: nucleus, Arrowhead: nuclear fossa.

Figure 12: Centriolar complex position in relation to the nuclear fossa. a) *Kronichthys heylandi*, state (0) centriolar complex or basal bodies totally inserted in the nuclear fossa; b) *Microglanis* aff. *parahybae*, state (1) proximal centriole inserted and basal body partially inserted in the nuclear fossa; c) *Trichomycterus* sp. 1, state (2) only proximal centriole inserted in the nuclear fossa; d) *Aspidoras poecilus*, state (4) proximal centriole and basal body totally outside of the nuclear fossa. (a) X 39.060; (b) X 28.560; (c) X 41.580; (d) X 17.000. D: distal centriole, P proximal centriole.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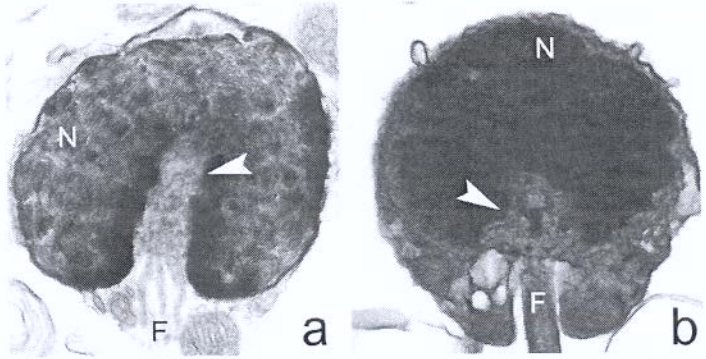
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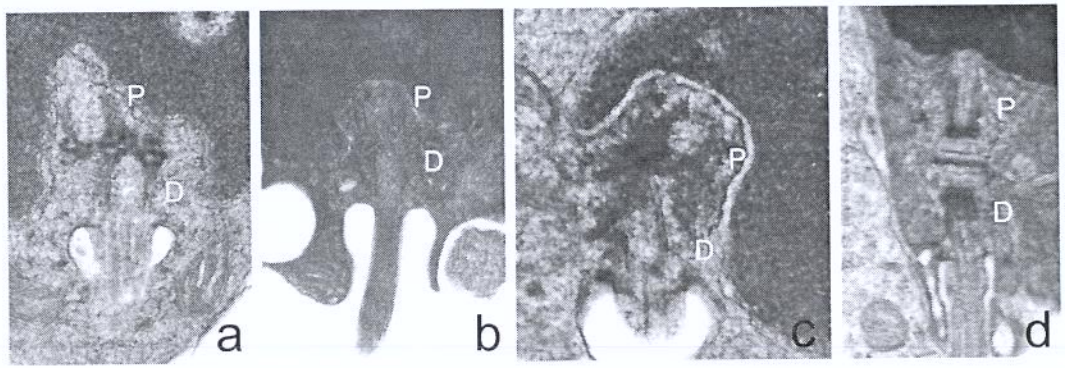
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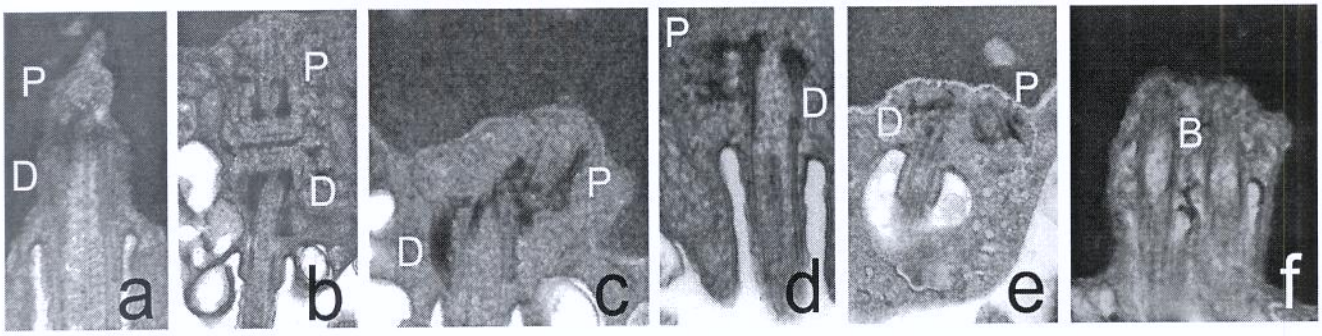
FIGURE CAPTIONS

Figure 13: Arrangement of the proximal and distal centrioles to each other. a) *Astroblepus* cf. *mancoi*, state (0) anterior and perpendicular; b) *Corydoras flaveolus*, state (1) co-axial; c) *Copionodon orthiocarinatus*, state (2) in obtuse angle; d) *Trichomycterus reinhardti*, state (3) lateral and perpendicular; e) *Hoplosternum littorale*, state (4) in acute angle; f) *Bunocephalus amazonicus*, state (5) paralell. (a) X 31.080; (b) X 23.000; (c) X 27.600; (d) X 28.560; (e) X 17.020; (f) X 23.000: B: basal bodies, D: distal centriole, P proximal centriole.

Figure 14: Midpiece symmetry in relation to the flagellum. a) *Pimelodus maculatus*, state (0) symmetric; b) *Corydoras aeneus*, state (1) asymmetric. (a) X 20.400; (b) X 17.480. F: flagellum, N: nucleus, Arrow: midpiece.

Figure 15: Cytoplasmic channel. a) *Pimelodus maculatus*, state (0) absent; b) *Astroblepus* cf. *mancoi*, state (1) single; c) *Bunocephalus amazonicus*, state (2) double. (a) X 41.580; (b) X 40.320; (c) X 34.960. F: flagellum, Arrow: cytoplasmic channel.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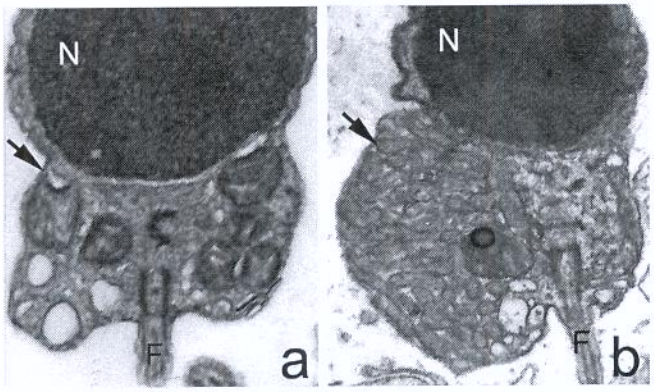
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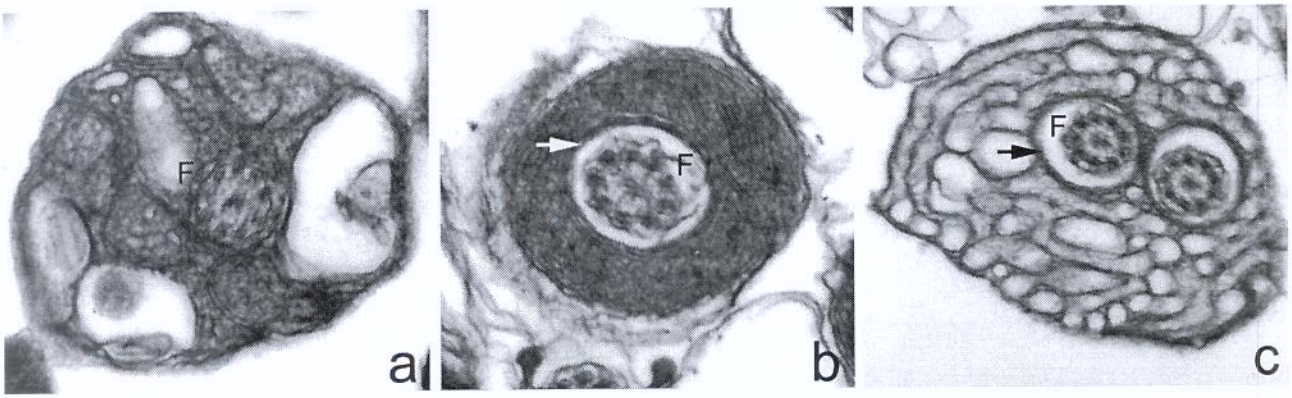
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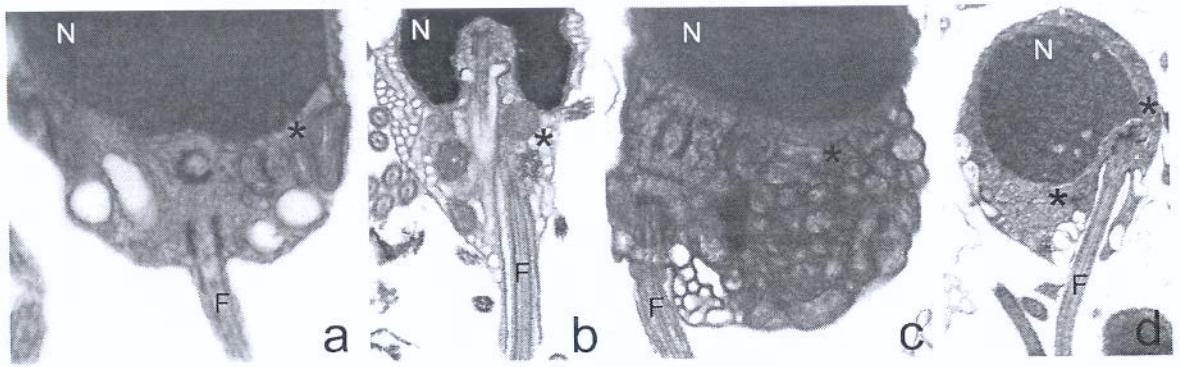
FIGURE CAPTIONS

Figure 16: Mitochondrial localization in the spermatozoon. a) *Pimelodus maculatus*, b) *Bunocephalus amazonicus*, and c) *Corydoras aeneus*, state (0) in different regions of the midpiece; d) *Trichomycterus* sp. 1, state (1) in all midpiece and around of the nucleus. (a) X 17.430; (b) X 16.560; (c) X 23.000; (d) X 13.600. F: flagellum, N: nucleus, Asterisk: mitochondria.

Figure 17: Mitochondrial amount in the midpiece. a) *Diplomystes mesembrinus*, state (0) single; b) *Kronichthys heylandi*, state (1) few (contable, usually less than 20); c) *Nematogenys inermis*, state (2) many (uncontable). (a) X 39.100; (b) X 28.980; (c) X 16.430. F: flagellum, N: nucleus, Asterisk: mitochondria.

Figure 18: Mitochondrial shape. a) *Cetopsis coecutiens*, state (0) rounded; b) *Loricariichthys platymetopon*, state (1) elongated; c) *Nematogenys inermis*, state (3) elongated and ramified. (a) X 23.000; (b) X 18.400; (c) X 16.430. F: flagellum, Asterisk: mitochondria.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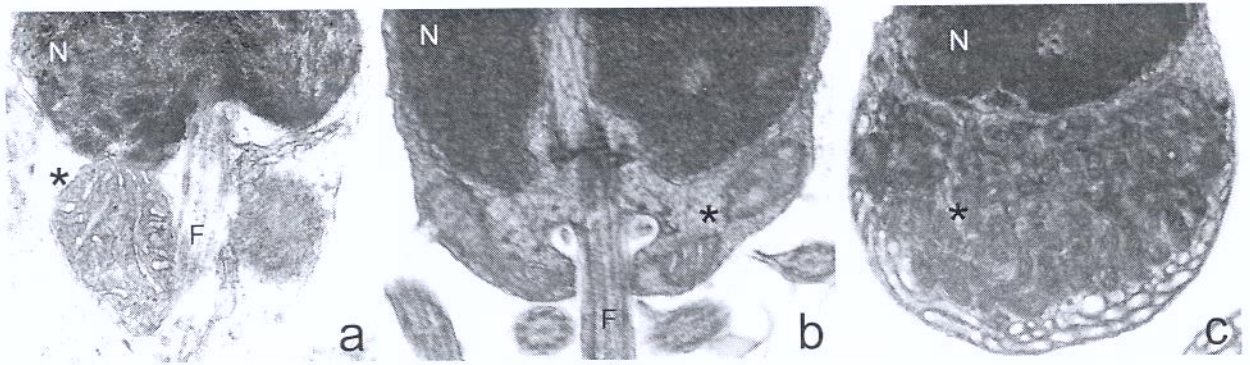
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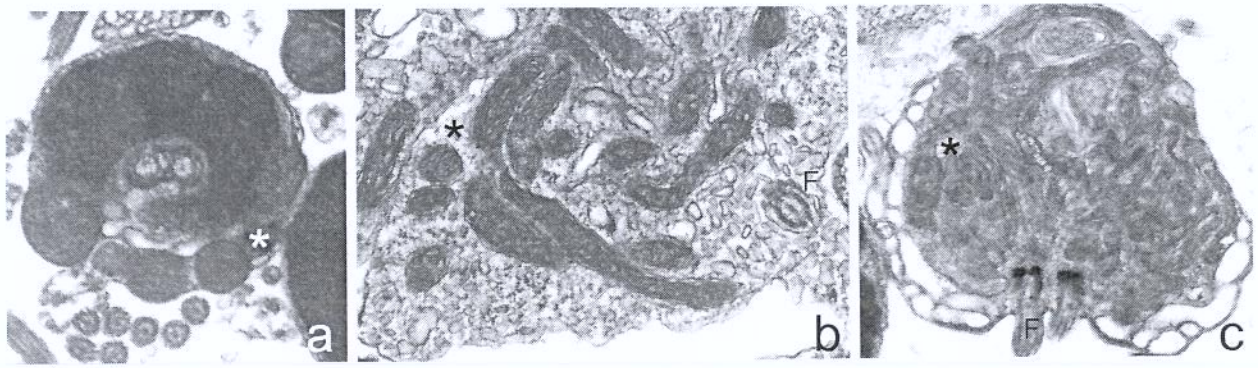
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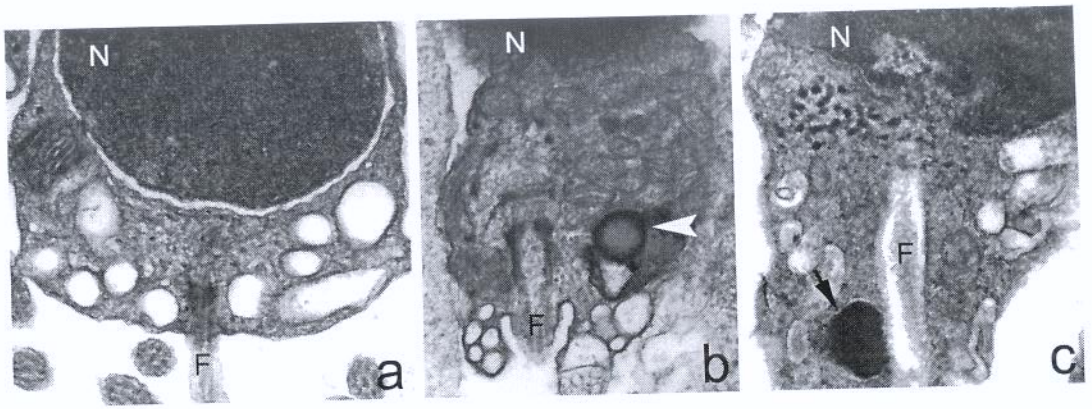
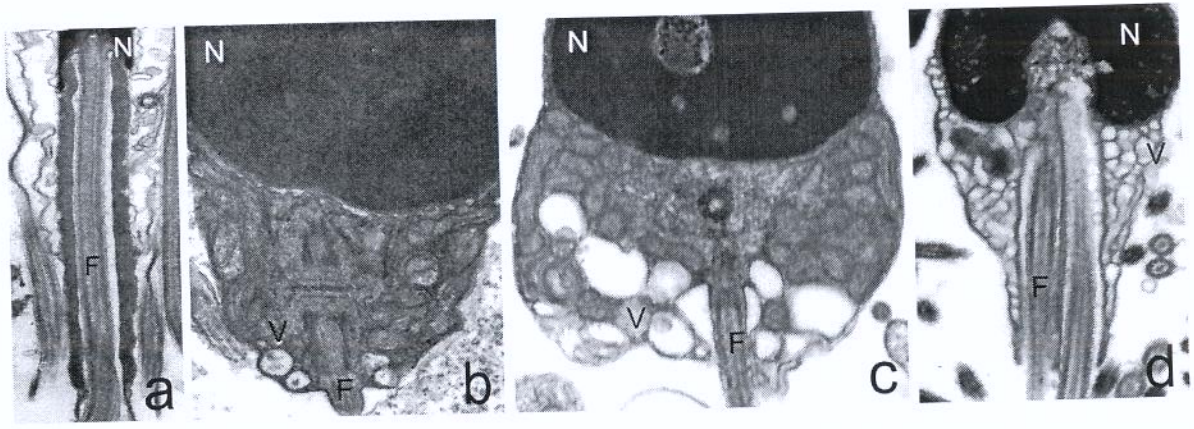
FIGURE CAPTIONS

Figure 19: Electron-dense spherical structure in the midpiece. a) *Pseudoplatystoma fasciatum*, state (0) absent; b) *Corydoras aeneus* and c) *Hisonotus* sp., state (1) present. (a) X 23.000; (b) X 25.200; (c) X 17.000. F: flagellum, N: nucleus, Arrowhead: electron-dense structure not surrounded by plasma membrane, Arrow: electron-dense structure surrounded by plasma membrane.

Figure 20: Vesicles in the midpiece. a) *Astroblepus* cf. *mancoi*, state (0) absent; b) *Corydoras flaveolus*, state (1) only in the basal region of the midpiece; c) *Loricariichthys platymetopon*, state (2) in the medial and basal regions of the midpiece; d) *Bunocephalus amazonicus*, state (3) in all midpiece. (a) X 17.000; (b) X 23.000; (c) X 20.400; (d) X 17.225. F: flagellum, N: nucleus, V: vesicles.

Figure 21: Vesicles amount in the midpiece. a) *Pseudoplatystoma fasciatum*, state (0) few (contable, usually less than 20); b) *Bunocephalus amazonicus*, state (1) many (uncontable). (a) X 23.000; (b) X 17.480. F: flagellum, N: nucleus, V: vesicles.


N
F
a
N
F
b
N
F
c


N
F
a
N
V
F
b
N
V
F
c
N
V
F
d

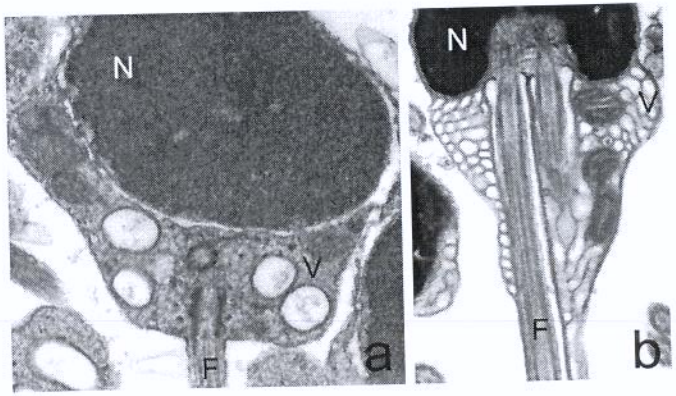
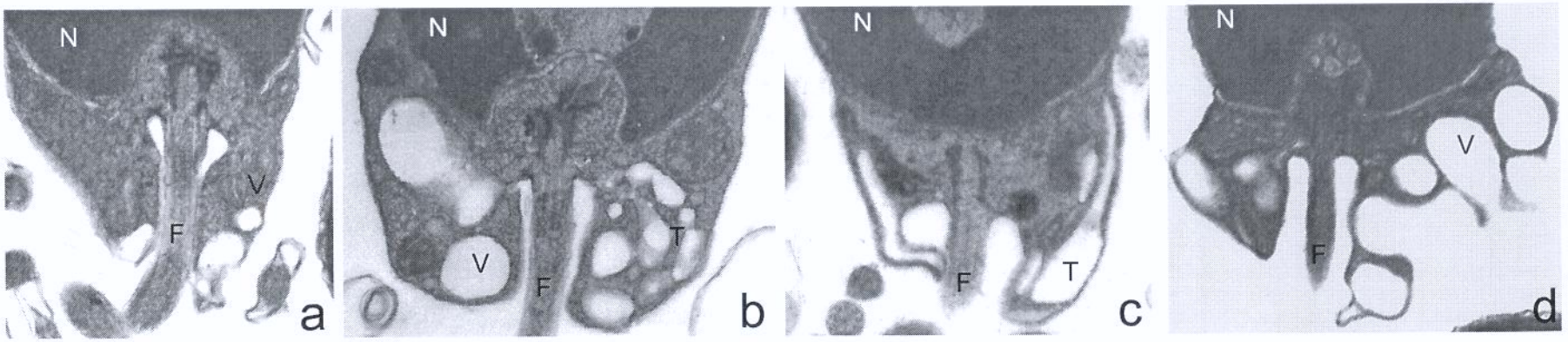

N
V
F
a
N
V
F
b

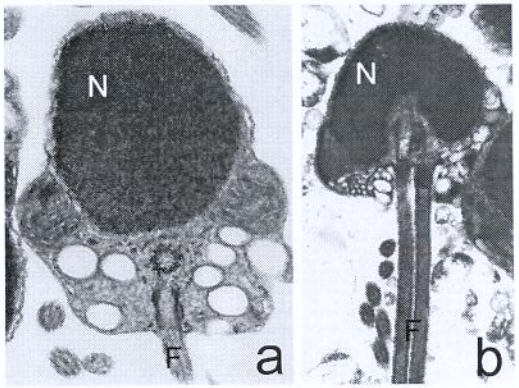
FIGURE CAPTIONS

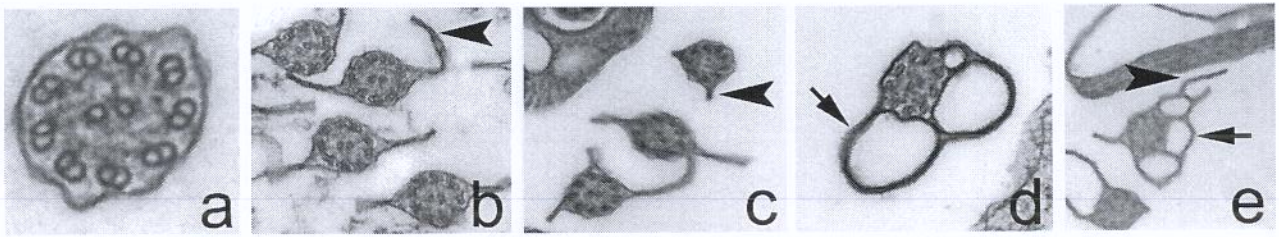
Figure 22: Characteristics of the vesicles and tubules presents in the midpiece. a) *Kronichthys heylandi* and b) *Copionodon orthiocarinatus*, state (0) small vesicles and tubules interconnected or not; c) *Rhamdia quelen*, state (1) only tubules interconnected to each other; d) *Microglanis* aff. *parahybae*, state (2) large and irregular vesicles interconnected or not among themselves and to plasma membrane. (a) X 22.100; (b) X 23.000; (c) X 19.720; (d) X 22.100. F: flagellum, N: nucleus, T: tubules, V: vesicles.

Figure 23: Flagellar number of the spermatozoa. a) *Pseudoplatystoma fasciatum*, state (0) one; b) *Cetopsis coecutiens*, state (1) two. (a) X 10.460; (b) X 17.000. F: flagellum, N: nucleus.

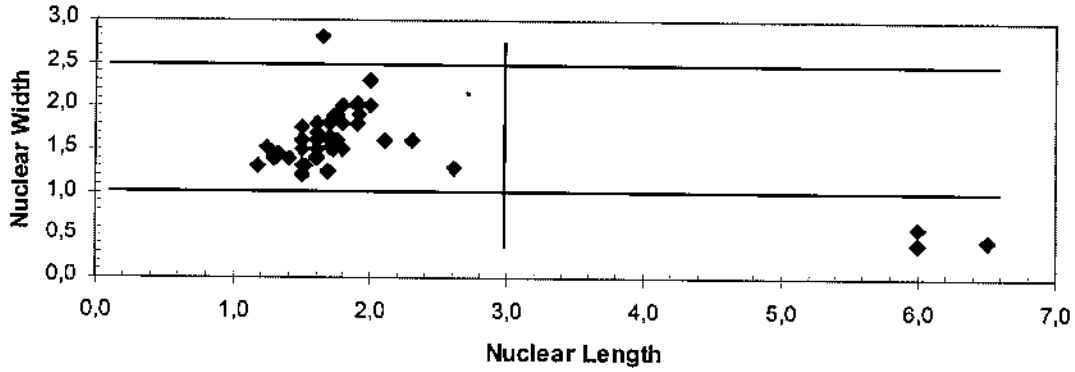
Figure 24: Flagellar membrane specializations. a) *Pseudoplatystoma fasciatum*, state (0) absent; b) *Trichomycterus areolatus*, state (1) present as two lateral projections or fins; c) *Trichomycterus* aff. *iheringi*, state (2) present as variable number lateral projections or fins; d) *Corydoras aeneus*, state (3) present as a membranous compartment; e) *Trichomycterus* sp. 2, state (4) present as variable number lateral projections or fins and membranous compartment. (a) X 92.000; (b) X 28.980; (c) X 23.800; (d) X 57.500; (e) X 29.900. Arrowhead: lateral projections, Arrow: membranous compartment.


N
V
F
a
N
V
F
T
b
N
F
T
c
N
V
F
d

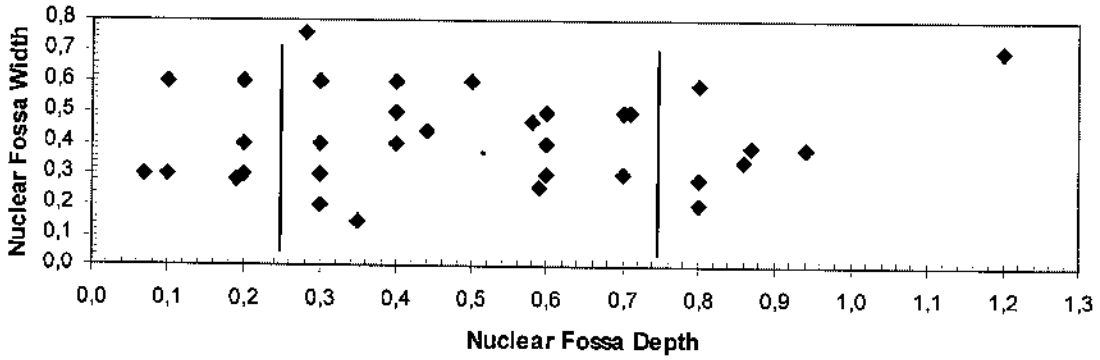

N
F
a
N
F
b


a
b
c
d
e

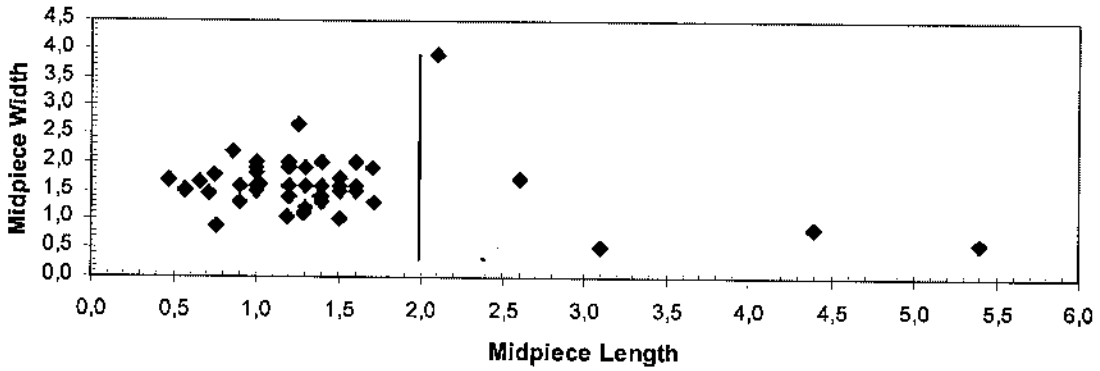
a


Nuclear Width
3,0
2,5
2,0
1,5
1,0
0,5
0,0
0,0
1,0
2,0
3,0
4,0
5,0
6,0
7,0
Nuclear Length

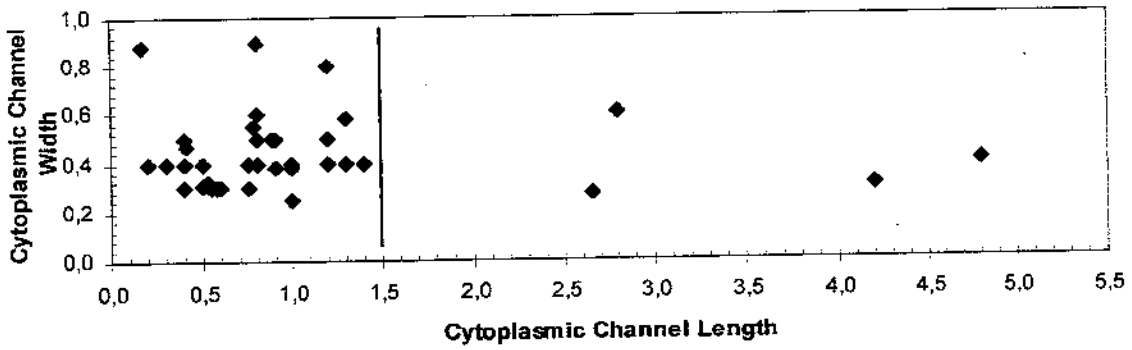
b


Nuclear Fossa Width
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0,0
0,0
0,1
0,2
0,3
0,4
0,5
0,6
0,7
0,8
0,9
1,0
1,1
1,2
1,3
Nuclear Fossa Depth

c


Midpiece Width
4,5
4,0
3,5
3,0
2,5
2,0
1,5
1,0
0,5
0,0
0,0
0,5
1,0
1,5
2,0
2,5
3,0
3,5
4,0
4,5
5,0
5,5
6,0
Midpiece Length

d



e

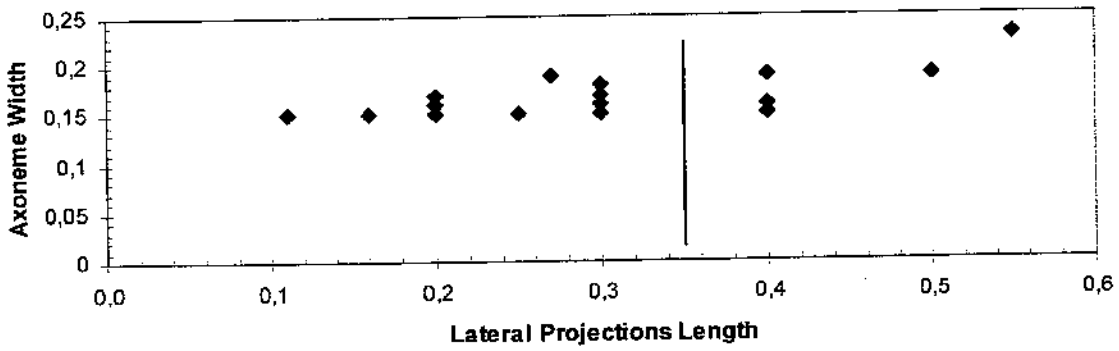


Figure 25: Relation between the length and width of the quantitative characters. a) Nucleus; b) Nuclear fossa; c) Midpiece; d) Cytoplasmic channel; e) Lateral projections. The vertical and horizontal lines indicate the interval, in micrometer, for each quantitative character-state. The losangue represent the siluriform species.

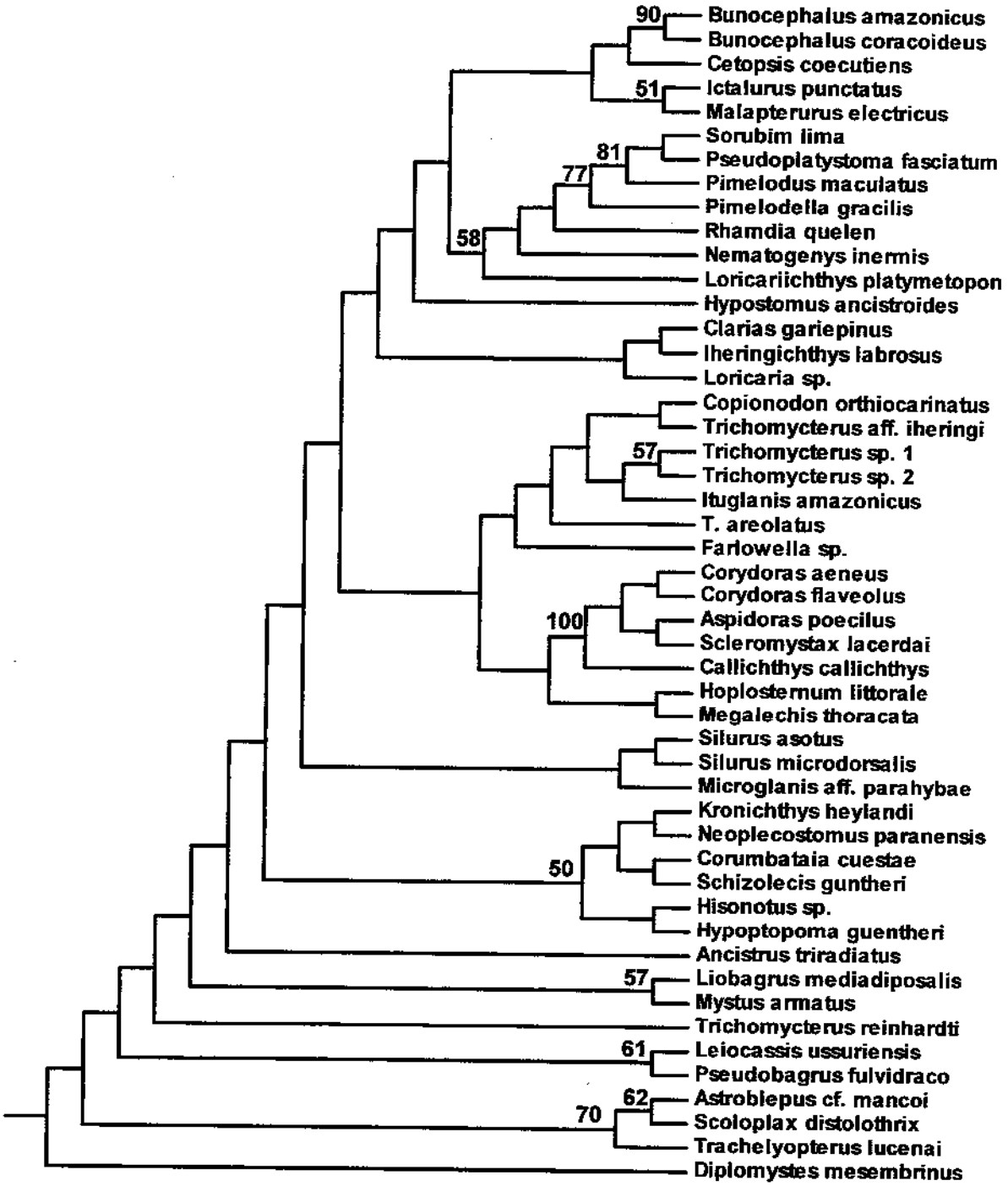


Figure 26: Majority-rule (50%) consensus tree showing the hypotheses of relationships among the Loricarioidea and the other siluriform families obtained from the analysis of 49 taxa and 32 characters of equal weight and "unord". Outgroup includes *Diplomystes mesembrinus*. (Trees 10.703, Tree length 171, Consistency index 0.40, Retention index 0.72). The numbers above of the branchs are bootstrap values.

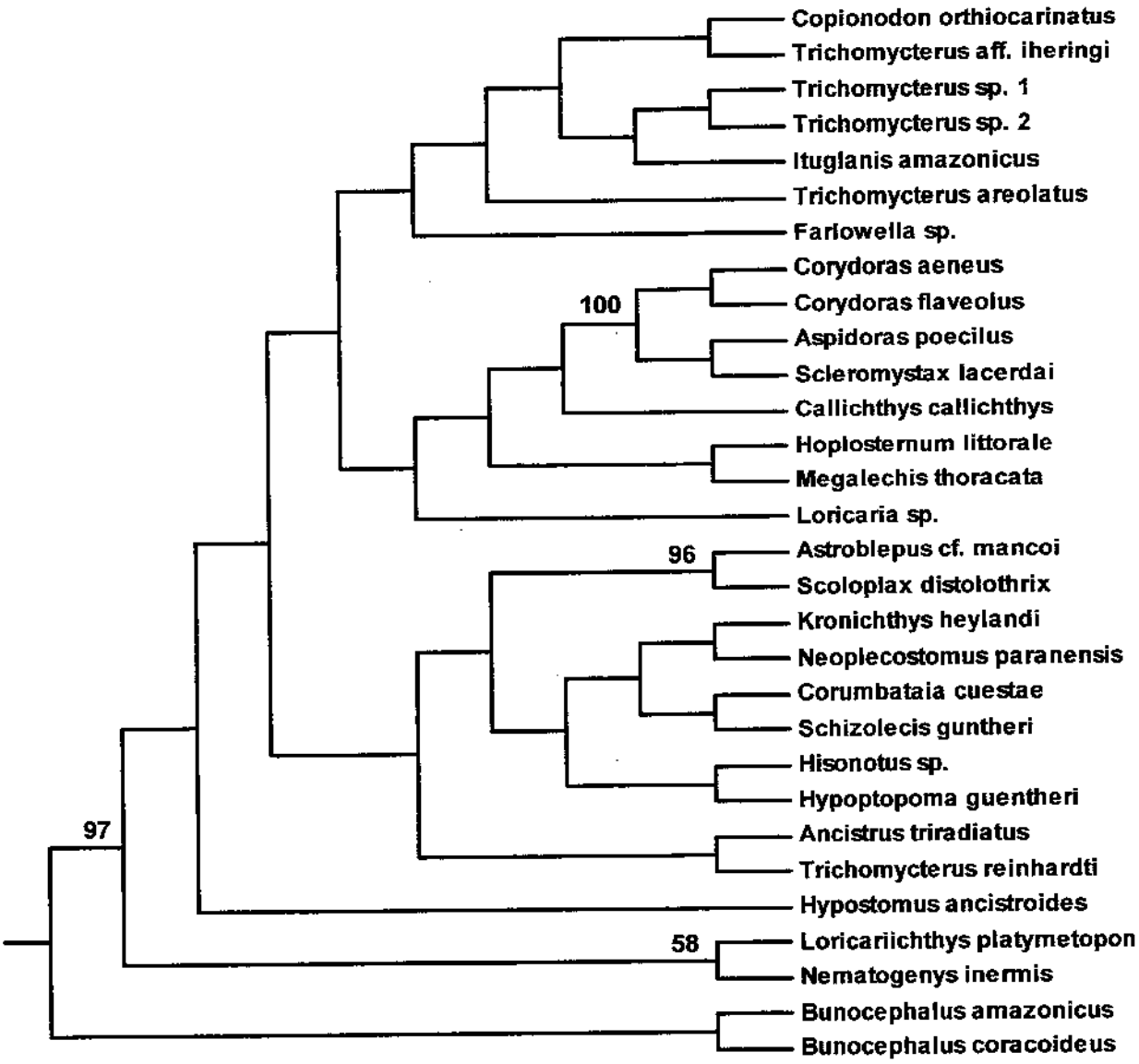


Figure 27: Majority-rule (50%) consensus tree showing the hypotheses of relationships among the species of Loricarioidea obtained from the analysis of 30 taxa and 32 characters of equal weight and "unord". Outgroup includes *Bunocephalus amazonicus*, *B. coracoideus*. (Trees 7.740, Tree length 104, Consistency index 0.53, Retention index 0.74). The numbers above of the branchs are bootstrap values.

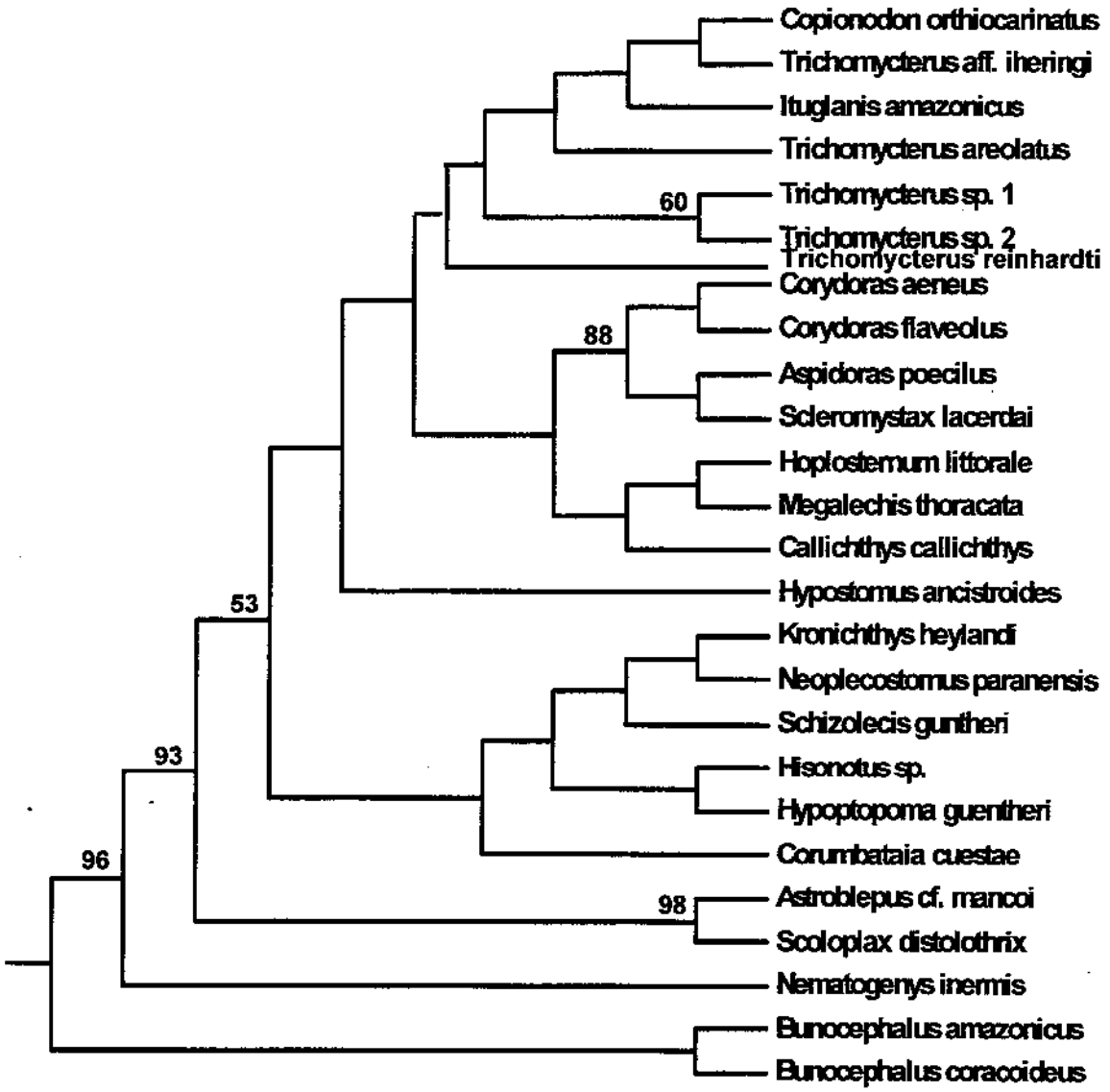


Figure 28: Majority-rule (50%) consensus tree showing the hypotheses of relationships among the most of the species of Loricarioidea obtained from the analysis of 26 taxa and 21 characters of equal weight, being 8 characters "ord" and 13 characters "unord". Outgroup includes *Bunocephalus amazonicus*, *B. coracoideus*. (Trees 21.494, Tree length 56, Consistency index 0.66, Retention index 0.82). The numbers above of the branchs are bootstrap values.

Table 1: Data matrix of 32 characters employed in the phylogenetic analysis of Loricarioidea, and among siluriform species. Characters ordered as in the character list. The "?" symbols represent the unavailable characters, and the "-" symbols, the no comparable characters.

Species	Characters																														
	0									1											2										3		
	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2
Diplomystes mesembrinus	0	1	1	1	2	0	1	0	0	1	0	0	0	1	0	2	0	0	0	0	0	-	0	0	2	0	0	-	-	0	1	0
Cetopsis coecutiens	1	0	0	2	0	0	1	2	0	1	0	0	0	1	0	1	0	5	0	0	1	0	0	1	0	0	3	1	1	1	0	-
Liobagrus mediadiposalis	?	?	?	?	?	?	?	1	0	1	1	0	0	1	1	1	0	5	0	0	2	0	0	1	0	0	0	-	-	1	1	0
Bunocephalus amazonicus	1	0	0	2	0	0	1	3	1	1	0	0	0	1	0	2	0	5	1	0	2	1	0	1	1	0	3	1	0	1	0	-
Bunocephalus coracoideus	?	?	?	?	?	?	?	2	1	1	0	0	0	1	0	2	0	5	0	0	2	1	0	1	1	0	3	0	0	1	0	-
Nematogenys inermis	1	0	0	2	0	1	0	5	0	2	1	0	0	0	-	-	-	5	1	0	1	0	0	2	3	0	1	1	1	1	0	-
Copionodon orthiocarinatus	0	1	1	1	1	1	1	0	0	1	1	1	0	1	0	1	2	2	0	1	1	0	1	1	1	0	3	1	0	0	2	0
Ituglanis amazonicus	0	1	1	1	1	1	1	4	0	1	1	1	1	1	0	1	0	3	0	1	1	0	1	1	1	0	3	1	0	0	2	1
Trichomycterus aff. *iheringi*	0	1	1	1	1	1	1	4	0	1	1	1	1	1	0	1	2	2	0	1	1	0	1	1	1	0	1	0	0	0	2	0
Trichomycterus areolatus	0	1	1	1	1	1	1	0	0	1	1	0	0	1	0	1	0	2	0	1	1	0	0	1	1	0	1	0	0	0	1	0
Trichomycterus reinhardti	?	?	?	?	?	?	?	0	0	1	1	0	1	1	0	1	2	3	0	1	1	0	0	1	1	0	0	-	-	0	1	0
Trichomycterus sp. 1	0	1	1	1	1	1	1	4	0	1	1	1	1	1	0	1	2	2	0	1	1	0	1	1	1	0	3	1	0	0	4	1
Trichomycterus sp. 2	0	1	1	1	1	1	1	0	0	1	1	1	0	1	0	1	2	2	0	1	1	0	1	1	1	0	3	1	0	0	4	0
Corydoras flaveolus	1	1	0	2	0	1	1	0	0	1	1	0	1	1	0	0	4	1	0	1	1	0	0	2	1	1	1	0	0	0	3	-
Corydoras aeneus	1	1	0	2	0	1	1	0	0	1	1	0	1	1	0	0	4	1	0	1	1	0	0	2	1	1	1	0	0	0	3	-
Scleromystax lacerdai	1	1	0	2	0	1	1	4	0	1	1	0	1	1	0	0	4	1	0	1	1	0	0	2	1	1	1	0	0	0	3	-
Aspidoras poecilus	1	1	0	2	0	1	1	4	0	1	1	0	1	1	0	0	4	1	0	1	1	0	0	2	1	1	1	0	0	0	3	-
Hoplosternum littorale	0	1	1	1	1	1	1	4	0	1	1	0	1	1	0	0	0	4	0	1	1	0	0	1	1	0	1	1	0	0	0	-
Megalechis thoracata	0	1	1	1	1	1	1	0	0	1	1	0	0	1	0	0	0	2	0	1	1	0	0	1	1	0	1	1	0	0	0	-
Callichthys callichthys	0	1	1	1	1	1	1	4	0	1	1	0	1	1	0	0	2	2	0	1	1	0	0	1	1	0	1	0	0	0	3	-
Scoloplax distolothrix	0	1	1	1	2	0	1	3	2	0	1	0	0	1	0	2	0	0	1	0	1	1	0	2	1	0	3	1	0	0	1	0
Astroblepus cf. *mancoi*	?	?	?	?	?	?	?	3	2	0	1	0	0	1	0	2	0	0	1	0	1	1	0	2	1	0	0	-	-	0	1	0
Kronichthys heylandi	0	1	1	1	2	1	1	0	0	1	1	0	0	1	0	1	0	2	0	0	1	0	0	1	1	1	3	0	0	0	1	0
Neoplecostomus paranensis	0	1	1	1	2	1	1	0	0	1	1	0	0	1	0	1	0	2	0	0	1	0	0	1	1	1	3	0	0	0	1	1
Corumbataia cuestae	0	1	1	1	2	1	1	4	0	1	1	0	0	1	0	1	0	0	0	0	1	0	0	1	1	1	1	0	0	0	1	1
Hisonotus sp.	0	1	1	1	2	1	1	0	0	1	1	0	0	1	0	1	0	0	0	0	1	0	0	1	1	1	1	0	0	0	1	0
Hypoptopoma guentheri	0	1	1	1	2	1	1	4	0	1	1	0	0	1	0	1	0	0	0	0	1	0	1	1	1	1	1	0	0	0	1	0
Schizolecis guntheri	?	?	?	?	?	?	?	0	0	1	1	0	0	1	0	1	0	0	0	0	1	0	1	1	1	1	1	0	0	0	1	0
Loricariichthys platymetopon	0	0	0	2	0	1	0	0	0	1	1	0	0	0	-	-	-	0	0	0	1	0	0	2	1	0	2	0	0	0	0	-
Farlowella sp.	0	1	1	1	1	1	1	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?

Table 1 (continued)

Species	Characters																													
	0									1										2									3		
	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2
Loricaria sp.	0	1	1	1	1	1	1	0	0	1	1	0	1	1	0	1	2	0	0	0	1	0	0	1	1	0	3	0	0	0	0	-
Hypostomus ancistroides	0	0	0	2	0	1	1	0	0	1	1	0	0	1	0	0	2	0	0	0	1	0	0	1	1	0	1	1	0	0	1	1
Ancistrus triradiatus	?	?	?	?	?	?	?	0	0	1	?	0	0	1	0	1	0	0	0	0	1	0	0	1	1	0	0	-	-	0	0	-
Clarias gariepinus	?	?	?	?	?	?	?	0	0	1	0	0	0	1	0	1	0	4	0	0	0	-	0	1	1	0	3	0	0	0	0	-
Silurus microdorsalis	?	?	?	?	?	?	?	0	0	1	1	0	0	1	0	2	1	0	0	0	1	0	0	1	0	0	3	0	0	0	0	-
Silurus asotus	?	?	?	?	?	?	?	0	0	1	1	0	0	1	0	1	1	2	0	0	1	0	0	1	1	0	2	0	1	0	0	-
Ictalurus punctatus	?	?	?	?	?	?	?	2	0	1	1	1	0	1	1	1	3	5	0	0	2	0	0	1	1	0	3	0	2	1	0	-
Malapterurus electricus	1	0	0	2	0	0	1	1	0	1	0	0	0	1	1	0	3	5	0	0	2	0	0	1	0	0	3	0	0	1	0	-
Microglanis aff. *parahybae*	0	1	1	1	2	1	1	0	0	1	1	0	0	1	0	1	1	2	0	1	1	0	0	1	1	0	3	1	2	0	0	-
Rhamdia quelen	0	0	0	2	0	1	0	4	1	1	1	0	0	0	-	-	-	3	0	0	1	0	0	1	1	0	2	0	1	0	0	-
Pimelodella gracilis	0	0	0	2	0	1	0	0	0	1	1	0	0	0	-	-	-	3	0	0	0	-	0	1	1	0	2	0	1	0	0	-
Trachelyopterus lucenai	?	?	?	?	?	?	?	3	2	0	1	0	0	1	0	1	1	0	1	1	1	1	0	1	1	0	0	-	-	0	0	-
Pimelodus maculatus	0	0	0	0	0	1	0	4	0	1	1	0	0	0	-	-	-	0	0	0	0	-	0	1	1	0	3	0	2	0	0	-
Pseudoplatystoma fasciatum	0	0	0	0	0	1	0	4	0	1	1	0	0	0	-	-	-	0	0	0	0	-	0	1	1	0	2	0	2	0	0	-
Sorubim lima	0	0	0	0	0	1	0	0	0	1	1	0	0	0	-	-	-	0	0	0	0	-	0	1	1	0	3	0	2	0	0	-
Iheringichthys labrosus	0	?	?	?	?	?	?	0	0	1	1	0	0	1	0	0	4	0	0	0	0	-	0	1	1	0	3	0	2	0	0	-
Mystus armatus	?	?	?	?	?	?	?	2	0	1	0	0	0	1	1	1	0	5	0	0	1	0	0	1	1	0	0	-	-	1	1	1
Pseudobagrus fulvidraco	?	?	?	?	?	?	?	2	0	1	1	0	0	1	0	2	0	1	0	0	1	0	0	1	0	0	0	-	-	0	1	0
Leiocassis ussuriensis	?	?	?	?	?	?	?	2	0	1	1	0	0	1	0	2	0	1	0	0	1	0	0	1	1	0	0	-	-	0	1	1

APPENDIX 1

Species	*Literature data*	*Analized data*
Diplomystidae		
Diplomystes mesembrinus	Quagio-Grassiotto et al., 2001	spermiogenesis and spermatozoa
Cetopsidae		
Cetopsis coecutiens	Spadella et al., 2006[a]	spermiogenesis and spermatozoa
Amblycipitidae		
Liobagrus mediadiposalis	Lee and Kim, 1999	spermatozoa
Aspredinidae		
Bunocephalus amazonicus	Spadella et al., 2006[a]	spermiogenesis and spermatozoa
Bunocephalus coracoideus	Mansour and Lahnsteiner, 2003	spermatozoa
Nematogenyidae		
Nematogenys inermis	Spadella et al., 2006a, submitted g	spermiogenesis and spermatozoa
Trichomycteridae		
Copionodon orthiocarinatus	Spadella et al., submitted d	spermiogenesis and spermatozoa
Ituglanis amazonicus	Spadella et al., submitted d	spermiogenesis and spermatozoa
Trichomycterus aff. *iheringi*	Spadella et al., submitted d	spermiogenesis and spermatozoa
Trichomycterus areolatus	Spadella et al., submitted d	spermiogenesis and spermatozoa
Trichomycterus reinhardti	Spadella et al., submitted d	spermatozoa
Trichomycterus sp. 1	Spadella et al., submitted d	spermiogenesis and spermatozoa
Trichomycterus sp. 2	Spadella et al., submitted d	spermiogenesis and spermatozoa
Callichthyidae		
Corydoras flaveolus	Spadella et al., submitted c	spermiogenesis and spermatozoa
Corydoras aeneus	Spadella et al., submitted c	spermiogenesis and spermatozoa
Scleromystax lacerdai	Spadella et al., submitted c	spermiogenesis and spermatozoa
Aspidoras poecilus	Spadella et al., submitted c	spermiogenesis and spermatozoa
Hoplosternum littorale	Spadella et al., submitted c	spermiogenesis and spermatozoa
Megalechis thoracata	Spadella et al., submitted c	spermiogenesis and spermatozoa
Callichthys callichthys	Spadella et al., submitted c	spermiogenesis and spermatozoa
Scoloplacidae		
Scoloplax distolothrix	Spadella et al., 2006b	spermiogenesis and spermatozoa
Astroblepidae		
Astroblepus cf. *mancoi*	Spadella et al., submitted f	spermatozoa
Loricariidae		
Kronichthys heylandi	Spadella et al., submitted e	spermiogenesis and spermatozoa
Neoplecostomus paranensis	Spadella et al., submitted e	spermiogenesis and spermatozoa
Corumbataia cuestae	Spadella et al., submitted e	spermiogenesis and spermatozoa
Hisonotus sp.	Spadella et al., submitted e	spermiogenesis and spermatozoa
Hypoptopoma guentheri	Spadella et al., submitted e	spermiogenesis and spermatozoa
Schizolecis guntheri	Spadella et al., submitted e	spermatozoa
Loricariichthys platymetopon	Spadella et al., submitted e	spermiogenesis and spermatozoa

Farlowella sp.	Spadella et al., submitted e	spermiogenesis
Loricaria sp.	Spadella et al., submitted e	spermiogenesis and spermatozoa
Hypostomus ancistroides	Spadella et al., submitted e	spermiogenesis and spermatozoa
Ancistrus triradiatus	Mansour and Lahnteiner, 2003	spermatozoa
Clariidae		
Clarias gariepinus	Mansour et al., 2002	spermatozoa
Siluridae		
Silurus microdorsalis	Lee and Kim, 2001	spermatozoa
Silurus asotus	Kwon et al., 1998	spermatozoa
Ictaluridae		
Ictalurus punctatus	Porier and Nicholson, 1982	spermatozoa
Malapteruridae		
Malapterurus electricus	Shahin, 2006	spermiogenesis and spermatozoa
Pseudopimelodidae		
Microglanis aff. *parahybae*	Quagio-Grassiotto et al., 2005	spermiogenesis and spermatozoa
Heptapteridae		
Rhamdia quelen	Quagio-Grassiotto et al., 2005	spermiogenesis and spermatozoa
Pimelodella gracilis	Quagio-Grassiotto et al., 2005	spermiogenesis and spermatozoa
Auchenipteridae		
Trachelyopterus lucenai	Burns et al., 2002	spermatozoa
Pimelodidae		
Pimelodus maculatus	Quagio-Grassiotto and Oliveira, submitted	spermiogenesis and spermatozoa
Pseudoplatystoma fasciatum	Quagio-Grassiotto and Oliveira, submitted	spermiogenesis and spermatozoa
Sorubim lima	Quagio-Grassiotto and Carvalho, 2000	spermiogenesis and spermatozoa
Iheringichthys labrosus	Santos et al., 2001	spermatozoa
Bagridae		
Mystus armatus	Mansour and Lahnsteiner, 2003	spermatozoa
Pseudobagrus fulvidraco	Lee, 1998	spermatozoa
Leiocassis ussuriensis	Kim and Lee, 2000	spermatozoa

5. CONCLUSÃO

A análise comparativa dos dados obtidos revela que o processo de espermatogênese, bem como de espermiogênese é variável entre as espécies de Loricarioidea. Em Nematogenyidae e Corydoradinae (subfamília de Callichthyidae), a espermatogênese é do tipo semi-cística, enquanto em Trichomycteridae, Callichthyinae (subfamília de Callichthyidae), Loricariidae e Scoloplacidae, esse processo é cístico. Quanto à espermiogênese, as famílias Trichomycteridae, Callichthyidae, Loricariidae e Scoloplacidae compartilham mais características em comum do que com Nematogenyidae. Considerando a morfologia dos espermatozóides em Loricarioidea, observa-se que cada família apresenta um padrão característico. No entanto, a maioria das características presentes em uma determinada família, não é exclusiva, podendo ser observáveis em outros Loricarioidea ou em outros grupos de Siluriformes. A comparação geral da ultra-estrutura dos espermatozóides em Loricarioidea mostra que as famílias Trichomycteridae, Callichthyidae e Loricariidae são mais similares entre si, sendo que o mesmo ocorre entre Scoloplacidae e Astroblepidae. A morfologia dos espermatozóides em Nematogenyidae é a que mais difere da observada nos outros Loricarioidea. A análise filogenética realizada com base nesses caracteres ultra-estruturais reprodutivos, visando elaborar hipóteses sobre a filogenia dos Loricarioidea e de seus grupos constituintes, mostrou que quando o uso desses caracteres se restringe aos relacionamentos inter e intra familiar em Loricarioidea, observa-se que eles podem ser mais informativos, podendo, inclusive, corroborar o monofiletismo de alguns grupos. Entretanto, quando esse conjunto de dados é empregado nas análises em nível de ordem, o mesmo não é informativo, uma vez que as topologias obtidas apresentam áreas muito incongruentes com as hipóteses atuais de relacionamento para a ordem Siluriformes. Portanto, essa classe de caracteres poderá ser empregada em estudos filogenéticos futuros, desde que seja criteriosamente aplicada, evitando-se interpretações equivocadas.

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CERTIFICADO

Certificamos que o Protocolo nº **52/07-CEEA**, sobre "Estudos filogenéticos na superfamília Loricarioidea (Teleostei, Siluriformes) com base na ultraestrutura dos espermatozóides", sob a responsabilidade de **MARIA ANGÉLICA SPADELLA**, está de acordo com os Princípios Éticos na Experimentação Animal adotado pelo Colégio Brasileiro de Experimentação Animal (COBEA) e foi aprovado "Ad referendum" da ***COMISSÃO DE ÉTICA NA EXPERIMENTAÇÃO ANIMAL*** (CEEA), nesta data.

Botucatu, 13 de julho de 2007.

Prof. Dr. MARCELO RAZERA BARUFFI
Presidente - CEEA

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Secretária – CEEA

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