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ANÁLISE COMPARADA DE CARACTERES REPRODUTIVOS E DIVERSIDADE DO GÊNERO *Brachyhypopomus* Mago-Leccia, 1994 NO RIO GRANDE DO SUL.

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“As coisas estão no mundo, só que eu preciso aprender”

Paulinho da Viola.

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Sumário

Resumo

A Ordem Gymnotiformes compreende os peixes popularmente conhecidos como “peixes elétricos”, os quais têm sua distribuição geográfica restrita a região Neotropical. Os Gymnotiformes podem ser encontrados na grande maioria dos ambientes aquáticos existentes em sua área de ocorrência, sendo especialmente diversos e abundantes em áreas alagadas com grandes quantidades de macrófitas aquáticas e no fundo dos canais de grandes rios. A família Hypopomidae está distribuída do rio la Plata, na Argentina (35°S) ao rio Tuira, no Panamá (8°N). A ecologia e história de vida da maioria das espécies de Hypopomidae permanecem praticamente desconhecidas, sendo talvez o gênero *Brachyhypopomus* o grupo mais estudado dentro da família. No Rio Grande do Sul, não existem estudos a respeito da diversidade de espécies pertencentes ao gênero *Brachyhypopomus* exceto por menções ocasionais em revisões e listas de espécies. Este trabalho visa contribuir para o conhecimento da diversidade do gênero *Brachyhypopomus* na região sul do Brasil, bem como para uma melhor compreensão da biologia reprodutiva e ecologia das espécies do gênero. O estudo visa ainda apresentar informações a respeito da morfologia das gônadas e da célula espermática das espécies de *Brachyhypopomus* ocorrentes no Estado. Os dois primeiros capítulos apresentam descrições de duas novas espécies pertencentes ao gênero *Brachyhypopomus* com ocorrência registrada para as bacias da laguna dos Patos, rio Tramandaí e rio Uruguai, no Rio Grande do Sul, além de localidades nos países do Paraguai e Uruguai. O terceiro capítulo aborda características da biologia reprodutiva das espécies *B.*

bombilla e *Brachyhypopomus* “G” n. sp. do Rio Grande do Sul tais como período reprodutivo e suas relações com fatores ambientais, fecundidade, tipo de desova, tamanho de primeira maturação gonadal, proporção sexual e dimorfismo sexual. O capítulo IV apresenta uma descrição histológica das gônadas de machos e fêmeas das espécies *B. bombilla* e *Brachyhypopomus* “G” n. sp. ao longo do ciclo reprodutivo das mesmas. O quinto capítulo contém a descrição da ultraestrutura dos espermatozóides das três espécies de *Brachyhypopomus* registradas para o sul do Brasil e das espécies *Eigenmannia trilineata* e *Gymnotus* aff. *carapo*, visando a observação das variações entre espécies de um mesmo gênero e a comparação dos caracteres descritos entre diferentes famílias de Gymnotiformes. Por fim, o capítulo VI reúne os resultados obtidos nos demais capítulos da tese juntamente com dados disponíveis na literatura com respeito à história de vida e morfologia do espermatozóide das espécies já estudadas da ordem Gymnotiformes, visando à construção de hipóteses sobre a existência de padrões reprodutivos e suas possíveis explicações filogenéticas.

Abstract

The order Gymnotiformes comprise the fishes popularly known as “electric fishes”, which have geographic distribution restricted to the Neotropical region. Gymnotiformes can be found in most aquatic habitats of their area of occurrence and are especially diverse and abundant in floodplains with great amounts of aquatic macrophytes and in deep river channels. The family Hypopomidae is distributed from rio la Plata, in Argentina (35°S) to rio Tuira, in Panamá (8°N). Ecology and life-history of the majority of Hypopomidae species remain mostly unknown. *Brachyhypopomus* is the most extensively studied genera of this family. In Rio Grande do Sul state, there have been no previous studies on *Brachyhypopomus* species diversity except for the occasional mention of the genus in checklists and reviews. This study aims to contribute for the increase on knowledge of *Brachyhypopomus* genus diversity in southern Brazil, as well as, for a better understanding of the reproductive biology and ecology of its species. The study also intends to present information concerning morphology of gonads and sperm cells of the species occurring in the state area. The first two chapters have descriptions of two new species included in the *Brachyhypopomus* genus distributed in the laguna dos Patos, rio Tramandaí and rio Uruguay river basins and also in localities from Uruguay and Paraguay. The third chapter regards features of reproductive biology of the species *B. bombilla* and *Brachyhypopomus* “G” n. sp. from Rio Grande do Sul, such as the reproductive period and its relations with environmental factors, fecundity, spawning type, first

gonadal maturation size, sex proportion and sexual dimorphism. Chapter IV presents a histological description of male and female gonads of the species *B. bombilla* and *Brachyhypopomus* “G” n. sp. throughout their reproductive cycles. The fifth chapter contains a description of the sperm ultrastructure of the three *Brachyhypopomus* species recorded for the southern Brazil and of the species *Eigenmannia trilineata* and *Gymnotus* aff. *carapo*. This chapter aim is to observe the variations among species from the same genus and to compare the described characters among different Gymnotiformes species. At last, chapter VI brings together the results obtained in the remaining chapters, as well as, data available in literature concerning life-history and spermatozoa morphology of the Gymnotiformes species currently studied, hypothesizing the existence of reproductive patterns and its possible phylogenetic explanations.

Introdução Geral

Introdução Geral

A Ordem Gymnotiformes compreende os peixes popularmente conhecidos como “peixes elétricos” assim chamados por serem capazes de gerar uma corrente elétrica, através de tecidos especializados, os quais formam o órgão elétrico. A partir das descargas do órgão elétrico (EODs) e de um sistema complementar de órgãos eletroreceptores distribuídos por quase toda superfície do corpo, o peixe pode perceber o ambiente utilizando estas informações para navegar, forragear e se orientar em águas de pouca visibilidade. As EODs também são determinantes na escolha do habitat por parte dos peixes (Crampton, 1998), em comportamentos predatórios (Westby, 1988), e interações intraespecíficas em geral (Kramer, 1983), especialmente no comportamento reprodutivo (Kramer, 1985), sendo atualmente usadas como base para análise das relações filogenéticas dentro do grupo (Alves-Gomes *et al.* 1995; Alves-Gomes, 2001), identificação de espécies morfologicamente crípticas e para muitas descrições de novas espécies. Sendo as EODs usadas em ambos navegação e reconhecimento de parceiros reprodutivos (isto é, isolamento reprodutivo pre-zigótico), elas possuem um papel central na diversificação evolutiva e especialização ecológica das espécies (Albert & Crampton, 2005).

Os gimnotiformes têm sua distribuição geográfica restrita a região Neotropical, ocorrendo do sul do México ao norte da Argentina (Mago-Leccia, 1994), sendo possível encontrá-los em córregos, pequenos canais e lagoas isoladas, onde podem suportar condições muito baixas de oxigênio durante os períodos de seca. Também são encontrados em lagunas, áreas inundadas nos períodos chuvosos e, principalmente, no fundo dos canais principais dos grandes rios, onde encontram alimento abundante durante todo o ano. A ordem é constituida por 117 espécies, 30 gêneros e 5 famílias (Reis *et al.,* 2003) e, segundo Albert & Crampton (2005), a mesma é consideravelmente mais diversa do que o anteriormente reconhecido, sendo que cerca de apenas 78% das espécies foram formalmente descritas e talvez metade delas permaneça ainda não descoberta na natureza. Os peixes elétricos atingem seu mais alto grau de diversidade nas bacias Amazônica e do Orinoco, formando um componente abundante e rico de espécies com amplo alcance de habitats (Crampton, 1996).

Na ultima década, têm sido realizados enormes avanços no conhecimento da diversidade e filogenia dos peixes gimnotiformes, novos campos sendo abertos para a pesquisa em ecologia e etologia dos sinais elétricos, bem como em evolução das diversas assembléias de espécies tropicais (Albert & Crampton, 2005).

Hypopomidae

A família Hypopomidae, a qual pertence o gênero em questão, foi proposta por Mago-Leccia (1978) e posteriormente revisada por Mago-Leccia (1994), Sullivan (1997), Albert & Campos-da-Paz (1998) e Albert (2001), o qual propôs como novo táxon, a tribo Brachyhypopominae. Albert & Crampton (2003) em recente lista de espécies da família, consideraram válidos um total de 6 gêneros e 25 espécies de hipopomideos, além de citarem a existência de 10 manuscritos em preparação sugerindo nomes de novas espécies.

Esta família está distribuída do rio la Plata, na Argentina (35°S) ao rio Tuira, no Panamá (8°N), estando presente em todos os países da América do Sul exceto o Chile (Albert, 2001). A ecologia e história de vida da maioria das espécies de Hypopomidae são praticamente desconhecidas, sendo talvez o gênero *Brachyhypopomus* o grupo mais estudado dentro da família (Albert & Crampton, 2003).

O gênero *Brachyhypopomus* foi descrito por Mago-Leccia (1994), tendo como espécie tipo *Rhamphichthys brevirostris* Steindachner, 1868 e compreendendo as seis espécies previamente incluídas no gênero *Hypopomus*. Atualmente o gênero é constituído por oito espécies sendo elas *B. brevirostris* (Steindachner, 1868), *B. occidentalis* (Regan, 1914), *B. beebei* (Schultz, 1944), *B. diazi* (Fernández-Yépez, 1972), *B. pinnicaudatus* (Hopkins, 1991), *B. janeiroensis* (Costa & Campos-da-Paz, 1992), *B. jureiae* Triques & Khamis, 2003, and *B. bombilla* Loureiro & Silva, 2006. As espécies do gênero *Brachyhypopomus* ocorrem em todas as maiores bacias hidrográficas Neotropicais e habitam uma grande variedade de ambientes aquáticos (Albert & Crampton, 2005). O gênero prefere águas relativamente rasas, onde exista vegetação mais ou menos densa que possa servir de abrigo ou locais com folhiço no fundo, sendo típico de águas calmas (Alves-Gomes, 1997). As espécies de *Brachyhypopomus* são altamente diversas e abundantes nas planícies de inundação amazônicas, onde constituem uma fração significativa da biomassa, apresentando-se ecologicamente importantes (Crampton, 1996).

No Rio Grande do Sul, não existem estudos a respeito da diversidade de espécies pertencentes ao gênero *Brachyhypopomus* exceto por menções ocasionais em revisões e listas de espécies. A citação mais antiga para o Estado foi feita por Ellis (1913) baseada em espécimes identificados como *Hypopomus brevirostris* (=*Brachyhypopomus brevirostris*) coletados por Herman von Ihering na bacia da laguna dos Patos (Malabarba, 1989). *Brachyhypopomus pinnicaudatus, B. beebei* e uma espécie não descrita do gênero foram citadas por Sullivan (1997) como amplamente distribuídas nas bacias hidrográficas da América do Sul cis-Andina, incluindo análise de material proveniente do Rio Grande do Sul. *Brachyhypopomus brevirostris* e *B. pinnicaudatus* foram citadas como ocorrendo na drenagem do rio de la Plata (Campos-da-Paz, 1997; Albert & Crampton, 2003) e *B. beebei* como ocorrendo na drenagem da laguna dos Patos (Campos-da-Paz, 1997). Após coletas exploratórias realizadas pela equipe do Laboratório de Ictiologia da UFRGS, esta identificou para o estado do Rio Grande do Sul a presença de três espécies do gênero *Brachyhypopomus*: *Brachyhypopomus bombilla,* uma segunda espécies não identificada, e uma terceira espécie que tem sido largamente identificada na literatura como *B. pinnicaudatus,* as duas últimas sendo distribuídas no sul do Brasil e Uruguai nas bacias do rio Uruguai, laguna dos Patos e rio Tramandaí.

Reprodução

A história de vida de um organismo foi conceituada por Begon *et al*. (1987) como sendo o padrão de seu crescimento, diferenciação e, especialmente, reprodução, ao longo de sua vida. De acordo com Nikolskii (1969), os problemas básicos para o estudo de peixes se apóiam entre outros fatores na dinâmica de populações, esta se relacionando com a solução de problemas ligados aos fatores que governam o desenvolvimento individual de peixes como o crescimento, a reprodução e a mortalidade. A reprodução é o processo que assegura a preservação e abundância de uma espécie. O conhecimento de sua biologia reprodutiva é de fundamental importância para a manutenção e proteção de seus estoques naturais, cultivo para fins econômicos e repovoamento de áreas (Agostinho & Júlio Jr, 1999).

Segundo Vazzoler (1996), praticamente todas as estratégias reprodutivas conhecidas podem ser encontradas em espécies de peixes. Diante disto, é possível afirmar que a definição das estratégias reprodutivas e padrões ecológicos apresentados pelos grupos de espécies fornece importantes informações não somente sobre a adaptação dos mesmos ao ambiente em curso, mas também sobre sua evolução.

Morfologia gonadal em nível anatômico e histológico tem sido estudada principalmente em espécies de peixes de interesse econômico com o intuito de identificar ciclos reprodutivos anuais, duração da estação reprodutiva, início da maturidade reprodutiva, ritmos de desova, fecundidade e vários outros aspectos da biologia reprodutiva que podem ser aplicados a questões ligadas a pesca

(Parenti & Grier, 2004). Ainda que existam alguns resultados disponíveis a respeito do desenvolvimento gonadal de gimnotiformes (Barbieri & Barbieri, 1984a, 1984b, 1985; Cognato & Fialho, 2006; Quintana *et al.,* 2004; Giora and Fialho, in press), o número de espécies estudadas e o nível de conhecimento atingido continua extremamente limitado.

Estudos a respeito da estrutura de espermatozóides de peixes teleósteos têm fornecido valiosas informações sobre modificações celulares associadas aos hábitos reprodutivos, sendo realizados principalmente para espécies com inseminação (Jamieson, 1991; Mattei, 1991; Burns *et al.*, 1998). Além disto, pesquisas em ultraestrutura do esperma têm revelado caracteres morfológicos utilizáveis na formação de hipóteses filogenéticas (Jamieson, 1991; Mattei, 1991). Ultraestrutura do espermatozóide de espécies da ordem Gymnotiformes foram realizados para *Apteronotus albifrons* (= *Sternarchus albifrons*) por Jamieson (1991), para *Gymnotus* cf. *anguilaris* e *Brachyhypopomus* cf. *pinnicaudatus* por França *et al*. (2007), e para *Rhamphichthys* cf. *hahni*, *Eigenmannia* cf. *virescens* e *Apteronotus cf. albifrons* por França (2006). Entretanto, muitas questões ainda permanecem e mais estudos são necessários para um melhor entendimento da evolução do espermatozóide em gimnotiformes (França, 2006).

Aspectos da biologia reprodutiva, ecologia e dinâmica populacional de espécies de gimnotiformes ainda têm sido relativamente pouco estudados (Hopkins, 1974a, 1974 b; Kirschbaum, 1975, 1979, 1984, 2000; Schwassmann, 1976; Barbieri & Barbieri, 1982, 1983a, 1983b, 1984a, 1984b, 1985; Provenzano, 1984; Hagedorn, 1988; Kirschbaum & Schugardt, 2002; Cognato & Fialho, 2006; Giora & Fialho in press), a falta de informações básicas a esse respeito, especialmente para espécies ocorrentes no limite sul da distribuição da ordem (sul do Brasil, Uruguai e Argentina) sendo um obstáculo para o melhor conhecimento destes peixes.

No estado do Rio Grande do Sul a ausência total de estudos a respeito do gênero *Brachyhypopomus* tem levado a subestimativa da diversidade do mesmo e a falta de subsídios para sua conservação e de seus habitats.

Objetivos

Este trabalho tem como objetivos:

- definir a diversidade de espécies do gênero *Brachyhypopomus* no Rio Grande do Sul através da descrição de novas espécies;
- estudar a reprodução das espécies *B. bombilla* e *Brachyhypopomus* “G” n. sp., analisando o período reprodutivo, fecundidade, proporção sexual, tamanho de primeira maturação gonadal e tipo de desova, além de estabelecer possíveis relações entre o ciclo reprodutivo das mesmas e aspectos abióticos e alimentares;
- descrever, através de análises histológicas, o desenvolvimento gonadal de machos e fêmeas das espécies *B. bombilla* e

Brachyhypopomus “G” n. sp. ao longo das diversas fases do período reprodutivo;

- descrever a ultraestrutura e a morfologia de espermatozóides das três espécies de *Brachyhypopomus* ocorrentes no Estado, além das espécies *Eigenmannia trilineata* e *Gymnotus* aff. *carapo* utilizando os métodos de microscopia eletrônica de transmissão (MET) e microscopia eletrônica de varredura (MEV);
- comparar a ultraestrutura e a morfologia dos espermatozóides das espécies de *Brachyhypopomus*, família Hypopomidae, com a das espécies *E. trilineata*, família Sternopygidae, e *G.* aff. *carapo*, família Gymnotidae, verificando possíveis padrões e/ou variações morfológicas;
- reunir estas informações com as já disponíveis para outras espécies, a fim de avaliar a evolução das características reprodutivas em um contexto filogenético e de fornecer subsídios para estudos sistemáticos em gimnotiformes.

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Capítulo I

Brachyhypopomus draco, a new sexually dimorphic species of Neotropical electric fish from southern South America (Gymnotiformes: Hypopomidae)

Brachyhypopomus draco, a new sexually dimorphic species of Neotropical electric fish from southern South America (Gymnotiformes: Hypopomidae)

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Brachyhypopomus draco, new species, is described from central, southern and coastal regions of Rio Grande do Sul state, Brazil, and Uruguay. It is diagnosed from congeners by, among other characters, the shape of the distal portion of the caudal filament in mature males, which during the reproductive period forms a distinct paddle shape structure.

Brachyhypopomus draco, espécie nova, é descrita para as regiões central, sul e costeira do estado do Rio Grande do Sul, Brasil, e Uruguai. Ela é diagnosticada de seus congêneres, entre outros caracteres, pelo formato da porção final do filamento caudal de machos maduros durante o período reprodutivo, que forma uma estrutura distinta em forma de remo.

Key words: Electric Organ Discharge, EOD, rio Uruguay, laguna dos Patos, rio Tramandaí.

Introduction

The family Hypopomidae, proposed by Mago-Leccia (1978), is widespread in South American countries, except Chile, and distributed from the río de la Plata in Argentina (35°S) to northern Panama (8°N) (Albert, 2001). The genus *Brachyhypopomus* was described by Mago-Leccia (1994) to comprise six species that previously belonged to *Hypopomus* and that share the characters: short snout, absence of mesocoracoid bridge, short and crescent-shaped maxillary bone, and posterior nares closer to eyes than to snout tip. Later, Albert (2001) redefined the genus based on four synapomorphies: premaxilla gracile with a curved anterior margin and forming a distinct angle with the maxilla in lateral view, dentary gracile, body cavity with 16-17 precaudal vertebrae, single transitional vertebra. Albert (2001) maintained the same six species cited by Mago-Leccia (1994) in the genus. Additionally he listed seven undescribed species and recognized five clades within *Brachyhypopomus* that were previously proposed by Sulivan (1997).

The six *Brachyhypopomus* species recognized as valid by Mago-Leccia (1994) and Albert (2001) are: *B. brevirostris* (Steindachner, 1868), which occurs in Eastern South America from the Orinoco to la Plata drainages; *B. occidentalis* (Regan, 1914), which occurs in Panama, and in several trans-Andean drainages of north eastern South America, including the Magdalena, Atrato, Catatumbo and Pacific slope rivers; *B. beebei* (Schultz, 1944), which is widespread through tropical areas east of Andes; *B. diazi* (Fernández-Yépez, 1972), which is restricted to the Caribbean littoral of Northwest Venezuela, from the río Tocuyo to río Alpargaton and also the northern Llanos in the Orinoco basin; *B. pinnicaudatus* (Hopkins, 1991), which is widespread through cis-Andean South America, including the Guianas, the Orinoco and Amazon basins, and much of the rio Paraná-Paraguay-Uruguay drainage as far south as the río de la Plata; and *B. janeiroensis* (Costa & Campos-da-Paz, 1992), which is known only from the São João and Paraíba do Sul river basins in Rio de Janeiro state (Albert & Crampton, 2003).

Subsequently, Triques & Khamis (2003) described *B. jureiae,* which is known to occur in the Una do Prelado river system in southern Brazil, and also from Ribeira de Iguape drainage, near Iguape, São Paulo (Triques & Khamis, 2003; F. Lima & W. Crampton pers. obs.). Loureiro & Silva (2006) described *B. bombilla*, which is distributed throughout the middle rio Uruguay tributaries and the laguna dos Patos and lagoa Mirim systems in eastern Uruguay.

In Rio Grande do Sul state, southern Brazil, there have been no previous studies of the species diversity of *Brachyhypopomus*, excepting the occasional mention of the genus in checklists and reviews. The oldest citation was by Ellis (1913), based on specimens identified as *Hypopomus brevirostris* (=*Brachyhypopomus brevirostris*) collected by Herman von Ihering in the laguna dos Patos drainage (Malabarba, 1989). *Brachyhypopomus pinnicaudatus, B. beebei* and an undescribed species have been quoted as widely distributed in cis-Andean South American basins by Sullivan (1997), including material from Rio Grande do Sul state. *Brachyhypopomus brevirostris* and *B. pinnicaudatus* have been cited to occur in the rio de la Plata drainage (Campos-da-Paz, 1997; Albert & Crampton, 2003), and *B. beebei* from laguna dos Patos drainage (Campos-da-Paz, 1997).

Based upon extensive sampling in Rio Grande do Sul, our studies revealed the presence of three *Brachyhypopomus* species in this region: *B. bombilla*; a second species that has been largely identified as *B. pinnicaudatus* in the literature; and a third and new species that is widespread in the rio Uruguay, laguna dos Patos and rio Tramandaí drainages in southern Brazil and Uruguay. This latter species is described herein.

Materials and Methods

Comparative data from *B. pinnicaudatus, B. bombilla* and *B. jureiae* were taken directly from the type specimens (see Comparative Material). Data from *B. brevirostris, B. occidentalis, B. beebei, B. diazi* and *B. janeiroensis* were compiled from original descriptions. Morphometric data were taken as point-to-point linear distances using digital calipers to the nearest 0.1 mm. Measurements were analyzed as percentage of length to the end of the anal fin (LEA), measured as the distance from tip of the snout to posterior end of anal fin base, or as percentage of head length (HL), measured from tip of snout to posterior margin of bony operculum. Total length was not used as a numerator in morphometric body relationships because of the high number of specimens with a damaged and/or regenerated caudal filament. Regenerated parts of the body were not measured and their meristic data were not recorded. The other measurements used were: caudal filament length, measured from the end of anal fin base to the tip of caudal filament; snout to anal-fin origin, from tip of snout to origin of anal-fin base; depth of caudal filament, measured at the posterior end of anal-fin base; longest anal-fin ray, from base to tip of the longest ray; pectoral-fin length, from the dorsal border of the fin base where it contacts the cleithrum to tip of the longest ray; body depth, measured at anal-fin origin; snout length, from tip of snout to anterior margin of orbit; gape width, between lateral margins of mouth gape; orbital diameter, between anterior and posterior margins of the orbit; interorbital distance, shortest distance between margins of orbits; posterior nare to eye, from posterior margin of posterior nare to anterior margin of orbit; branchial aperture, from posterodorsal to anteroventral extent of branchial fold; head width at operculum, largest width measured at bony operculum; head width at eyes, head width measured at center of eyes. Pectoral and anal-fin ray counts were taken directly from ethanol preserved specimens under a stereo-microscope. Vertebrae were counted from radiographed specimens including the four vertebrae of the Weberian Apparatus. Apparently, Albert (2001) did not include the vertebrae of the Weberian Apparatus in the precaudal counts; therefore, four vertebrae were added to Albert's (2001) counts for comparison. Osteological data were taken from cleared-and-stained specimens following Taylor & Van Dyke (1985). Sex and the stage of sexual maturity were determined by dissection, following Cognato & Fialho (2006).

Fish specimens belong to the collections of Departamento de Zoologia, Universidade Federal do Rio Grande do Sul, Porto Alegre (UFRGS), Museu de Ciências e Tecnologia, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre (MCP), Universidade Federal de Minas Gerais, Belo Horizonte (DZUFMG), Museu de Zoologia, Universidade de São Paulo, São Paulo (MZUSP), Museu Nacional, Rio de Janeiro (MNRJ), Faculdad de Ciências Naturales, Montevideo (ZVC-P), Academy of Natural Sciences of Philadelphia, Philadelphia (ANSP), National Museum of Natural History, Washington D.C. (USNM), California Academy of Sciences, San Francisco (CAS), University of Michigan Museum of Zoology, Ann Arbor (UMMZ).

Electric Organ Discharge (EOD) recordings were taken from fishes obtained from rio Uruguay, rio Tramandaí and laguna dos Patos drainages. Specimens were held in water from the capture locality at 27ºC +/- 0.5 ºC for at least 12 hours before the recording. All recordings were made in an aquarium maintained at 27ºC +/- 0.1 ºC, containing water from the capture locality (or nearby). Each specimen was allowed 10 minutes to acclimate before recording.

Signals were picked up from silver/silver-chloride electrodes placed posterior and anterior to the fish and with a ground contact in the center. Individual fish were placed within a nylon-mesh sock. The electrodes were connected to a custom-built AC-coupled differential amplifier with a frequency response of +/- 3 dB from 0.2 Hz-110 kHz (Wells & Crampton, 2006). EODs were digitized using an Edirol UA5 A-D converter at a sampling rate of 96 kHz and resolution of 24 bits. Recordings of resting repetition rate were taken during daylight hours between 1000 and 1400 from fish held in nylon-mesh socks under subdued lighting. Recordings of nocturnal activity were taken from 1-3 hrs after sunset from single specimens held in 10 l buckets containing water from the source locality, and water hyacinths. An electrode was placed in the center of this tank and the fish was allowed unrestricted movement before and during the recording. All signal analyses were conducted using custom-written MATLAB or Java software designed by W. Crampton. EOD durations were calculated with the beginning and end of the EOD taken at a 1% threshold of the amplitude of the dominant positive phase (P1) following normalization to the P1 amplitude. EODs recorded from specimens with more than 10% damage to the caudal filament were not included for waveform analyses.

Calculations of EOD repetition rate were taken by measuring all successive inter-pulse intervals over one minute recordings. The EOD rate for each fish was characterized by three parameters. 1. The mean pulse rate in Hz calculated as the reciprocal of the mean of all IPIs (in seconds). 2. The standard deviation (SD) around the mean pulse rate in Hz. This measure of variance indicates the stability of the EOD repetition rate. 3. The coefficient of variance (CV) calculated as a percentage (mean/SD*100). Because the CV is weighted by the mean, it is used for standardized comparisons of the relative stability of the pulse rate over a wide range of rates. The mean and SD of these three measures were then calculated among all recorded adult or post-larval specimens, and are presented here to summarize diurnal and nocturnal EOD activity.

Results

Brachyhypopomus draco, new species

(Figs. 1, 2)

Holotype. MCP 41540, 1 (male, 137.39 mm LEA), Brazil, Rio Grande do Sul, Parque Estadual de Itapuã, lagoa Verde (30°22’52.4”S - 51°01’25”W), 12 Jan 2004, D. Cognato, L. R. Malabarba, C. E. Machado, R. Q. Carvalho.

Paratypes. All from Brazil, Rio Grande do Sul. **Laguna dos Patos drainage:** MCP 41539, 1 (male, 147.12 mm LEA), collected with the holotype. UFRGS 8888, 1 (male, 126.43 mm LEA), same locality of the holotype, 18 Jan 2006, J. Giora, A. P. Dufech & J. Ferrer. MNRJ 30916, 2 (1 male, 151.87 mm LEA, 1 female, 128.42 mm LEA), same locality of the holotype, 9 Feb 2004, D. Cognato, M. Azevedo, A. Schaan & C. Hiroshi. MCP 41538, 2 (1 male, 88.49 mm LEA, 1 female, 91.76 mm LEA), same locality of the holotype, 30 Jun 2004, D. Cognato & A. Schaan. MNRJ 30917, 1 (male, 150.27 mm LEA), MZUSP 94429, 1 (male, 139.13 mm LEA), same locality of the holotype, 9 Sep 2004, D. Cognato, M. Schossler & A. Schaan. MCP 41537, 1 (female, 108.40 mm LEA), same locality of the holotype, 10 Oct 2003, D. Cognato & A. Schaan. MNRJ 30918, 1 (male, 138.20 mm LEA), same locality of the holotype, 11 Nov 2003, D. Cognato, J. Giora, J. Anza & A. P. Dufech. UFRGS 732, 1 (female, 78.02 mm LEA), Viamão, arroio Alexandrina (30°06’00”S - 50°57’00”W), 7 Sep 1980, L. R. Malabarba. UFRGS 820, 1 (male, 93.77 mm LEA), Guaíba, açude near BR290 highway (30°07’00”S - 51°20’00”W), 4 Nov 1979, L. R. Malabarba, C. F. M. Santos & P. A. Buckup. UFRGS 822, 1 (male, 92.56 mm LEA), Guaíba, açude in BR290 highway, near junction with BR116 highway (30°03’00”S - 51°21’00”W), 18 Jan 1980, P. A. Buckup & C. Souto. UFRGS 6526, 1 (male, 102.21 mm LEA), UFRGS 6525, 1 (male, 100.69 mm LEA), Eldorado do Sul, artificial canal near BR290 highway (30°02’55”S - 51°23’34”W), 10 Dec 2003, W. Crampton & L. R. Malabarba. UFRGS 4317, 1 (male, 50.01 mm LEA), Viamão, arroio Itapuã (30°15’00”S - 51°02’00”W), 01 Mar 1988, F. Becker & L. R. Malabarba. **Rio Tramandaí drainage:** UFRGS 6486, 1 (male, 85.37 mm LEA), UFRGS 6488, 1 (male, 98.12 mm LEA), UFRGS 6489, 1 (male, 100.80 mm LEA), UFRGS 6490, 1 (female, 95.54 mm LEA), UFRGS 6491, 1 (male, 80.94 mm LEA), Capão da Canoa, creek parallel to Estrada do Mar road (29°43’09”S - 50°56’00”W), 20 Nov 2003, L. R. Malabarba, J. Giora, J. Anza & D. Cognato. MNRJ 30915, 2 (1 male, 69.93 mm LEA, 1 female, 85.29 mm LEA), Cidreira, lagoa Fortaleza (30°08’58”S

- 50°14’30”W), 11 Jul 2003, L. R. Malabarba. UFRGS 6671, 3 (2 males, 85.75-107.03 mm LEA, 1 female, 96.37 mm LEA), Terra de Areia, creek into rio Três Forquilhas, along the road RS486 (29°33’22”S - 50°04’19”W), 20 Nov 2003, L. R. Malabarba, J. Giora, J. Anza & D. Cognato. **Rio Uruguay drainage:** UFRGS 6748, 1 (male, 90.84 mm LEA), Rosário do Sul, Sanga do Jacaré, BR290 (30°12’04”S - 55°03’17”W), 18 Jun 2004, D. Cognato, W. Crampton, J. Giora & D. Rocha. MZUSP 94428, 8 (2 males, 95.64-126.77 mm LEA, 6 females, 51.97-103.94 mm LEA), UFRGS 6753, 1 (male, 88.35 mm LEA), Rosário do Sul, stream tributary of arroio Gueromana, BR290 (30°01’00”S - 55°23’18”W), 19 Jun 2004, D. Cognato, W. Crampton, J. Giora & D. Rocha. UFRGS 6785, 4 (2 males, 79.31-105.13 mm LEA, 2 females, 84.54-85.32 mm LEA), UFRGS 6494, 1 (male, 92.56 mm LEA), UFRGS 6497, 1 (male, 90.12 mm LEA), Rosário do Sul, sanga do Jacaré, BR290 (30°12’42”S - 55°03’17”W), 26 Nov 2003, L. R. Malabarba, J. Giora, D. Cognato, G. Neves & J. Ferrer. UFRGS 6789, 4 (2 males, 106.52-126.36 mm LEA, 2 females, 71.58-80-06 mm LEA), Rosário do Sul, stream tributary of arroio Gueromana, BR290 (30°01’00”S - 55°23’18”W), 26 Nov 2003, L. R. Malabarba, J. Giora, D. Cognato, G. Neves & J. Ferrer. UFRGS 6507, 1 (male, 92.94 mm LEA), UFRGS 6509, 1 (male, 92.56 mm LEA), UFRGS 6511, 1 (male, 98.32 mm LEA), São Gabriel, stream tributary of arroio Piraí, BR290 (30°18’56”S - 54°24’22”W), 26 Nov 2003, L. R. Malabarba, J. Giora, D. Cognato, G. Neves & J. Ferrer.

Non-type material. The following specimens were used to record the distribution of the new species, but are not used in the species description and are not part of the type series. However, they do not differ in morphometric or meristic characteristics from the type specimens. **Brazil, Rio Grande do Sul:** MCP 20215, 5, and MCP 20217, 3, Eldorado do Sul; MCP 20711, 1, Osório; UFRGS 7686, 30, Viamão, and UFRGS 7707, 3, Viamão; UFRGS 8475, 4, Pantano Grande - Rio Pardo; UFRGS 8938, 1, rio Tramandaí; UFRGS 6780, 11, São Gabriel; UFRGS 6750, 14, Rosário do Sul - Alegrete; UFRGS 8263, 28, Charqueadas. **Paraguay:** USNM 181483, 5, Pueblo Ybytymi, Departamiento Paraguarí; ANSP 170412, 1, Deparatamiento Missiones; ANSP 175180, 1, Departamiento Missiones. **Uruguay**: ZVC-P 2727, 1, Departamiento Artigas, lagoa Redonda.

Diagnosis. *Brachyhypopomus draco* can be distinguished from all described congeners by the extreme broadening of the distal portion of the caudal filament in males during the reproductive period to form a distinct paddle shaped structure (*vs.* moderately broadened distally in *B. pinnicaudatus* and *B. brevirostris* into paddle-shaped structures, and versus broadened along most of the length of the caudal filament in *B. occidentalis*, *B. diazi, B. beebei, B. janeiroensis*, and in several undescribed species of the genus). *Brachyhypopomus draco* can be further diagnosed from other species of the genus by the following characters: caudal filament length 17.3-35.2% of LEA (*vs.* 36.5-50.0% in *B. jureiae*; 33.0-50.0% in *B. janeiroensis*, and 13.3-20.0% in *B. bombilla*); pectoral fin ray length 4.1-5.8% of LEA (*vs.* 6.7-7.9% in *B. pinnicaudatus*); body depth 8.8-12.2% of

LEA (*vs.* 12.1-16.2% in *B. pinnicaudatus*); snout length 20.7-30.0% of HL (*vs.* 31.7-32.2% in *B. jureiae*); gape width 9.5-16.5% of HL (*vs.* 17.8-23.1% in *B. pinnicaudatus*); interorbital distance 22.7-35% of HL (*vs.* 14.9-20.5% in *B. bombilla*); branchial aperture 16.5-26% of HL (*vs.* 24.9-31.0% in *B. pinnicaudatus*); head width at operculum 48.4-65% of HL (*vs.* 60.0-73.0% in *B. pinnicaudatus*); head width at eyes 31.3-45.6% of HL (*vs.* 24.7-31.7% in *B. bombilla*); number of anal fin rays 155-198 (*vs.* 188-211 in *B. jureiae*; 251-295 in *B. brevirostris*; 214-228 in *B. beebei*, and 200-240 in *B occidentalis*); upper jaw equal to lower jaw (*vs.* upper jaw slightly longer than lower jaw in *B. bombilla, B. occidentalis, B. diazi, B. brevirostris* and *B. jureiae*).

Description. Morphometric data are presented in Table 1. Head conical, nearly triangular in lateral view, snout short, eyes small, mouth terminal with upper jaw equal to lower jaw, no teeth in both jaws. Body slender, slightly laterally compressed. Dorsal profile straight from snout to supraoccipital, slightly convex posteriorly to caudal filament. Ventral profile gently convex from lower jaw to anal-fin origin, slightly convex to nearly straight along anal-fin base. Highest body depth located posterior to anal-fin origin. Body depth increasing smoothly from head to body region near the 30th anal-fin ray, clearly gradually decreasing from that point to caudal filament. Caudal filament moderately short, slender and laterally compressed in females, juveniles and males in non-reproductive period; caudal filament paddle-shaped in sexually mature males (see sexual dimorphism, below). Cycloid scales covering uniformly the body, except head and fins. Scales smaller on all anterior quarter portion of body; posterior scales at dorsal and medial body regions two to four times larger in diameter than anterior ones; smallest scales covering region of anal-fin pterygiophores. First anterior perforated scale of lateral line above pectoral fin origin and lateral line extending to caudal filament tip, hardly discernible. Branchial aperture small and slightly anterior to pectoral fin origin. Anus with the presence of urogenital papilla in males and females, although less developed in juveniles. Pectoral fins rounded with pigmented rays and perpendicular insertion; pectoral-fin rays i-ii + 13-15 (15-17 total pectoral-fin rays, n = 47, median = 16). Anal fin relatively long with vii-xii + 148-186 rays (155-198 total anal-fin rays, n = 41, mean = 181.3) which are pigmented. Anal-fin origin located posterior to posterior edge of pectoral fin. Precaudal vertebrae 21-23 (20-22 anterior, 1-2 transitional; n= 6).

Coloration in life. General body color brownish varying from dark brown to yellowish brown, dark brown near dorsal midline and clearing ventrally. A highly variable number of dark brown bands, well delineated or not, nearly perpendicular or oblique to longitudinal body axis, running posterodorsally from base of anal-fin rays to nearby lateral line and occurring from head to tip of caudal filament. Anal and pectoral-fin rays speckled brown, with hyaline inter-radial membranes. Adults and juveniles exhibit the same coloration pattern.

Secondary sexual dimorphism. During reproductive period, established through monthly variation analysis of male and female gonads in a *B. draco* population, males undergo hypertrophy of the distal portion of caudal filament. This has been observed between August and December in a population studied from the type-locality (A. Schaan, J. Giora and C. Fialho, in preparation). Extremely vertical broadening and lateral compression give the caudal filament a paddle-like shape. After the reproductive period, this structure regresses until the caudal filament resembles those of females and juveniles. In addition, adult males are significantly larger than females.

Electric organ discharge. *Brachyhypopomus draco* generates a continuous train of pulse-type EODs. Adult specimens (with developing or fully developed gonads) (72.6-105 mm LEA) exhibited the following EOD parameters. The mean EOD repetition rate (per individual) during the day ranged from 15.7-24.6 Hz (mean among all specimens, 19.6 Hz, SD 2.5, n = 11 individuals fishes), with a standard deviation (SD) of 0.4-1.2 Hz (mean 0.7, SD 0.3, n = 11), and coefficient of variance of 1.8-7.0 % (mean 3.9 %, SD 1.88, n = 11). The lowest and highest absolute pulse rates recorded from all diurnal recordings were 13.7 and 29.2 Hz respectively. During the hours of peak foraging activity, 1-3 hours after sunset, the mean EOD repetition rate (per individual) ranged from 34.2-45.8 Hz (mean among all specimens 38.6 Hz, SD 5.2, n = 6) with a standard deviation of 2.8-10.8 Hz (mean 2.9, SD 1.1, n = 6) and a coefficient of variation of 8.3-27.3 % (mean 20.7 %, n = 6). The lowest and highest absolute pulse rates recorded from all nocturnal recordings were 8.4 and 80.2 Hz respectively.

In sum, adult specimens of *B. draco* exhibited a distinct increase in pulse rate from the resting day-time state (mean 19.6 Hz) to the nocturnal active state (mean 38.6 Hz). The coefficient of variation of pulse rate during nocturnal activity (mean 20.7 %) was considerably higher than during the day (mean 3.9%) reflecting the greater variability in pulse rate during foraging. Disturbances such as minor vibrations provoked novelty responses, sudden increases in the resting pulse rate from the baseline rate (Fig. 3). The approximately stable resting diurnal EOD punctuated by novelty responses results in a right-skewed distribution of inter-pulse intervals in a histogram (Fig. 3). Some specimens of *B. draco* displayed spontaneous complete cessations of the EOD for periods of up to 1 minute. Normal EOD activity was resumed after these interruptions.

The EODs waveforms of adult specimens of *B. draco* comprised two components of alternating polarity (P1 and P2) *sensu* Crampton & Albert (2006), and varied in duration from 1.172-1.992 ms (mean 1.578, SD 0.147, n = 23) (Fig. 4). The Peak Power Frequency (PPF) (see Fig. 4) of these EODs varied from 0.6958-0.8942 kHz (mean 0.7707, SD 0.0534, n = 23).

We did not encounter evidence for obvious sexual differences in the EOD waveforms of *B. draco*, despite evidence for this phenomenon in other species of *Brachyhypopomus* (*e.g*. *B. occidentalis*, Hagedorn, 1985, Shumway & Zelick, 1988; *B. pinnicaudatus* Stoddard *et al*., 2003; *B. brevirostris*, Kawasaki & Heiligenberg, 1989, Crampton, 1996). Where a sexual difference exists, this is manifest as an elongation of the second negative waveform phase, P2 and a corresponding reduction in the PPF of the EOD. We observed no consistent EOD waveform shape differences. Likewise, we observed no significant difference between the PPF of five sexually mature males (0.7504-0.8942 kHz, mean 0.8178, SD 0.0675) and 2 sexually mature females (0.7385-0.7949 kHz, mean 0.7667, SD 0.0399) (2-sample T-test, df = 5, n = 7, P = 0.38). The observation of a lower PPF in mature females than males contrasts with cases of sexual EOD differences in *Brachyhypopomus*, where sexually mature males invariably display lower PPFs. Nonetheless, we did note that the Spectral Power Densities of the two sexually mature females exhibited a more rapid decline from the PPF than in the five sexually mature males (Fig. 4). Males and females with developing gonads also exhibited no obvious difference in waveform shape, and no significant difference in the PPF (males: 0.6958-0.8362 Hz, mean 0.7518, SD 0.0460, n = 10; females: 0.7111-0.8194 kHz, mean 0.7642, SD 0.0422, n = 6) (2-sample T-test, df = 14, n = 16, P = 0.59). All recorded specimens had been held in social isolation for several days before they were recorded. This is known to minimize the hormonally-induced modulation of the P2 phase in sexually mature males (Stoddard *et al*., 2003). Further investigation is required to explore whether EOD differences emerge in males that are exposed to normal social stimuli (*i.e.* the presence of sexually mature conspecifics).

All recordings presented here were made during the southern summer (December) when ambient water temperature is typically in the range 24 – 30º C. During the winter, water temperatures in Rio Grande do Sul decline to as low as 10º C. We noted that these seasonal temperature changes have a significant impact on the waveform duration and shape of the EODs of *B. draco*. The PPF of the EOD declines substantially, and the P2 phase becomes diminished in relative amplitude. We will present detailed observations on this phenomenon elsewhere. Loureiro & Silva (2006) discuss the effect of temperature on the EODs of *B. bombilla*.

Distribution. *Brachyhypopomus draco* is widely known from central, southern and coastal regions of Rio Grande do Sul state, Brazil, and Uruguay. It is known from three drainages: laguna dos Patos, rio Uruguay and rio Tramandaí, and was also found at two localities in Paraguay (USNM 181483; ANSP 170412; ANSP 175180).

Habitat. *Brachyhypopomus draco* inhabits river edges, slow-moving creeks, lagoons and flooded areas with muddy or sandy bottom and abundant emergent or floating vegetation. The species was particularly abundant in the type locality, a lagoon (30°22’52, 4”S e 51°01’25”W) inside a state preserved area, Parque Estadual de Itapuã in Rio Grande do Sul, Brazil. The lagoon is approximately 4 hectares in area and reaches 1 meter in depth. It has a muddy bottom, abundant macrophytes and vegetal material in various stages of decomposition, and is surrounded by psammophilous forest with strong vegetational influence from Atlantic forest formations (Fig. 6). At the type locality, *B. draco* was found occurring together with *Gymnotus* aff. *carapo*. At other collecting sites throughout Rio Grande do Sul state the species was collected along with the gymnotiforms

Eigenmannia trilineata, *B. bombilla* and another undescribed *Brachyhypopomus* species (see Comparative Material).

Etymology. Name “draco”, from the Greek “drakon” meaning dragon, in reference to the shape of distal portion of caudal filament in mature males, similar to that illustrated in these imaginary creatures.

Discussion

The new species is referred to the genus *Brachyhypopomus*, since it conforms to diagnoses of the genus proposed by Mago-Leccia (1994) and Albert (2001). The new species lacks a mesocoracoid bridge, presents a short snout, a short and crescent-shaped maxillary bone, and posterior nares closer to eyes than to snout tip, as proposed in Mago-Leccia’s (1994) diagnosis.

Albert (2001) considered the characters used by Mago-Leccia (1994) ambiguous in his analysis, and proposed four other characters to diagnose the genus. *Brachyhypopomus draco* possesses two of these: the premaxilla gracile with a curved anterior margin and forming a distinct angle with the maxilla in lateral view, and the dentary gracile. The two other characters used by Albert (2001) were the presence of a single transitional vertebra and body cavity with 16-17 precaudal vertebrae (20-21, with the addition of C1-C4).

We observed 21-23 precaudal vertebrae in *B. draco* (20-22 anterior, 1-2 transitional; n= 6). The number of transitional vertebrae exhibited variability, with three of six specimens bearing one transitional vertebra; two bearing one transitional vertebra, and one vertebra associated to a rib (but not completely developed, and not attached to the vertebra); and one bearing two transitional vertebrae. Variability in the number of precaudal and transitional vertebrae has also been observed in *B. bombilla* (Loureiro & Silva, 2006).

From a study of both morphological and molecular sequence data, Sullivan (1997) diagnosed five species groups within *Brachyhypopomus*, discussed later by Albert (2001): the *B. brevirostris* group, consisting of *B. brevirostris* and one undescribed species; the *B. beebei* group, including *B. beebei, B. pinnicaudatus, B. janeiroensis,* and two undescribed species, and the *B. occidentalis* group, including *B. occidentalis* and *B. diazi.* Within the *B. beebei* species group, two subgroups were established: one clade consisting of two undescribed species, and another consisting of *B. beebei* and *B. pinnicaudatus. Brachyhypopomus draco* can be included in the *B. beebei* species group*,* sharing all the group characteristics: lower jaw equal to upper jaw; ventral ethmoid reduced; fourth supraorbital lateral line pore close to vertical through posterior nares; and supraorbital lateral line canal not attached to frontal rostral to the orbit. Nevertheless, *B. draco* cannot be included in any of the *B. beebei* subgroups because it does not share any of their characteristics.

Although *B. draco* is more closely related to *B. beebei, B. pinnicaudatus,* and *B. janeiroensis*, included in the *B. beebei* group, it can be easily distinguished from these species. In addition to the presence of the paddle-shaped tail in mature males and the morphometric and meristc characters presented in diagnosis, the coloration pattern of *B. draco* also differs from these *Brachyhypopomus* species. According to the diagnoses presented by Sullivan (1997), *B. beebei* has a narrow depigmented stripe running along the posterior half of upper dorsum, *B. pinnicaudatus* has a nearly solid dark brown pigment over the dorsum broken by fine unpigmented reticulations, and *B. janeiroensis* lacks lateral bands along sides of the body in mature individuals. All these color patterns diverge from *B. draco* that presents a dark brown dorsal surface with a variable number of darker bands, nearly perpendicular or oblique to the longitudinal body axis. Moreover, the clade consisted of *B. beebei* and *B. pinnicaudatus* is diagnosed by the presence of a tiny hook-like ossification medial to first branchiostegal ray (Sullivan, 1997; Albert, 2001), which is absent in *B. draco*.

The undescribed species referred herein as occurring in Rio Grande do Sul can also be diagnosed from *B. draco* by the lack of a paddle-shaped distal portion of caudal filament in mature males, and by the coloration pattern, showing dorsal surface of the body with chocolate-brown stains that form a reticulated drawing contrasting with a yellow background. Furthermore, *B. draco* has the anal-fin origin located posterior to posterior edge of the pectoral fin (versus anal-fin origin located at the same line of posterior edge of the pectoral fin in the undescribed species).

Comparative material. *Brachyhypopomus jureiae*: DZUFMG 011, 2, Paratype, Brazil, São Paulo, rio do Descavado, Juréia Ecological Station; *Brachyhypopomus bombilla:* ZVC-P 6287, 1, Holotype, Uruguay, Rocha Departamiento, rio Cuatro Palmas; ZVC-P 5688, 1, Paratype, Uruguay, rio Quebracho; ZVC-P 5686, 10, 3 CS, Uruguay, Rocha Departamiento, rio San Luiz; ZVC-P 5685, 1, Uruguay, Tacuarembo Departamiento, rio Batovi; MNHN 3210, 1, Paratype, Uruguay, Rocha Departamiento, rio San Luiz; UFRGS 9282, 15, UFRGS 9273, 18, UFRGS 9284, 17, UFRGS 6741, 1, UFRGS 6742, 1, UFRGS 6745, 1, UFRGS 6496, 1, UFRGS 6495, 1, Brazil, Rio Grande do Sul, Rosário do Sul; *Brachyhypopomus pinnicaudatus:* ANSP 163463, Holotype, 1, French Guiana; ANSP 163464, Paratype, 1, French Guiana; USNM 301966, Paratype, 1, French Guiana; USNM 301967, Paratype, 1, French Guiana; USNM 301968, 1, French Guiana; UMMZ 216032, Paratype, 3, French Guiana; *Brachyhypopomus* sp.: UFRGS 6520, 1, UFRGS 6524, 1, UFRGS 8932, 1, Brazil, Rio Grande do Sul, Viamão, Parque Estadual de Itapuã, lagoa Negra; UFRGS 2219, 2, Brazil, Rio Grande do Sul, Santo Antônio da Patrulha; UFRGS 3844, 2, Brazil, Rio Grande do Sul, Capão da Canoa, lagoa dos Quadros; UFRGS 6499, 1, UFRGS 6503, 1, UFRGS 6504, 1, UFRGS 6505, 1, Brazil, Rio Grande do Sul, São Gabriel.

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Table 1. Morphometric data for *Brachyhypopomus draco*. Length to the end of anal fin (LEA); Head length (HL).

Character	Holotype	n	Minimum	Maximum	Mean	L1	L2	S.D.
						95% confidence limit		
LEA (mm)	137.39	44	50	151.9	96.9			24.559
				Percents of LEA				
HL	10.1	44	9.4	14.2	11.4	11.084	11.694	1.004
Caudal filament length	29.1	34	17.3	35.2	25.1	23.324	26.863	5.152
Snout to anal fin origin	18.3	44	17.9	23	20.3	19.93	20.703	1.272
Depth of caudal filament	2.7	44	1.4	3.1	2.1	2.01	2.244	0.386
Longest anal fin ray	3.7	43	2.9	4.4	3.6	3.528	3.739	0.347
Longest pectoral fin ray	4.1	41	4.1	5.8	5	4.852	5.168	0.507
Body depth	9.4	44	8.8	12.2	10.8	10.521	11.085	0.926
				Percents of HL				
Snout length	26.5	50	20.7	30	25.6	25.128	26.086	1.686
Gape width	14.5	50	9.5	16.5	13.7	13.322	14.148	1.454
Orbital diameter	9.5	50	9.4	14.4	12.3	11.903	12.641	1.3
Interorbital distance	23.4	50	22.7	35	28.4	27.602	29.257	2.913
Posterior nare to eye	3.4	49	2.9	5.4	4.2	4.044	4.343	0.526
Branchial aperture	23.3	50	16.5	26	19.9	19.312	20.448	1.999
Head width at operculum	54.1	50	48.4	65	57	55.969	57.962	3.507
Head width at eyes	37.2	50	31.3	45.6	39.9	39.091	40.636	2.718

Fig. 1. Holotype (MCP 41540, male, 137.39 mm LEA) (above) and paratype (MCP 41537, female, 108.40 mm LEA) (below) of *Brachyhypopomus draco*, from Parque Estadual de Itapuã, Rio Grande do Sul, Brazil.

Fig. 2. *Brachyhypopomus draco,* MCP 41540, male, 137.39 mm LEA; a, head; b, tail.

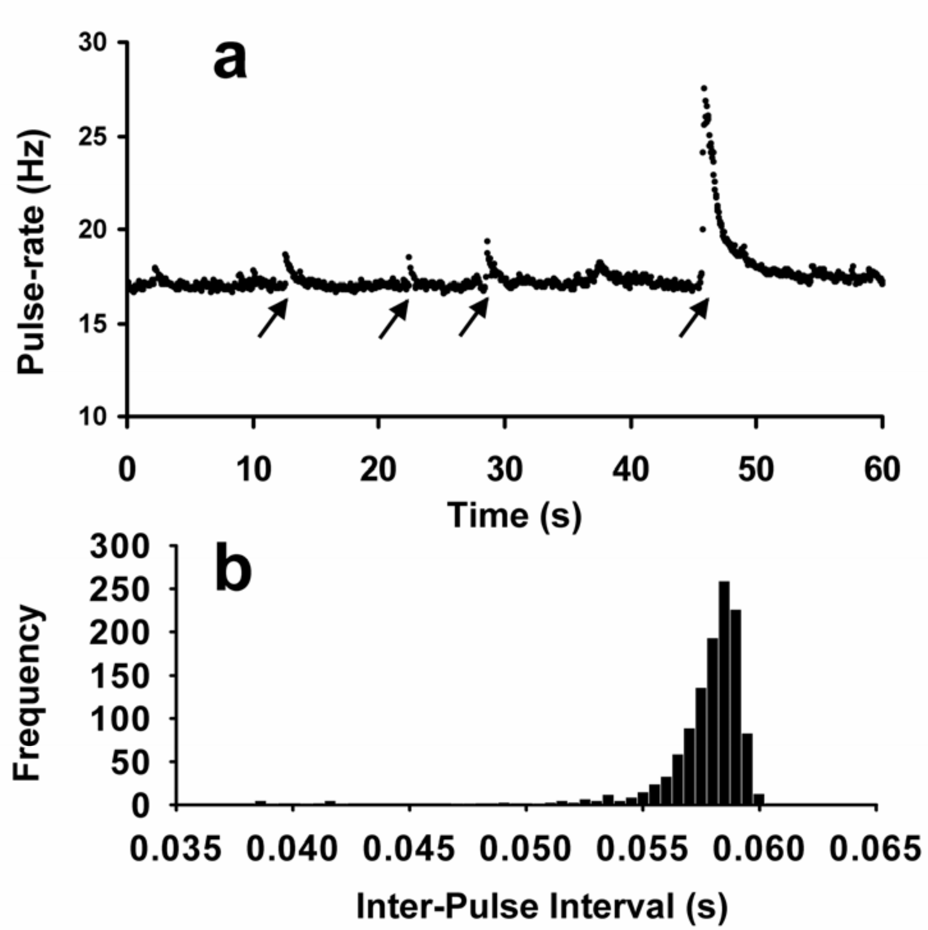

Fig. 3. a. Resting diurnal Electric Organ Discharge (EOD) repetition rate of a single specimen of *Brachyhypopomus draco* (UFRGS 6487). Repetition rate in Hz (pulses per second) is plotted for a 60 s recording. Each point represents the distance from an EOD to the preceding one (Inter-Pulse Interval, IPI). Note the intermittent upward modulations of repetition rate (arrows) in response to disturbances such as minute vibrations. These ‘novelty responses’ are a normal part of EOD activity in the wild. b. Histogram of 1218 IPIs for the 60 s recording, exhibiting a typical right-skewed distribution due to the novelty responses. Repetition rate (Hz) is the reciprocal of Inter-pulse interval (*e.g.* 1/20 Hz = 0.05 s)

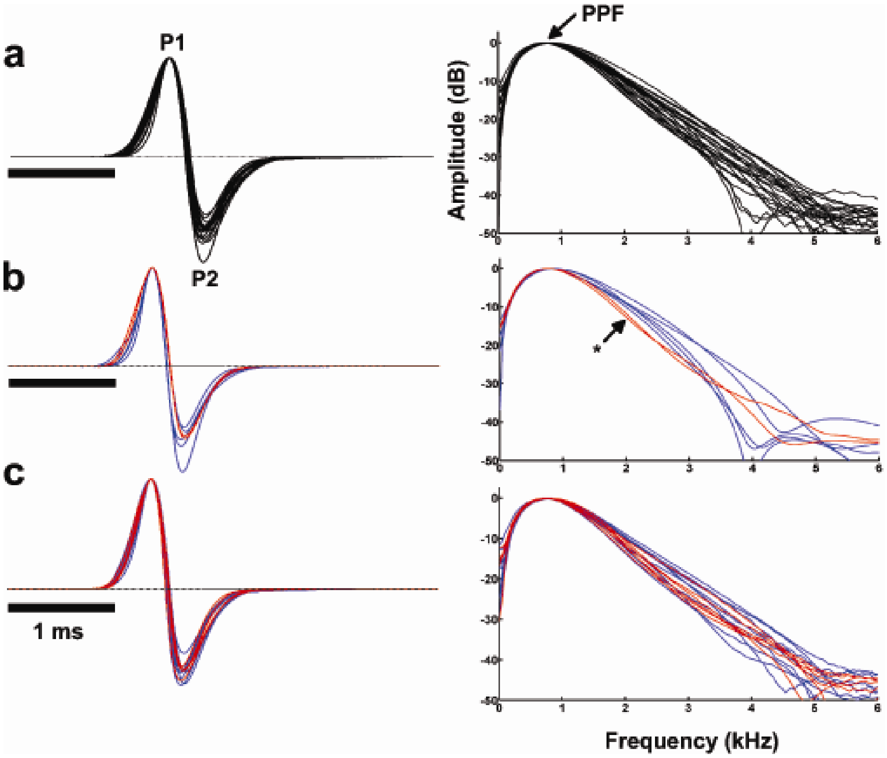

Fig. 4. Electric Organ Discharge (EOD) waveform (left) and Spectral Power Density (right) of adult specimens of *Brachyhypopomus draco* from Rio Grande do Sul state, Brazil. a: All 23 recorded specimens with developing or fully developed gonads (86-135 mm). b: 7 adult specimens with fully mature gonads; 5 male (blue) 2 female (red). c: 16 adult specimens with developing gonads; 10 males (blue), 6 females (red). EODs plotted with head positivity upwards, normalized to the amplitude of the dominant positive phase (P1), and aligned at the P1 peak. Scale bar = 1 ms. Spectral Power Densities (SPDs) were computed from a 65, 536-point Fast Fourier Transform and the Peak Power Frequency (PPF) scaled to 0 dB. Upper and lower ranges of the PPF are marked with arrows. Note the more rapid decline of the SPDs of the two sexually mature females versus males (labeled with asterisk in c) but no obvious sexual difference in EOD waveform shape or PPF.

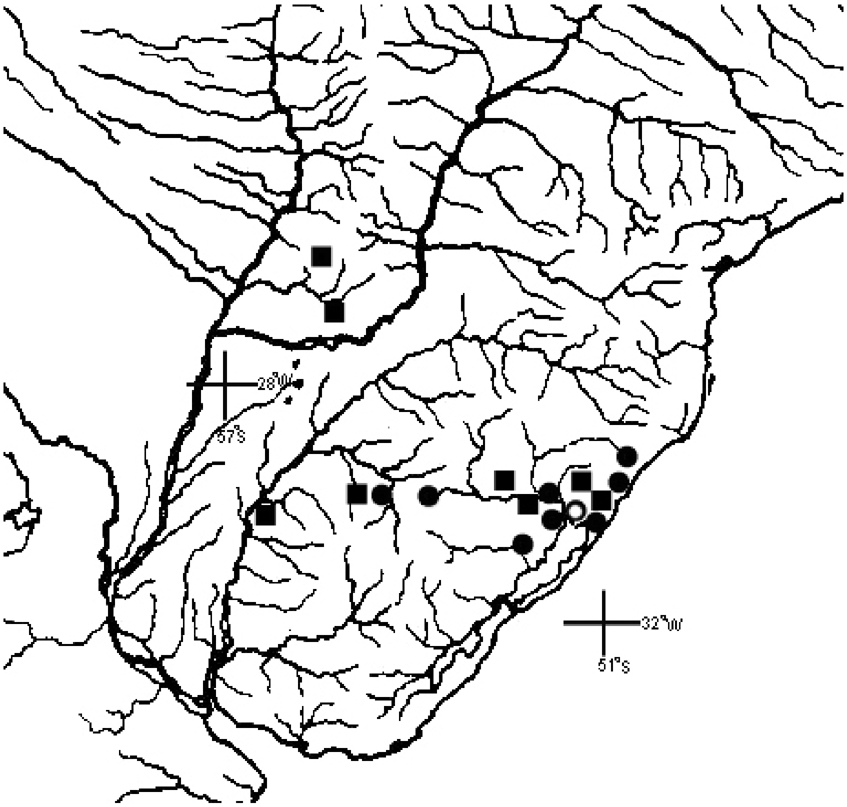

Fig. 5. Map of Southern Brazil, Uruguay, Argentina and Paraguay with the distribution of *Brachyhypopomus draco*. Squares represent non-type specimens. Empty circle represents the type locality.

Fig. 6. Typical habitat of *Brachyhypopomus draco*; marshland in the Parque Estadual de Itapuã, Rio Grande do Sul, Brazil.

Capítulo II

A new species of the Neotropical electric fish genera *Brachyhypopomus* from southern South America (Gymnotiformes: Hypopomidae).

A new species of the Neotropical electric fish genera *Brachyhypopomus* from southern South America (Gymnotiformes: Hypopomidae).

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A new species of *Brachyhypopomus*, from central, southern and coastal regions of Rio Grande do Sul state, Brazil, Uruguay, and Paraguay is described. It is diagnosed from the congeners on the basis of body coloration, meristic and morphometric characters, such as the number of anal-fin rays, position of anal-fin origin in relation to pectoral-fin, distal portion of caudal filament of mature males, and body proportions. The new species has been formerly identified as *B. pinnicaudatus* and is herein distinguished from it.

Uma nova espécie de *Brachyhypopomus* das regiões central, sul e costeira do estado do Rio Grande do Sul, Brasil, Uruguai e Paraguai é descrita. Ela é diagnosticada de seus congêneres baseado em coloração do corpo, caracteres merísticos e morfológicos tais como número de raios da nadadeira anal, posição da origem da nadadeira anal em relação à nadadeira peitoral, porção distal do filamento caudal de machos maduros e proporções corporais. A espécie nova tem sido identificada como *B. pinnicaudatus* e é aqui distinguida da mesma.

Key words: *Brachyhypopomus*, rio Uruguay, laguna dos Patos, rio Tramandaí.

Introduction

The genus *Brachyhypopomus* was described by Mago-Leccia (1994) to comprise six species that previously belonged to *Hypopomus*: *B. occidentalis* (Regan, 1914), *B. beebei* (Schultz, 1944), *B. brevirostris* (Steindachner, 1868), *B. diazi* (Fernández-Yépez, 1972), *B. pinnicaudatus* (Hopkins, 1991), and *B. janeiroensis* (Costa & Campos-da-Paz, 1992). All these quoted species were described for the northern area of Neotropical region, except for *B. janeiroensis*, which occurs in São João and Paraíba do Sul river basins in Rio de Janeiro state (Albert & Crampton, 2003). Recently, with the increase of studies especially on southern Brazil and Uruguay, additional species have been described. *Brachyhypopomus jureiae* Triques & Khamis, 2003 was described as occurring only in rio Una do Prelado system in southern Brazil, *B. bombilla* Loureiro & Silva, 2006 as distributed throughout middle rio Uruguay tributaries and the laguna dos Patos and lagoa Mirim systems in eastern Uruguay, and *B. draco* Giora, Malabarba & Crampton, in press as widely distributed in Rio Grande do

Sul state, southern Brazil, Uruguay, and also in Paraguay. Nevertheless, the low number of studies on Gymnotiformes from southern South America still resulting in the underestimation of the species diversity in this area.

The species *B. pinnicaudatus* was described by Hopkins (1991) on the basis of one population from French Guiana; however, the author has considered the species to be widespread throughout tropical South America. Sullivan (1997), in an unpublished review, considered *B. pinnicaudatus* widely distributed in the Amazon and Paraná/Paraguay drainage basins as well as coastal drainages of southern Brazil and Uruguay, including material from Rio Grande do Sul state. Campos-da-Paz (1997) and Albert & Crampton (2003) referred *B. pinnicaudatus* as distributed throughout eastern South America from rio Catumbo basin, Orinoco and Guianas to rio de la Plata basin.

Based upon extensive sampling in Rio Grande do Sul state, our studies revealed the presence of three *Brachyhypopomus* species in this region: *B. bombilla*, *B. draco*, and a third species, widespread in rio Uruguay, laguna dos Patos and rio Tramandaí drainages in southern Brazil and Uruguay, which has been largely identified as *B. pinnicaudatus* in the literature. We have compared this population identified as *B. pinnicaudatus* with the type series and found it to be a new species. The misapplication of names and the existence of groups of species concealed under a unique specific name has been a problem for a better understanding of the real diversity of species in almost all gymnotiform genera. The present study aims to describe this new *Brachyhypopomus* species from southern Brazil.

Materials and Methods

Comparative data from *B. pinnicaudatus, B. bombilla*, *B. draco* and *B. jureiae* were taken directly from the type specimens (see Comparative Material). Data from *B. brevirostris, B. occidentalis, B. beebei, B. diazi* and *B. janeiroensis* were compiled from original descriptions. Morphometric data were taken as point-to-point linear distances using digital calipers to the nearest 0.1mm. Measurements were analyzed as percents of length to the end of the anal-fin (LEA), measured as the distance from tip of the snout to posterior end of anal-fin base, or as percents of head length (HL), measured from tip of snout to posterior margin of bony operculum. The total length was not used as a numerator in morphometric body relationships because of the high number of specimens with a damaged and/or regenerated caudal filament. Regenerated parts of the body were not measured and their meristic data were not recorded. The other measurements used were taken following Giora *et al.* (in press). Pectoral and anal-fin ray counts were taken directly from ethanol preserved specimens under a stereo-microscope. Vertebrae were counted from radiographed specimens including the four vertebrae of the Weberian Apparatus. Apparently, Albert (2001) did not include the vertebrae of the Weberian Apparatus in the precaudal counts; therefore, four vertebrae were added to Albert's (2001) counts for comparison. Osteologic data were taken from cleared-and-stained specimens following Taylor & Van Dyke (1985). Sex and the stage of sexual maturity were determined by dissection, following Cognato & Fialho (2006).

Fish specimens belong to the collections of the Departamento de Zoologia, Universidade Federal do Rio Grande do Sul, Porto Alegre (UFRGS), Museu de Ciências e Tecnologia, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre (MCP), Universidade Federal de Minas Gerais, Belo Horizonte (DZUFMG), Museu de Zoologia, Universidade de São Paulo, São Paulo (MZUSP), Museu Nacional, Rio de Janeiro (MNRJ), Faculdad de Ciências Naturales, Montevideo (ZVC-P), Academy of Natural Sciences of Philadelphia, Philadelphia (ANSP), National Museum of Natural History, Washington D.C. (USNM), California Academy of Sciences, San Francisco (CAS), University of Michigan Museum of Zoology, Ann Arbor (UMMZ).

Results

Brachyhypopomus "G" n. sp., new species

(Fig. 1)

Brachyhypopomus pinnicaudatus [in part]. Sullivan (1997: 243-248) [specimens of *Brachyhypopomus* "G" n. sp. listed under *B. pinnicaudatus*].

Brachyhypopomus sp. Giora *et al.* (in press) [listed in comparative material of *B. draco*].

Holotype: UFRGS 9229 (male, 149.03 mm LEA), Palmares do Sul, chanel connecting lagoa do Casamento and lagoa dos Gateados (aprox. 30°28'S, 50°40'W), 28 Sep 2006, F. Becker.

Paratypes: All from Brazil, Rio Grande do Sul. **Laguna dos Patos drainage:** UFRGS 9580, 1 (male, 139.62 mm LEA), Charqueadas, floaded area near arroio dos Ratos creek (29°57'31.9"S, 51°33'10.1"W), 28 Nov 2005, J. Giora, D. Roca, A. Ribeiro. UFRGS 9581, 2 (1 male, 128.68 mm LEA, 1 female, 126.36 mm LEA), Charqueadas, floaded area near arroio dos Ratos creek (29°57'31.9"S, 51°33'10.1"W), 22 Sep 2005, J. Giora, D. Rocha, R. Hirano. UFRGS 6520, 1 (female, 124.32 mm LEA), UFRGS 6524, 1 (male, 125,82 mm LEA), Viamão, Parque Estadual de Itapuã, lagoa Negra (30°21'35"S, 50°58'34"W), 11 Nov 2003, J. Anza, D. Cognato, A. Dufech & J. Giora. UFRGS 8932, 1 (female, 133.35 mm LEA), Viamão, Parque Estadual de Itapuã, Lagoa Negra (30°21'35"S, 50°58'34"W), 6 Jan 2003, D. Cognato, T. Dias & J. Giora. UFRGS 4240, 5 (3 males, 74.40-97.24 mm LEA, 2 females, 75.04-78.09 mm LEA), Barra do Ribeiro, along the road between BR 116 and Barra do Ribeiro city (30°17'00"S, 51°21'00"W), 21 Oct 1987, L. Malabarba, F. Becker & M. Schneider. UFRGS 5641, 2 (males, 81.73-126.29 mm LEA), Viamão, Parque Estadual de Itapuã, lagoa do Palácio (30°24'10"S, 50°57'25"W), 26 Nov 2002, J. Anza, L. Malabarba, T. Gonçalves & T. Hasper. UFRGS 2219, 2 (1 male, 120.87 mm LEA, 1 female, 80.03 mm LEA), Santo Antônio da Patrulha, creek between BR 290 and Santo Antônio da Patrulha city (29°52'00"S, 50°35'00"W), 3 Mar 1983, L. Malabarba, C. M. Malabarba & R. Reis. **Rio Tramandaí drainage:**

UFRGS 8933, 1 (female, 101.64 mm LEA), Arroio do Sal, creek parallel to Estrada do Mar road (29°30’33”S, 49°53’34”W), 11 Mar 2005, J. Anza, J. Giora, L. R. Malabarba. UFRGS 3844, 2 (1 male, 91.62 mm LEA, 1 female, 83.11 mm LEA), Capão da Canoa, lagoa dos Quadros (29°42’00”S, 50°06’00”W), 14 Nov 1988, L. Malabarba. **Rio Uruguay drainage:** UFRGS 6499, 1 (male, 102.13 mm LEA), UFRGS 6503, 1 (female, 84.12 mm LEA), UFRGS 6504, 1 (male, 123.16 mm LEA), UFRGS 6505, 1 (male, 135.79 mm LEA), UFRGS 6781, 15 (8 males, 59.83-127.17 mm LEA, 7 females, 78.09-127.17 mm LEA), São Gabriel, stream tributary of arroio Piraí, BR290 (30°18’56”S, 54°24’22”W), 26 Nov 2003, L. Malabarba, J. Giora, D. Cognato, G. Neves & J. Ferrer.

Non-type material: The following specimens were used to record the distribution of the new species, but are not used in the species description and are not part of the type series. However, they do not differ in morphometric or meristic characteristics from the type specimens. **Brazil, Rio Grande do Sul:** MCP 9710, 1, Porto Alegre, Foz do Arroio da Faxina; MCP 14177, 1, Eldorado do Sul; MCP 14459, 2, Eldorado do Sul; MCP 14606, 2, Guaíba; MCP 15326, 2, Eldorado do Sul; MCP 11135, 1, Santo Ângelo; USNM 191645, 1, Porto Alegre. **Uruguay:** ZVC – P 5002, 1, Paso Manzagano, Rio Negro; ZVC – P 1992, 1, Paso del Sauce. **Paraguay:** ANSP 185112, 1, Departamiento Cordillera; ANSP 185116, 5, Departamiento Boqueron; ANSP 185114, 1, Presidente Haynes; UMMZ 207817, 1, Concepcion; USNM 232235, 3, Presidente Haynes.

Diagnosis: *Brachyhypopomus* “G” n. sp. is diagnosed from all congeners by the following combination of features: body depth 11.0-14.1% of LEA (*vs*. 10.9-11.0% in *B. jureiae,* 10.1-10.9% in *B. janeiroensis*); branchial aperture 20.4-28.1% of LEA (*vs*. 19.0-19.8% in *B. jureiae*); caudal filament length 19.6-32.2% of LEA (*vs*. 42.3-45.8% in *B. jureiae*, 34.0-39.6% in *B. janeiroensis*); anal-fin ray length 3.6-5.1% of LEA (*vs*. 3.3-3.4% in *B. jureiae*); pectoral-fin length 4.9-6.4% of LEA (*vs*. 4.3-4.6% in *B. jureiae*); snout to anal-fin origin 17.0-21.2% of LEA (vs. 16.1-17.4% in *B. beebei*); number of anal-fin rays 180-216 (*vs*. 214-228 in *B. beebei*, 200-240 in *B. occidentalis*, 251-295 in *B. brevirostris*); upper jaw equal to lower jaw (*vs*. upper jaw slightly longer than lower jaw *B occidentalis, B. diazi, B. brevirostris* and *B. jureiae*).

Brachyhypopomus “G” n. sp. is diagnosed from *B. pinnicaudatus* by the following characters: caudal filament length 19.6-32.2% of LEA (*vs*. 32.3-42.3%); pectoral-fin length 4.9-6.4% of LEA (*vs*. 6.7-7.9%); head length 10.0-12.7% of LEA (*vs*. 12.4-15.6%); number of anal fin rays 180-216 (*vs*. 176-185).

Brachyhypopomus “G” n. sp. is diagnosed from the syntopic species *B. draco* and *B. bombilla* by the following characters: dorsal surface of the body with chocolate-brown stains that are connected forming a reticulated drawing contrasting with the yellow background, *vs*. dark brown dorsal surface with a variable number darker bands, nearly perpendicular or oblique to longitudinal body axis, never being interconnected in *B. draco*, and body background light brown with an irregularly scattered darker brown pigmentation more dense in dorsal half of body in *B. bombilla*; anal-fin origin located at same line of posterior edge of pectoral-fin, *vs*. anal-fin origin located posterior to posterior edge of pectoral-fin in *B. draco*; distal portion of caudal filament moderately broadened vertically and laterally compressed in sexually mature males, *vs*. extreme broadened and forming a distinct paddle shaped structure in *B. draco*, and not broadened in *B. bombilla*; body depth 11.0-14.1% of LEA (*vs*. 8.8-12.2% in *B. draco*, 9.4-11.0% in *B. bombilla*); branchial aperture 20.4-28.1% of LEA (*vs*. 13.8-18.3% in *B. bombilla*); gape 14.4-18.5% of HL (*vs*. 9.5-16.5% in *B. draco*); head width at operculum 52.4-73.2% of HL (*vs*. 48.4-65.0% in *B. draco*, 43.6-51% in *B. bombilla*); head width at center of eyes 31.1-48.1% of HL (*vs*. 24.7-31.7% in *B. bombilla*); interorbital distance 23.3-30.4% of HL (*vs*. 14.9-20.5% in *B. bombilla*); number of anal-fin rays 180-216 (*vs*. 155-198 in *B. draco*); upper jaw equal to lower jaw (*vs*. upper jaw slightly longer than lower jaw in *B. bombilla*); precaudal vertebrae 20-21, 18-19 anterior and 1-2 transitional (*vs.* 21-23, 20-22 anterior and 1-2 transitional in *B. draco*).

Description: Morphometric data are presented in Table 1. Head conical, nearly triangular in lateral view, snout short and blunt, eyes small, mouth terminal with upper jaw length equal to lower jaw, no teeth in both jaws. Body laterally compressed. Dorsal profile convex from snout to caudal filament, most notably at anterior half of body. Ventral profile convex from lower jaw to anterior anal-fin base; nearly straight from that point to caudal filament. Highest body depth located at anal-fin origin. Body depth increasing from head to anal-fin origin, remaining nearly same depth to close to the 40th anal-fin ray, sharply decreasing from that point to caudal filament. Caudal filament moderately short, slender and nearly cylindrical in females, juveniles and males in non-reproductive period; caudal filament vertically broadened and laterally compressed in sexually mature males (see sexual dimorphism, below). Cycloid scales covering body, except head and fins. Scales smaller on anterior portion of body; five to seven series of large scales along mid-lateral portion of body, reducing to two or three series posteriorly. Mid-lateral scales nearly twice larger than dorso-lateral and dorsal scale and four times larger in diameter than anterior ones; smallest scales covering region of anal-fin pterygiophores. First anterior perforated scale of lateral line above pectoral-fin origin; lateral line irregular, not extending to caudal filament. Branchial aperture small and slightly anterior to pectoral-fin origin. Anus with the presence of urogenital papilla in males and females, although less developed in juveniles. Pectoral fins rounded and with perpendicular insertion; pectoral-fin rays i-ii + 13-15 (15-17 total pectoral-fin rays, n = 38, mean = 15.9). Anal-fin relatively long with vi-x + 173-206 rays (180-216 total anal-fin rays, n = 38, mean = 196.2). Anal-fin origin located at same line of posterior edge of pectoral-fin. Precaudal vertebrae 20-21 (18 -19 anterior, 1-2 transitional; n= 5).

Color in Life: General body color varying from light brown to yellow. Head darker than rest of body. Dorsal surface with chocolate-brown stains that are connected forming reticulated drawing contrasting with yellow background. Variable number of brown stripes running from dorsal stains to base of anal fin, roughly defined in middle of body and occurring from head to tip of caudal filament. Anal and pectoral fins speckled with dark brown pigment, with hyaline inter-radial membranes.

Secondary Sexual Dimorphism: The caudal filament of dominant mature males is laterally compressed, especially on its distal portion, differing from that of females, juveniles and non-dominant males, which is usually cylindrical (J. Giora and C. Fialho, in preparation).

Distribution: *Brachyhypopomus* “G” n. sp. is known from laguna dos Patos, rio Uruguay and rio Tramandaí drainages in Rio Grande do Sul state, Brazil, being widely distributed from central, southern and coastal regions of the state. It also occurs in rio Uruguay basin in Uruguay (ZVC–P 5002; ZVC–P 1992) and in rio Paraguay basin in Paraguay (ANSP 185112; ANSP 185116; ANSP 185114; UMMZ 207817; USNM 232235).

Habitat: *Brachyhypopomus* “G” n. sp. inhabits river banks, slow-moving creeks, lagoons, and flooded areas with muddy or sandy bottom; the occurrence of the species is always associated with abundant emergent or floating vegetation. *Brachyhypopomus* “G” n. sp. is sympatric and commonly syntopic with *B. draco*, and more rarely syntopic with *B. bombilla*. Throughout Rio Grande do Sul state the species was also collected along with the gymnotiforms *Eigenmannia trilineata* and *Gymnotus* aff. *carapo*.

Discussion

Brachyhypopomus “G” n. sp. is included in the genus *Brachyhypopomus* by sharing the synapomorphies proposed by Mago-Leccia (1994): lack of mesocoracoid bridge, short snout, short and crescent-shaped maxillary bone, and posterior nares closer to eyes than to snout tip. Moreover, the new species shares two of the four diagnostic features defined by Albert (2001) for *Brachyhypopomus*: the premaxilla gracile with a curved anterior margin and forming a distinct angle with the maxilla in lateral view, and the dentary gracile.

Regarding the species-group established by Sullivan (1997) and latter discussed by Albert (2001), *Brachyhypopomus* “G” n. sp. can be included in the *B. beebei* species group by the presence of lower jaw equal to upper jaw, fourth supraorbital lateral-line pore close to vertical through posterior nares, and supraorbital lateral-line canal not attached to frontal rostral to the orbit. The authors have also described the presence of ventral ethmoid reduced as a diagnostic character for this group of species; although there is no clear definition of what “reduced” means, we found the ventral ethmoid small and possibly comparable to that described condition. The new species cannot be included in any of the *B. beebei* subgroups because it does not share any of their characteristics. *Brachyhypopomus pinnicaudatus* and *B. beebei* were quoted by Sullivan (1997) and Albert (2001) as integrating a clade within *B. beebei* species group diagnosed by the presence of a tiny hook-like ossification medial to first branchiostegal ray (homologous to second brachiostegal ray in species with five rays). *Brachyhypopomus* “G” n. sp. does not show this hook-like ossification and possesses fifth branchiostegal ray differing from the two species mentioned. Moreover, Albert (2001) states that this character is polymorphic in *B. pinnicaudatus*, which can indicate the presence of more than one species referred to this one.

Sullivan (1997) diagnosed *B. pinnicaudatus* by the nearly solid dark brown pigment over dorsum, broken by fine depigmented reticulations, with narrow yellow streak threaded through reticulated pattern, and by the posttemporal completely fused to supracleithrum. *Brachyhypopomus* “G” n. sp. differs from this diagnosis by presenting the posttemporal completely independent from the supracleithrum. From the specimens referred by Sullivan (1997) as *B. pinnicaudatus* from southern South America, at least two lots of specimens belong to *Brachyhypopomus* “G” n. sp. species (UMMZ 207818; USNM 232235, see non-type material), both from Paraguaian locations. *Brachyhypopomus* “G” n. sp. has also been identified as *B. pinnicaudatus* by Albert & Crampton (2003), Campos-da-Paz (1997) and many lists of species from Rio Grande do Sul state river basins (pers. obs.). Nevertheless, the new species herein described can be easily distinguished from *B. pinnicaudatus* by four characters: caudal filament length, pectoral fin length, head length, and number of anal-fin rays. Through analysis of specimens from Peru, French Guiana, Guyana, Bolivia, Paraguay, Uruguay, and Brazil – especially southern and northern Brazilian regions – we have identified a great number of recognizable morphotypes referred as *B. pinnicaudatus*. The results point to a necessity of a review of the species considered to be widespread throughout the Neotropical region as a matter of avoiding misapplied names and underestimation of species diversity. Moreover, the description of a new species with distribution restricted to southern South America reinforces the importance of continuing taxonomic work on Gymnotiformes species from this region, and demonstrates that low latitude areas may be more diverse than previously supposed.

Comparative material: *Brachyhypopomus jureiae*: DZUFMG 011, 2, Paratype, Brazil, São Paulo, rio do Descavado, Jureia Ecological Station; *Brachyhypopomus bombilla:* ZVC-P 6287, 1, Holotype, Uruguay, Rocha Departamiento, rio Cuatro Palmas; ZVC-P 5688, 1, Paratype, Uruguay, rio Quebracho; ZVC-P 5686, 10, 3 CS, Uruguay, Rocha Departamiento, rio San Luiz; ZVC-P 5685, 1, Uruguay, Tacuarembo Departamiento, rio Batovi; MNHN 3210, 1, Paratype, Uruguay, Rocha Departamiento, rio San Luiz; UFRGS 9282, 15, UFRGS 9273, 18, UFRGS 9284, 17, UFRGS 6741, 1, UFRGS 6742, 1, UFRGS 6745, 1, UFRGS 6496, 1, UFRGS 6495, 1, Brazil, Rio Grande do Sul, Rosário do Sul; *Brachyhypopomus pinnicaudatus:* ANSP 163463, Holotype, 1, French Guiana; ANSP 163464, Paratype, 1, French Guiana; USNM 301966, Paratype, 1, French Guiana; USNM 301967, Paratype, 1, French Guiana; USNM 301968, 1, French Guiana; UMMZ 216032, Paratype, 3, French Guiana; *Brachyhypopomus draco*: MCP 41540, Holotype; 1, MCP 41539, Paratype, 1, MNRJ 30916, Paratype, 2, UFRGS 8888, Paratype, 1, MCP 41538, Paratype, 2, MNRJ 30917, Paratype, 1, MZUSP 94429, Paratype, 1, MCP 41537, Paratype, 1, MNRJ 30918, Paratype, 1, Brazil, Rio Grande do Sul, Parque Estadual de Itapuã; UFRGS 732, Paratype, 1, Brazil, Rio Grande do Sul, Viamão; UFRGS 820, Paratype, 1, UFRGS 822, Paratype, 1, Brazil, Rio Grande do Sul, Guaíba; UFRGS 6526, Paratype, 1, UFRGS 6525, Paratype, 1, Brazil, Rio Grande do Sul, Eldorado do Sul; UFRGS 4317, Paratype, 1, Brazil, Rio Grande do Sul, Viamão; UFRGS 6486, Paratype, 1, UFRGS 6488, Paratype, 1, UFRGS 6489, Paratype, 1, UFRGS 6490, Paratype, 1, UFRGS 6491, Paratype, 1, Brazil, Rio Grande do Sul, Capão da Canoa; MNRJ 30915, Paratype, 2, Brazil, Rio Grande do Sul, Cidreira; UFRGS 6671, Paratype, 3, Brazil, Rio Grande do Sul, Terra de Areia; UFRGS 6748, Paratype, 1, MZUSP 94428, Paratype, 8, UFRGS 6753, Paratype, 1, UFRGS 6785, Paratype, 4, UFRGS 6494, Paratype, 1, UFRGS 6497, Paratype, 1, Brazil, Rio Grande do Sul, Rosário do Sul; UFRGS 6507, Paratype, 1, UFRGS 6509, Paratype, 1, UFRGS 6511, Paratype, Brazil, Rio Grande do Sul, São Gabriel.

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Table 1. Morphometric data for *Brachyhypopomus* “G” n. sp. Length to the end of anal fin (LEA); Head length (HL).

Character	Holotype	n	Minimum	Maximum	Mean	L1	L2	S.D.
						95% confidence limit		
LEA (mm)	149.03	38	50	151.9	96.9			24.559
				Percents of LEA				
HL	10.57	38	10.0	12.7	11.3	11.100	11.568	0.713
Caudal filament length	20.15	36	19.6	32.2	24.7	23.644	25.770	3.142
Snout to anal fin origin	17.64	38	17.0	21.2	18.9	18.589	19.263	1.026
Depth of caudal filament	2.31	38	1.6	2.4	2.0	1.966	2.114	0.224
Longest anal fin ray	4.10	38	3.6	5.1	4.5	4.385	4.585	0.304
Longest pectoral fin ray	5.07	38	4.9	6.4	5.6	5.488	5.685	0.300
Body depth	13.12	38	11.0	14.1	12.9	12.593	13.158	0.859
				Percents of HL				
Snout length	25.02	38	23.3	28.2	26.4	25.99	26.724	1.117
Gape width	18.35	38	14.4	18.5	16.2	15.838	16.585	1.138
Orbital diameter	9.65	38	9.0	12.1	10.8	10.496	11.005	0.774
Interorbital distance	30.38	38	23.3	30.4	26.4	25.868	26.902	1.574
Posterior nare to eye	6.30	37	3.4	6.3	5.0	4.755	5.217	0.693
Branchial aperture	27.85	38	20.4	28.1	25.5	24.796	26.246	2.206
Head width at operculum	67.24	38	52.4	73.2	64.9	63.233	66.653	5.202
Head width at eyes	46.22	38	31.1	48.1	41.2	38.899	42.404	3.811

Fig. 1. Holotype (UFRGS 9229, male, 149.03 mm LEA) of *Brachyhypopomus* “G” n. sp., from Palmares do Sul, Rio Grande do Sul, Brazil.

Fig. 2. Sexually dimorphic caudal filament of *Brachyhypopomus* “G” n. sp. (CHAR 0805, male, 139.62 mm LEA, caudal filament length 44.89 mm).

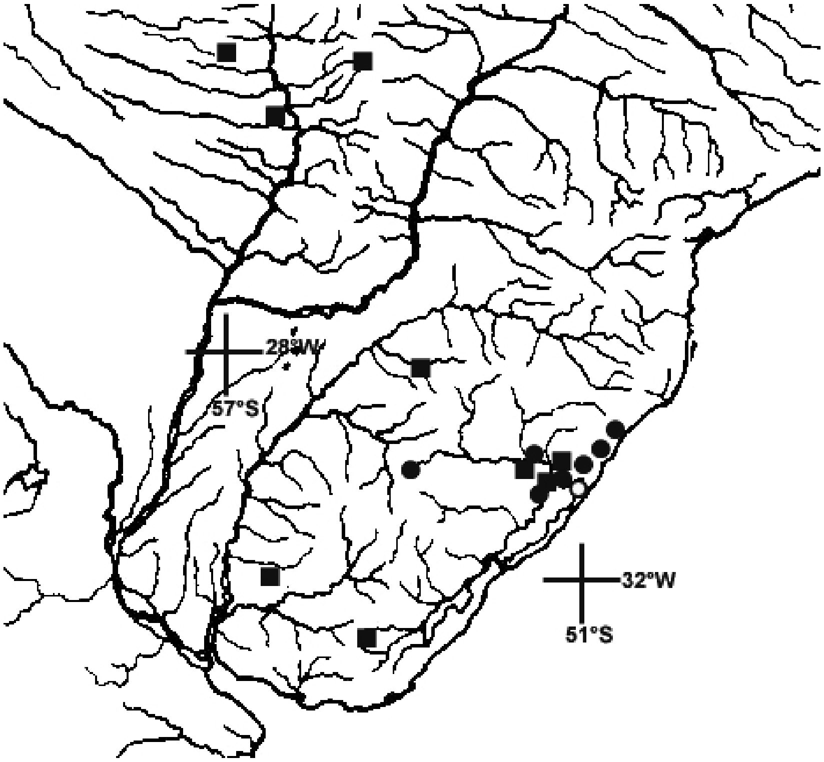

Fig. 3. Map of Southern Brazil, Uruguay, Argentina and Paraguay with the distribution of *Brachyhypopomus* “G” n. sp. Squares represent non-type specimens. Empty circle represents the type locality.

Fig. 4. Typical habitat of *Brachyhypopomus* “G” n. sp.; floaded area near arroio dos Ratos creek, Charqueadas, Rio Grande do Sul, Brazil.

Capítulo III

Reproduction of two species of the Neotropical electric fish *Brachyhypopomus* (Teleostei: Hypopomidae) from southern Brazil.

Reproduction of two species of the Neotropical electric fish *Brachyhypopomus* (Teleostei: Hypopomidae) from southern Brazil.

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This study describes the reproductive biology of the electric fishes *Brachyhypopomus bombilla* and *Brachyhypopomus* “G” from southern Brazil. The reproductive period of *B. bombilla* lasted from October/2004 to January/2005 being related to the increase in photoperiod, whereas male GSI is also related to the oxygen variation. *Brachyhypopomus* “G” reproductive period has occurred from October/2005 to February/2006 being also related to the increase in photoperiod. The relative fecundity was established as 0.21 oocytes per mg total weight for *B. bombilla* and 0.20 oocytes for *Brachyhypopomus* “G”. Both species have shown fractioned spawning. First maturation size estimated as 97.6 mm for females and 93.7 mm for males of *B. bombilla*, and as 104.5 mm for females and 108.0 mm for males of *Brachyhypopomus* “G”. Sex ratio did not differ from 1:1 under an χ^2 test (α= 0.01) in the two species. No sexual dimorphism related to total length was observed; however, a modification on caudal filament was verified in males of *Brachyhypopomus* “G”.

Este estudo descreve a biologia reprodutiva dos peixes elétricos *Brachyhypopomus bombilla* e *Brachyhypopomus* “G” do sul do Brasil. O período reprodutivo da espécie *B. bombilla* se estendeu de Outubro/2004 a Janeiro/2005, sendo relacionado ao aumento do fotoperíodo, tendo o IGS dos machos também sido relacionado às variações de oxigênio. O período reprodutivo de *Brachyhypopomus* “G” ocorreu de Outubro/2005 a Fevereiro/2006, sendo também relacionado ao aumento do fotoperíodo. A fecundidade relativa foi estabelecida como 0.21 ovócitos por mg de peso para *B. bombilla* e 0.20 ovócitos para *Brachyhypopomus* “G”. Ambas espécies apresentaram desova parcelada. O tamanho de primeira maturação gonadal de *B. bombilla* foi estimado como 97.6 mm para fêmeas e 93.7 mm para machos, sendo estimado como 104.5 mm para fêmeas e 108.0 mm para machos de *Brachyhypopomus* “G”. A proporção sexual não diferiu de 1:1 em nenhuma das espécies de acordo com a análise do teste χ^2 (α= 0.01). Não foi observado dimorfismo sexual relacionado ao comprimento total, entretanto foi verificada uma modificação no filamento caudal de machos de *Brachyhypopomus* “G”.

Key words: Reproductive biology, Gymnotiformes, Hypopomidae, *Brachyhypopomus*, environmental factors.

Introduction

Gymnotiformes have a wide geographical range throughout South and Central America, occurring in an incredible diversity of aquatic habitats including river channels, flood-plains, flooded forests, forest streams, cataracts, swamps, coastal creeks, and estuarine reaches (Albert & Crampton, 2003). Of the three South American ostariophysan orders, the Gymnotiformes comprises the smallest number of species and is also the least investigated group as far as systematics and ecology are concerned (Kirschbaum, 1995).

The genus *Brachyhypopomus* is included in the family Hypopomidae, which is widespread from río de la Plata in Argentina (35°S) to northern Panama (8°N) (Albert, 2001). The specimens of the genus prefer slow-moving and shallow waters with dense floating vegetation that can be used as shelter, as well as, sites with leaf mats on the bottom (Alves-Gomes, 1997). *Brachyhypopomus* species are highly diverse and abundant in the Amazon flood-plains, where they constitute a significant biomass portion (Crampton, 1996).

Few studies have brought gymnotiform reproductive aspects (Hopkins, 1974a e b; Kirschbaum, 1975, 1979, 1984, 2000; Schwassmann, 1976; Barbieri & Barbieri, 1982, 1983a, 1983b, 1984a, 1984b, 1985; Provenzano, 1984; Hagedorn, 1988; Kirschbaum & Schugardt, 2002; Cognato & Fialho, 2006; Giora & Fialho in press). The lack of basic information about species distribution, natural history, behavior, ecology and population dynamics, especially on the extreme southern boundary of the Neotropical electric fishes distribution (southern Brazil, Uruguay, and Argentina), is still an obstacle for a better understanding of this order of fishes. With this mind, the present study aims to establish characteristics of the reproductive period, spawning type and fecundity of *Brachyhypopomus bombilla* and *Brachyhypopomus* “G” populations from southern Brazil. Moreover, it aims to test the reproductive period relationships with environmental and alimentary factors, as well as, characteristics of the species population structure such as sex ratio, sexual dimorphism, individual recruitment time and first gonadal maturation size.

Material and Methods

Brachyhypopomus bombilla specimens were sampled monthly from July/2004 to June/2005 in the arroio do Jacaré (30°12’42.8”S 55°03’17.5”W) in Rosário do Sul Municipality, which is inserted in the rio Uruguai drainage. The creek shows muddy bottom and dark water with constant flow and goes through a considerable depth variation during rainy and dry periods. The site has a great number of floating vegetation mainly composed by *Pistia stratiotis*, and edge vegetation mostly composed by reeds and grass. The gymnotiforms *Eigenmannia trilineata, Brachyhypopomus draco* and *Gymnotus* aff*. carapo* could be sampled along with *B. bombilla* at this collect place.

Brachyhypopomus “G” specimens were sampled monthly from April/ 2005 to March/2006 in a flooded area near arroio dos Ratos creek (29°57’31.9”S 51°33’10.1”W) in Charqueadas Municipality, which is inserted in the laguna dos

Patos drainage. The site shows dark and slow-moving water, with hardly any flow, muddy bottom, and depth ranging from 1 to 1.5 meters without great depth variation along the rainy and dry periods. The collect place also possesses substantial amounts of aquatic vegetation such as *Pistia stratiotis, Salvinia auriculata,* and *Polygonum* sp. The marginal vegetation is formed by reeds and bushes. At this sample location, *Brachyhypopomus "G".* could be collected along with the gymnotiforms *Eigenmannia trilineata, Brachyhypopomus draco* and *Gymnotus* aff*. carapo.*

In both sites, the collection was executed under floating vegetation using a dip net and an electric fish finder (Crampton *et al.*, 2007). The specimens were fixed in the field in 10% formalin solution. Water temperature, water conductivity, pH and dissolved O_2 were recorded at the time of collection. Rainfall data were obtained from the Meteorology District of Porto Alegre. Photoperiod was obtained with the Skymap software correlating dates of collection with collecting place coordinates.

In the laboratory, fishes were transferred to 70% ethanol and total length (Lt) in millimetres and total weight (Wt) in grams were measured. Individuals were dissected to record stomach (Ws) and gonad (Wg) weight and to establish the gonadal maturation stage of males and females. Voucher specimens were catalogued in the fish collection of the Departamento de Zoologia, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil (*Brachyhypopomus bombilla* – UFRGS 9284; *Brachyhypopomus* "G" – UFRGS 9200).

Stomach repletion index (RI) and gonadossomatic index (GSI) were estimated following the formula adapted from Santos (1978). These indexes represent the percentile organ weight related to fish total weight: RI = Ws x 100/Wt, and GSI= Wg x 100/Wt. Ws corresponds to stomach weight, Wg to gonad weight and Wt to total weight.

The reproductive period for males and females of both *Brachhyhypopomus* species was established through the analyses of monthly variation of the mean GSI values. The multiple regression with analysis of variance (ANOVA) was applied to verify possible dependence between abiotic (rainfall, photoperiod, temperature, conductivity, pH and dissolved O_2) and the reproductive period as well as alimentary factor (RI) and the reproductive period (Zar, 1999).

The absolute fecundity was estimated counting all vitellogenic oocytes present in the ovaries of females of both species with the highest GSI values. The relative fecundity was determined by the number of counted oocytes per female milligram of weight (Adebisi, 1987). For the determination of the spawning type, the same gonads selected for fecundity analysis were used. A sub-sample of 150 oocytes was removed from each selected gonad and the largest possible oocyte diameter was obtained with observation on a stereomicroscope with a millimetred ocular (Vazzoler, 1996).

The sex ratio was determined by the distribution of male and female frequency during the sampled period. The χ^2 test ($p = 0.05$) was applied to verify the existence of significant differences between the number of males and females of the studied species. The first gonadal maturation size of both *Brachyhypopomus* species males and females was estimated from the distribution of juvenile and adult relative frequencies for total length classes (Vazzoler, 1996). The curve obtained was adjusted according to the expression: Rf = 1- (e^{-aLtb}). Rf corresponds to the relative frequency of adults, *e* to the natural logarithm base, Lt to total length (mm), and *a* and *b* to estimated constants related to curve adjustment. The first gonadal maturation size is considered as corresponding to a frequency of 0.5 (50%) of the adult individuals.

For determination of new individual breeding period, months when larvae were sampled have been recorded. The distribution of relative frequencies of males and females in different total length classes was analysed and tested under χ^2 test ($p = 0.05$) to observe possible sexual dimorphism related to specimen lengths. Relative frequency of *Brachyhypopomus* “G” specimens with vertically broadened and laterally compressed distal portion of the caudal filament was calculated to observe the development of this sexually dimorphic feature along the reproductive period.

Results

Overall 241 specimens of *B. bombilla* were collected: 128 males with total length ranging from 42.08 mm to 146.04 mm, 113 females ranging from 40.21 mm to 132.68 mm, and three larvae ranging from 22.86 mm to 36.94 mm.

Additionally, a total of 211 *Brachyhypopomus* “G” specimens were sampled: 108 males with total length ranging from 42.96 mm to 188.80 mm, 102 females ranging from 45.65 mm to 175.36 mm, and one larva with 24.23 mm of total length.

The reproductive period estimated for *B. bombilla* lasted from October/2004 to January/2005, with the GSI peak occurring in October and November/2004 for males and in November/2004 for females (Fig. 1). For the species *Brachyhypopomus* “G”, the reproductive period was estimated as lasting from October/2005 to February/2006, with male GSI peak occurring in November/2005 and female GSI peak occurring in October/2005 (Fig. 2).

The GSI of *B. bombilla* and *Brachyhypopomus* “G” males and females did not exhibited significant relation to the repletion index (RI). Monthly variation of RI are summarised in Table 1 for *B. bombilla* and in Table 2 for *Brachyhypopomus* *“*G”. Among the tested abiotic factors, the GSI of *B. bombilla* males has presented significant relation to photoperiod and dissolved oxygen variations (photoperiod: F= 11.417, t= 4.437, p = 0.002; dissolved oxygen: F= 13.069, t= 2.722, p = 0.024), while for females it has been significantly related only to photoperiod (F= 12.144, t= 3.485, p = 0.006). The photoperiod variation was also related to *Brachyhypopomus* “G” male and female GSI (males: F= 114.970, t= 10.722, p = 0.000; females: F= 27.006, t= 5.197, p = 0.000). Monthly data of water temperature, pH, conductivity, dissolved oxygen, rainfall, and photoperiod are summarised in Table 3 for *B. bombilla* collecting site and in Table 4 for *Brachyhypopomus* “G” collecting site.

The absolute fecundity had an average value of 587.33 oocytes for the species *B. bombilla* (ranging from 369 to 773 oocytes) for females with total length ranging from 101.13 to 137.41 mm (Table 5). The average relative fecundity of this species was estimated as 0.21 oocytes per mg total weight (Table 5). For *Brachyhypopomus* “G”, average absolute fecundity was established as 589.44 oocytes (ranging from 299 to 799 oocytes) for females with total length from 85.42 a 149.0 mm (Table 6). The average relative fecundity of *Brachyhypopomus* “G” was estimated as 0.20 oocytes per mg total weight (Table 6).

In the two *Brachyhypopomus* species studied herein, analysis of the absolute frequency distribution of vitellogenic oocyte diameter conforms to that of species with oocyte development synchronic in more than two groups, and with a fractional spawning (*B. bombilla* Fig. 3; *Brachyhypopomus* “G” Fig. 4). In both analyses there is a high frequency of store oocytes that will only mature in the next reproductive period, followed by oocyte shares in successive maturation stages, which are eliminated at different times in the reproductive period. The first gonadal maturation size was estimated for *B. bombilla* males as 93.7 mm and for females as 97.6 mm (Fig. 5); and for *Brachyhypopomus* “G” males as 108.0 mm and for females as 104.5 mm (Fig. 6).

The χ^2 test results ($p<0.05$) demonstrate for *B. bombilla* a sex ratio of 1:1 during all sampled months except July/2004, when males were found in higher number than females. Analysing the total number of male and female sampled of this species, the sex ratio was determined as 1:1. *Brachyhypopomus* “G” studied population has shown a sex ratio of 1:1 during all sampled months, as well as in the analyses of total number of males and females. The time of new individual breeding was estimated for the species *B. bombilla* as beginning in December/2004 and lasting until March/2005, these being the months when larvae (December/2004 and March/2005) and male and female included in the lowest length classes were collected. The time of new specimens breeding of *Brachyhypopomus* “G” was established as occurring from December/2005 to March/2006, since one larva was collected in February/2006 and male and female included in the lowest length classes were collected during all these quoted months.

Significant differences in total length related to sexual dimorphism were not observed for any of the studied species. The total length class represented by the 85-mm mean point has been the only class that has differed from 1:1 male and female proportion in *B. bombilla* analyses, females being more abundant than males at this length class (Fig. 7). None of the length classes defined for *Brachyhypopomus* “G” has exhibited significant differences in male and female proportion according to χ^2 test results ($p<0.05$) (Fig. 8).

Males of *Brachyhypopomus “*G” with hypertrophy of the distal portion of caudal filament – vertical broadening and lateral compression of the distal portion of caudal filament – were sampled during all year months except February and March/2006 when no males larger than 130 mm were collected. The highest relative frequencies of males with broadened caudal filament occurred in October and November/2005 (Fig. 9). Total length of specimens with caudal filament hypertrophy ranged from 145.38 mm to 188.8 mm. In spite of it, males with total length included in this range and without hypertrophied caudal filament could be collected along with males with hypertrophy of this structure. No females were observed with caudal filament modification.

Discussion

Determination of the reproductive period is fundamental to the establishment of all other aspects of the biology of the species and population dynamics. According to Provenzano (1984), reproduction in gymnotiforms is controlled by periodic gonadal maturation and regression. *Brachyhypopomus bombilla* and *Brachyhypopomus* “G” have shown cyclical reproductive periods lasting for four and five months respectively, and both periods corresponding to the Southern Hemisphere spring and summer.

Successful reproduction requires an organism to synchronize its reproductive physiology and behavior with events in its environment (Moore & Marler, 1988). Gymnotiformes reproductive cycles have been related to seasonal environment variations typical of the tropical zone of Neotropical area, most species breeding during the rainy season, in which the increase of rainfalls determines high water levels and low water conductivities (Hopkins, 1974a, 1974b; Kirschbaum, 1975, 1979, 1984, 2000; Schwassmann, 1976; Hagedorn, 1988; Kirschbaum & Schugardt, 2002). However, when Gymnotiformes are studied as inhabitants of the temperate zone, close to the southern boundary of their continental distribution, different biogeographical and ecological conditions must be taken into account to understand their natural history (Silva *et al.*, 2007). The reproductive seasonality in fishes of temperate environments, where longer rainfall periods are not defined, is mainly related to temperature, photoperiod and food availability (McKayne, 1984; Payne, 1986). For *Brachyhypopomus pinnicaudatus* and *Gymnotus carapo* from Uruguay, gonad recrudescence can be observed in natural populations coinciding with high temperature means and extreme photoperiod (Silva *et al.*, 2003). In captivity experiments with both species quoted above (Silva *et al.,* 1999; Silva *et al.*, 2002; Ardanaz *et al.,* 2001), as well as, with *B. pinnicaudatus* only (Quintana *et al.*, 2004) also from Uruguay, the gonadal maturation could be achieved through high water temperature aclimatation. Male and female reproductive period of both *Brachyhypopomus* species studied herein have been related to photoperiod variations of the collecting site agreeing with results obtained for other gymnotiform species from Rio Grande do Sul state (Giora & Fialho in press; Cognato & Fialho, 2006). Moreover, reproductive period of Characiformes species from southern Brazil have also been associated with photoperiod increase (Azevedo *et al.*, 2000; Oliveira, 2003; Lampert, 2003; Gonçalves *et al*., 2005). Even though there is a coincidence between both *Brachyhypopomus* reproductive seasons and the year period with the highest temperature means, there was no significant relation between the species GSI variations and water temperatures. According to Köppen system, Brazilian southern region exhibits Cfa climate (humid subtropical) without a well defined rain season – with rains slightly more abundant during winter months - hot summers, and mild winters. For these reasons, circannual patterns of species distributed on this region seem to be more properly comparable with those of temperate climate regions. However, the differences between this two climate zones must be considered to avoid generalizations and waste of important information concerning ecological diversity.

Dissolved oxygen availability is a very important factor for Neotropical electric fishes, being recognized as the principal environmental element that influences the distribution and migration of these fishes (Kramer *et al.*, 1978, Crampton, 1998). For males of *B. bombilla* the GSI was positively related with variation of dissolved oxygen, although it does not appear to be as important for the reproductive period establishment as the photoperiod variations.

Despite the fact that no statistically significant relation between the gonadossomatic and replection indexes was found for both species, *B. bombilla* males and females have shown extremely low values for RI within the months before and after the established reproductive period (July, August, September, May, and June), which are the coldest months of the sampled year. In a study about feeding habits of the same *B. bombilla* population (J. Giora, H. T. Moraes & C. Fialho, in preparation), it was reported that the fishes starve during the months quoted above, since not only extremely low RI indexes were determined but also no alimentary items were found in the specimen stomachs along this period. Moreover, throughout these starving months specimens were found embedded in the creek muddy bottom. For all that, *B. bombilla* can be considered a very seasonal species, which presents hardly any feeding and reproductive activity during winter months.

The absolute and relative fecundities established for both species were quite similar. For *Brachyhypopomus draco*, a relative fecundity of 0.17 oocytes per mg of female weight has been determined (A. Schaan, J. Giora and C. Fialho, in preparation), which is slightly lower than those of the species studied here. According to two other studies with gymnotiforms from southern Brazil, *Gymnotus* aff. *carapo* has shown relative fecundity of 0.20 oocytes (Cognato & Fialho, 2006), which is the same as *Brachyhypopomus* “G”, and *Eigenmannia trilineata* has shown the highest relative fecundity of 0.27 oocytes per mg of female weight (Giora & Fialho, in press). Among several other factors, fecundity depends on the coelomatic cavity capacity of lodge ripe oocytes and of oocytes size (Vazzoler, 1996). Since absolute fecundity only considers the total number of oocytes in the fish gonads, it cannot be used for comparisons among different size species. The great majority of studies regarding reproduction of Gymnotiformes species presents data about absolut fecundity only (Barbieri & Barbieri, 1982; Assunção e Schwassmann, 1995; Kirschbaum & Schugardt, 2002) Due to that, more results concerning relative fecundity of Gymnotiformes are needed for a better discussion and comparison of the results.

Multiple spawning in the same reproductive period can result in a much higher annual reproductive effort than is possible with single spawning (Burt *et al.*, 1988). Besides that, species with fractional spawning are better adapted to unfavourable environmental conditions and could solve problems of competition for spawning sites among females of the same population (Nikolsky, 1969). At the present time, all studied gymnotiform species are considered to be fractional spawners (Barbieri & Barbieri, 1982; Assunção & Schuwasmann, 1995; Kirschbaum & Schugardt, 2002; Quintana *et al.*, 2004; Cognato & Fialho, 2006; A. Schaan, J. Giora and C. Fialho, in preparation; Giora & Fialho, in press). The present work is in agreement with that statement, since *B. bombilla* and *Brachyhypopomus* “G” were also defined as fractional spawners.

According to Kirschbaum & Schugardt (2002), *B. pinnicaudatus* has a quick growth, growing one mm per day over the first months of its life, obtaining minimum size of 10-12 cm for first reproduction after four-five months. The relatively high sizes of first gonadal maturation also explicit a quick growth for males and females of the two studied species, which can be defined as a reproductive strategy of the genus. For a population of *E. trilineata,* in which males reach a total of length of 247.8 mm and females 170.0 mm, the first gonadal maturation sizes were defined as 63.5 mm and 80.5 mm respectively (Giora & Fialho, in press). This fact leads to a much lower growth rate for *E. trilineata* population in comparison with *Brachyhypopomus* species.

Both *Brachyhypopomus* species did not show a predominance of one of the sexes and had 1:1 sex ratio along almost all sampled months and on total number of collected specimens. The same result has been obtained for *B. draco* (A. Schaan, J. Giora and C. Fialho, in preparation) pointing to a possible pattern for the genus. No sexual dimorphism related to the fish total length has been documented; however, *Brachyhypopomus* “G” has exhibited a sexually dimorphic shape of the caudal filament distal portion. The majority of the *Brachyhypopomus* species – such as *B. pinnicaudatus, B. beebei, B. brevirostris, B. occidentalis,* and *B. draco* - possesses some kind of broadening, compression, and/or elongation of the mature male caudal filaments (Hagedorn & Carr, 1985; Hagedorn, 1988; Hopkins *et al.*, 1990; Rapp-Py-Daniel & Cox-Fernandes, 2005; A. Schaan, J. Giora and C. Fialho, in preparation). These morphological modifications of caudal filament can permit the males to have greater eletrocytes than the females (Hagedorn & Carr, 1985) and to accrue more matings (Hagedorn, 1988). It has been reported for a *B. draco* population that males undergo hypertrophy of the distal portion of caudal filament during the reproductive period, after that regressing this structure until the caudal filament resembles those of females and juveniles (A. Schaan, J. Giora and C. Fialho, in preparation). The existence of similar caudal filament regression cannot be established for *Brachyhypopomus* “G”, since males with hypertrophy of this structure could be found during all months in which large males were sampled. The highest frequency of specimens with broadened filament along the reproductive period months shows that the development of this structure probably occurs concomitantly with male gonadal maturation. However, the presence of males with the highest total lengths exhibiting caudal filaments identical to those of females and juveniles suggests a possible dominance system among males, only dominant males undergoing caudal filament hypertrophy. Dominance among males of a same breeding group has been also observed for the species *B. brevisrostris* during agnostic behavior observed in captivity (Kirschbaum & Schugardt, 2002).

The presented data indicate that some reproductive patterns for the order Gymnotiformes and more particularly for the genus *Brachyhypopomus* can be established, although there is great diversity of reproductive strategies among the different genera and species of Neotropical electric fishes. Thus far, more data on additional species mainly from the southern boundary of gymnotiform distribution are needed for a more complete understanding of the reproductive biology of this order of fishes.

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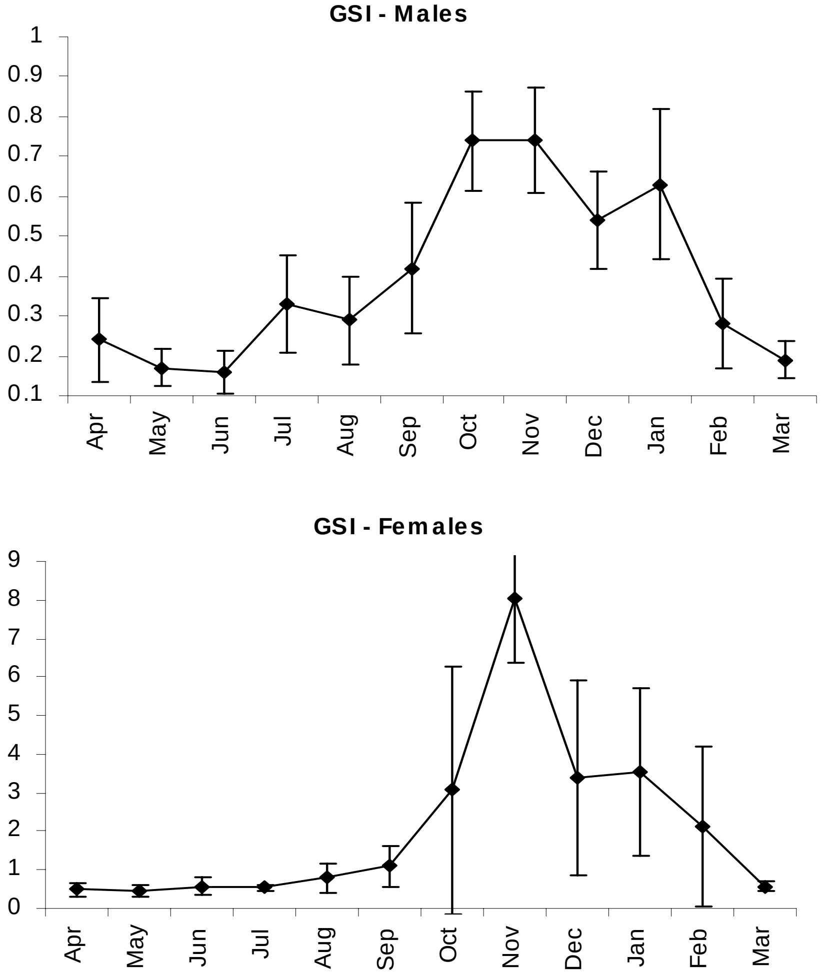

Fig. 1. Monthly variation of mean gonadossomatic index (GSI) for *Brachyhypopomus bombilla* males and females from July/2004 to June/2005. Vertical bars represent the standard deviation.

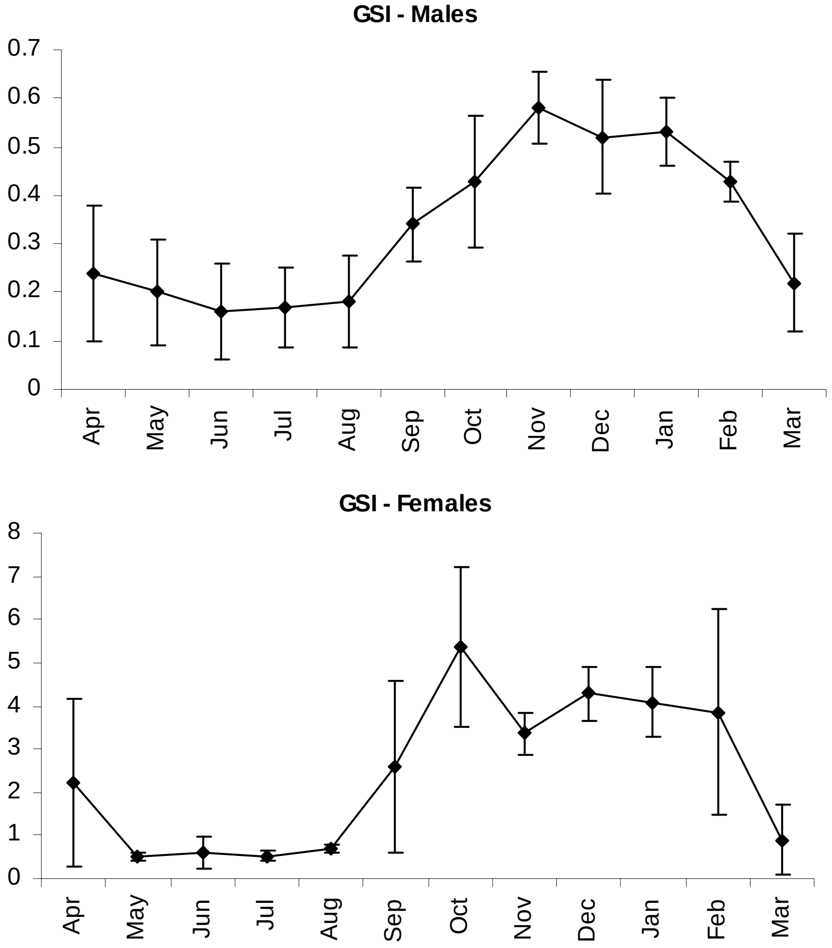

Fig. 2. Monthly variation of mean gonadossomatic index (GSI) for *Brachyhypopomus* “G” males and females from Apryl/2005 to Marh/2006. Vertical bars represent the standard deviation.

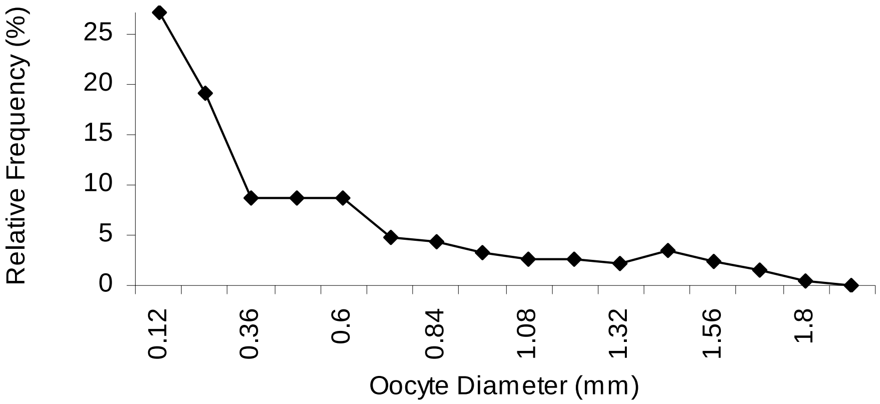

Fig. 3. Relative frequency distribution of oocyte diameters of *Brachyhypopomus bombilla*.

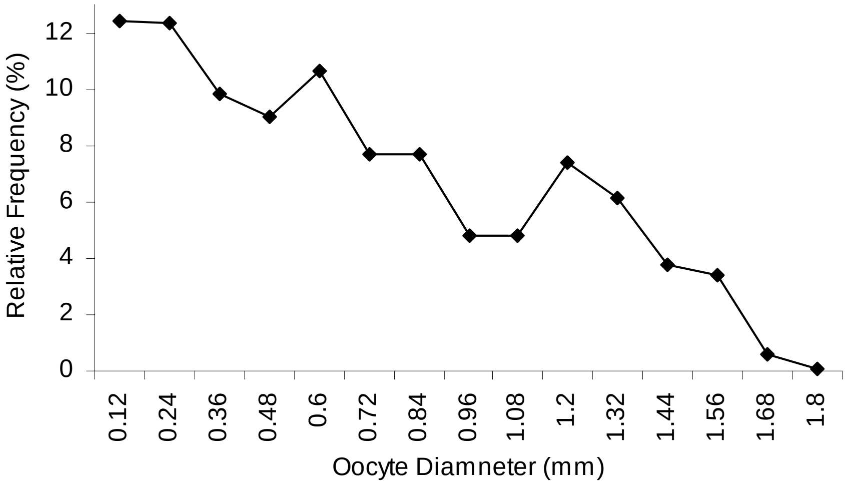

Fig. 4. Relative frequency distribution of oocyte diameters of *Brachyhypopomus* “G”.

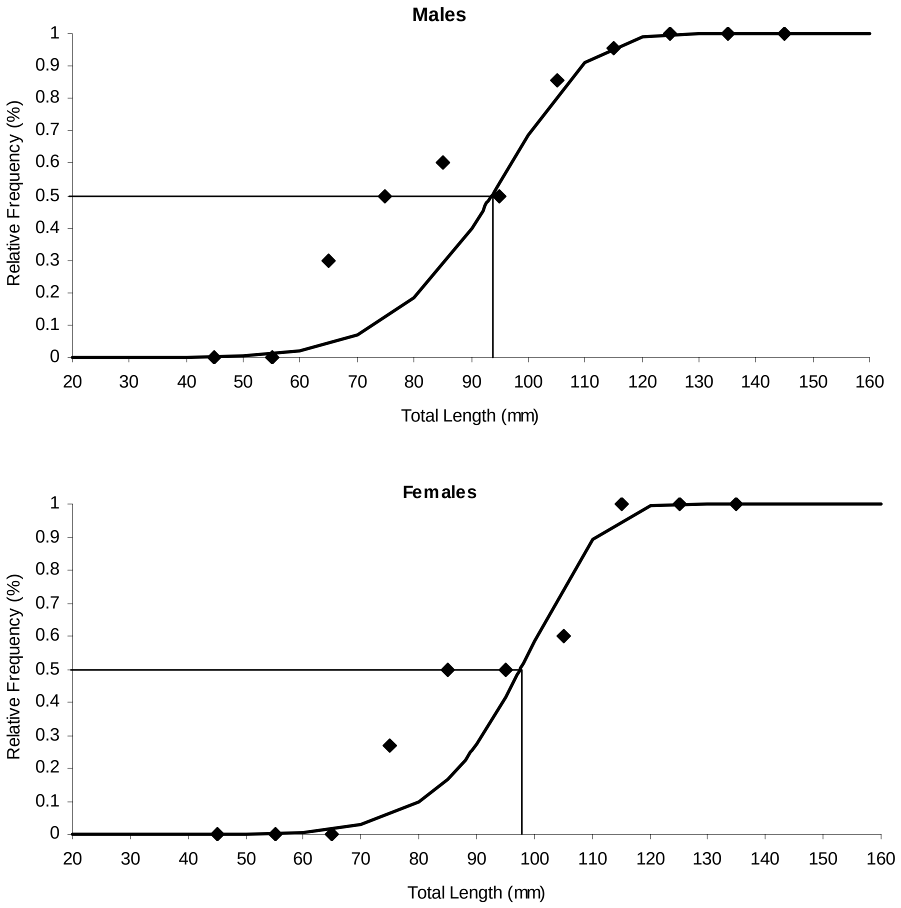

Fig. 5. Distribution of *Brachyhypopomus bombilla* male and female relative frequencies for total length classes. The lines show the point at which 50% of the individuals are considered adults.

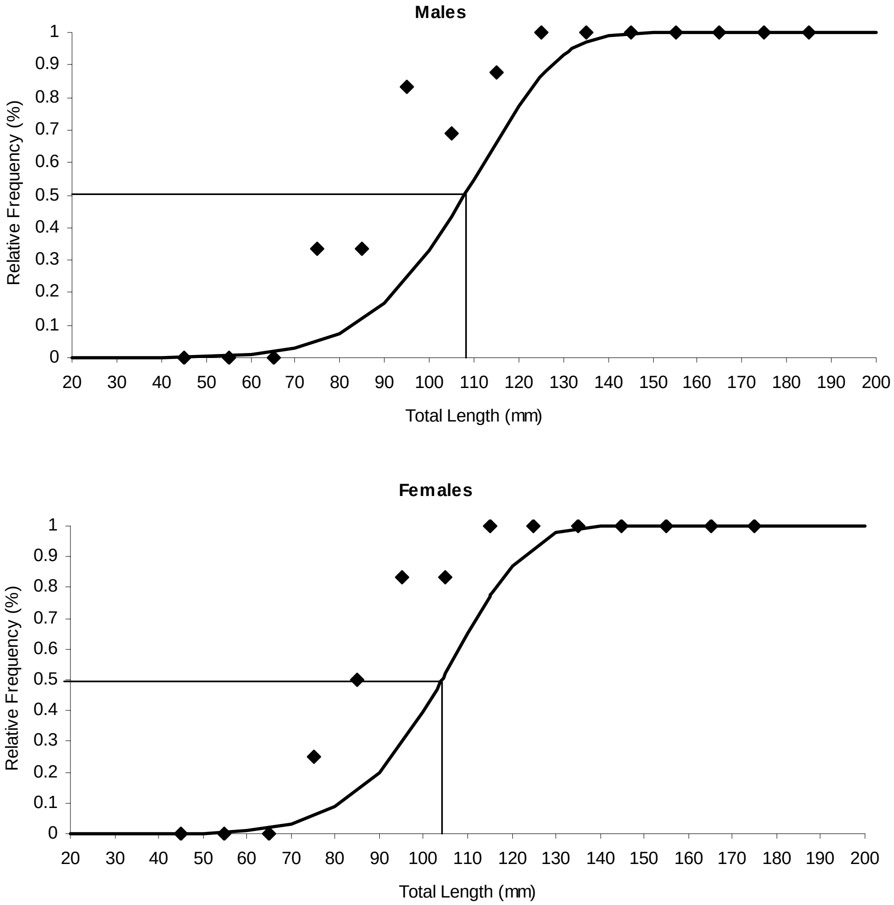

Fig. 6. Distribution of *Brachyhypopomus* “G” male and female relative frequencies for total length classes. The lines show the point at which 50% of the individuals are considered adults.

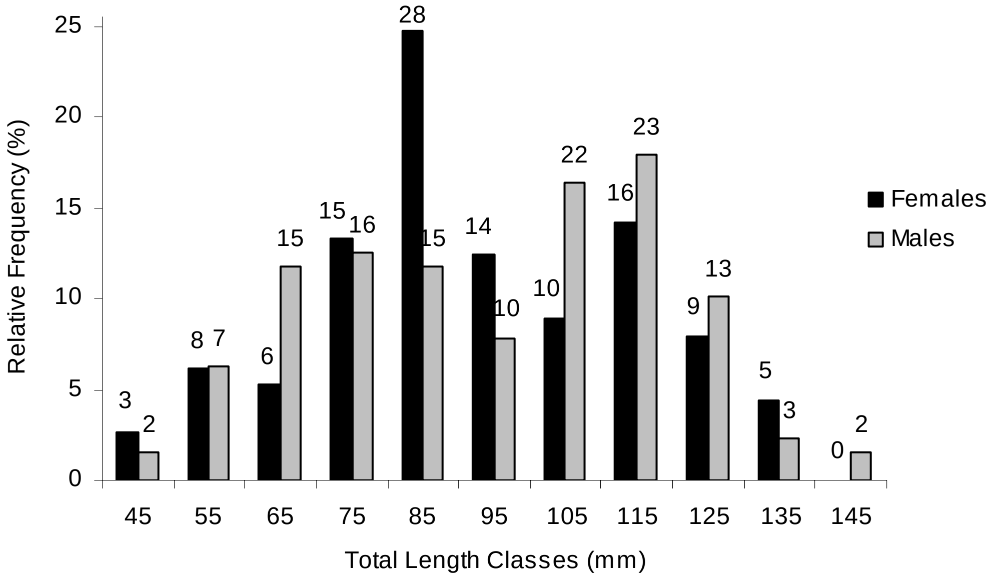

Fig. 7: Relative frequency distribution of *Brachyhypopomus bombilla* males and females for total length classes. Numbers above the columns represent the absolut values.

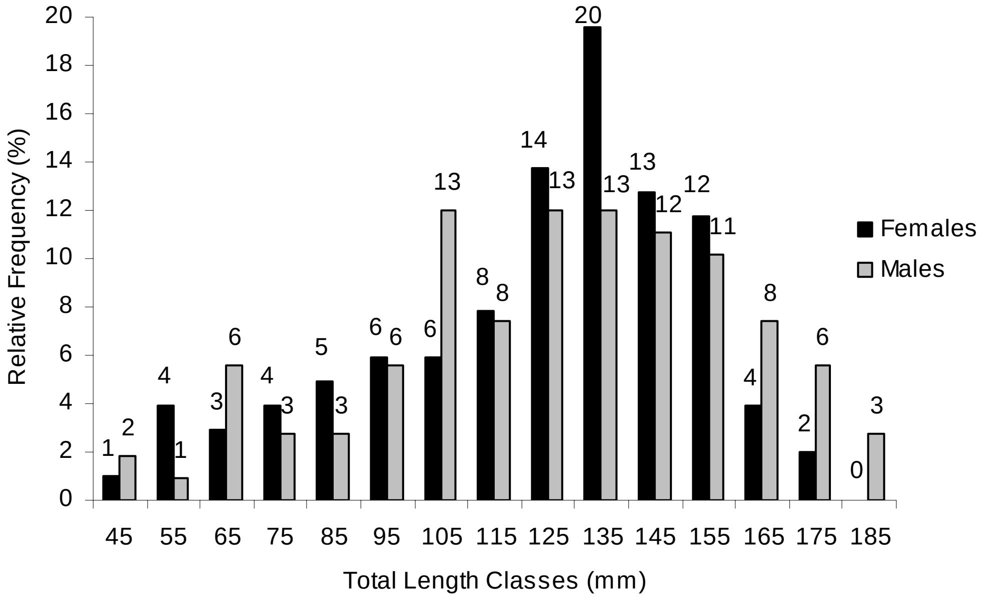

Fig. 8. Relative frequency distribution of *Brachyhypopomus* "G" males and females for total length classes. Numbers above the columns represent the absolut values.

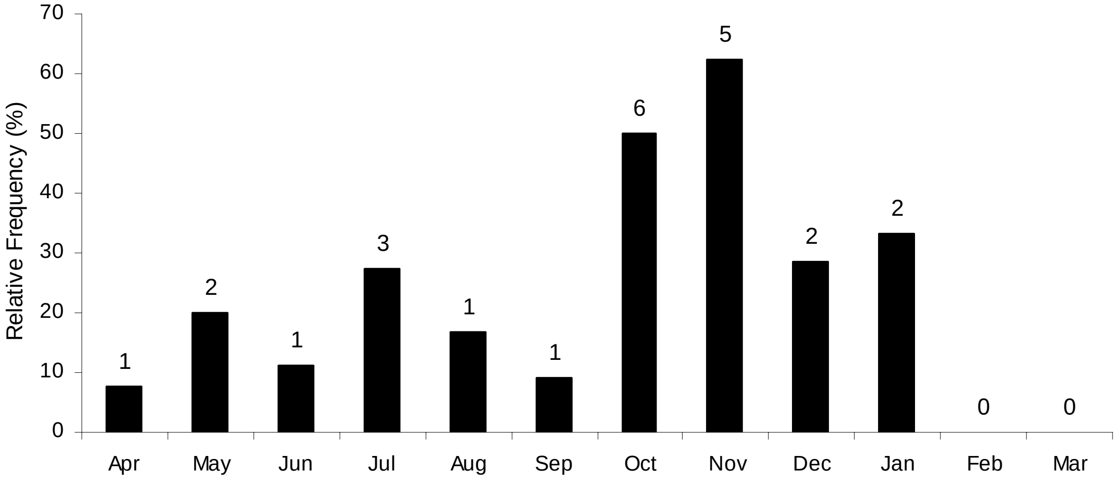

Fig 9. Relative frequency of *Brachyhypopomus* "G" males with hypertrophy of caudal filament distal portion. Numbers above the columns represent the absolut values.

Table 1. Monthly variation of *Brachyhypopomus bombilla* male and female replection indexes (RI).

	RI - Males	RI - Females
Apr	3.26	2.42
May	0.91	0.83
Jun	0.48	0.45
Jul	0.67	0.68
Aug	0.63	0.66
Sep	0.83	0.8
Oct	1.72	1.7
Nov	1.91	2.24
Dec	1.21	1.6
Jan	1.71	2.32
Feb	2.06	1.92
Mar	1.4	1.82

Table 2. Monthly variation of *Brachyhypopomus* "G" male and female replection indexes (RI).

	RI - Males	RI - Females
Apr	1.82	1.47
May	1.92	2.55
Jun	1.72	1.85
Jul	1.24	2.07
Aug	1.07	1.05
Sep	1.98	1.85
Oct	2.01	2.46
Nov	1.11	1.64
Dec	2.24	2.14
Jan	2.37	2.58
Feb	3.14	3.21
Mar	3.3	3.19

Table 3. Monthly variation of the water temperature (ºC), pH, conductivity (μs/cm), dissolved oxygen (mg/l), rainfall (mm), and photoperiod (min) values in the *Brachyhypopomus bombilla* collect site (arroio do Jacaré, Rosário do Sul Municipality).

	Temperature	pH	Conductivity	Diss. Oxygen	Rainfall	Photoperiod
Jul	12.6	7.07	44.1	7.94	66.8	628
Aug	16.9	7.17	42.5	7.77	14.1	663
Sep	17.3	7.18	38.4	7.69	151.5	713
Oct	20.9	7.25	34.3	7.6	117.1	767
Nov	23.1	7.31	53	6.51	155.7	816
Dec	26.7	7.33	51.8	5.03	91.9	845
Jan	24.9	7.29	13.1	1.22	67.3	829
Feb	25.1	7.34	10.32	2.12	22.8	784
Mar	22.8	7.24	22.8	1.67	76.9	736
Apr	20.4	7.03	14.87	4.11	167.9	686
May	18.1	7.07	11.62	1.74	161.9	649
Jun	20.4	7.07	11.24	1.03	93.1	615

Table 4. Monthly variation of the water temperature (ºC), pH, conductivity (μs/cm), dissolved oxygen (mg/l), rainfall (mm), and photoperiod (min) values in the *Brachyhypopomus* “G” collect site (flooded area near arroio dos Ratos creak, Charqueadas Municipality).

	Temperature	pH	Conductivity	Diss. Oxygen	Rainfall	Photoperiod
Apr	–	–	–	–	145.8	700
May	19.2	7.22	13.53	0.95	153.7	650
Jun	18.7	7.08	13.34	0.89	34.7	615
Jul	15.3	7.16	13.02	0.89	57.7	619
Aug	17	7.08	12.08	0.9	155.9	668
Sep	18.5	7.22	10.01	1	164.3	727
Oct	20.9	7.17	8	0.8	271.1	784
Nov	24.8	7.25	48.6	1.7	79.3	833
Dec	29.7	7.32	52.7	0.6	56	845
Jan	26	7.29	57.2	0.44	174.2	818
Feb	26	7.29	55.8	0.25	88.9	775
Mar	27	7.27	13.7	0.7	81.2	720

Table 5. Total length (TL), total weight (TW), gonadossomatic index (GSI), absolute fecundity (AF) and relative fecundity (RF) of *Brachyhypopomus bombilla* females.

	TL (mm)	TW (g)	GSI	AF	RF
	101.13	2.02	7.01	443	0.22
	105.86	1.97	7.67	497	0.25
	114.03	2.42	7.77	546	0.23
	116.5	2.14	6.43	369	0.17
	120.37	3.17	7.96	528	0.17
	121.33	2.90	6.61	619	0.21
	130.11	3.44	6.89	773	0.22
	130.47	3.46	7.52	762	0.22
	137.41	3.11	11.30	749	0.24
Mean	119.69	2.74	7.68	587.33	0.21

Table 6. Total length (TL), total weight (TW), gonadossomatic index (GSI), absolute fecundity (AF) and relative fecundity (RF) of *Brachyhypopomus* “G” females.

	TL (mm)	TW (g)	GSI	AF	RF
	85.42	1.12	6.29	299	0.27
	112.72	1.57	6.62	466	0.29
	131.05	2.90	6.40	540	0.19
	133.08	3.19	5.84	574	0.18
	133.15	4.23	5.01	734	0.17
	135.13	3.86	4.65	616	0.16
	135.59	3.79	8.93	637	0.17
	145.57	4.65	5.84	799	0.17
	149.1	4.10	5.50	640	0.16
Mean	128.98	3.27	6.12	589.44	0.20

Capítulo IV

Oogenesis and spermatogenesis in two species of *Brachyhypopomus* (Gymnotiformes: Hypopomidae) from Southern Brazil.

Oogenesis and spermatogenesis in two species of *Brachyhypopomus* (Gymnotiformes: Hypopomidae) from Southern Brazil.

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The process of oogenesis and spermatogenesis in the species *Brachyhypopomus bombilla* and *Brachyhypopomus* “G” from Southern Brazil is described through the establishment of gonadal maturation stages. The development of germ cells was classified in five growth phases for females and in six for males of both species. The gonadal maturation stages defined for females were: early maturation, late maturation, mature, spawning and spent. The maturation stages defined for males were: early maturation, mid maturation, late maturation, regression and regressed. As opposed to the homogeneity on cell types and stages of gonad maturation, the two studied *Brachyhypopomus* species have shown differences in the frequencies of maturation stages along the year, *B. bombilla* possessing a more seasonal gonadal development than *Brachyhypopomus* “G”.

O processo da oogênese e da espermatogênese foi descrito para as espécies *Brachyhypopomus bombilla* e *Brachyhypopomus* “G” através do estabelecimento de estádios de maturação gonadal. O desenvolvimento das células germinativas foi classificado in cinco estágios de desenvolvimento para fêmeas e seis para machos de ambas as espécies. Os estádios de maturação gonadal definidos para fêmeas foram: maturação inicial, maturação final, desovando e esgotado. Os estádios de maturação gonadal definidos para machos foram: maturação inicial, maturação intermediaria, maturação final, regredindo e regredido. Contrariamente a homogeneidade apresentada com relação aos tipos celulares e estádios de maturação gonadal, as duas espécies de *Brachyhypopomus* estudadas demonstraram diferenças nas freqüências dos estádios de maturação ao longo do ano, *B. bombilla* possuindo um desenvolvimento gonadal mais sazonal do que *Brachyhypopomus* “G”.

Key words: Oogenesis, spermatogenesis, *Brachyhypopomus*, southern Brazil.

Introduction

The South American electric fishes (Gymnotiformes) are a very successful group found in all types of aquatic habitats, including river channels, flood-plains, flooded forests, forest streams, waterfalls, swamps, coastal creeks and estuaries (Crampton, 1998). The family Hypopomidae, first proposed by Mago-Leccia

(1978), is widespread in South American countries, with the exception of Chile, and distributed from the río de la Plata in Argentina (35°S) to Northern Panama (8°N) (Albert, 2001). The genus *Brachyhypopomus* was described by Mago-Leccia (1994) to comprise six species that previously belonged to *Hypopomus*. Species of *Brachyhypopomus* are especially diverse and abundant in Amazon flood-plains, where they constitute a significant part of the biomass, thus being ecologically important (Crampton, 1996).

Teleost fishes are predominantly dioecious and with cyclic reproductive processes, during which their gonads undergo a series of morphological and physiological modifications (Nagahama, 1983). In its broadest sense, oogenesis is the process by which primordial germ cells become ova that are ready to be fertilized (Patino & Sullivan, 2002). Spermatogenesis, the formation of sperm, is a complex process in which spermatogonia divide and differentiate into spermatozoa (Chaves-Pozo *et al.*, 2005). According to Garcia *et al.* (2001), in order to preserve aquatic ecosystems it is necessary, among other things, to understand the reproductive cycles of their ichthyofauna, and one of the initial steps in this process is the study of gametogenesis.

In spite of the results presented by Barbieri & Barbieri (1984, 1985) and Cognato & Fialho (2006) for *Gymnotus* aff. *carapo,* by Quintana *et al.* (2004) for *Brachyhypopomus pinnicaudatus*, and by Giora and Fialho (in press) for *Eigenmannia trilineata*, data on gametogenesis of gymnotiform fishes are still very limited. Therefore, this work aims to study the process of oogenesis and spermatogenesis in two species of *Brachyhypopomus* from southern Brazil through descriptions of gonadal maturation stages.

Material and Methods

Specimens of both species were collected monthly during the period of one year. Fishes were located using an electric fish finder (Crampton *et al.*, 2007) and captured under floating vegetation by means of a dip net. Specimens of *Brachyhypopomus bombilla* were collected from July/2004 to June/2005 at a creek that is part of rio Uruguay drainage (30°12’42.8”S 55°03’17.5”W), near to the Municipality of Rosário do Sul. Specimens of *Brachyhypopomus* “G” were collected from April/2005 to March/2006 at a flooded area near the arroio dos Ratos (29°57’31.9”S 51°33’10.1”W), Municipality of Charqueadas, laguna dos Patos drainage.

Fishes were fixed in 10% formalin throughout the period of field work. In the laboratory, the specimens were transferred to 70% ethanol, their total lengths measured, and gonads removed. Gonads were dehydrated in an ethanol series and infiltrated and embedded in glycol methacrylate. Tissues were sectioned at 3.5 µm on a Sorvall Type JB-4 microtome and stained with toluidine blue. The slides were photographed under a Nikon E200 microscope. Voucher specimens were catalogued in the fish collection of the Departamento de Zoologia, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil

(*Brachyhypopomus* “G” – UFRGS 9200; *Brachyhypopomus bombilla* – UFRGS 9284).

Results

A total of 29 *Brachyhypopomus* “G” females were analyzed, with total lengths ranging from 55.63 mm to 167.85 mm; 31 males of this species were analyzed, total lengths ranging from 60.96 mm to 146.04 mm. A total of 37 females of *B. bombilla* were analyzed, ranging in total length from 54.04 mm to 132.68 mm; 30 males of this species were analyzed, total lengths ranging from 49.42 mm to 176.80 mm.

As it is typical for Gymnotiformes, both *Brachyhypopomus* species have two ovaries or testes located in an extremely ventral position, with gonoducts from each ovary or testis joining in a common gonoduct anterior to the cloaca. The two ovaries are anteriorly united forming a common structure especially after early maturation stage; mature ovaries occupy a great part of the body cavity and their volume is noticeable in live fish observations. Testes are elongate, varying from translucent to yellowish organs of nearly equal length; the proximal portion of the testis is slender and connected to the gonoduct, while the distal portion is broader mainly during reproductive period. Both species showed cyclical gonadal development with distinct, recognizable maturation stages that are herein described for females as: early maturation, late maturation, mature, spawning and spent; and for males as: early maturation, mid maturation, late maturation, regression, and regressed.

Female gonadal maturation stages

The germ cells and structures observed in the ovaries during oogenesis are characterized henceforth. Oogonia are rounded cells, each with a large central nucleus containing a single nucleolus, and located clumped within the ovarian lamellae (Fig 1 A, B; Fig 2 A, B). Near the oogonia, there are other cells, alone or forming nests, with oogonia-like aspect that can be identified as oocytes that have not begun primary growth (Fig 1 A, B; Fig 2 A, B). Primary growth phase oocytes are surrounded by squamous follicle cells, and contain a large central nucleus with multiple nucleoli. As the oocyte in primary growth phase enlarges, these nucleoli increase in number and size and migrate to the periphery of the nucleus (Fig 1 A, C; Fig 2 A, C). Throughout this oocyte growth phase it is possible to see “Balbiani bodies” or “yolk nuclei” that are a circular portions of coarse-granulated cytoplasm, lightly stained, and located adjacent to the nucleus (Fig 1 A; Fig 2 A). Previtellogenic oocytes exhibit an increase in cytoplasmic volume, which becomes more granular in appearance, with clear lipid vesicles at the periphery (Fig 1 D, E F; Fig 2 C, D, E, I). The nuclear outline is less defined and the number of nucleoli increases. Surrounding the previtellogenic oocytes, a distinct zona radiata becomes evident. This consists of a thin, continuous acellular layer located between the oocyte membrane and the layer of squamous follicle cells surrounding it. Vitellogenic oocytes exhibit a great increase in the number of lipid vesicles, as well as the beginning of yolk deposition (Fig 1 E; Fig 2 D). Moreover, vitellogenic oocytes have a contracted nucleus with a very irregular outline and a thicker zona radiata (Fig 1 E; Fig 2 D). Mature oocytes show rapid enlargement and a complete change in the character of the cytoplasm due to the massive increase of yolk granules, which are spherical on the periphery of the oocyte and fused in the interior (Fig 1 G, H; Fig 2 E, F, G). The nuclei of oocytes at this maturation stage remain contracted and lose the spherical shape (Fig 1 H; Fig 2 G), while the zona radiata and cuboidal follicle cells show an occasional undulation pattern. Post-ovulatory follicles are collapsed structures formed by rows of cuboidal cells that originally surrounded the mature oocyte before its liberation. Atretic follicles are oocytes that undergo degenerative processes because of non-ovulation or another physiological reason and are reabsorbed by the ovaries (Fig 1 I; Fig 2 H, I). Oocytes undergoing atresia lose their tumescence, yolk granules start to fuse with one another and the zona radiata shows invaginations that result in its eventual rupture.

The early maturation stage of both species is characterized by the presence of cells that represent oogonia and oocytes that have not begun primary growth (oogonia-like oocytes). Clumps of these cells are located within the ovarian lamellae among the abundant primary growth phase oocytes (Fig 1 A, B; Fig 2 A, B). For both *Brachyhypopomus* species, specimens whose ovaries were in the early maturation stage included those with the smallest total lengths representing fishes maturing for the first time, as well as larger fishes whose ovaries are undergoing recrudescence and having been active during the previous reproductive period. In the smaller specimens, gonads are compact with very little space between oocytes and the ovarian lamellae (Fig 1 A, B; Fig 2 A, B). In the larger specimens, on the other hand, gonads are less compact with distended ovarian lamellae, as well as post-ovulatory follicles indicative of a prior reproductive season (Fig 1 C).

The late maturation stage of both species studied is characterized by the presence of fewer oogonia/oogonia-like oocytes, abundant oocytes in primary growth phase, and the appearance of previtellogenic oocytes (Fig 1 D, E, F; Fig 2 C, D). At the end of this stage, the ovary also contains vitellogenic oocytes (Fig 1 E; Fig 2 D). As with the previous gonadal stage, specimens of *B. bombilla* and *Brachyhypopomus* “G” maturing for the first time may have ovaries in the late maturation characterized by small, compact gonads. Specimens that had already spawned during a prior reproductive season and are currently maturing anew have more flaccid gonads containing some post-ovulatory follicles. These post-ovulatory follicles are rare in late maturation gonads at the beginning of this maturation stage and cannot be found later on.

Gonads in the mature stage are characterized by the presence of very scarce oogonia/oogonia-like oocytes, few oocytes in primary growth phase, and a substantial number of vitellogenic and mature oocytes that occupy a major part of the gonad (Fig 2 E). At this stage, atretic follicles are rarely observed. Fishes with the greatest total lengths can be found in the maturation stage. No specimens of *B. bombilla* at the mature stage were identified during this analysis.

The spawning maturation stage of *B bombilla* and *Brachyhypopomus* “G” is similar to the mature stage, being characterized by the presence of very scarce oogonia/oogonia-like oocytes, few previtellogenic oocytes and oocytes in primary growth phase and a high number of vitellogenic and mature oocytes. The appearance of post-ovulatory follicles at this stage indicates the beginning of release of mature oocytes (Fig 1 G, H; Fig 2 F, G). Some ovaries in the spawning stage also contain atretic follicles. At the beginning of this stage the gonads are large and compact and contain few post-ovulatory follicles. As spawning proceeds, the ovaries become more flaccid with a lower number of mature oocytes and a higher number of post-ovulatory follicles.

The spent stage of both *B. bombilla* and *Brachyhypopomus* “G” is characterized by the presence of large numbers of post-ovulatory follicles and oocytes in primary growth phase, few oogonia/oogonia-like oocytes, and quite often atretic follicles (Fig 1 I; Fig 2 H, I). Gonads in the spent stage are small, flaccid, and show extremely distended ovarian lamellae (Fig 2 H, I). At the end of this stage the ovaries begin to become reorganized, with an increase in the number of oogonia/ oogonia-like oocytes and the complete degeneration and reabsorption of atretic follicles, characterizing the reentry into the early maturation stage.

Although the two *Brachyhypopomus* species herein analyzed show the same female gonadal maturation stages, they do not have the same relative frequencies during the observed months. During most months, *Brachyhypopomus bombilla* exhibited the early maturation stage at high frequencies; the late maturation stage occurs during six months of the year; spawning and spent stages occur during fewer months within a limited period of the year; the mature stage was not observed (Fig. 3). *Brachyhypopomus* “G” exhibited the late maturation and spawning stages at high frequencies during six months of the year; early maturation and spent stages occur at lower frequencies during fewer months; the mature stage occurs in September only (Fig. 3).

Male gonadal maturation stages

The germ cells observed in the testis during spermatogenesis are characterized henceforth. Spermatogonia are the largest cells found in the testes and they exist either single or in small groups. Primary spermatogonia are pale-staining spherical cells with a large central nucleus which contains one or two nucleoli (Fig 4 A, H, I; Fig 5 A, I). As spermatogenesis develops, primary spermatogonia undergo mitotic divisions originating the secondary spermatogonia increasing in number forming cysts, and becoming smaller, with smaller nucleus and not so evident nucleoli (Fig 4 C, F; Fig 5 A, B, C, F, I). Primary spermatocytes are slightly smaller than spermatogonia and have smaller coarser nucleus without the presence of nucleolus. These spermatocytes are arranged in cysts of a great number of cells and are located adjacent to the spermatogonia (Fig 4 C, F, G, I; Fig 5 A, B, C, E, F, I). Secondary spermatocytes exist in cysts with approximately twice the number of germ cells than primary spermatocytes cysts. They differ from their immediate precursors by the much smaller size and condensed nucleus with clumped chromatin (Fig 4 C, F, G; Fig

5 B, C, E, I). Spermatids are nearly half the size of secondary spermatocytes and have condensed and strongly-staining nucleus. These cells generally occupy the peripheral zone of the seminiferous lobule lumen (Fig 4 C, F, G; Fig 5 B, C, F). Spermatozoa are the smallest germ cell in the testis. They have condensed strongly-staining spherical nucleus, and are found in the central region of the seminiferous lobule and throughout the elaborate duct system (Fig 4 C, D, F, G, I; Fig 5 B, C, E, F, G, I).

The early maturation stage is defined by the massive presence of spermatogonia in the testes (Fig 4 a; Fig 5 A). Initially, primary and secondary spermatogonia are the only germ cells in the gonad, and are presented compacted occupying the entire testes area. Subsequently, scattered cysts of primary and later secondary spermatocytes begin to appear, whereas spermatogonia still is the most abundant germ cell of the testis. Macroscopically, gonads in early maturation stage are thin, nearly translucent to whitish, and, mainly for *B. bombilla* species, associated to fine layers of lipid cells. *Brachyhypopomus bombilla* was found in high relative frequencies on this maturation class during five months of the year, before and after the reproductive period (Fig 6). *Brachyhypopomus* “G” was also found on this class during five months of the year, although in much lower relative frequencies and always in a more advanced phase with the continuous presence of spermatocyte cysts dispersed between spermatogonia (Fig 6). For both *Brachyhypopomus* species on early maturation stage, there has been found the smallest total length fishes maturing for the first time as well as larger fishes, which have participated on the last reproductive period and are restarting the gonad maturation process.

The mid maturation stage is characterized by the existence of spermatogonia and spermatocytes at nearly the same proportion in the testes (Fig 4 B, C; Fig 5 B, C). Spermatids are also found in the gonads on this stage but in lower number, and as maturation progresses and the spermatogonia number decreases, groups of spermatozoa start to emerge. Lobules near the distal portion of the testis tend to be at an earlier period of development than those in the proximal portion of the gonad nearer the gonoduct. For this reason, during mid maturation spermatids and spermatozoa are more frequent in the testicular proximal part (Fig 4 D), spermatogonia and spertocytes being always more abundant in the opposite side. During this stage, the gonads increase in size and volume starting to look more turgid. Both *Brachyhypopomus* species were found in mid maturation stage during seven months of the sampled year (Fig 6). However, *Brachyhypopomus* “G” has shown much higher relative frequencies of fishes on this maturation class, reaching 100% of the analyzed fishes in four of the months, and has been the most constant gonad developing stage for this species (Fig 6). Fishes of all sizes can be found on this gonad maturation class, except the ones included in the lowest length class analyzed (lower than 70 mm).

The late maturation stage is distinguished by a steady decline of spermatogonia number and a remarkable increase of spermatozoa (Fig 4 E; Fig 5 D). Initially, in the distal testicular portion there is a considerable amount of primary and secondary spermatogonia together with primary and secondary spermatocytes cysts. Whereas in the testis proximal portion the seminiferous lobule function starts to switch from sperm production to sperm storage with a massive presence of spermatozoa between small cysts of secondary spermatocytes and spermatids. As late maturation progresses, the totality of testicular lobule lumens is fully filled with spermatozoa and spermatogonia become scarcely scattered or absent among spermatocyte cysts (Fig 5 E). At the end of this maturation stage, primary spermatogonia still exist only on the periphery of extreme distal testis portion. Late maturation gonads can be observed in fishes with the highest total lengths, and can be macroscopically described as turgid enlarged and white to yellowish colored organs. *Brachyhypopomus* “G” and *B. bombilla* were found in this testicular maturation class during two of the sampled months, which are corresponding to the beginning of the reproductive period for both species (Fig 6).

The regression maturation stage is characterized by a great spermatogenesis reduction and by the presence of an increasing amount of spermatogonia, which were rare or absent during late maturation stage (Fig 4 F, G; Fig 5 F, H). In the proximal portion of the testes, the lobule lumens are completely filled with spermatozoa and outlined by clusters of spermatogonia and few spermatocyte and spermatid cysts. The testicular ducts are always filled up with spermatozoa (Fig 4 G). In the distal testis portion there are greater primary spermatogonia amounts that start to take spermatozoa place in the lobules. Primary and mainly secondary spermatocytes are much more frequent during this maturation class in *Brachyhypopomus* “G” than in *B. bombilla* species (Fig 4 F, G; Fig 5 H). Macroscopically, gonads in regression stage are pretty similar to those in late maturation, as well as the total length of fishes on this stage that have the same range of fishes on previous maturation stage. *Brachyhypopomus* “G” and *B. bombilla* can be observed in regression class during three and four of the analyzed months respectively (Fig 6).

The regressed stage is defined by the return of primary spermatogonia as the principal germ cells in the testes (Fig 4 H, I; Fig 5 I). Residual spermatozoa produced during the former reproductive period are observed in small groups spread among the spermatogonia (Fig 4 H, I; Fig 5 I). In *B. bombilla* regressed gonads, primary spermatocytes and secondary spermatocytes are very infrequent and found as residual clusters (Fig 4 I). As it was observed on early maturation and regression stages, *Brachyhypopomus* “G” testes on the regressed class have shown a much higher frequency of spermatocytes than that exhibited for *B. bombilla*. Although primary spermatocytes have been hardly found, cysts of secondary spermatocytes are pretty numerous in *Brachyhypopomus* “G” regressed testes (Fig 5 I). At macroscopic analyzes, testes on this maturation stage are flaccid whitish and much smaller than those on previous development class. Both analyzed species were found on this maturation class during three of the sampled months, which are at the end of reproductive season (Fig 6).

Discussion

Gonadal maturation in fishes is a continuous process, even though it can be classified in stages that vary according to the criteria used (Chini *et al*., 2001). Because the establishment of gonad maturation stages results in stagnant maturation scales, the histological analysis has been recommended as a reliable method to avoid misclassification (Dias *et al*., 1998). Although macroscopic features are quite important for gonad's morphological characterization, the definition of maturation classes based only on this sort of features can lead to mistakes and misclassification. Gonadal maturation stages in female and male *B. bombilla* and *Brachyhypopomus* "G" were defined based upon changes in germ cell development classes and gonadal cycles through individual reproductive events.

According to female gonad evaluations, it is possible to affirm that both *Brachyhypopomus* species show successive spawning events during one reproductive period, which is validated by the definition of the spawning maturation stage. The presence of post-ovulatory follicles in the gonads and the definition of a spawning maturation class have been identified as an indication of fractional spawning - where more than one oocyte lot is liberated during one reproductive period - for teleosts (Vazzoler, 1996) and, more specifically, for Gymnotiformes (Barbieri & Barbieri, 1985; Cognato & Fialho, 2006; Giora & Fialho, in press). From the analysis of both ovary and testis development cycles, it is also evident that the studied species have life period longer than a year, since adult fishes presenting gonads matured in a later period were found in early and late maturation stages of females and in early and mid maturation of males being prepared for a new reproductive season.

Based on ultrastructural studies (Wallace & Selman, 1981), the “Balbiani body”, herein described as present in primary growth stage oocytes, was considered as a complex composed of cytoplasmic organelles including mitochondria, Golgi elements, multivesicular bodies, and lipid granules, depending on the species. Moreover, the “Balbiani body” has been proposed to function as an mRNA transport organizer that organizes and mediates the delivery of RNAs and germinal granules to the vegetal pole of the egg (Kloc, *et al.*, 1998).

The origin of the new spermatogonia that restart spermatogenesis during the following reproductive cycle has been widely discussed and is still a matter of controversy (Chaves-Pozo *et al.*, 2005). Two hypotheses have been suggested to explain this germ cells renewal: some authors affirm that primary spermatogonia arise from an extra-testicular source and they annually migrate into the seminiferous tubules and lobules (van den Hurk *et al.*, 1978); other studies suggested that there is a residual population of spermatogonia within testis after breading season that serve to repopulate the gonad (Burke & Leatherland, 1983; Ruby & McMillan, 1970; Grier & Taylor, 1998; Chaves-Pozo *et al.*, 2005). In the two studied *Brachyhypopomus* species due to the presence of spermatogonia in the testes throughout the year – although in low frequency during late maturation stage – it is probable that the new generation of germ cells derive from mitotic division of spermatogonia that already exist in the seminiferous lobules.

Although the wide range of gonadal morphologies reflects the complexity of teleost reproduction, basic features (i.e. the structure of germ cells and different somatic cell elements constituting the gonadal tissue) are similar (Nagahama, 1983). In relation to the cell types described herein, there is no significant difference in comparison with the female (Wallace & Selman, 1981; Nagahama, 1983; Vazzoler, 1996; Garcia *et al.*, 2001) and male germ cells (Burke & Leatherland, 1983; Selman & Wallace, 1986; Grier & Taylor, 1998; Reygadas & Escorcia, 1998; Brown-Peterson *et al.*, 2002) exhibited for the majority of teleosts. The maturation stages described for *Brachyhypopomus* female gonads are similar to those described previously by Barbieri & Barbieri (1985) and Cognato & Fialho (2006) for *Gymnotus* aff. *carapo*, and by Giora & Fialho (in press) for *Eigenmannia trilineata.* The same occurred with *Brachyhypopomus* male gonad maturation stages, which were quite similar to those described previously for two Perciform species by Grier & Taylor (1998) and Lo Nostro *et al.* (2000), for *Synbranchus marmoratus* by Brown-Peterson *et al.* (2002), and also for *Gymnotus* aff. *carapo* by Barbieri & Barbieri (1984) and Cognato & Fialho (2006). These statements are suggesting that gonad development classification based on changes in the testicular and ovarian germ cells may be applicable to a great range of Gymnotiformes species, even though many interspecific differences can be observed.

As opposed to the homogeneity on cell types and stages of gonad maturation, the two studied *Brachyhypopomus* species have shown differences in the frequencies of maturation stages along the year. From the ovaries analyzes it can be observed that *B. bombilla* remains in early maturation stage during great part of the year and exhibits gonads in spawning stage during a short and well defined period. This information leads to a fast gonad development just before reproductive period - which proved to be relatively brief - and to a low gonad development rate during the months between two reproductive seasons. The lack of analyzed *B. bombilla* female specimens in the mature stage is in agreement with this statement, since a very fast preparation for spawning would reduce the possibilities of fully matured female capture in monthly samples. Concerning *Brachyhypopomus* "G" females, the gonad development appeared to be more continuous and regular with uninterrupted gonad growth before, during, and after the reproductive season. The major differences between *Brachyhypopomus* "G" and *B. bombilla* testis maturation classes concern the frequencies of fishes on early and mid maturation classes. In spite of occurring during the same number of months along the studied year, the frequency of *Brachyhypopomus* "G" on mid maturation was higher than *B. bombilla*, whereas, on early maturation, the opposite was true for *B. bombilla.* The explanation for these divergences may be associated to the higher number of spermatocytes in testes of *Brachyhypopomus* "G" during the majority of maturation stages, which shows a more constant spermatogenesis than *B. bombilla* along the reproductive cycle.

These divergences between the two *Brachyhypopomus* species ovaries and testes development are evidencing the establishment of distinct reproductive strategies. Life-history strategy is defined as a complex pattern of co-evolved life-history traits designed for a particular environment (Rochet, 2000), where as reproductive strategy is the whole of characteristics assumed for a species with the aim of reproductive success and population balance (Wootton, 1984). The changes on a species resource allocation from reproductive to competitive activities can only occur in habitats where it may increase the future generation survival (Lowe-McConnell, 1999). Studies on feeding habits of *B. bombilla* (J. Giora, H. T. Moraes & C. Fialho, in preparation) have demonstrated that the species fasts during the six months between the end of a reproductive period and the beginning of the next one. Therefore, these results are indicating that feeding dynamics may be interfering on gonad development of this species, which has proved to possess a more seasonal gonadal development than *Brachyhypopomus* "G". After all, the quoted imbalance between frequencies of maturation classes and the establishment of different reproductive strategies for the species are evidencing the elevated gonad development diversity that can be found even among the same genus species.

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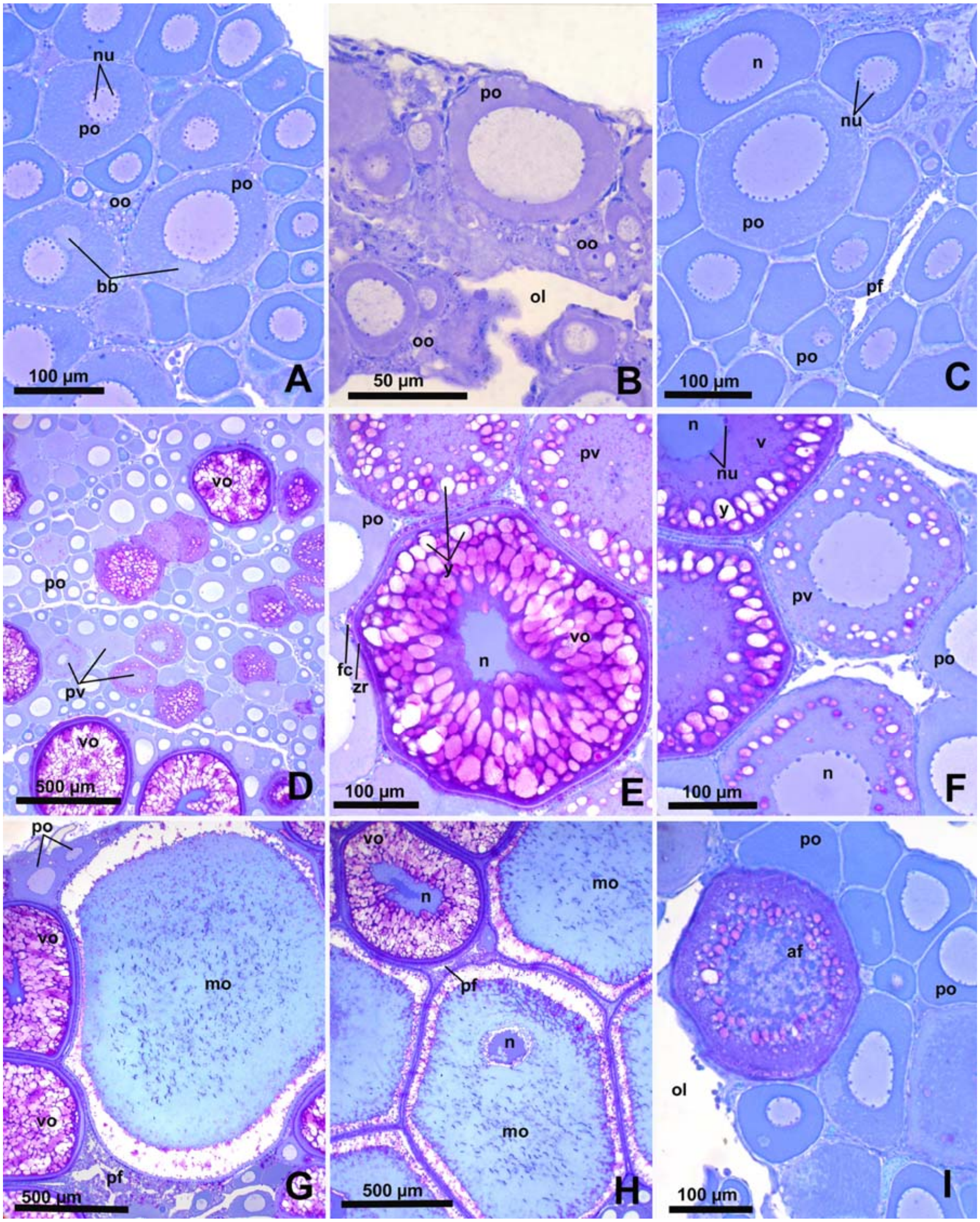

Fig. 1. Female gonads of *Brachyhypopomus bombilla*. A, B, C - early maturation stage; D, E, F – late maturation stage; G, H – spawning stage; I – spent stage.

oo = oogonia/oogonia-like oocytes; po = primary growth phase oocyte; pv = previtellogenic oocyte; vo = vitellogenic oocyte; mo = mature oocyte; pf = post-ovulatory follicle; af = atretic follicle; n = nucleus; nu = nucleolus; y = yolk; zr = zona radiata; fc = follicle cells; ol = ovarian lamellae; bb = balbiani body.

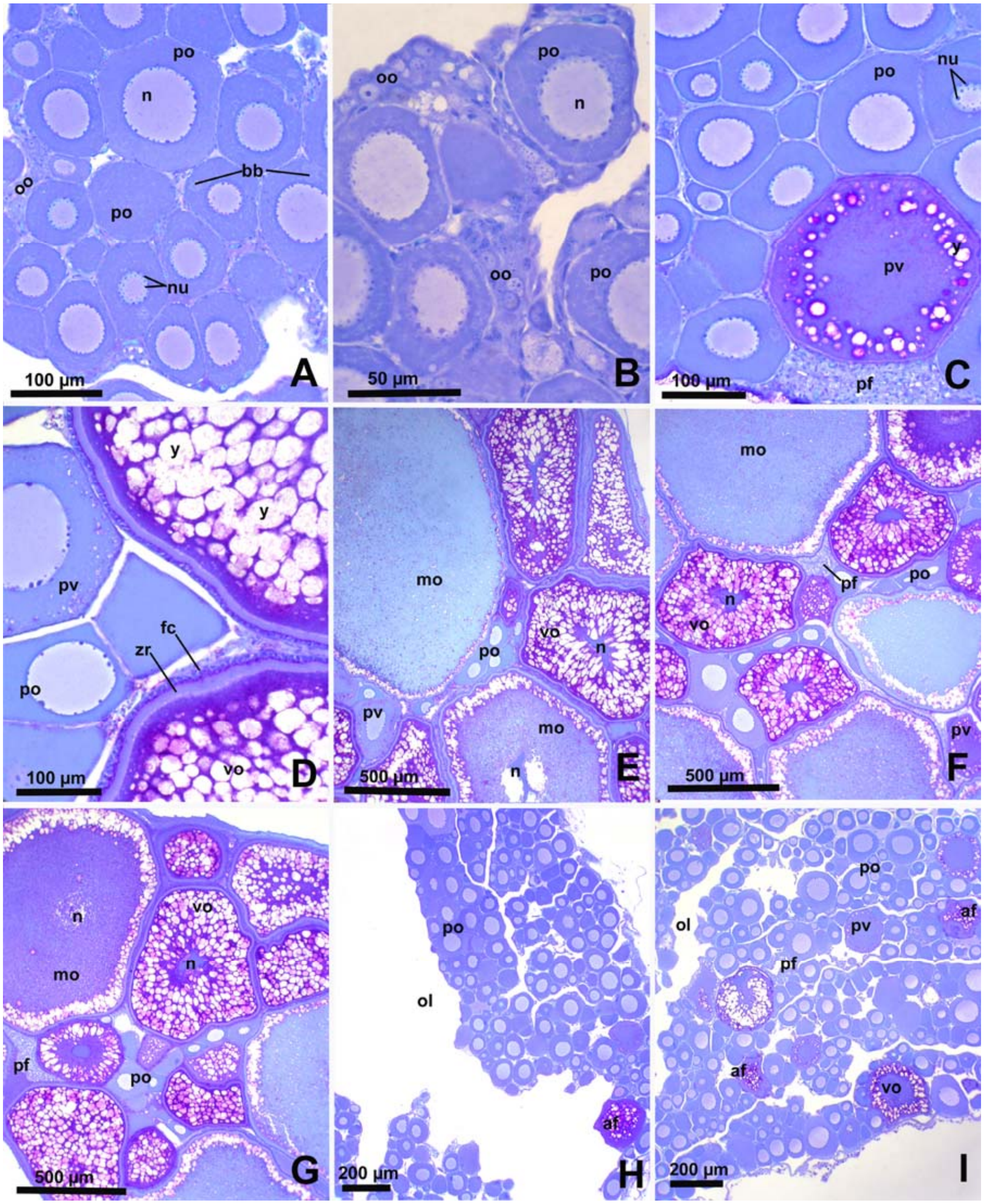

Fig. 2. Female gonads of *Brachyhypopomus* “G”. A, B - early maturation stage; C, D – late maturation stage; E – mature stage; F, G – spawning stage; H, I - spent stage oo = oogonia/oogonia-like oocytes; po = primary growth phase

oocyte; pv = previtellogenic oocyte; vo = vitellogenic oocyte; mo = mature oocyte; pf = post-ovulatory follicle; af = atretic follicle; n = nucleus; nu = nucleolus; y = yolk; zr = zona radiata; fc = follicle cells; ol = ovarian lamellae; bb = balbiani body.

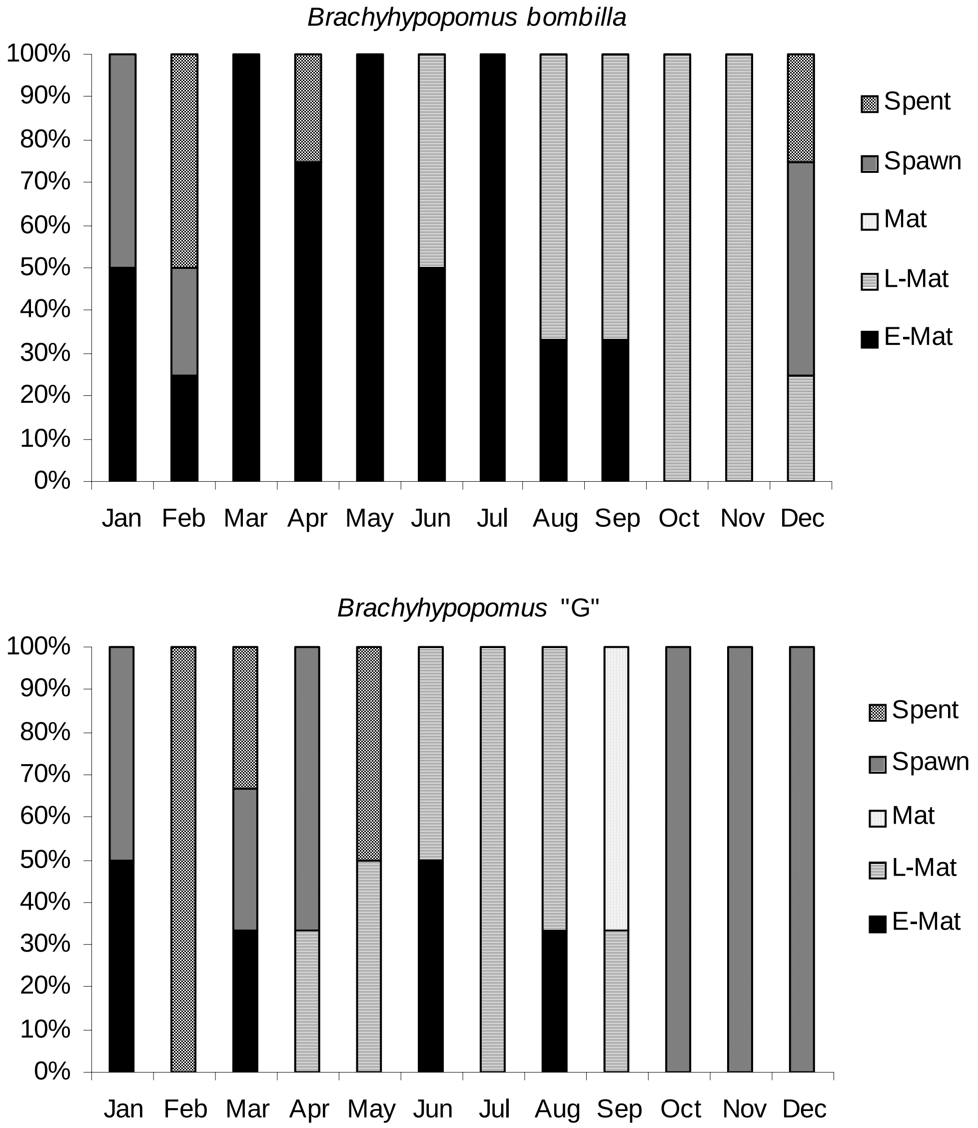

Fig. 3. Monthly variation of *Brachyhypopomus bombilla* and *Brachyhypopomus* “G” female gonadal stage frequencies. Spent = spent stage; Spawn = spawning stage; Mat = mature stage; L-Mat = late maturation stage; E-Mat = early maturation stage.

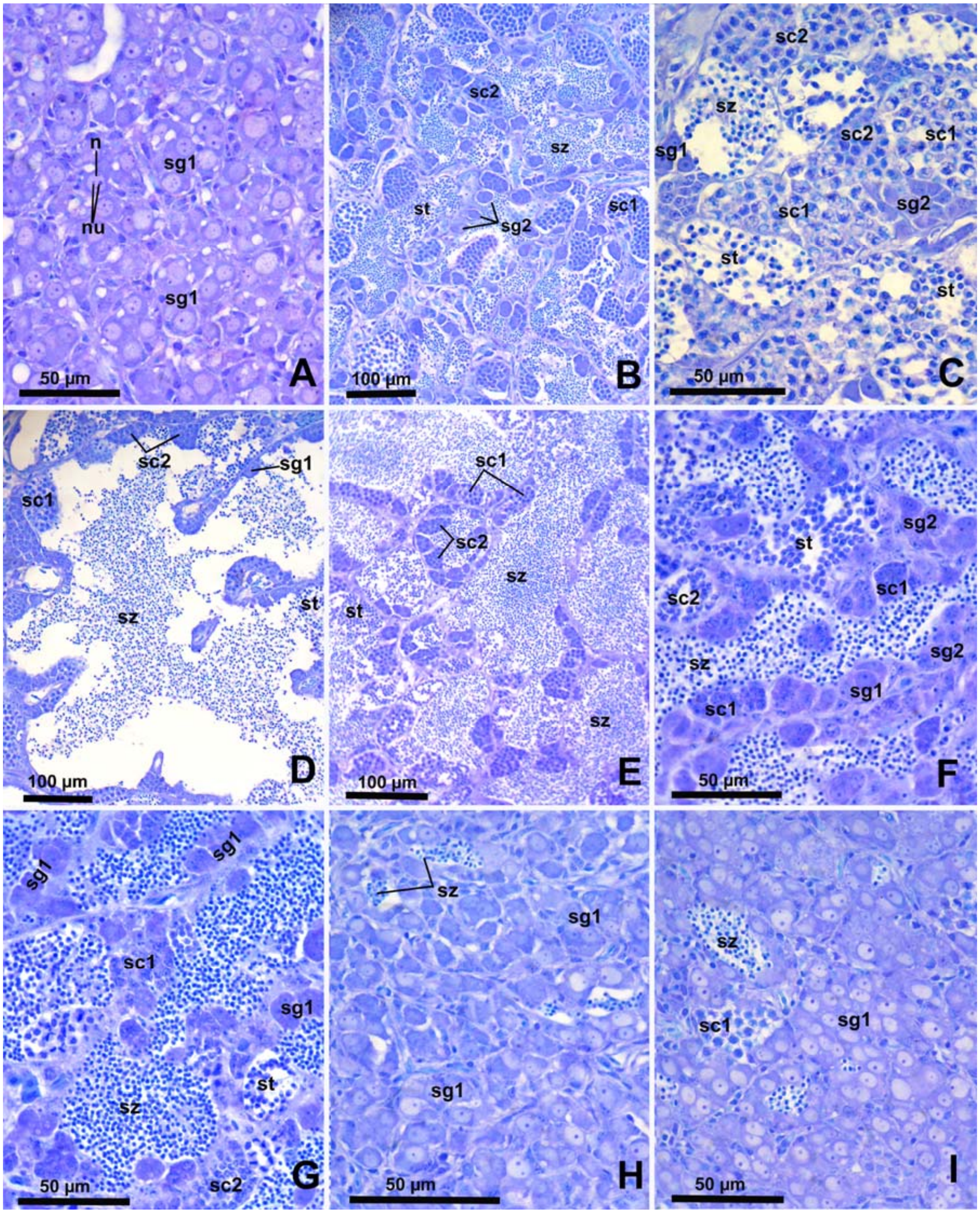

Fig. 4. Male gonads of *Brachyhypopomus bombilla*. A - early maturation stage; B, C, D – mid maturation stage; E – late maturation stage; F, G – regression maturation stage; H, I – regressed maturation stage. sg1 = primary

spermatogonia; sg2 = secondary spermatogonia; sc1 = primary spermatocyte; sc2 = secondary spermatocyte; st = spermatid; sz = spermatozoa; n = nucleus; nu = nucleolus.

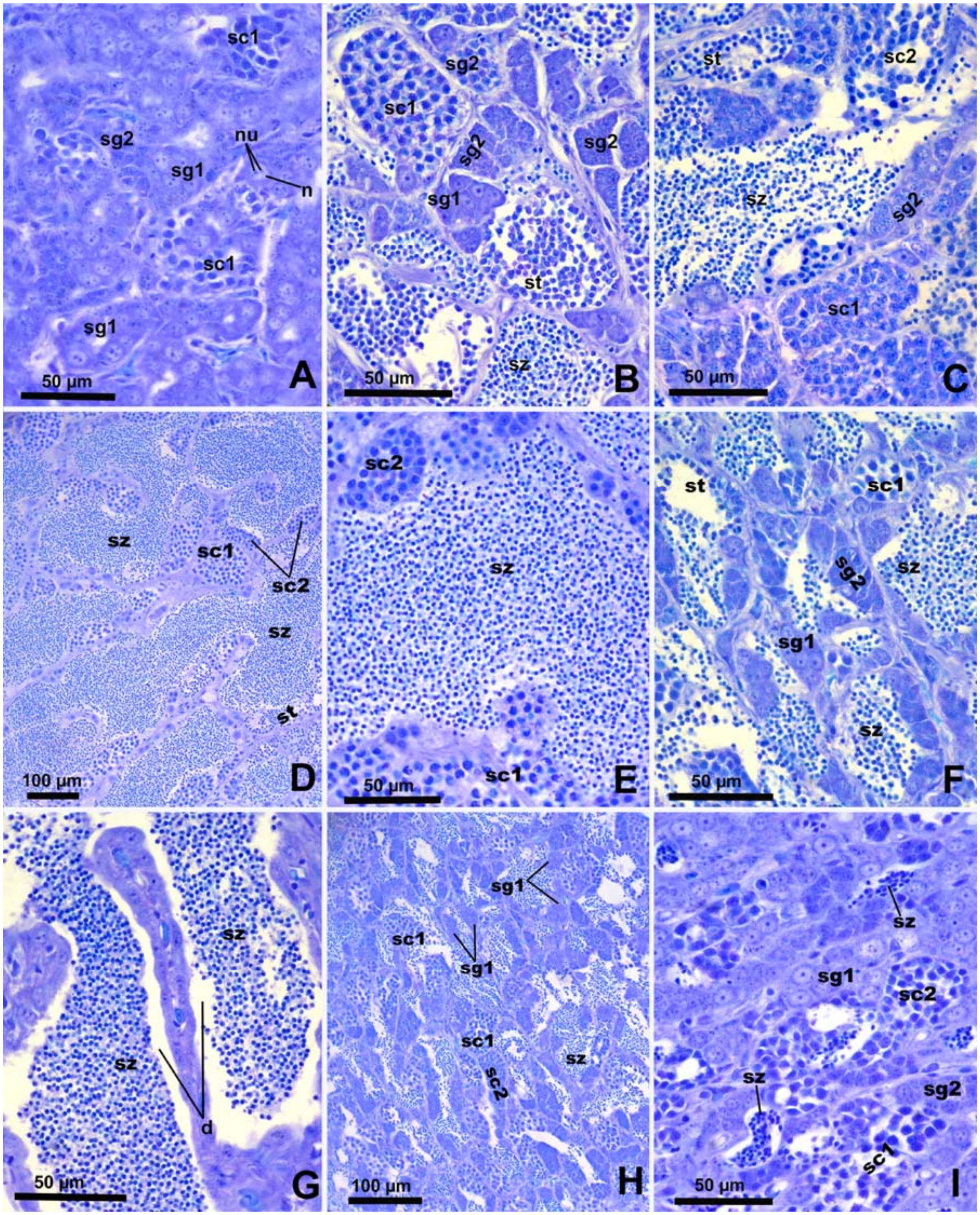

Fig. 5. Male gonads of *Brachyhypopomus* "G". A - early maturation stage; B, C – mid maturation stage; D, E – late maturation stage; F, G, H – regression maturation stage; I – regressed maturation stage. sg1 = primary spermatogonia;

sg2 secondary spermatogonia; sc1 = primary spermatocyte; sc2 = secondary spermatocyte; st = spermatid; sz = spermatozoa; n = nucleus; nu = nucleolus; d = testicular ducts.

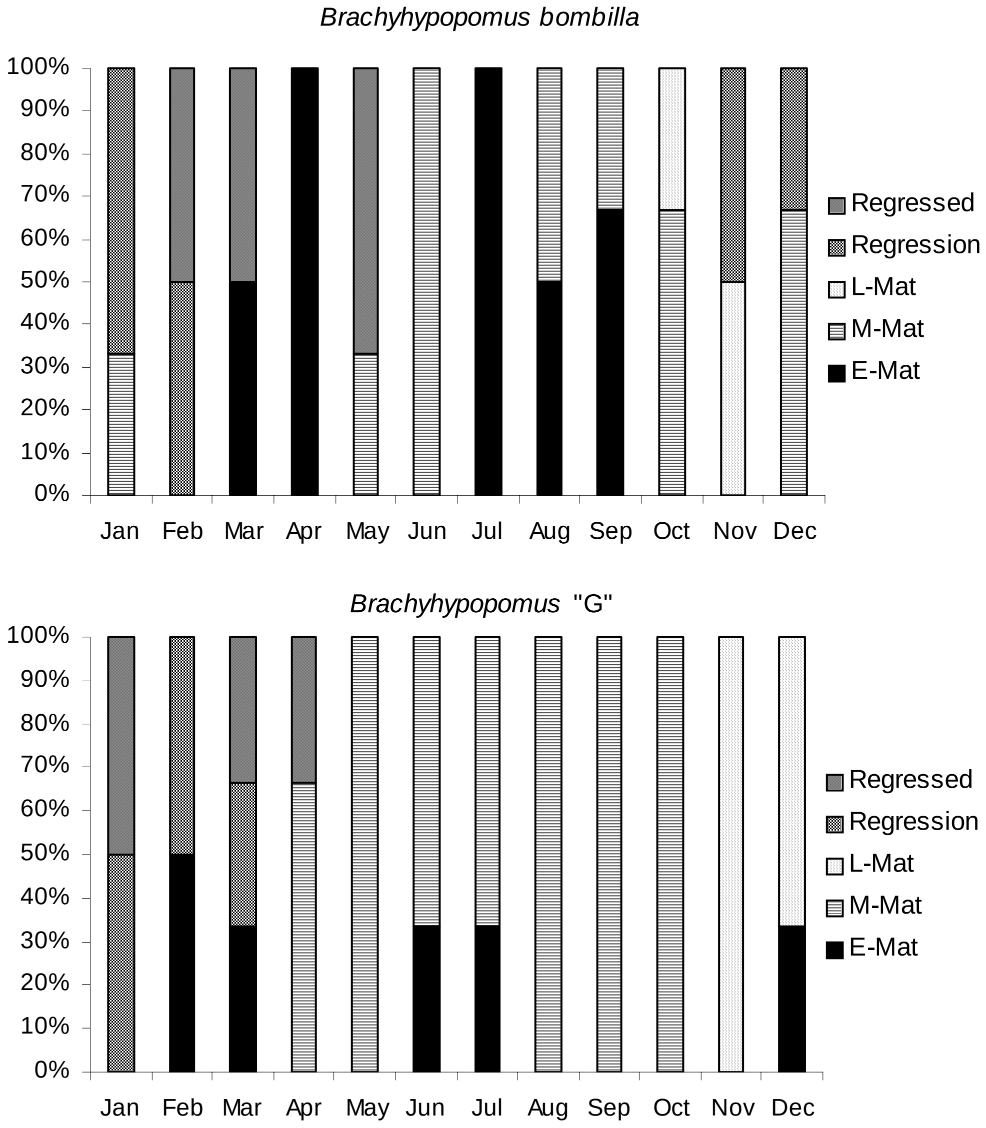

Fig. 6. Monthly variation of *Brachyhypopomus bombilla* and *Brachyhypopomus* “G” male gonadal maturation stage frequencies. Regressed = regressed maturation stage; Regression = regression maturation stage; L-Mat = late maturation stage; M-Mat = mid maturation stage; E-Mat = early maturation stage.

Capítulo V

Sperm ultrastructure in three different families of weakly electric fishes (Teleostei: Gymnotiformes).

Sperm ultrastructure in three different families of weakly electric fishes (Teleostei: Gymnotiformes).

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This study presents details of the spermatozoa ultrastructure of *Gymnotus* aff. *carapo* (family Gymnotidae), *Eigenmannia trilineata* (family Sternopygidae), and three *Brachyhypopomus* species (*B. draco, B. bombilla,* and *Brachyhypopomus* "G" – family Hypopomidae) from southern Brazil. Some differences were found between the representatives of the different families, such as the presence of nuclear rotation, observed in *E. trilineata* and in the *Brachyhypopomus* species, or its absence, observed in *Gymnotus* aff. *carapo*, and the presence of axonemal-fins, only observed in *E. trilineata*. Some intraspecific variations could also be noticed among the analyzed *Brachyhypopomus* species, such as nuclear density and vacuoles number and organization level. Most of the characters found in the spermatozoa of the species studied herein are shared with species of Gymnotiformes previously analyzed.

Este estudo apresenta detalhes sobre a ultraestrutura do espermatozóide de *Gymnotus* aff. *carapo* (família Gymnotidae), *Eigenmannia trilineata* (família Sternopygidae), e três espécies de *Brachyhypopomus* (*B. draco, B. bombilla,* e *Brachyhypopomus* “G” – família Hypopomidae) do sul do Brasil. Algumas diferenças foram encontradas entre os representantes das diferentes famílias, tais como a presença de rotação nuclear, observada em *E. trilineata* e nas três espécies de *Brachyhypopomus*, e a presença de aletas laterais no flagelo, observadas somente em *E. trilineata*. Algumas variações intraespecíficas também puderam ser registradas entre as espécies de *Brachyhypopomus* analisadas, tais como a densidade nuclear e o número e nível de organização dos vacúolos. A maioria das características encontradas nos espermatozóides das espécies aqui analisadas são compartilhadas com as demais espécies de Gymnotifomes previamente analisadas.

Key words: Spermatozoa, Hypopomidae, Sternopygidae, Gymnotidae.

Introduction

The order Gymnotiformes is restricted to Neotropical freshwaters occurring from Guatemala to Argentina, and also in the Caribbean island of Trinidad (Mago-Leccia, 1978). The order is constituted by 117 species, 30 genera, and five families (Reis *et al.,* 2003) and, although, there are many hypotheses on the relationships among its families (Triques, 1993; Mago-Leccia, 1994; Alves-Gomes *et al.*, 1995, Albert and Crampton, 2005), they are still unsolved.

According to Mattei (1991), since the fishes have demonstrated great structural diversity at all taxonomic levels, it is not possible to construct spermatic models, even for monophyletic groups as Actinopterygii or Teleostei. For this reason, results on ultrastructure of sperm cell have provided valuable information on cellular modifications associated with reproductive habits and uncovered morphological characters useful in hypothesizing phylogenetic relationships (Baccetti, 1987; Burns *et al*., 1998; Burns *et al*., 2002).

Ultrastructural studies on Gymnotiformes spermatozoa were done for the species *Apteronotus albifrons* (cited as *Sternarchus albifrons*) by Jamieson (1991), for *Gymnotus* cf. *anguilaris* and *Brachyhypopomus* cf. *pinnicaudatus* by França *et al*. (2007), and for *Rhamphichthys* cf. *hahni*, *Eigenmannia* cf. *virescens* and *Apteronotus* cf. *albifrons* by França (2006). However, there are many remaining questions and more studies are needed for a better understanding of the spermatozoa evolution in the Gymnotiformes order (França, 2006). The purpose of the present study is to provide details of spermatozoa ultrastructure in *Gymnotus* aff. *carapo*, *Eigenmannia trilineata*, and three *Brachyhypopomus* species (*B. draco, B. bombilla,* and *Brachyhypopomus* “G”) from southern Brazil.

Material and Methods

The present study was conducted on adult males of *Brachyhypopomus bombilla, Brachyhypopomus draco, Brachyhypopomus* “G”*,* and *Eigenmannia trilineata* and *Gymnotus* aff*. carapo* collected in Rio Grande do Sul State, southern Brazil. *Brachyhypopomus draco, Brachyhypopomus* “G”*,* and *G.* aff. *carapo* were collected at a flooded area near the arroio dos Ratos creek (29°57’31.9”S 51°33’10.1”W), Charqueadas Municipality, laguna dos Patos drainage. *Brachyhypopomus bombilla* and *E. trilineata* were collected at a creek inserted on rio Uruguay drainage (30°12’42.8”S 55°03’17.5”W), near Rosario do Sul Municipality. The fishes were sacrificed by severing the spinal cord. Immediately afterwards, small pieces of testes were placed in modified Karnovsky's fixative (Ito and Karnosvsky, 1968) and kept under refrigeration until the start of further processing. The fishes were deposited on fish collection of the Universidade Federal do Rio Grande do Sul (*Brachyhypopomus* “G” – UFRGS 9200; *Brachyhypopomus bombilla* – UFRGS 9284, *Brachyhypopomus draco* – UFRGS 6750, *Eigenmannia trilineata* – UFRGS 6635, *Gymnotus* aff. *carapo* – UFRGS 6859).

Testes were dehydrated in an ethanol series towards the critical-point dried for scanning electron microscopy (SEM). The dried tissue was then attached to stubs with carbon double-stick tape and teared apart with needles. The sample was sputter-coated with carbon and gold and viewed in a LEO 1430VP scanning electron microscope. For transmition electron microscopy (TEM), testes were cut in small pieces (±1 mm^3), rinsed in phosphate buffer and post-fixed in 1% osmium tetroxide in phosphate buffer. After that testes were rinsed in phosphate buffer, dehydrated in an ethanol series, infiltrated, and embedded in Araldite 502. Ultrathin sections were cut on a Sorvall MT5000 ultramicrotome, mounted on grids, and stained with aqueous uranyl acetate and lead citrate. Sections were examined with a JEOL JEM 1200 transmission electron microscope.

Results

Spermatozoa of *Brachyhypopomus draco, Brachyhypopomus bombilla* and *Brachyhypopomus* "G".

The spermatozoa of the three analyzed species are constituted by well defined head, midpiece, and flagellum, with spherical nucleus. They have electron-dense nucleus, *B. bombilla* showing more flocular chromatin with some lighter spots scattered, while *B. draco* and *Brachyhypopomus* "G" show peripheral electro-density with less condensed chromatin in the center of the nucleus. All these *Brachyhypopomus* species have nuclear rotation. The centrioles are in a perpendicular orientation and are completely contained within the nuclear fossa. *Brachyhypopomus draco* possesses a very deep fossa, which is the deepest between these three species. The midpiece contains a great number of vacuoles and elongated mitochondria, which are irregularly distributed. The midpiece of the three species also possesses a cytoplamic channel. The species *B. draco* has many ramified vacuoles forming a membranous structure differing from the other *Brachyhypopomus* herein analyzed. The flagellum has the classical 9 + 2 microtubular pattern and it does not show intratubular differentiation, both tubules of each peripheral doublet being not electron-dense. The flagellar membrane does not have lateral projections or fins, however, *B. bombilla* possesses an enlarged membrane in comparison with the other two studied species.

Spermatozoa of *Gymnotus aff. carapo.*

The spermatozoa of *Gymnotus* aff. *carapo* are constituted by head, with spherical nucleus, well defined midpiece and a single flagellum. The nucleus is peripherally electron-dense, with less condensed chromatin in its center and does not show nuclear rotation, the flagellum remaining lateral in relation to the nucleus. The spermatozoa have a very deep nuclear fossa and the centrioles are arranged in perpendicular orientation. Although the centrioles are located very near the nucleus, they are not contained within the nuclear fossa. The midpiece has rounded mitochondria, which have an irregular distribution, and a great number of vacuoles mostly concentrated at last portion of midpiece. The midpiece also possesses a cytoplasmic channel. The flagellum has a 9 + 2 microtubular arrangement and it does not show intratubular differentiation, the entire flagellum being not electron-dense. The flagellar membrane does not have lateral projections or fins.

Spermatozoa of *Eigenmannia trilineata*

The spermatozoa of *E*. *trilineata* are constituted by well defined head, midpiece, and flagellum with spherical nucleus slightly flattened. The nucleus is electron-dense with dense masses of chromatin in a flocular matrix. The *E. trilineata* spermatozoa also show nuclear rotation. The centrioles are in a perpendicular orientation and are contained within the nuclear fossa that is lower than in the other analyzed species. In the midpiece the spermatozoa has a cytoplasmic collar attached along most of the nucleus, small number of vacuoles and large mitochondria irregularly distributed. The flagellum has the usual 9 + 2 microtubular arrangement with no intratubular differentiation. The flagellar membrane has lateral projections that constitute long axonemal-fins along the entire flagellum length.

Discussion

The shape, length, and width of the sperm cell nucleus show great variation between species, and these characteristics are frequently associated with the fertilizing type. The basic structure of the spermatozoon of species that are externally fertilizing is an anacrosomal aquasperm defined as having a spherical to ovoid nucleus and a short midpiece (Jamieson, 1991). The species herein analyzed, as all studied Gymnotiformes species (Jamieson, 1991; França *et al*., 2007; França, 2006), have shown the aquasperm structure suggesting the external fertilization of the species. Although the representatives of the three studied families possess the same spermatozoon basic structure, there are some differences, especially on nuclear position related to the flagellum, centriolar complex position, nuclear density, vacuoles conformation, and the presence of axonemal fins.

According to Mattei (1970), there are two basic types of Teleostei spermatozoa due to the process of nuclear rotation during the spermiogenesis. In type I the nucleus has a 90° rotation in relation to the flagellar axis with the flagellum located perpendicularly to the nucleus whereas the centrioles are placed inside the nuclear fossa. In type II the nuclear rotation does not occur with the flagellum located laterally to the nucleus whereas the centrioles are placed outside the nuclear fossa. *Eigenmannia trilineata* and the three *Brachyhypopomus* species analyzed have shown type I spermatozoa, the most common type of spermiogenesis and spermatozoa between the Teleostei

(Mattei, 1970), which is observed in almost all Characiformes species (Quagio-Grassiotto *et al.,* 2003), as well as, in Cypriniformes (Bacceti *et al*., 1984) and Siluriformes (Poirer & Nicholson, 1982) and it is presumed to be a primitive condition within Teleostei (Peccio, 2003). *Gymnotus* cf. *carapo* has shown the type II spermatozoa, exhibiting not only the nucleus without rotation, but also the centriolar complex outside the nuclear fossa. Type II spermiogenesis has only been described in the characiform family Acestrorhynchidae (Matos *et al*., 2000) and in the inseminated species of the subfamily Gladulocaudinae (Burns & Weitzman, 2005). More recently, the type II spermiogenesis has been also described for another *Gymnotus* species (França *et al*., 2007).

The spermatozoa nucleus of *E. trilineata* possesses dense masses of chromatin in a flocular matrix being similar in density to the nucleus described for *Rhamphichthys* cf. *hahni*, *Eigenmannia* cf. *virescens,* and *Apteronotus* cf. *albifrons* (França, 2006), and for *Apteronotus albifrons* (cited as *Sternarchus albifrons*) (Jamieson, 1991). The species *B. draco*, *Brachyhypopomus* “G” and *G.* aff. *carapo* have shown spermatozoa nucleus very similar in density to those described for *Gymnotus* cf. *anguilaris* and *Brachyhypopomus* cf. *pinnicaudatus* (França *et al*., 2007), while *B. bombilla* has a more flocular chromatin, being more similar to the nucleus of species of the families Sternopygidae, Rhamphichthyidae and Apteronotidae than to those of Hypopomidae or even Gymnotidae.

The vacuoles are located in midpiece and exhibit great variation of position, number, distribution, and connection between them (Spadella, 2004). All the species analyzed herein have demonstrated the presence of vacuoles in the midpiece, even though the number, organization level, and distribution around the midpiece have shown variation between the different families, as well as within the genus *Brachyhypopomus*.

Up to the present, the presence of axonemal-fins have not been reported for any Ostariophysi taxon, being its absence interpreted as a secondary reduction and an apomorphic character for this group of Teleostei (Jamieson, 1991). Most recently, Quagio-Grassioto *et al.* (2001) have described similar fins on the flagellar membrane of the species *Diplomystes mesembrinus* which is considered a Siluriformes primitive representative, and Pecio (2003) has reported for the first time for a Characiformes species the presence of this structure. In Gymnotiformes, the presence of axonemal-fins has been described for representatives of the families Rhamphichthyidae and Sternopygidae (França, 2006) and their absence for Apteronotidae (França, 2006; Jamieson, 1991), Hypopomidae and Gymnotidae (França *et al*., 2007). The results obtained for *E. trilineata*, *G.* aff. *carapo*, *B. draco*, *B. bombilla* and *Brachyhypopomus* “G” about the presence of axonemal-fins corroborate these studies; although, the enlargement of the flagellar membrane in *B. bombilla* had represented a differing character between all the *Brachyhypopomus* species analyzed until today.

This study exemplifies the diversity of sperm ultrastructure characters not only between the families of Gymnotiformes, but also within its genera, indicating that this information can be useful to define phylogenetic relationships at all taxonomic levels.

Acknowledgements

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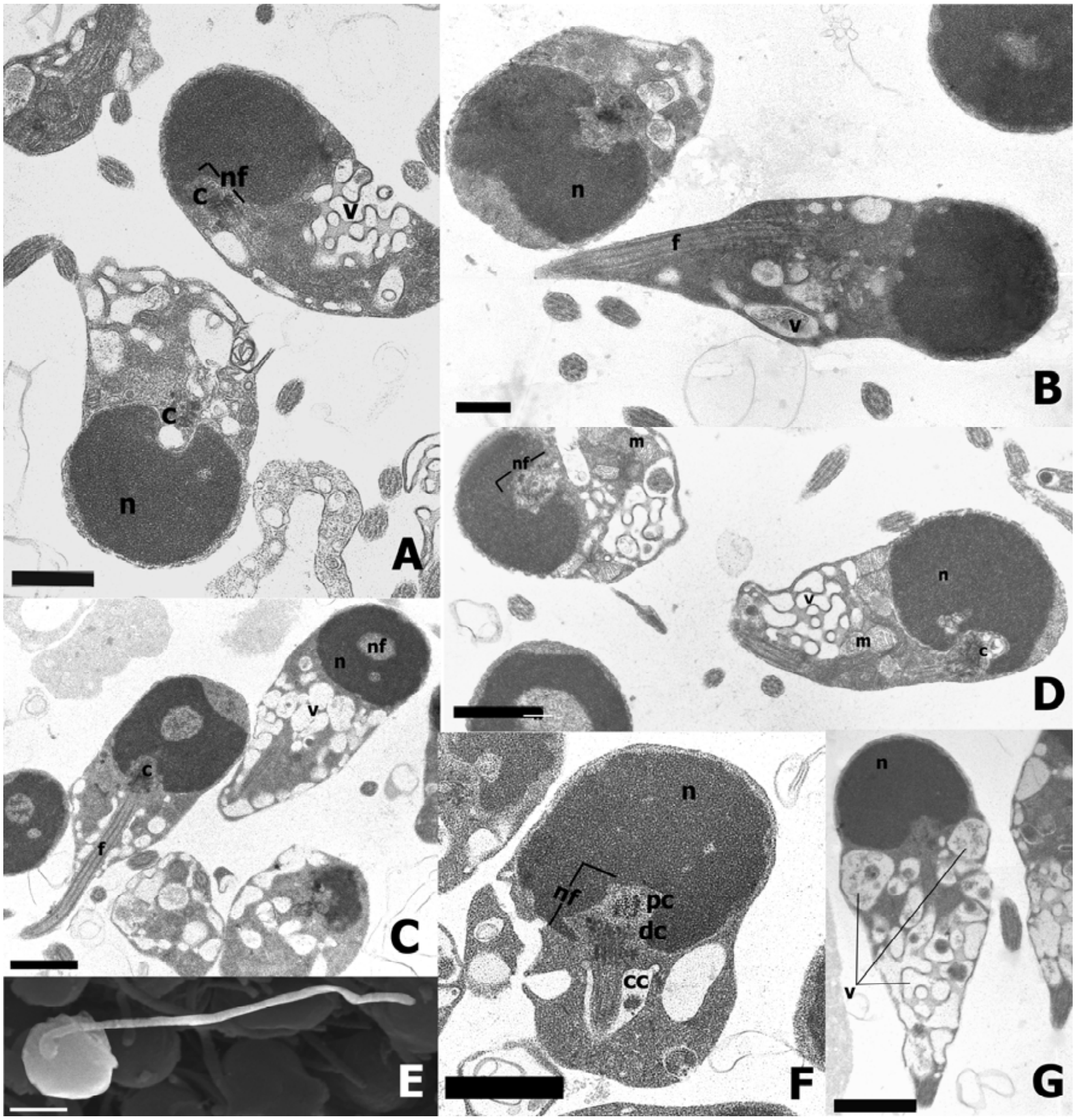

Fig. 1. A, B, C, D, F, G – Transmition electron microscopy of *Brachyhypopomus draco* spermatozoa; E – Scanning electron microscopy of *B. draco* spermatozoa. n= nucleus; c= centriole; pc= proximal centriole; dc= distal centriole, cc= citoplasmic chanel; v= vacuole; m= mitochondria; nf= nuclear fossa; f= flagellum. A and B scale bar= 500 nm; C-G scale bar= 1μm.

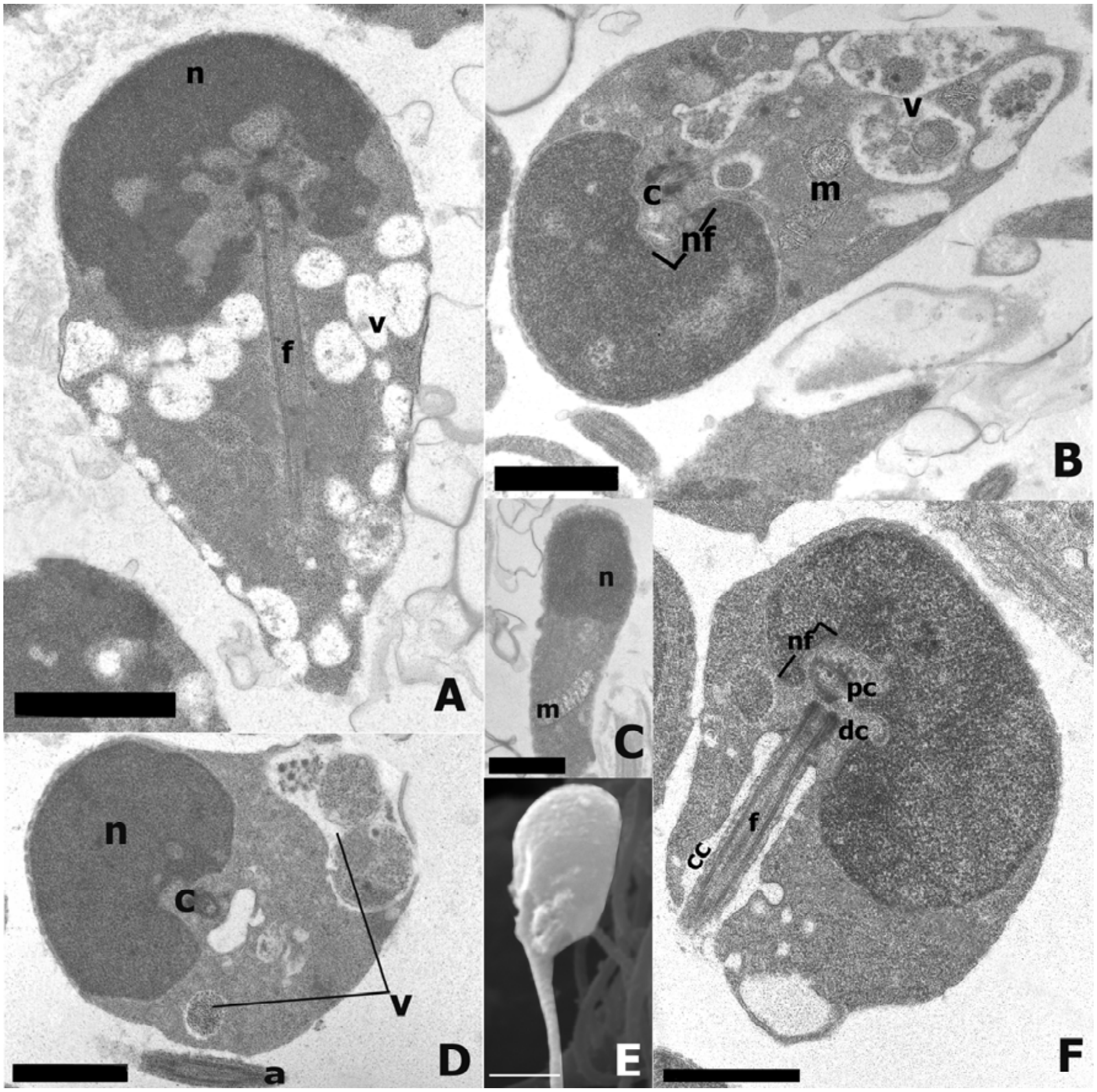

Fig. 2. A, B, C, D, F - Transmition electron microscopy of *Brachyhypopomus* “G” spermatozoa; E – Scanning electron microscopy of *Brachyhypopomus* “G” spermatozoa. n= nucleus; c= centriole; pc= proximal centriole; dc= distal centriole, cc= citoplasmic chanel; v= vacuole; m= mitochondria; a= axoneme; nf= nuclear fossa; f= flagellum. A and B scale bar= 500 nm; C-G scale bar= 1µm.

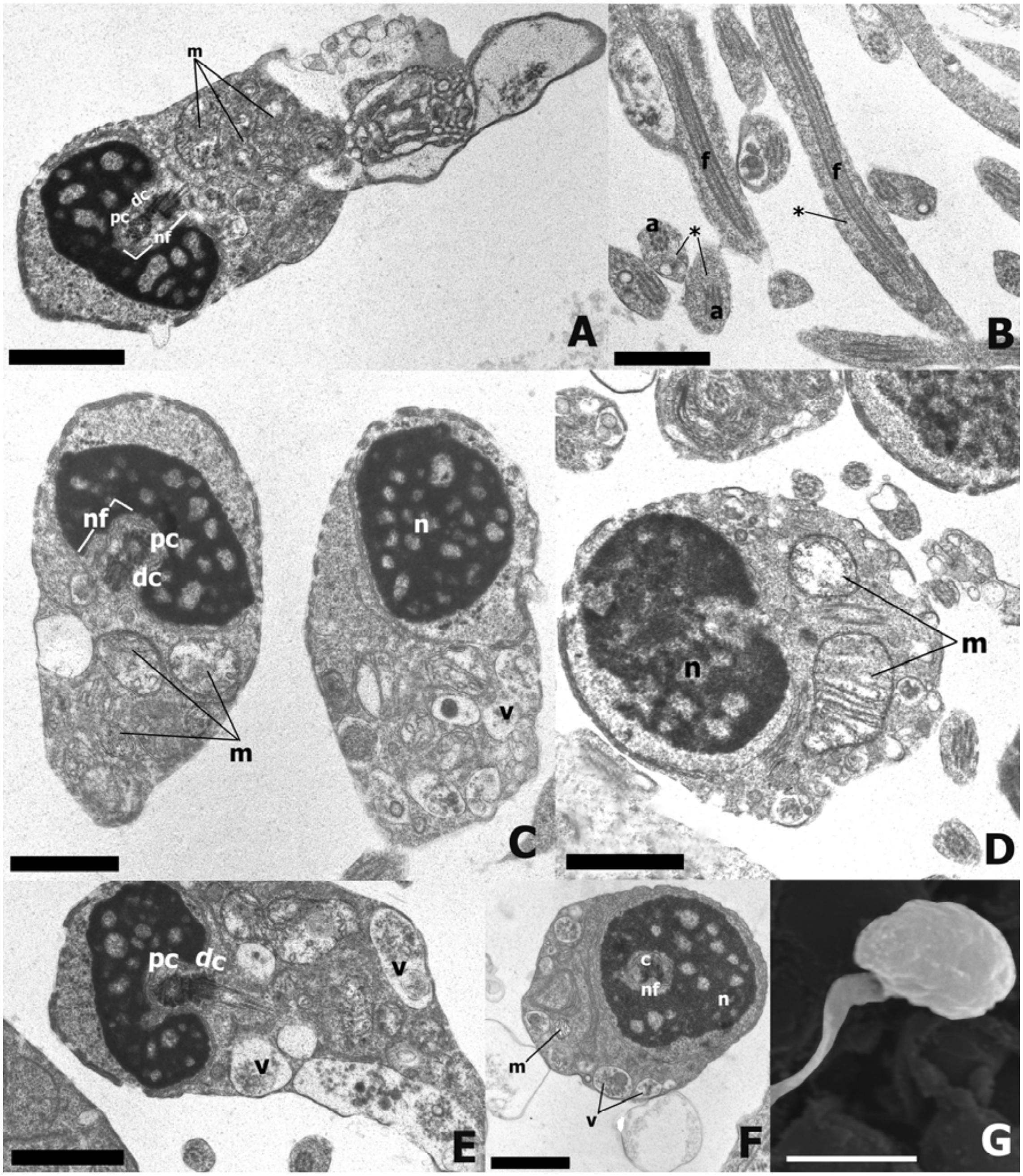

Fig. 3. A, B, C, D, E, F - Transmition electron microscopy of *Brachyhypopomus bombilla* spermatozoa; G – Scanning electron microscopy of *B. bombilla* spermatozoa. n= nucleus; c= centriole; pc= proximal centriole; dc= distal centriole; v= vacuole; m= mitochondria; a= axoneme; nf= nuclear

fossa; f= flagellum; asterisk= flagellar membrane. A-F scale bar= 1µm; G scale bar= 2 µm.

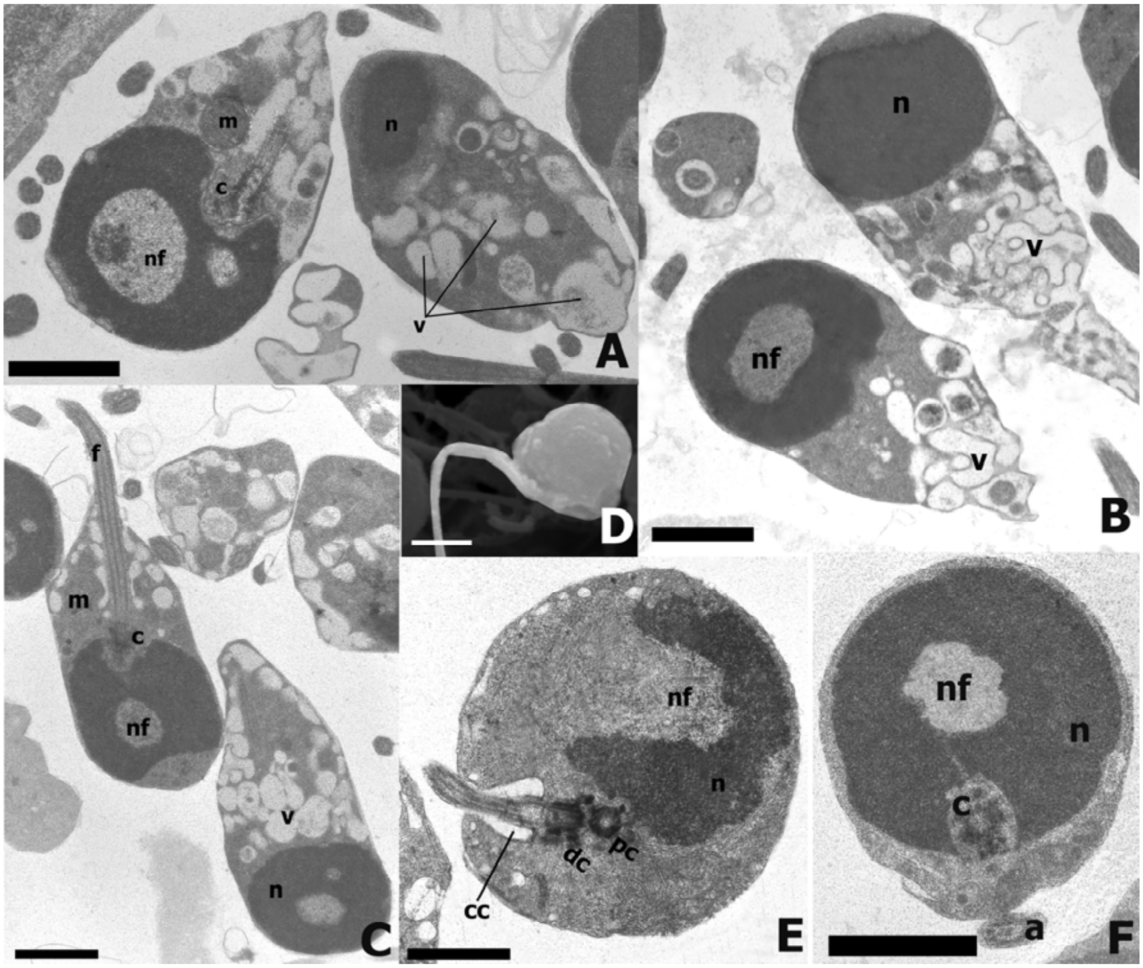

Fig. 4. A, B, C, E, F - Transmition electron microscopy of *Gymnotus* aff. *carapo* spermatozoa; D – Scanning electron microscopy of *G.* aff. *carapo* spermatozoa*.* n= nucleus; c= centriole; pc= proximal centriole; dc= distal centriole; v= vacuole; m= mitochondria; a= axoneme; nf= nuclear fossa; cc=citoplasmic chanel f= flagellum. A-F scale bar= 1µm.

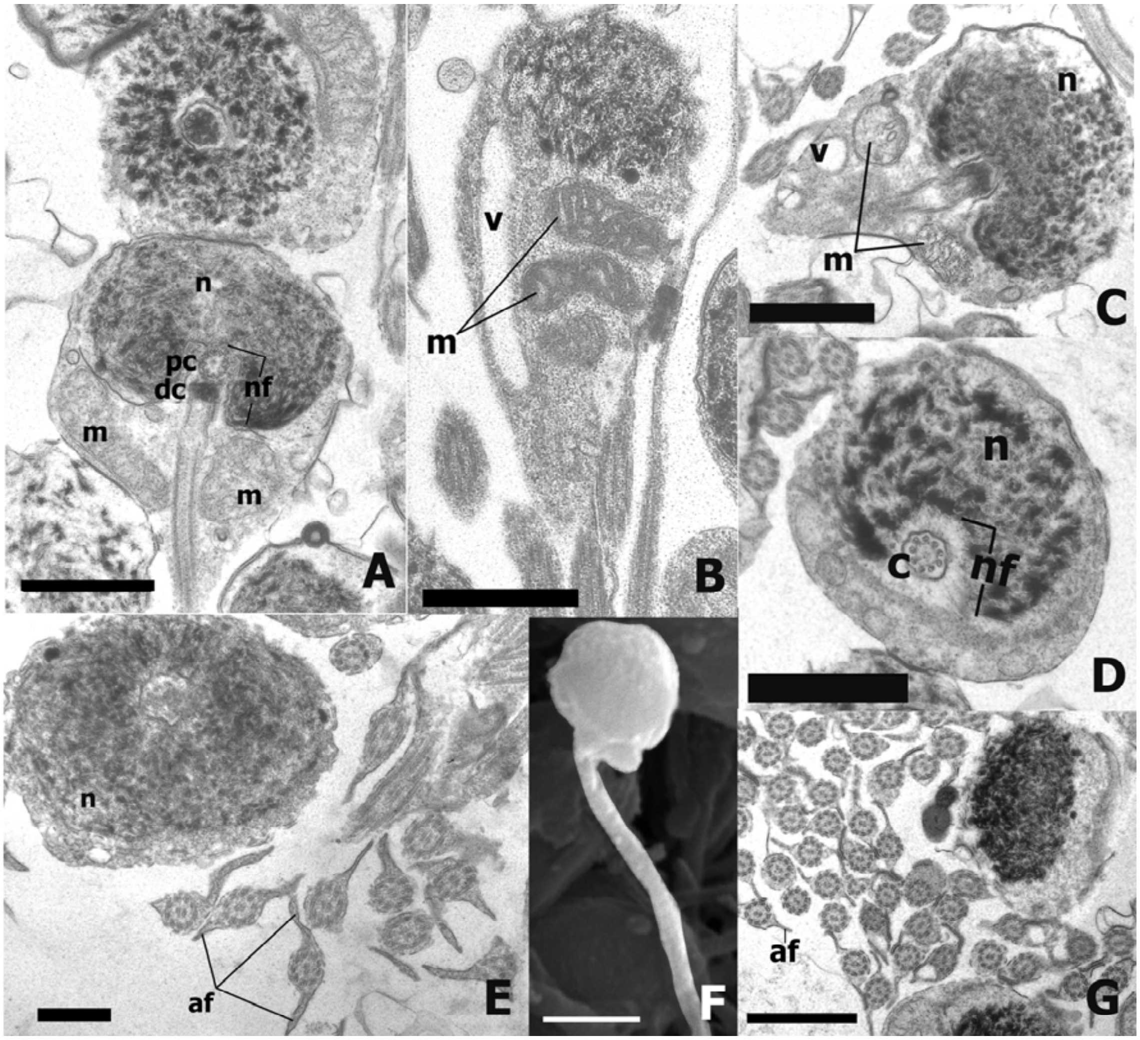

Fig. 5. A, B, C, E, G - Transmition electron microscopy of *Eigenmannia trilineata* spermatozoa; F – Scanning electron microscopy of *E. trilineata* spermatozoa*.* n= nucleus; c= centriole; pc= proximal centriole; dc= distal centriole; v= vacuole; m= mitochondria; af= axonemal fin; nf= nuclear fossa. A-F scale bar= 1µm.

Capítulo VI

Reproductive characters and spermatozoa structure in Gymnotiformes species (Teleostei: Ostariophysi) with phylogenetic considerations.

Reproductive characters and spermatozoa structure in Gymnotiformes species (Teleostei: Ostariophysi) with phylogenetic considerations.

The organism evolutionary history is associated with climate and geography changes occurred throughout the planet history; as a consequence, the elucidation of phylogenetic relationships among the organisms allow the study of their evolution and are the base for biogeographic and ecological studies (Futuyma, 1992). The knowledge concerning biological diversity is the starting point for all studies related to biological sciences, ecology, behavior, evolution, and species recognition and description (Savage, 1995).

Fishes represent the oldest and most diverse group among all vertebrates. About 24% of the fish species occurs in Neotropical freshwaters (Vari & Malabarba, 1998). As amazing as the diversity of Neotropical fishes is, also is their huge diversity of forms, behaviors, and life-histories, which allow them to occupy a great variety of habitats.

From all this diversity, representing only a small percentage of all fish species (about 1.3%) there are the species endowed with the ability to generate electricity through specialized electric organs (Moller, 1995). The ability to generate electricity from such organs evolved several times independently in the marine electric rays, in several Siluriformes, in a few species of marine stargazers, in the African Mormyriformes, and in the South American

Gymnotiformes. The last two correspond to the so-called weakly electric teleosts, the appearance of electric organs in these two distantly related groups being a good example of convergent evolution (Kirschbaum, 1984). Both Gymnotiformes and Mormyriformes species emit continuously electric organ discharges that can be either pulse or wave-type and that are monitored by electroreceptors, modified lateral line organs. These impressive electrosensory similarities are in many species also followed by similarities in morphology (Lissmann, 1958), maybe arising as adaptations to similar feeding behaviors (Roberts, 1972, Marrero & Winemiller, 1993).

Gymnotiformes is a clade of ostariophysan fishes most closely related to catfishes (Siluriformes), with which they share the presence of a passive electrosensory system (Fink and Fink, 1981, 1996; Finger, 1986). The Neotropical electric fishes constitute a group of rather specialized fishes. They exhibited a peculiar external morphology: in most species the body is very elongated, compressed laterally, without dorsal and pelvic-fins, and with a long anal fin that is responsible for the fish swimming. The caudal-fin is replaced by the caudal filament except for the families Apteronotidae and early stages of Sternopygidae, which possesses a reduced caudal-fin (Mago-Leccia, 1994). All gymnotiforms possess an extremely well-developed ability to regenerate the caudal parts of their body when damaged or injured (Ellis, 1913, Cognato *et al*., 2007).

The order is constituted by 117 species, 30 genera, and five families (Reis *et al.,* 2003); however, according to Albert & Crampton (2005) just about 78% of the known gymnotiform species have been formally described, and perhaps half again as many species remain undiscovered in the wild. Although there are many hypotheses on the relationships between gymnotiform families (Triques, 1993; Mago-Leccia, 1994; Alves-Gomes *et al.*, 1995, Albert and Crampton, 2005), these relationships are still unsolved.

Life-history x phylogeny classification

Continuous adaptation of organisms to long term environmental change remains to be one of the basic concepts of evolution (Futuyma, 1998; Pigliucci, 1996; Rose & Lauder, 1996) involving genetic change and speciation. On the other hand, individuals also must cope with short term variation of the same parameters (temperature, pressure, O_2 levels, etc.) within and/or between alternative habitats. In both cases, functional response involves metabolic and physiological processes which depend on the genetic make-up and may result in anatomical and morphological variation (Almeida-Val *et al.,* 1999).

The organisms can present life-history features owing either to ecological adaptations or to its historical legacy (phylogeny). For this reason, some organism life-history characteristics may not be representing an environmental adaptation, but a legacy of an ancestral taxa or population (Coddington, 1988).

Relations among life-history and phylogeny have been studied by some authors (Kramer, 1978; Wooton, 1984; Winemiller, 1989; Rochet, 2000; Becker *et al.*, 2003; Charnov & Gillooly, 2004). In these studies the main biological characteristics discussed are length of the breeding season, fecundity, parental care, and first maturation size, all features concerning the species reproduction. According to Winemiller (1989), traits associated with reproduction should be subjected to intense natural selection as these directly affect the individual’s selection. The great majority of the studies about fish life-history with an evolutionary aim analyzes the possible ancestry of the reproductive features exhibited for the studied group. However, due to the lack of knowledge on most part of the groups of fishes, hardly any of these studies actually use the established patterns as a systematic analysis tool (Azevedo, 2004).

Phylogenetic hypotheses deliver an indispensable perspective to comparative studies, not only by permitting the study of character evolution within a proper evolutionary framework of sister and outgroup relationships, but also by bringing into the study the concept of relative time of how such changes occur (Alves-Gomes, 1999). According to Kochmer & Handel (1986), phylogeny can be used as the “null hypothesis” for ecological model tests. With this mind, the interpretation of life-history patterns in a phylogenetic perspective can be not just a new source of systematic characters, but also a way to provide better understanding of the taxa life-history and behavior evolution.

Gonadal and germ cell morphology x phylogeny classification

Despite a few efforts aimed at using reproductive characters in comprehensive classifications of bony fishes, these areas of research remain relatively independent (Parenti & Grier, 2004). The wide range of gonadal morphologies reflects the complexity of teleost reproduction; however, basic features (i.e. the structure of germ cells and different somatic cell elements constituting the gonadal tissue) are similar (Nagahama, 1983).

Fish female gonads have been far more studied than male gonads. It can be explained due to females determine the spawning period and the number of descendants in the population and for the fact that oogenesis show cellular modifications more evidently than spermatogenesis (Silveira *et al.*, 1995). As opposed to it, the testes, and more specifically the sperm cell morphology, can also provide important information regarding reproductive and evolutionary aspects.

According to Mattei (1991), since the fishes have demonstrated great structural diversity at all taxonomic levels, it is not possible to construct spermatic models even for monophyletic groups as Actinopterygii or Teleostei. Notwithstanding, spermatozoa structure has been reported to be quite constant among same family species (Baccetti, 1987; Jamieson, 1991). For all these reasons, results on ultrastructure of sperm cell have provided valuable information on cellular modifications associated with reproductive habits and uncovered morphological characters useful in hypothesizing phylogenetic relationships (Baccetti, 1987; Jamieson, 1991; Burns *et al*., 1998, 2002).

In spite of the studies realized by Jamieson (1991) and Mattei (1988, 1991), which provide data on sperm ultrastructure of species representing all major groups of fishes, sperm morphology of freshwater Neotropical Ostariophysi are still poorly known. Concerning gymnotiform species, the current low number of studies (Jamieson,1991; França *et al*., 2007; França, 2006) and analyzed species do not allow the arising of phylogenetic hypothesis based just on spermatozoa ultrastructure.

Life-history and sperm ultrastructure of gymnotiform fishes

Investigations into phylogenetic, biogeographic, and ecological aspects of gymnotiform diversity are accelerating and the actual dimensions of the fauna now coming to be more fully appreciated (Albert & Crampton, 2005). The data available in literature concerning life-history and reproductive aspects of Gymnotiformes species are compiled in Table 1. Data concerning spermatozoa ultrastructure features of Gymnotiformes are compiled in Table 2.

Based on the presented results, it is possible to suggest that the fractional spawning type and the seasonal reproductive cycle are a constant strategy assumed by all Gymnotiformes species throughout all their geographical distribution. According to Nikolsky (1969), species with fractional spawning are better adapted to unfavourable environmental conditions and could solve problems of competition for spawning sites among females of the same population. In accordance with this statement and with the fact that gymnotiform species are known to mostly inhabit unstable habitats, the quoted reproductive strategies have been probably assumed as a matter of adaptation.

Among several other factors, fecundity depends on the coelomatic cavity capacity of lodge ripe oocytes and on the oocytes size (Vazzoler, 1996). The relative fecundity is obtained by the total number of oocytes per female milligram of weight. Relative fecundity is a quite relevant information that allows comparisons among the fecundity of different species and estimations on the energetic effort applied on oocyte production. Since absolute fecundity only considers the total number of oocytes in the fish gonads, it cannot be used for comparisons among different size species. Due to that, and considering the great range of body size achieved by gymnotiform species, only a low percentage of the studies presented here are meaningful for fecundity comparisons.

Patterns of diversity in Gymnotiformes suggest that the evolution of body size is influenced by EOD type, species with pulsetype EODs tending to be smaller on average than species with wave-type EODs, and exhibiting more size and habitat diversity (Albert & Crampton, 2005). Adult total length in Gymnotiformes ranges over an order of magnitude from as small as 50 mm in mature specimens of an undescribed *Hypopygus* from the Venezuelan Amazon (Albert & Crampton, 2005) to more than 2235.2 mm in *Electrophorus electricus* (Elis, 1913). The species included in Table 1 have ranged from 129 mm (*Brachyhypopomus occidentalis*) to 1870 mm (*Electrophorus electricus*) in total length, but in contrast with this great size variation, the number of analyzed species is evidencing that just an increase on the number of studies would make possible the verification of relations between size and reproductive strategies.

According to the compiled data, parental care is reported only for three gymnotiform genera: *Gymnotus, Electrophorus* and *Sternopygus*. From these three genera, *Gymnotus* and *Electrophorus* are integrating the same family, Gymnotidae (Albert, 2001), which can be indicating the establishment of this behavior as a pattern for all species of the family. Despite of that, up until now *Sternopygus macrurus* is an exception among the Sternopygidae species.

Moreover, parental care has been studied in several families of Siluriformes – the sister group of Gymnotiformes (Fink & Fink, 1996) – being undocumented among extant basal groups and sporadic documented in derived groups (Crampton & Hopkins, 2005).

Gymnotiform reproductive cycles have been related to environmental factors associated with the rainy and dry seasons, which are seasonal variations typical of the tropical zone of Neotropical area (Hopkins, 1974; Kirschbaum, 1975, 1979, 1984, 2000; Hagedorn, 1988; Assunção & Schwassmann, 1995; Kirschbaum & Schugardt, 2002; Crampton & Hopkins, 2005). However, the reproductive seasonality in fishes of temperate environments, where longer rainfall periods are not defined, is mainly related to temperature, photoperiod and food availability (McKayne, 1984; Payne, 1986). Studies with species from the genus *Brachyhypopomus*, *Gymnotus,* and *Eigenmannia* from southern South America have defined photoperiod and temperature as the environmental clues that trigger the breeding period (Silva *et al.*, 2002; 2003; Quintana *et al*., 2004; Cognato & Fialho, 2006; Giora & Fialho, in press; A. Schaan, J. Giora and C. Fialho, in preparation; Giora & Fialho, Chapter III). As a consequence of this discrepancy, the environmental factors related with the trigger of reproductive cycle seem to be a life-history feature owing more to ecological adaptations than to the phylogeny of genus and families.

The characters of sperm cell ultrastructure have appeared to be highly informative since patterns can be observed as well as differences and exceptions. The Gymnotiformes species currently analyzed (Jamieson, 1991;

França *et al*., 2007; França, 2006; Giora & Fialho, Chapter V) have shown the aquasperm structure of the spermatozoa as it was defined by Jamieson (1991), proving the external fertilization of the species.

Until recently, the presence of axonemal-fins had not been reported for any Ostariophysi taxon, the absence of these structures being interpreted as a secondary reduction and apomorphic character of this group of teleost (Jamieson, 1991). Quagio-Grassioto *et al.* (2001) reported the presence of similar fins for *Diplomystes mesembrinus*, believed to be a primitive representative of Siluriformes, and Pecio (2003) documented the existence of these structures for the first time in a representative of the Characiformes. Nowadays, the axonemal-fins are also known to be present in spermatozoa of fishes from the gymnotiform families Ramphichthyidae and Sternopygidae (França, 2006; Giora *et al.*, Chapter V).

According to the presence (type I) or absence (type II) of nuclear rotation, two types of spermiogenesis have been described in teleostei (Mattei, 1970). Type I is the most common type of spermiogenesis and spermatozoa among the teleostei (Mattei, 1970), which is observed in almost all Characiformes species (Quagio-Grassiotto *et al.,* 2003), as well as, in Cypriniformes (Bacceti *et al*., 1984) and Siluriformes (Poirer & Nicholson, 1982) and it is presumed to be a primitive condition within teleostei (Peccio, 2003). Type II has only been described in the characiform family Acestrorhynchidae (Matos *et al*., 2000) and in the inseminated species from the subfamily Gladulocaudinae (Burns & Weitzman, 2005). From the gymnotiform species currently studied, type II spermatozoa have been reported just for species of the genus *Gymnotus*. The centriolar complex location is associated with the presence or absence of nuclear rotation. Because of that, these two characters can be analyzed together.

Additionally, through this data compilation, it is possible to define the presence and organization of the cytoplasmatic vacuoles as a character that can be phylogenetically informative.

Besides the low number of analyzed species concerning reproductive strategies and germ cell morphology, the great number of gymnotiform species that remain undescribed or is said to have extremely wide distributions across multiple river basins in South America is also a problem for the establishment of patterns and tests of phylogenetic hypotheses. The reproductive patterns and morphological aspects of gonads and germ cells are not necessarily representing characters that are useful in analyzing systematics, although they are showing that this information is potentially meaningful for the understanding of the relationships among the groups.

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Table 1. Data available in literature concerning life-history and reproductive aspects of Gymnotiformes species.

	Highest Total Length	Absolute Fecundity	Relative Fecundity	Spawning Type	First Maruration Size	Sexual Dimorphism - Size	Reproductive Sazonality	Months of Reproductive Period	Environmental Factor Related to Reproductive Period	Parental Care	Reference
Family Gymnotidae											
Gymnotus* aff. *carapo	males = 280.7 mm females = 281.8 mm	915.3 ± 202	0.2	fractional	male = 146 mm female = 141 mm	no	yes	November to March	male = incresing O_2, decreasing conductivity female = increasing temperature and photoperiod, decreasing O2	–	Cognato & Fialho (2006); Giora *et al*. (Chapter V)
***Gymnotus carapo* I**	–	about 200-247 eggs per spawning	–	fractional	about 1 year of age or slightly earlier	–	yes	–	male and female = decreasing conductivity	yes, male presenting mouth breeding	Kirschbaum & Schugardt (2002)
***Gymnotus carapo* II**	male = 335 mm	–	–	–	–	–	yes	–	–	yes, male presenting nesting construction and parental care	Crampton & Hopkins (2005)
***Gymnotus carapo* III**	–	–	–	–	–	–	yes	–	increasing temperature and photoperiod	–	Silva *et al.* (2002; 2003)
***Gymnotus carapo* IV**	male = 520 mm female = 480 mm	2192 - 1791	–	fractional	248 mm - between 1 and 2 years of age	–	yes	October to December	increasing photoperiod, O2 and conductivity	–	Barbieri & Barbieri (1982, 1983a, 1983b, 1984a, 1984b, 1985)
Gymnotus mamiraua	male = 270 mm	–	–	fractional	–	–	yes	Februart to August	rising and high-water perriod	yes, male presenting nesting construction and parental care	Crampton & Hopkins (2005)
Electrophorus eletricus	male = 1870 mm female = 1150 mm	17000	–	fractional	female = 68.5 cm, about 3.5 years of age	yes, males assuming the highest lengths	yes	September to December	beginning of dry season	yes, male presenting nesting construction and parental care	Assunção & Schwassmann (1995)

Family Ramphichthyidae											
***Rhamphichthys* sp.**	–	about 500 to 1000 eggs per spawning	–	fractional	–	–	yes	–	male and female = increasing water level, decreasing conductivity	no	Kirschbaum & Schugardt (2002)
Family Hypopomidae											
Brachyhypopomus draco	male = 212.34 female = 176.79 mm	935.8 (576-1391)	0.173	fractional	–	yes, males assuming the highest lengths	yes	August to December	male = increasing O2 and deepth, decreasing conductivity female = increasing deepth	–	A. Schaan, J. Giora and C. Fialho (in prep); Giora *et al*. (Chapter V)
Brachyhypopomus bombilla	male = 146.04 mm female = 132.68	587.33 (369-773)	0.21	fractional	male = 93.7 mm female = 97.6 mm	no	yes	October to January	male = increasing O2 and photoperiod female = increasing photoperiod	–	Giora & Fialho (Chapter III); Giora *et al*. (Chapter V)
***Brachyhypopomus* "G" new sp**	male = 188.8 mm female = 175.36 mm	589.44 (299-799)	0.20	fractional	male = 108.0 mm female = 104.5 mm	no	yes	October to February	male and female = increasing photoperiod	–	Giora & Fialho (Chapter III); Giora *et al*. (Chapter V)
***Brachyhypopomus occidentalis* (cited as *Hypopomus occidentalis*)**	male = 150.9 mm female =129 mm	–	–	–	–	yes, males assuming the highest lengths	yes	December to April	transition of the wet-dry season	no	Hagedorn (1988)
***Brachyhypopomus pinnicaudatus* I**	–	about 70 eggs per spawning	–	fractional	10-12 cm (4-5 months)	–	yes	–	male and female = decreasing conductivity	–	Kirschbaum & Schugardt (2002)
***Brachyhypopomus pinnicaudatus* II**	180 mm	–	–	fractional	male and female = 90 mm	–	yes	November to January	increasing temperature and photoperiod	–	Quintana *et al.* (2004); Silva *et al.* (2002; 2003)
Brachyhypopomus brevirostris	–	about 50 eggs per spawning	–	fractional	–	–	yes	–	male and female = decreasing conductivity	–	Kirschbaum & Schugardt (2002)

Family Sternopygidae											
Eigenmannia trilineata	male = 247.79 mm female = 170 mm	1196.06 (744-2217)	0.27	fractional	male = 6.35 female = 8.05	yes, males assuming the highest lengths	yes	October to February	male and female = increasing photoperiod, decreasing conductivity	–	Giora & Fialho (in press); Giora *et al.* (Chapter V)
Eigenmannia virescens	male = 330 mm female = 200 mm	100 to 200 eggs per spawning	–	fractional	female = 15 cm	yes, males assuming the highest lengths	yes	–	male and female = increasing water level and rain imitation, decreasing conductivity and pH	–	Kirschbaum (1975, 1979, 1984, 2000)
Eigenmannia lineata	–	about 65-162 eggs per spawning	–	fractional	–	–	–	–	–	no	Kirschbaum & Schugardt (2002)
Sternopygus macrurus	male = 546.5 mm female = 460 mm	about 300 eggs per spawning	–	fractional	about 1 year of age or slightly earlier	–	yes	–	male and female = decreasing conductivity (before the first flood of the season)	yes, male guarding the eggs deposited on substract	Hopkins (1974); Kirschbaum & Schugardt (2002)
Family Apteronotidae											
Apteronotus leptorhynchus	–	1 to 105 eggs per spawning	–	fractional	about 1 year of age or slightly earlier	–	yes	–	male and female = increasing water level, decreasing conductivity	–	Kirschbaum & Schugardt (2002)

Table 2. Data available in literature concerning spermatozoa ultrastructures of Gymnotiformes spescies.

	Sperm Nucleus	Nuclear Shape	Centriole Orientation	Nuclear Rotation	Axonemal Fins	Extensive Cytoplasmatic Vacuoles	Cytoplasmatic Collar Attached to the Nucleus	Centriolar Complex Location	Citoplasmatic Chanel	Reference
Family Gymnotidae										
Gymnotus aff. _carapo_	dense masses of chromatin - peripherically electron dense	spherical	perpendicular	no	absent	yes - mainly on midpiece posterior portion	no	outside nuclear fossa	present	Cognato & Fialho (2006); Giora *et al*. (Chapter V)
Gymnotus cf. _anguillaris_	condensed chromatin in juxtaposed filament	semi-arch		no	absent	yes - mainly on midpiece perifery	–	outside nuclear fossa	present	França *et al*. (2007)
Family Ramphichthyidae										
Rhamphichthys cf. _hahni_	condensed chromatin in filamentous axis	semi-arch	slightly eccentric	yes	present	yes - throughout the entire midpiece	–	within nuclear fossa	–	França (2006)
Family Hypopomidae										
Brachyhypopomus draco	dense masses of chromatin - peripherically electron dense	spherical	perpendicular	yes	absent	yes - forming a membranous structure	no	within nuclear fossa	present	A. Schaan, J. Giora and C. Fialho (in prep); Giora *et al*. (Chapter V)
Brachyhypopomus bombilla	dense masses of chromatin in a granular matrix	spherical	perpendicular	yes	absent	yes	no	within nuclear fossa	present	Giora & Fialho (Chapter III); Giora *et al*. (Chapter V)
Brachyhypopomus "G" new sp	dense masses of chromatin - peripherically electron dense	spherical	perpendicular	yes	absent	yes	no	within nuclear fossa	present	Giora & Fialho (Chapter III); Giora *et al*. (Chapter V)

Brachyhypopomus* cf. *pinnicaudatus	condensed chromatin in juxtaposed filament	spherical	slightly eccentric	yes	absent	yes - mainly on midpiece perifery	–	within nuclear fossa	absent	França *et al*. (2007)
Family Sternopygidae										
Eigenmannia trilineata	dense masses of chromatin in a granular matrix	sub-spheroidal	perpendicular	yes	present	yes	yes	within nuclear fossa	present	Giora & Fialho (in press); Giora *et al.* (Chapter V)
Eigenmannia* cf. *virescens	condensed chromatin in filamentous axis	semi-arch	slightly eccentric	yes	present	yes - mainly on midpiece perifery	–	within nuclear fossa	present	França (2006)
Family Apteronotidae										
Apteronotus albiforns **(cited as *Sternarchus albifrons*)**	dense masses of chromatin in a granular matrix	sub-spheroidal	perpendicular	yes	absent	–	yes	within nuclear fossa	present	Jamieson (1991)
Apteronotus* cf. *albiforns	condensed chromatin in filamentous axis	semi-arch	perpendicular	yes	absent	yes - throughout the entire midpiece	–	within nuclear fossa	present	França (2006)

Síntese dos Resultados

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- Duas novas espécies do gênero *Bracyhypopomus* são descritas para as regiões central, sul e costeira do estado do Rio Grande do Sul, Brasil, e Uruguai, *Bracyhypopomus* “G” também tendo ocorrência registrada para o Paraguai.

- *Bracyhypopomus draco* é diagnosticada de seus congêneres, entre outros caracteres, pelo formato da porção final do filamento caudal de machos maduros durante o período reprodutivo, que forma uma estrutura distinta em forma de remo.

- *Bracyhypopomus* “G” é diagnosticada de seus congêneres baseado em coloração do corpo, caracteres merísticos e morfológicos tais como número de raios da nadadeira anal, posição da origem da nadadeira anal em relação à nadadeira peitoral, porção distal do filamento caudal de machos maduros e proporções corporais.

- *Bracyhypopomus* “G” tem sido identificada como *B. pinnicaudatus* e é agora distinguida da mesma.

- O período reprodutivo da espécie *B. bombilla* se estendeu de Outubro/2004 a Janeiro/2005, sendo relacionado ao aumento do fotoperíodo, tendo o IGS dos machos também sido relacionado às variações de oxigênio. O período reprodutivo de *Brachyhypopomus* “G” ocorreu de Outubro/2005 a Fevereiro/2006, sendo também relacionado ao aumento do fotoperíodo.

- A fecundidade relativa foi estabelecida como 0.21 ovócitos por mg de peso para *B. bombilla* e 0.20 ovocitos para *Brachyhypopomus* “G”.

- *B. bombilla* e *Brachyhypopomus* “G” apresentaram desova parcelada.

- O tamanho de primeira maturação gonadal de *B. bombilla* foi estimado como 97.6 mm para fêmeas e 93.7 mm para machos, sendo estimado como 104.5 mm para fêmeas e 108.0 mm para machos de *Brachyhypopomus* “G”.

- A proporção sexual não diferiu de 1:1 nas populações estudadas de *B. bombilla* e *Brachyhypopomus* “G” de acordo com a analise do teste χ^2 (p= 0.01).

- Não foi observado dimorfismo sexual relacionado ao comprimento total, para *B. bombilla* e *Brachyhypopomus* “G”, entretanto foi verificada uma modificação no filamento caudal de machos de *Brachyhypopomus* “G”.

- O desenvolvimento das células germinativas de *B. bombilla* e *Brachyhypopomus* “G” foi classificado in cinco estágios de desenvolvimento para fêmeas e seis para machos.

- Os estádios de maturação gonadal definidos para fêmeas de *B. bombilla* e *Brachyhypopomus* “G” foram: maturação inicial, maturação final, desovando e esgotado.

- Os estádios de maturação gonadal definidos para machos de *B. bombilla* e *Brachyhypopomus* “G” foram: maturação inicial, maturação intermediaria, maturação final, regredindo e regredido.

- Contrariamente a homogeneidade apresentada com relação aos tipos celulares e estádios de maturação gonadal, as duas espécies de *Brachyhypopomus* estudadas demonstraram diferenças nas freqüências dos estádios de maturação ao longo do ano, *B. bombilla* possuindo um desenvolvimento gonadal mais sazonal do que *Brachyhypopomus* “G”.

- Algumas diferenças foram encontradas entre a ultraestrutura do espermatozóide das espécies analisadas representando as famílias Gymnotidae, Setrnopygidae e Hypopomidae: presença de rotação nuclear, observada em *E. trilineata* e nas três espécies de *Brachyhypopomus*; presença de aletas laterais no flagelo, observadas somente em *E. trilineata*.

- Algumas variações intraespecificas com respeito a ultraestrutura do espermatozóide das espécies de *Brachyhypopomus* analisadas foram registradas, tais como a densidade nuclear e o número e nível de organização dos vacúolos.

- A maioria das características encontradas nos espermatozóides de *Gymnotus* aff. *carapo*, *Eigenmannia trilineata*, *B. draco, B. bombilla,* e *Brachyhypopomus* "G" são compartilhadas com as demais espécies de Gymnotifomes atualmente analisadas.

- Através da compilação dos dados atualmente disponíveis na literatura a respeito da reprodução e história de vida de espécies de Gymnotiformes é possível estabelecer padrões utilizáveis em análises evolutivas e filogenéticas.

- Alguns aspectos potencialmente utilizáveis em análises comparativas baseadas nos dados já existentes para a reprodução espécies de Gymnotiformes são: tipo de desova, fecundidade, tamanho corporal, cuidado parental.

- Aspectos da ultraestrutura do espermatozóide tem se mostrado informativo para a formação de hipóteses filogenéticas.

- Alguns aspectos potencialmente utilizáveis em análises comparativas baseadas nos dados já existentes de ultraestrutura do espermatozóide para espécies de Gymnotiformes são: presença ou ausência de aletas laterais no flagelo, rotação nuclear, localização do complexo centriolar, numero e organização dos vacúolos celulares.

- O pequeno número de espécies ainda analisadas com relação a aspectos reprodutivos e de morfologia do espermatozóide, e o grande número de espécies ainda não descritas ou identificadas como possuindo uma distribuição extremamente ampla entre muitas bacias hidrográficas da América do Sul, tem sido um problema para o estabelecimento de padrões utilizáveis em análises comparativas e filogenéticas.