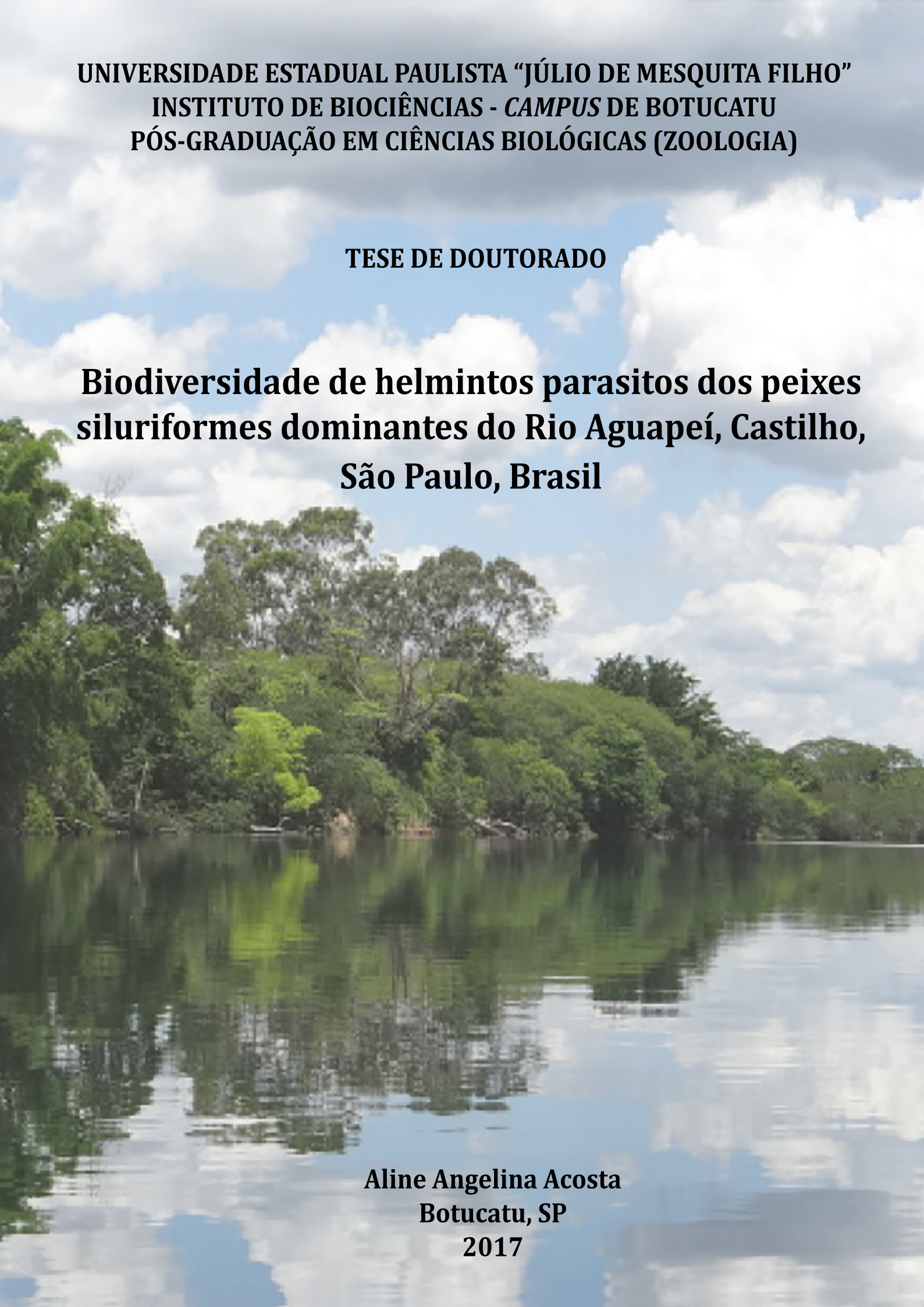




**UNIVERSIDADE ESTADUAL PAULISTA “JÚLIO DE MESQUITA FILHO”
INSTITUTO DE BIOCÊNCIAS - *CAMPUS* DE BOTUCATU
PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS (ZOOLOGIA)**

TESE DE DOUTORADO

**Biodiversidade de helmintos parasitos dos peixes
siluriformes dominantes do Rio Aguapeí, Castilho,
São Paulo, Brasil**

**Aline Angelina Acosta
Botucatu, SP
2017**

UNIVERSIDADE ESTADUAL PAULISTA
“JÚLIO DE MESQUITA FILHO”
INSTITUTO DE BIOCÊNCIAS - *CAMPUS* DE BOTUCATU
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS
BIOLÓGICAS (ZOOLOGIA)

**Biodiversidade de helmintos parasitos dos peixes
siluriformes dominantes do Rio Aguapeí, Castilho, São
Paulo, Brasil**

Aline Angelina Acosta

Orientador: Prof. Tit. Reinaldo José da Silva

Tese apresentada ao Programa de Pós-graduação em Ciências Biológicas (Zoologia) do Instituto de Biociências da Universidade Estadual Paulista, UNESP, Campus de Botucatu-SP, como parte dos requisitos para obtenção do título de Doutor em Ciências Biológicas (Zoologia).

Botucatu, SP
2017

FICHA CATALOGRÁFICA ELABORADA PELA SEÇÃO TÉC. AQUIS. TRATAMENTO DA INFORM.
DIVISÃO TÉCNICA DE BIBLIOTECA E DOCUMENTAÇÃO - CÂMPUS DE BOTUCATU - UNESP
BIBLIOTECÁRIA RESPONSÁVEL: ROSEMEIRE APARECIDA VICENTE-CRB 8/5651

Acosta, Aline Angelina.

Biodiversidade de helmintos parasitos dos peixes
siluriformes dominantes do Rio Aguapeí, Castilho, São
Paulo, Brasil / Aline Angelina Acosta. - Botucatu, 2017

Tese (doutorado) - Universidade Estadual Paulista
"Júlio de Mesquita Filho", Instituto de Biociências de
Botucatu

Orientador: Reinaldo José da Silva
Capes: 20400004

1. Bagre (Peixe). 2. Biodiversidade. 3. Parasito.
4. Peixes. 5. Taxonomia numérica. 6. Aguapeí, Rio (SP).

Palavras-chave: Biodiversidade; Parasitos; Peixes;
Siluriformes; Taxonomia.

***“The river may be wide, but it can always be crossed”
African Proverb***

Agradecimentos

AGRADECIMENTOS

Agradeço a Deus por ter me dado força, coragem e perseverança ao longo destes quatro anos.

Aos meus pais, Ana Luiza e Amilton, meus tios Ernesto, Patrícia, Adriane e Marcos por me apoiarem em minhas decisões e guiar meus passos sempre que preciso. Aos meus irmãos, que me fazem lembrar todos os dias da pessoa que quero ser para eles, e a buscar essa pessoa com sabedoria e determinação. A minha família de coração, Lou, Pawel e Nya, por terem me acolhido em suas vidas há oito anos e por ainda fazerem questão de me manter nelas. Ao Vanderlei por ter me acompanhado e apoiado nestes quatro anos.

Ao professor Reinaldo pelos seis anos de orientação, período o qual pude crescer profissionalmente e pessoalmente graças ao seu apoio, paciência e ensinamentos.

A Fundação de Amparo à Pesquisa do Estado de São Paulo – FAPESP, (2012/22895–7/2015/22382–8) pelo apoio científico e financeiro. A Companhia Energética de São Paulo – CESP, pelo apoio logístico nos trabalhos de campo. Aos pescadores Gilberto, Silvio e Hércules pelo apoio fundamental nas coletas.

Aos colegas e funcionários do Departamento de Parasitologia. Aos colegas e amigos do Laboratório de Parasitologia de Animais Silvestres (LAPAS) que alegam os meus dias de trabalho. Agradeço especialmente a colaboração e amizade de Maria Isabel, Fábio, Priscilla, Lidianne Franceschini e Aline Zago, e aos “lapianos” presentes e ausentes Érica, Lidi, Drausio, Mariana, Rodrigo, Alison, Gislayne, Heleno e Jaciara.

Ao Tomáš Scholz por ter me acolhido em seu laboratório e ter me dado a oportunidade de crescer e expandir meus horizontes na taxonomia e sistemática, e pelo seu apoio e ajuda durante minha estadia em seu laboratório. Aos funcionários e colegas do Laboratory of Helminthology do Institute of Parasitology, Biology Center, Czech Academy of Science (IPCAS). Agradeço especialmente ao Philippe pela sua indispensável ajuda e colaboração enquanto estive no IPCAS, também a Simona, Mirka, David e família, Jesus, Camila, Honza, Roman, Aneta, Martina, Blanka, pela ajuda e companhia durante minha estadia. A Isabel Blasco-Costa por ter me acolhido no Muséum d'histoire naturelle (MHN), e em sua casa e principalmente pelos ensinamentos de filogenia.

Finalmente, agradeço àqueles que fizeram e fazem de Botucatu um lugar feliz e que se tornaram minha família: Mariana, Priscila, Jamile, Bel, Jairon, Dani, Pomba, Isabela, Naná, Katharinne e Marciano.

Sumário

Resumo	1
Abstract	3
1. Introdução geral	5
1.1. Parasitos e a biodiversidade	6
1.2. Parasitos de peixe como indicadores de impacto ambiental	7
1.3. Monogeneas da região Neotropical: diversidade e estudos filogenéticos de dactilogirídeos de siluriformes	8
1.4. Área de estudo	9
1.5. Hospedeiros investigados	10
1.6. Referências	14
2. Objetivos	19
2.1. Objetivo geral	20
2.2. Objetivos específicos	20
3. Helminth parasites of eight siluriform species from the Aguapeí River, Upper Paraná river basin, São Paulo State, Brazil	22
Abstract	23
3.1. Introduction	24
3.2. Materials and Methods	26
3.3. Results	29
3.4. Discussion	108
3.5. References	126
4. Added resolution to circumscription of some gill monogeneans species (Dactylogyridae) of Neotropical catfishes including description of a new <i>Demidospermus</i> species and erection of a new genus	139
Abstract	140
4.1. Introduction	141

4.2. Materials and Methods	142
4.3. Results	152
4.4. Discussion	165
4.5. References	167
 5. A synoptic review of <i>Heteropriapulus</i> spp. (Monogenea: Dactylogyridae) from loricariid catfishes in South America including description of five new species and first molecular phylogeny	172
Abstract	173
5.1. Introduction	174
5.2. Materials and Methods	176
5.3. Results	180
5.4. Discussion	200
5.5. References	201
 6. <i>Spinitectus aguapeiensis</i> n. sp. (Nematoda: Cystidicolidae) from <i>Pimelodella avanhandavae</i> (Siluriformes: Heptapteridae) in the Aguapeí River, Upper Paraná River basin, Brazil	209
Abstract	210
6.1. Introduction	211
6.2. Materials and Methods	212
6.3. Results	212
6.4. Remarks	217
6.5. References	218
 7. Considerações finais	221

Resumo

Peixes da ordem Siluriformes, conhecidos como bagres ou catfishes, apresentam distribuição mundial com mais de 2.800 espécies reconhecidas. Aproximadamente 1.700 espécies são encontradas nas Américas, principalmente na região Neotropical. Apesar desta alta diversidade, abundância e importância econômica, tanto como alimento e ornamental, dos siluriformes, o conhecimento sobre seus helmintos parasitos é fragmentado e insuficiente. Oito espécies de siluriformes (*Pterodoras granulosus*, *Trachydoras paraguayensis*, *Pimelodella avanhandavae*, *Loricariichthys platymetopon*, *Pterygoplichthys ambrosettii*, *Rhinelepis aspera*, *Hemisorubim platyrhynchos* e *Sorubim lima*) do rio Aguapeí, bacia do alto rio Paraná, município de Castilho, São Paulo, Brasil, foram investigados quanto a sua fauna de helmintos parasitos. Cinquenta e quatro *taxa* de helmintos parasitos foram encontrados: 25 monogeneas, 15 digenéticos, 11 nematoides e três cestoides. Com base em novos dados moleculares (13 novas sequências de rDNA 28S de dactilogirídeos) e morfológicos, é proposta uma revisão da diagnose do gênero *Demidospermus* com descrição de uma nova espécie, também é proposto um novo gênero de dactilogirídeo com uma descrição de espécie nova, e discute-se sobre a questão do posicionamento de gênero de algumas outras espécies cujos dados moleculares estão disponíveis. O gênero *Heteropriapulus* é revisado, com descrição de cinco novas espécies de loricarídeos, e ainda dados moleculares preliminares (sequências de rDNA 28S) são apresentadas para três *taxa* incluindo a espécie tipo *Heteropriapulus heterotylus*. Uma nova espécie de nematoide do gênero *Spinitectus* é descrita com base em observações morfológicas. *Spinitectus aguapeiensis* n. sp. é o primeiro registro de uma nova espécie para o siluriforme *Pimelodella avanhandavae*, o quarto registro desta espécie na bacia do Alto Paraná e a sexta espécie do gênero descrita para América do Sul. Apresentando novos registros de hospedeiros, novos registros de localidade, e possíveis novas espécies para a ciência, o inventário da helmintofauna destas espécies de hospedeiros aumentou consideravelmente o conhecimento da vasta e ainda desconhecida diversidade de parasitos de peixes siluriformes da região Neotropical.

Palavras-chave: Bacia do rio Alto Paraná, bagres, morfologia, filogenia, Dactylogyridae, diversidade.

Abstract

Fishes of the order Siluriformes, known as catfishes, have a worldwide distribution with more than 2,800 recognized species. Approximately 1,700 species are found in America, mainly in the Neotropical region. Despite being highly diverse, abundant, and of economic importance as food and ornamental fishes, the knowledge about their helminth parasites is fragmentary and insufficient. Eight species of catfishes (*Pterodoras granulosus*, *Trachydoras paraguayensis*, *Pimelodella avanhandavae*, *Loricariichthys platymetopon*, *Pterygoplichthys ambrosettii*, *Rhinelepis aspera*, *Hemisorubim platyrhynchos*, and *Sorubim lima*) from the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo State, Brazil, were surveyed for helminth parasites. Fifty-four helminth parasite taxa were found: 25 monogeneans, 15 digeneans, 11 nematodes, and three cestodes. Based on new molecular (13 new dactylogyrids sequences of the 28S rDNA) and morphological data, a diagnosis amendment of the dactylogyrid genus *Demidospermus* (with a new species description) is proposed, a new dactylogyrid genus is erected with description of a new species is also proposed, and generic assignment of unidentified taxa for which molecular data are available is briefly discussed. The dactylogyrid genus *Heteropriapulus* is reviewed, five newly described species from loricariids are described, and also first molecular data (partial sequences of the 28S rRNA gene) are provided for three taxa including the type species *Heteropriapulus heterotylus*. A new nematode species of *Spinitectus* is described based on morphological observations. *Spinitectus aguapeiensis* n. sp. is the first record of a nematode species in *Pimelodella avanhandavae*, the fourth record of this genus in the Paraná River basin, and the sixth species of this genus in South America. By providing new host records, new geographical records, and putative new species to science, the survey of the helminthfauna of these host species has considerably increased the knowledge on the vast and far to be known diversity of Neotropical siluriform fish parasites.

Key-words: Upper Paraná river basin, catfishes, morphology, phylogeny, Dactylogyridae, diversity.

1. Introdução geral¹

1.1. Parasitos e a biodiversidade

A diversidade biológica, a despeito da sua notória complexidade científica, foi definida pela Convenção sobre Diversidade Biológica, adotada em 1992, por ocasião da Conferência das Nações Unidas sobre Meio Ambiente e Desenvolvimento no Rio de Janeiro, como: “A variabilidade de organismos vivos de todas as origens, compreendendo, dentre outros, os ecossistemas terrestres, marinhos e outros ecossistemas aquáticos e os complexos ecológicos de que fazem parte, compreendendo ainda a diversidade dentro de espécies, entre espécies e de ecossistemas”. Segundo Boero (2010) a diversidade biológica está intrinsecamente associada à identificação dos seres vivos, através da taxonomia, uma vez que o conhecimento das espécies biológicas define o grau de sua diversidade.

Os parasitos têm sido reconhecidos como importante parte da biodiversidade global. Considerando-se o papel dos parasitos nos ecossistemas naturais, é de extrema importância identificar pontos de acesso de alta e baixa diversidade parasitária a fim de compreender o funcionamento da biosfera. Assim, a biodiversidade de parasitos pode ser muito importante já que o parasitismo possui papel chave nos ecossistemas, como regular a abundância ou densidade das populações de hospedeiros, estabilizar cadeias tróficas e estruturar comunidades animais (POULIN e MORAND, 2004; LUQUE e POULIN, 2007). Um dos principais passos a ser tomado para conservação da biodiversidade é a realização de inventários, no entanto, os parasitos foram incorporados em avaliações de biodiversidade apenas recentemente. Logo, a fauna parasitária de grandes áreas tem sido um componente extremamente subestimados da biodiversidade mesmo reconhecendo-se que os parasitos representam uma grande fração desta biodiversidade total do planeta (ANON, 2000; POULIN e MORAND, 2004; LUQUE e POULIN, 2007; DOBSON et al., 2008; LAFFERTY, 2012; POULIN, 2014).

Mais de 6.000 espécies de peixes de água doce são reconhecidas na América do Sul (REIS, 2013), assim espera-se também uma grande diversidade de parasitos de peixes para esta região. Neste contexto, estudos de parasitos de peixes na América do Sul é de

¹ Esta seção foi elaborada de acordo com a Associação Brasileira de Normas Técnicas, ABNT/NBR 6023, e citações de acordo com ABNT/NBR 10520.

grande relevância para que se possa compreender os papéis chave destes no ecossistema assim como para desenvolver melhores medidas de conservação da biodiversidade. Este conhecimento também pode ser relevante para outras áreas, como impacto do parasitismo em pisciculturas e zoonoses transmitidas por parasitos de peixe (LUQUE et al., 2016).

Menos de 10% do total de espécies de peixe conhecidas na América do Sul tiveram sua fauna parasitária investigada. Assim, mais estudos possibilitariam a criação de mais base de dados a fim de que se teste com consistência, diferentes hipóteses sobre biodiversidade e distribuição de espécies de helmintos parasitos em peixes Neotropicais (LUQUE e POULIN, 2007; LUQUE et al., 2016).

1.2. Parasitos de peixe como indicadores de impacto ambiental

Os parasitos podem indicar alguns aspectos da biologia de seus hospedeiros, como dieta, migração, recrutamento, desagregação de população e filogenia. Alterações na biodiversidade de comunidades parasitárias podem ser utilizadas como indicadoras da saúde de sistemas ecológicos já que estas refletem impactos ambientais mediante suas respostas às alterações no hábitat, como mudança na fisiologia, composição química e prevalência ou intensidade (VIDAL-MARTÍNEZ et al., 2010; MADI e UETA, 2012). Assim, muitos estudos têm sido realizados na América Latina a respeito do uso de parasitos de peixe como bioindicadores de impactos ambientais (VIDAL-MARTÍNEZ e WUNDERLICH, 2016).

Monogeneas ectoparasitos de peixes estão em contato direto com o ambiente, logo são mais sensíveis a qualquer mudança nos parâmetros das águas (NACHEV, 2010; BAYOUMY et al., 2008). Devido a este fato, estudos têm sido realizados utilizando monogeneas como biomarcadores em diferentes aspectos, como: avaliar efeitos da exposição a altas concentrações de efluentes, efeito do ciclo hidrológico de seca e cheia, acumuladores de metais pesados, efeito de reservatórios com diferentes características tróficas e efeitos do represamento em um ambiente natural (SIDDAL et al., 1997; VITAL, 2008; BAYOUMY et al., 2008; MADI e UETA, 2009; ACOSTA et al., 2013).

1.3. Monogeneas da região Neotropical: diversidade e estudos filogenéticos de dactilogirídeos de siluriformes

A classe Monogenea pertence ao filo Platyhelminthes e inclui em sua maioria ectoparasitos e ocasionalmente endoparasitos, com especificidade de hospedeiros vertebrados aquáticos (peixes), e podem ser também encontrados em anfíbios, répteis, e, pelo menos uma espécie, em mamíferos. Nos peixes são encontrados nas brânquias, pele, cavidade nasal, ureteres e alguns nos dutos intestinais. Em anfíbios e répteis podem estar na boca, cloaca e bexiga urinária. Em mamíferos, existem apenas registro de uma espécie, *Oculotrema hippopotami* Stunkard, 1924 (Polystomatidae), parasitando hipopótamos. Em invertebrados, foram relatados em lula e copépodes (RUPERT et al., 2005; THATCHER, 2006). A classe monogenea é monofilética, e seus membros são divididos em duas subclasses: Polyopisthocotylea e Monopisthocotylea. Indivíduos do grupo Polyopisthocotylea se alimentam de sangue enquanto que os Monopisthocotylea se alimentam de células epiteliais e muco. Membros da família Dactylogyridae pertencem a subclasse Monopisthocotylea (BUCHMANN e BRESCIANI, 2006; BRUSCA e BRUSCA, 2007).

Uma análise de dados de todos os registros de helmintos parasitos de peixes na América do Sul demonstrou que Monogenea representa o grupo mais diverso com 835 espécies registradas de diferentes países e 1133 associações parasito-hospedeiro (LUQUE et al. 2016). Takemoto et al. (2009) analisou dados de helmintos parasitos de 72 espécies de peixes coletadas durante sete anos na bacia do Alto do Paraná e neste estudo a classe Monogenea foi o grupo que apresentou o maior número de espécies. Membros da família Dactylogyridae representam os *taxa* mais abundantes nos ambientes aquáticos continentais na América do Sul (THATCHER, 2006). Neste contexto, a fauna global de monogeneas da família Dactylogyridae parasitos de siluriformes é bastante diversa, com cerca de 380 espécies pertencentes a 31 gêneros, e quase metade destes gêneros e cerca de 80 espécies estão distribuídas na região Neotropical (COHEN et al., 2013; BRAGA et al., 2014; BRANCHES e DOMINGUES, 2014; MENDOZA-PALMERO et al., 2015).

Apesar dessa grande diversidade de dactilogirídeos parasitando peixes siluriformes na região Neotropical, até o momento somente o estudo de Mendoza-Palmero et al. (2015) analisou as relações filogenéticas destas monogeneas, com o objetivo de reconstruir a história evolutiva destes parasitos baseados em sequências parciais de 28S large subunit rDNA (LSU) e baseado nos resultados, estes autores

sugerem a hipótese de que monogeneas parasitos de peixes siluriformes podem representar um grupo independente dentro de Dactylogyridae.

1.4. Área de estudo

O presente estudo foi realizado no Rio Aguapeí, na porção localizada na Reserva Particular do Patrimônio Nacional (RPPN) Foz do rio Aguapeí. Esta RPPN foi instaurada em 2006 pela Companhia Energética do Estado de São Paulo (CESP), como condicionante da mitigação dos impactos da usina Hidrelétrica de Três Irmãos e também para fins de conservação da biodiversidade local (SMA-SP, 2013). A reserva é banhada pelo rio Aguapeí e compreende os municípios de Paulicéia, Castilho e São João do Pau d'Alho, Noroeste Paulista. Esta unidade de conservação encontra-se nos domínios fito-ambientais Mata Atlântica e Cerrado, e é caracterizada por uma fauna adaptada aos ciclos hidrológicos de cheia e seca bem definidos da região. Esse ecossistema possui grande semelhança com o Pantanal Sul-Mato-Grossense, por estar periodicamente inundado. Esse processo está associado ao regime hidrológico, que provoca a expansão, contração e fragmentação dos sistemas aquáticos, além de interferir no grau de conectividade entre as partes do sistema (RODRIGUES et al., 2005). Durante a inundação, os sistemas aquáticos expandem suas áreas, inundando porções da planície e assim estabelecem ligações entre o rio e planície, rio e lagoa, lagoa e planície, bem como a troca de organismos entre os ambientes (NEIFF, 1996). A fauna silvestre da região da RPPN Foz do Rio Aguapeí é bastante diversa com muitas espécies de mamíferos, aves, répteis, anfíbios e peixes (CESP, 2014; MATSUNAKA et al., 2014).

O Rio Aguapeí (Figuras 1 e 2) pertence a Unidade de Gerenciamento de Recursos Hídricos 20 (UGRHI 20), de acordo com a divisão do Sistema Integrado de Gerenciamento de Recursos Hídricos do Estado de São Paulo. A UGRHI do Rio Aguapeí possui área de drenagem de 13.196 Km², limitando-se ao Norte com a Bacia do Rio Tietê, a Oeste com o Estado do Mato Grosso do Sul, tendo como divisa o Rio Paraná, a Leste é limitado pela Serra dos Agudos e ao Sul encontra-se com a Bacia do Rio do Peixe. É formada pelo Rio Feio, que nasce a uma altitude de 600 metros, entre as cidades de Gália e Presidente Alves, e pelo Rio Tibiriçá, que nasce a uma altitude de 480 metros, junto à cidade de Garça. A bacia possui extensão aproximada de 420 Km até sua foz no Rio Paraná, a uma altitude de 260 metros, entre o Porto Labirinto e o Porto Independência. Os principais afluentes da margem esquerda do Rio Aguapeí são o Rio Tibiriçá e o Córrego Sete de Setembro e os da margem direita são os Ribeirões Claro e Grande. O

Rio Aguapeí apresenta 857 km² de vegetação natural remanescente que ocupa, aproximadamente, 6,5% da área da UGRHI. As categorias de maior ocorrência são Floresta Estacional Semidecidual e Formação Arbórea/Arbustiva em Região de Várzea. Possui a floresta ciliar mais preservada e contínua na paisagem de sua bacia hidrográfica, porém apresenta alguns trechos fragmentados com presença de gado até as margens do rio (PORTAL SIGRH, 2016).

Dados dos levantamentos ictiofaunísticos do rio Aguapeí presentes nos planos de manejo do Parque Estadual do Aguapeí mostram predominância das ordens Siluriformes e Characiformes. Levantamentos realizados em 2008 no rio Aguapeí registraram para a ordem Characiformes 10 famílias com 35 espécies e para a ordem Siluriformes 9 famílias com 22 espécies (SMA-SP, 2012). No entanto, levantamentos de 2011 e 2012 mostraram dominância da ordem Siluriformes em número de espécies, representando 45,2% da ictiofauna (VILELA et al., 2015). Apesar desta considerável diversidade de peixes siluriformes, somente duas espécies, *Auchenipterus osteomystax* (Miranda Ribeiro, 1918) e *Trachelyopterus galeatus* (Linnaeus, 1766), tiveram recentemente sua fauna de helmintos parasitos investigada (YAMADA et al., 2017). Para espécies de peixes de outras ordens no rio Aguapeí ainda não há nenhum estudo de helmintos parasitos.

1.5. Hospedeiros investigados

Dentre os organismos vivos conhecidos cerca de 28.000 são peixes (NELSON, 2006), riqueza esta que também é refletida em diversidade morfológica e ecológica (LANGEANI et al., 2007). Grande parte desta riqueza encontra-se em águas tropicais, especialmente na região Neotropical, onde pode-se encontrar mais de 6.000 espécies incluindo espécies válidas e as que são reconhecidas, porém não descritas (LOWE-MCCONNELL, 1999; REIS et al., 2003; REIS, 2013). A América do Sul compreende a maior parte desta diversidade na bacia Amazônica e do Paraná. A bacia do Alto do Paraná possui registro de 310 espécies de peixe, com 11 ordens e 38 famílias, das quais a maior riqueza é encontrada em Siluriformes e Characiformes, representando 80% das espécies dos grupos dominantes (LANGEANI et al., 2007).

A ordem Siluriformes é um grupo monofilético com mais de 2800 espécies com distribuição cosmopolita, agrupadas em 35 famílias e 446 gêneros cujos membros são popularmente conhecidos como “bagres” e “catfishes” (NELSON, 2006). Aproximadamente 1.700 espécies, descritas e não descritas, ocorrem na América e sua maioria encontra-se na região Neotropical (ALBERT e REIS, 2011). Peixes siluriformes

são amplamente apreciados por aquaristas, além disso muitas espécies de grande porte são utilizadas para consumo. Mesmo sendo um grupo bastante diverso e de importância econômica, menos de 10% das espécies de siluriformes na região Neotropical tiveram sua fauna de helmintos parasitos analisadas (MORAVEC, 1998; THATCHER, 2006; LUQUE e POULIN, 2007; DE CHAMBRIER et al., 2014).

No rio Aguapeí, os siluriformes mais abundantes pertencem às famílias Doradidae Bleeker, 1858 – *Pterodoras granulosus* (Valenciennes, 1821) (n=59) e *Trachydoras paraguayensis* (Eigenmann & Ward, 1907) (n=70); Heptapteridae – *Pimelodella avanhandavae* Eigenmann, 1917 (n=40); Loricariidae Rafinesque, 1815 – *Loricariichthys platymetopon* Isbrücker & Nijssen, 1979 (n=80), *Pterygoplichthys ambrosettii* (Holmberg, 1893) e *Rhinelepis aspera* Spix & Agassiz, 1829 (n=30); e Pimelodidae Bonaparte, 1835 – *Hemisorubim platyrhynchos* (Valenciennes, 1840) (n=21) e *Sorubim lima* (n=25) (Bloch & Schneider, 1801). Consequentemente, foram as espécies avaliadas neste estudo quanto a sua fauna parasitária (Figura 3; Tabela I).

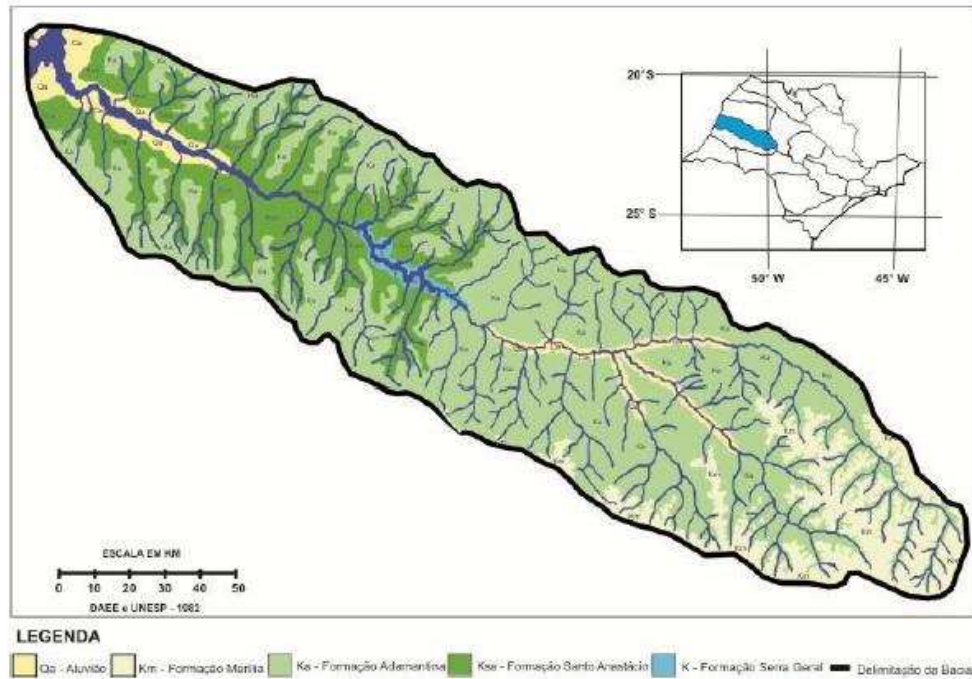


Figura 1. Mapa da bacia hidrográfica do rio Aguapeí (Fonte: Acervo do Saber/Unesp - https://edutec.unesp.br/publicador/index.php/conteudo/visualizar?pid=353&Conteudo_page=5).



Figura 2. Rio Aguapeí, localizado na RPPN Foz do Rio Aguapeí, município de Castilho, São Paulo, Brasil.

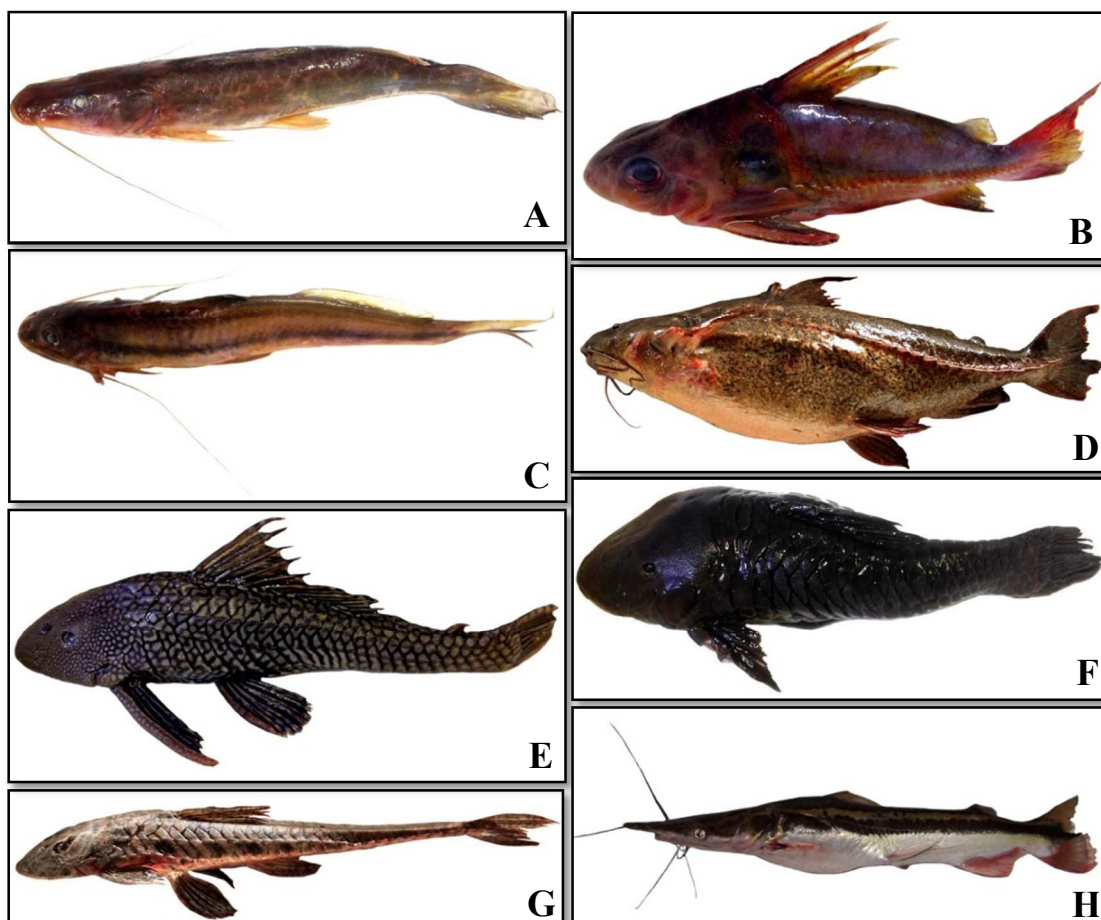


Figura 3. Espécies de peixes coletados do Rio Aguapeí, RPPN Foz do Rio Aguapeí, município de Castilho, São Paulo, Brasil. (A) *Hemisorubim platyrhynchos*; (B) *Trachydoras paraguayensis*; (C) - *Pimelodella avanhandavae*; (D) *Pterodoras granulosus*; (E) *Pterygoplichthys ambrosettii* (F) *Rhinelepis aspera*; (G) *Loricariichthys platymetopon*; e (H) *Sorubim lima*.

Tabela 1. Dados biométricos dos exemplares das oito espécies de peixes analisados neste estudo do rio Aguapeí, bacia do Alto Paraná, município de Castilho, São Paulo, Brasil. Comprimento padrão em centímetros e peso em gramas. Valores mostrados como média $\pm$ desvio padrão (mínimo – máximo).

Hospedeiro	Comprimento padrão	Peso
Doradidae		
<i>Pterodoras granulosus</i>	30,4 $\pm$ 8 (7,3–64)	740 $\pm$ 870,2 (9,2–5500)
<i>Trachydoras paraguayensis</i>	9,5 $\pm$ 1 (6,2–11,5)	30 $\pm$ 9 (15,9–51,6)
Heptapteridae		
<i>Pimelodella avanhandavae</i>	12,4 $\pm$ 2,4 (9–17,5)	23,5 $\pm$ 15 (7,8–58,5)
Loricariidae		
<i>Loricariichthys platymetopon</i>	23,4 $\pm$ 3,3 (14,3–28,7)	99,3 $\pm$ 38 (20,1–180)
<i>Pterygoplichthys ambrosettii</i>	28,9 $\pm$ 4,6 (20,5–45)	492,3 $\pm$ 205,5 (191,5–1520)
<i>Rhinelepis aspera</i>	31,6 $\pm$ 3 (25–38)	881,5 $\pm$ 246,7 (466,5–1495)
Pimelodidae		
<i>Hemisorubim platyrhynchos</i>	28,5 $\pm$ 3 (23–36,5)	310,8 $\pm$ 107,3 (141,4–641,4)
<i>Sorubim lima</i>	37,3 $\pm$ 6 (29,2–51,5)	489,7 $\pm$ 274,2 (190,4–1175)

1.6. Referências

- ACOSTA, A.A.; QUEIROZ, J.; BRANDÃO, H.; CARVALHO, E.D.; SILVA, R.J. Helminth of *Steindachnerina insculpta* in two distinct stretches of the Taquari River, state of São Paulo, Brazil. **Revista Brasileira de Parasitologia Veterinária**, v. 22, n. 4, p. 539-547, 2013.
- ALBERT, J.S.; REIS, R.E. Introduction of Neotropical Freshwater. In: ALBERT, J.S.; REIS, R.E. (Eds). **Historical Biogeography of Neotropical Freshwater Fishes**. California: University of California Press, 1 ed., 2011. p. 3-19.
- ANON. **The Global Taxonomy Initiative: Using Systematic Inventories to Meet Country and Regional Needs**. The Center for Biodiversity and Conservation, American Museum of Natural History, New York.
- BAYOUMY, E.M.; OSMAN, H.A.M.; EL-BANA, L.F.; HASSANAIN, M.A. Monogenean Parasites as Bioindicators for Heavy Metals Status in Some Egyptian Red Sea Fishes. **Global Veterinaria**, v. 2, n. 3, p. 117-122, 2008.
- BRAGA, M.P.; ARAUJO, S.B.L.; BOEGER, W.A. Patterns of interaction between Neotropical freshwater fishes and their gill Monogenoidea (Platyhelminthes). **Parasitology Research**, v. 113, p. 481-490, 2014.

- BRUSCA, R. C.; BRUSCA, G. J. **Invertebrados**. 2. ed. Rio de Janeiro: Guanabara Koogan S.A., 2007.
- BUCHMANN, K.; BRESCIANI, J. Monogenea (Phylum Platyhelminthes), v. 1. In: WOO, P. T. K. (Org.) **Fish Diseases and Disorders: Protozoan and Metazoan Infections**. 2. ed. Wallingford, Oxon: CABI Publishing, 2006. p. 294-341.
- BOERO, F. The study of species in the era of biodiversity: A tale of stupidity. **Diversity**, v. 2, p. 115-126, 2010.
- BRANCHES, B.; DOMINGUES, M.V. A new species of *Unilatus* (Platyhelminthes: Monogeneoidea) from the gills of *Leporacanthicus galaxias* Isbrücker et Nijssen (Siluriformes: Loricariidae) from Brazil. **Acta Parasitologica**, v. 59, p. 91-97, 2014.
- CESP – Companhia Energética de São Paulo. **Unidades de conservação**. Disponível em: http://www.cesp.com.br/portalCesp/portal.nsf/V03.02/MeioAmbiente_Conservacao_Texto?OpenDocument&Highlight=0,UNIDADES,DE,CONSERVA%C7%C3O,Unidades,De,Conserva%E7%E3o,unidades,de,conserva%E7%E3o. Acesso em: dez. 2016.
- COHEN, S.C.; JUSTO, M.C.N.; KOHN, A. **South American Monogeneoidea parasites of fishes, amphibians and reptiles**. Rio de Janeiro: Fundação Oswaldo Cruz e Conselho Nacional de Desenvolvimento Científico e Tecnológico, 2013. 663p.
- DE CHAMBRIER, A.; SCHOLZ, T.; KUCHTA, R. Taxonomic status of Woodland's enigmatic tapeworms (Cestoda: Proteocephalidea) from Amazonian catfishes: back to museum collections. **Systematic Parasitology**, v. 87, p. 1-19, 2014.
- DOBSON, A.P.; LAFFERTY, K.D.; KURIS, A.M.; HECHINGER, R. F.; JETZ, W. Homage to Linnaeus: how many parasites? How many hosts? **Proceedings of the National Academy of Sciences**, v. 105, p. 482-489, 2008.
- LAFFERTY, K.D. Biodiversity loss decreases parasite diversity: theory and patterns. **Philosophical Transactions of the Royal Society B: Biological Sciences**, v. 367, p. 2814-2827, 2012.
- LANGEANI, F.; CASTRO, R.M.C.; OYAKAWA, O.T.; SHIBATTA, O.A.; PAVANELLI, C.S.; CASATTI, L. Diversidade da ictiofauna do Alto Rio Paraná: composição atual e perspectivas futuras. **Biota Neotropica**, v. 7, n. 3, p. 181-197, 2007.
- LOWE-MCCONNELL, R.H. **Estudos ecológicos de comunidades de peixes tropicais**. Edusp, São Paulo: Edusp. 1999. 584p.

- LUQUE, J.L.; POULIN, R. Metazoan parasite species richness in Neotropical fishes: hotspots and the geography of biodiversity. **Parasitology**, v. 134, p. 865-78, 2007.
- LUQUE, J.L.; PEREIRA, F.B.; ALVES, P.V.; OLIVA, M.E.; TIMI, J.T. Helminth parasites of South American fishes: current status and characterization as a model for studies of biodiversity. **Journal of Helminthology**, pp. 1–15, 2016. DOI: 10.1017/S0022149X16000717
- MADI, R. R.; UETA, M.T. Parasitas de peixes como indicadores ambientais. In: Silva-Souza, A. T.; Lizama, M. A. P.; Takemoto, R. M. (Eds.). **Patologia e Sanidade de Organismos Aquáticos**. Maringá: Massoni, 2012. p 33-58.
- MATSUNAKA, A.M.; SANTOS, R.M.; POSSO, S.R. Abundância e riqueza da avifauna nos buritizais da RPPN Foz do rio Aguapeí, Paulicéia/SP. **X Fórum Ambiental da Alta Paulista**, v. 10, n. 3, 2014, p. 42-55, 2014.
- MENDOZA-PALMERO, C.A.; BLASCO-COSTA, I.; SCHOLZ, T. Molecular phylogeny of Neotropical monogeneans (Platyhelminthes: Monogenea) from catfishes (Siluriformes). **Parasites and Vectors**, v. 8, p. 164, 2015.
- MORAVEC, F. **Nematodes of freshwater fishes of the Neotropical Region**. Praga: Academia, 1998. 464p.
- NACHEV, M. **Bioindication capacity of fish parasites for the assessment of water quality in the Danube River**. 2010. Thesis. Germany: Universität Duisburg-Essen.
- NEIFF, J.J. Large rivers of South America: toward the new approach. **Verhandlungen des Internationalen Verein Limnologie**, n. 26, p. 167-180, 1996.
- NELSON, J. S. **Fishes of the world**. 4.ed. New York: John Wiley & Sons, 2006. 601p.
- PORTAL SigRH. Sistema Integrado de Gerenciamento de Recursos Hídricos do Estado de São Paulo. **Comitê da Bacia Hidrográfica dos rios Aguapeí e Peixes**. Disponível em: <http://www.sigrh.sp.gov.br/cbhap/apresentacao>. Acesso em: Dez. 2016.
- POULIN, R. Parasite biodiversity revisited: frontiers and constraints. **International Journal for Parasitology**, v. 44, p. 581-589, 2014.
- POULIN, R.; MORAND, S. **Parasite Biodiversity**. Washington: Smithsonian Books, 2004. 216p.
- REIS, R.E.; KULLANDER, S.O.; FERRARIS-JR., C.J. (Orgs.). **Check list of the freshwater fishes of South and Central America**. Porto Alegre: EDIPUCRS, 2003. 729p.

- REIS, R.E. Conserving the freshwater fishes of South America. **International Zoo Yearbook**, v. 47, p. 67-70, 2013.
- RODRIGUES, M. T. The conservation of brazilian reptiles: challenges for a megadiverse country. **Conservation Biology**, v. 19, n. 3, p. 659-664, 2005.
- RUPERT, E. E.; FOX, R. S.; BARNES, R. D. **Zoologia dos invertebrados: uma abordagem funcional-evolutiva**. 7. ed. São Paulo: Roca, 2005.
- SECRETARIA DO MEIO AMBIENTE DO ESTADO DE SÃO PAULO – SMA. **Plano de Manejo – Parque Estadual do Aguapeí (Encarte 3)**. Disponível em: http://observatorio.wwf.org.br/site_media/upload/gestao/planoManejo/PM_PEAguapei_parte_002.pdf. Acesso em: Dez. 2016.
- SECRETARIA DO MEIO AMBIENTE DO ESTADO DE SÃO PAULO – SMA. **Anuário das Reservas Particulares do Patrimônio Natural instituídas pela Secretaria do Meio Ambiente do Estado de São Paulo**. Governo do Estado de São Paulo, 2013. 84p.
- SIDDALL, R.; KOSKIVAARA, M.; VALTONEN, E.T. *Dactylogyrus* (Monogenea) infections on the gills of roach (*Rutilus rutilus* L.) experimentally exposed to pulp and paper mill effluent. **Parasitology**, v. 114, n. 5, p. 439-446, 1997.
- TAKEMOTO, R.M.; PAVANELLI, G.C.; LIZAMA, M.A.P.; LACERDA, A.C.F.; YAMADA, F.H.; MOREIRA, L.H.A.; Ceschini, T.L.; BELLAY, S. Diversity of parasites of fish from the Upper Paraná River floodplain, Brazil. **Brazilian Journal of Biology**, v. 69, n. 2, p. 691-705, 2009.
- THATCHER, V. E. **Amazon Fish Parasites**. 2.ed. Bulgaria: Pensoft Publishers, 2006. 509p.
- VIDAL-MARTÍNEZ, V.M.; PECH, D.; SURES, B.; PURUCKER, T.; POULIN, R. Can parasites really reveal environmental impact? **Trends in Parasitology**, v. 26, n. 1, p. 44-51, 2010.
- VIDAL-MARTÍNEZ, V.M.; WUNDERLICH, A.C. Parasite as bioindicator of environmental degradation in Latin America: A meta-analysis. **Journal of Helminthology**, 2016.
DOI: 10.1017/S0022149X16000432
- VILELA, M.J.; DUTRA, M.A.; MARQUES, H.; DIAS, J.H.P.; LANGEANI, F.; CARVALHO, F.R.; MARTINS, F.O. **Resultados parciais do inventário da ictiofauna do rio Aguapeí, na área da RPPN Foz do Aguapeí, São Paulo, Bacia do Alto Paraná**. XX Encontro Brasileiro de Ictiologia, Maringá, 2013.

- VITAL, J.F. **Diversidade de Parasitas de *Pygocentrus nattereri* (Kner, 1858) (Characiformes: Characidae) durante o ciclo hidrológico em um lago de várzea e seu potencial como indicadora de qualidade ambiental.** 51f. Dissertação (Mestrado) – Universidade Federal do Amazonas, Instituto Nacional de Pesquisas da Amazônia, Manaus, 2008.
- YAMADA, P.O.F.; YAMADA, F.H.; SILVA, R.J.; ANJOS, L.A. Ecological implications of floods on the parasite communities of two freshwater catfish in a Neotropical floodplain. **Acta Parasitologica**, v. 62, n.2. DOI: 10.1515/ap-2017-00

2. Objetivos

2.1. Objetivo geral

Dada a escassez de estudos de helmintofauna para peixes siluriformes na região Neotropical, somando-se a escassez de estudos de ictioparasitologia para peixes do rio Aguapeí, o presente estudo teve como objetivo inventariar os helmintos parasitos de peixes siluriformes desta região.

2.2. Objetivos específicos

2.2.1. Helminth parasites of eight siluriform species from the Aguapeí River, Upper Paraná river basin, São Paulo State, Brazil

Caracterizar a fauna de helmintos parasitos de oito espécies de siluriformes dominantes no rio Aguapeí, bacia do Alto Paraná, municipalidade Castilho, estado de São Paulo, Brasil. Estas espécies de peixes foram pouco estudadas a respeito de suas helmintofauna, especialmente em se tratando de monogeneas.

2.2.2. Added resolution to circumscription of some gill monogenean species (Dactylogyridae) of Neotropical catfishes including description of a new *Demidospermus* species and erection of a new genus

Apresentar, com base em novos dados moleculares (13 novas sequências de 28S rDNA) e morfológicos, uma diagnose amendada do gênero *Demidospermus* com descrição de uma nova espécie, descrever um novo gênero de dactilogirídeo e propor a descrição de uma nova espécie, e discutir sobre a questão do posicionamento de gênero de algumas outras espécies cujos dados moleculares estão disponíveis.

2.2.3. A synoptic review of *Heteropriapulus* spp. (Monogenea: Dactylogyridae) from loricariid catfishes in South America including description of five new species and first molecular phylogeny

Descrever cinco novas espécies do gênero *Heteropriapulus* encontrados nas brânquias dos hospedeiros loricarídeos *Pterygoplichthys ambrosettii* e *Rhinelepis aspera*. Adicionalmente, apresentar dados moleculares e de relações filogenéticas para espécies deste gênero de dactilogirídeo, incluindo a espécie tipo *Heteropriapulus heterotylus*.

2.2.4. *Spinitectus aguapeiensis* n. sp. (Nematoda: Cystidicolidae) from *Pimelodella avanhandavae* (Siluriformes: Heptapteridae) in the Aguapeí River, Upper Paraná River basin, Brazil

Descrever uma nova espécie do gênero *Spinitectus*. *Spinitectus aguapeiensis* n. sp. é o primeiro registro de uma nova espécie para o siluriforme *Pimelodella avanhandavae*, o quarto registro desta espécie na bacia do Alto Paraná e a sexta espécie do gênero descrita para América do Sul.

***Helminth parasites of eight siluriform
fishes from the Aguapeí River, Upper
Paraná River basin, São Paulo State, Brazil***

3. Helminth parasites of eight siluriform fishes from the Aguapeí River, Upper Paraná River basin, São Paulo State, Brazil¹

Abstract

Fishes of the order Siluriformes, known as catfishes, have a worldwide distribution with more than 2,800 recognized species. Approximately 1,700 species are found in America, mainly in the Neotropical region. Despite being highly diverse, abundant, and of economic importance as food and ornamental fishes, the knowledge about their helminth parasites is fragmentary and insufficient. Eight species of catfishes (*Pterodoras granulosus*, *Trachydoras paraguayensis*, *Pimelodella avanhandavae*, *Loricariichthys platymetopon*, *Pterygoplichthys ambrosettii*, *Rhinelepis aspera*, *Hemisorubim platyrhynchos*, and *Sorubim lima*) from the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo State, Brazil, were surveyed for helminth parasites. Fishes were placed individually in plastic bags, frozen, and transported to the Department of Parasitology, Institute of Bioscience, São Paulo State University (UNESP), municipality of Botucatu, São Paulo State, Brazil. In the laboratory, they were defrosted, weighed, measured, and examined for helminth parasites following classic methodology. The ecological analyses were carried out at the component community level and at the infracommunity level. Fifty-four helminth parasite *taxa* were found: 25 monogeneans, 15 digeneans, 11 nematodes, and three cestodes. By providing new host records, new geographical records, and putative new species to science, the survey of the helminthfauna of these host species has considerably increased the knowledge on the vast and far to be known diversity of Neotropical siluriform fish parasites.

Key-words: Neotropical region, catfishes, parasite diversity, helminthological survey.

¹ Manuscript to be submitted to the journal Acta Parasitologica.

3.1. Introduction

Brazil ranks the fifth place in size with more than 9,000,000 km², encompassing a wide variety of landscapes and climates, and holding 12% of the freshwater in the world (Bizerril and Primo, 2001). The heterogeneity of habitats leads to an increase of species diversity (MacArthur and MacArthur, 1961), as seen in the ecosystems of South America, in which fish species exhibit high morphological, physiological, ecological, and reproductive diversity (Aguiar, 2008). The amount of the world's freshwater fish species is estimated in 13,000 and 31% of this diversity can be found in the Neotropical region (Crispim and Stevaux, 2003).

Biodiversity in freshwaters has been widely studied and it becomes more and more apparent that parasites are a fundamental part of this diversity. Parasites constitute one of the least studied groups in most biodiversity studies, even though they play a key role in functioning of ecosystems as an integral component of trophic webs. In addition, parasites are indicators of several biological aspects of their hosts, and can also be used in the assessment of environmental quality and richness (Brooks and Hoberg, 2001; Poulin and Morand, 2004). Adequate understanding of the role of parasites in natural ecosystems as well as the identification of spots of high parasite diversity are important to fully understand how the biosphere works (Lafferty, 1997; Luque and Poulin, 2007). Consequently, knowledge of the diversity of parasites is crucial for any ecological and evolutionary studies because parasitism plays an important role in ecosystems by regulating the abundance or density of the host populations, establishing food chains, and structuring animal communities (Lafferty and Kuris, 2005; Thomas *et al.*, 2005).

Fishes of the order Siluriformes, known as catfishes, have a worldwide distribution with more 2,800 species. Approximately 1,700 species (60.7%) are found in America, mainly in the Neotropical region (Nelson, 2006; Albert and Reis, 2011). Despite being highly diverse, abundant, and of economic importance as food and ornamental fishes, the knowledge about their helminth parasites is fragmentary and insufficient (Mendoza-Palmero *et al.*, 2012; de Chambrier *et al.*, 2015). Ichthyofaunistic surveys of the Aguapeí River, showed a dominance of the fish orders Characiformes and Siluriformes in 2008, and in 2011 and 2012 such studies showed a dominance of siluriforms representing 45.2% of the ichthyofauna (SMA-SP, 2012; Vilela *et al.*, 2013). However, despite this considerable diversity of siluriform fishes in this river, only two species, *Auchenipterus osteomystax* (Miranda Ribeiro, 1918) and *Trachelyopterus*

galeatus (Linnaeus, 1766), have had their helminth fauna recently surveyed (Yamada *et al.*, 2017). There is not any other study on helminth parasites for fish species of other orders from the Aguapeí River.

Among helminth parasites infecting catfishes, monogeneans represent economically the most important group because they reproduce fast (they have direct life cycles, with no intermediate host) and may cause losses or even mass mortality in heavily infected fishes (Thatcher, 2006). Most monogeneans are ectoparasites of fishes, with majority attached to the gills of their hosts (family Dactylogyridae) (Eiras *et al.*, 2010). Monogeneans are rather diverse regarding the host groups that they parasitize, their morphology and ecology (Poulin, 2002). They are usually highly host specific, with most species infecting only one or very few host species (oioxenous or stenoxenous) (Poulin, 1992; Sasal *et al.*, 1998). Luque *et al.* (2016) gathered data of all records of helminth parasites of fishes in South America and showed that monogeneans represent the most diverse group with 835 species recorded from different countries and 1,133 host-parasite associations. Takemoto *et al.* (2009) analyzed data of helminth parasites of 72 fish species sampled during seven years in the Upper Paraná River basin and the class Monogenea also showed the highest number of species recovered. From this diversity, members of the family Dactylogyridae are the most abundant in freshwaters of South America (Thatcher, 2006).

Over 350 species of 31 genera of gill monogeneans of the family Dactylogyridae have been reported from catfishes (Mendoza-Palmero *et al.*, 2015) and about 75 species in 14 genera are known from the Neotropical region. However, this number in fact represents only a negligible part of the actual species diversity of dactylogyrids in the Neotropical region, for instance, Mendoza-Palmero *et al.*, 2012 listed about 60 morphotypes representing putative new species found on pimelodid catfishes in the Peruvian Amazon.

Therefore, the aim of this study is to characterize the helminth fauna of eight siluriform dominant species collected in the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo State, Brazil. These fish species have had their helminthfauna poorly studied over the years, particularly regarding that of monogeneans.

3.2. Materials and Methods

3.2.1. Study area

Refer back to section 1.4.1

3.2.2. Sampling of fishes and their helminth parasites

Four collecting trips were carried out, two during the rainy season (Jan/Nov 2014) and two during the dry season (Aug/2013, Jun/2014). Eight species of catfishes were collected using gill nets, multifilament cast nets and fish rods, and examined for helminth parasites (see Table I for number of fishes collected by period). Fishes were placed individually in plastic bags, frozen, and transported to the Department of Parasitology, Institute of Bioscience, São Paulo State University (UNESP), municipality of Botucatu, São Paulo State, Brazil. In the laboratory, they were defrosted, weighed, measured, and examined for helminth parasites.

The body, fins, nasal cavity, eyes and inner face of the operculum of the fish hosts were examined for ectoparasites. Gills were removed and washed using 53 and 75 micrometers sieves and placed in Petri dishes. After external examination, a longitudinal incision in the ventral surface was made and all inner organs were removed and separated. The visceral cavity and all organs were examined using a stereomicroscope. The helminths collected were preserved in 70% alcohol and some were preserved in ethanol absolute for molecular analyses. For species identification, monogeneans were taken from the gills and some specimens collected were mounted on slides with Hoyer's or Grey and Wess' medium, and a mixture of glycerin-ammonium picrate (GAP) to study sclerotized structures while some specimens were stained with Gomori's trichrome to study internal organs (Ergens, 1969; Humason, 1979; Kritsky *et al.*, 1986). Digeneans and cestodes were stained with carmine and cleared with clove oil; and the nematodes were diaphonized with lactophenol (Eiras *et al.*, 2006). The parasites were identified based mainly on identification keys and reference guides (Moravec, 1998; Gibson *et al.*, 2002, 2005, 2008; Kohn *et al.*, 2007; Cohen *et al.*, 2013) and analyzed using the Qwin Lite 3.1 (Leica) computerized system for image analysis with differential interference contrast (DIC) and an Olympus BX51 microscope equipped with DIC and phase contrast optics. Illustrations were made with the aid of a drawing tube (camera lucida) mounted on an Olympus BX51 microscope.

Voucher species will be deposited in the Helminthological Collection of the Institute Oswaldo Cruz (CHIOC), Helminthological Collection of the Instituto de Biociências de Botucatu (CHIBB), and Helminthological Collection of the Institute of Parasitology, České Budejovice, Czech Republic (IPCAS).

Table I. Number of fishes collected and sampling periods in the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo State, Brazil.

Host species	Aug/13	Jan/14	Jun/14	Nov/14	Total
Siluriformes					
Doradidae					
<i>Pterodoras granulosus</i> (Valenciennes, 1821)	19	20	-	20	59
<i>Trachydoras paraguayensis</i> (Eigenmann and Ward, 1907)	20	20	20	10	70
Heptapteridae					
<i>Pimelodella avanhandavae</i> Eigenmann, 1917	20	-	-	20	40
Loricariidae					
<i>Loricariichthys platymetopon</i> Isbrücker and Nijssen, 1979	20	20	20	20	80
<i>Pterygoplichthys ambrosettii</i> (Holmberg, 1893)	20	20	20	20	80
<i>Rhinelepis aspera</i> Spix and Agassiz, 1829	3	3	5	19	30
Pimelodidae					
<i>Hemisorubim platyrhynchos</i> (Valenciennes, 1840)	4	8	8	1	21
<i>Sorubim lima</i> (Bloch and Schneider, 1801)	7	8	4	6	25
Total	113	99	77	116	405

3.2.3. Statistical analyses

The ecological terminologies of prevalence, mean intensity of infection/infestation, and mean abundance were determined following Bush *et al.* (1997). The ecological analyses were carried out at the component community level (all helminths in all hosts) and at the infracommunity level (all helminths in each individual) (Holmes and Price, 1986). The infracommunity components were classified according to

Bush and Holmes (1986) in core species (prevalence > 66.6%), secondary species (prevalence >33.3% and <66.6%) and satellite species (prevalence <33.3%). Species accumulation curves were generated to demonstrate the cumulative number of species recovered relative to the host sampling effort (Colwel and Coddington, 1994).

To determine ecological attributes of the helminth communities the following indexes were calculated: (a) Shannon-Wiener diversity index that was calculated at component community level using the formula $H' = -\sum(p_i \ln p_i)$, in which H' = species diversity; p_i = proportion of species i in the community, in which $p_i = (n_i/N)$; n_i = individual numbers of i species and N = total number of individuals (Krebs, 1989; Begon *et al.*, 2007); (b) Simpson index that was calculated at component community level ranging from 0 (low diversity) to 1 (high diversity) following the formula $1 - D = 1 - \sum (P_e)^2$ in which P_e is the proportion of species e in the community; (c) Berger-Parker index ($d = N_{\max}/N$) that was calculated for each helminth *taxa* to measure the dominance of the species, in which $N_{\max}$ = number of individuals of each abundance species, N = total number of individuals of all species (Krebs, 1989); and (d) Discrepancy index (D), described by Poulin (1993), that was used to evaluate the spatial distribution of parasites based on their abundance. This index ranges from 0 to 1, and can be interpreted as: $D = 0$, all hosts harboring the same number of parasites; $D = 1$, all parasites found in a single host. This analysis was calculated using the Quantitative Parasitology 3.0 software (Rózsa *et al.*, 2000). Shannon-Wiener, Simpson and Berger-Parker indexes were calculated using the software PAST (version 4.3).

3.2.4. Molecular analyses

For molecular identification of cestodes, a small piece of the posterior end of the specimens was cut and placed in molecular-grade 96–99% ethanol for sequencing of the LSU (large subunit) of the 28S rRNA gene. Genomic DNA was extracted using DNeasy® Blood and Tissue Kit (Qiagen) according to manufacturer's protocol. The D1–D3 lsrDNA region was amplified by PCR using the primers and conditions described in Brabec *et al.* (2012). Amplicons were Sanger sequenced at GATC Biotech (Konstanz, Germany) using the PCR primers and the internal primers 900F (5'-CCGTCTTGAAACACGGACCAAG-3') and 400R (5'-GCAGCTTGACTACACCCG-3') (Olson *et al.*, 2003). Contiguous sequences were assembled using Geneious version

R8 (<http://www.geneious.com/>; Kearse *et al.*, 2012) and then submitted to BLAST to confirm the species identity.

3.3. Results

A total of 405 specimens of eight siluriform fish species (*Hemisorubim platyrhynchos*, *Loricariichthys platymetopon*, *Pimelodella avanhandavae*, *Pterodoras granulosus*, *Pterygoplichthys ambrosettii*, *Rhinelepis aspera*, *Sorubim lima*, and *Trachydoras paraguayensis*) was surveyed for helminth parasites.

Table II presents a list of the hosts and their respective helminth parasite species found in this study followed by their authors' descriptions. Table II also indicates the respective Figure number of each helminth species and whether each helminth found represents new host record, new geographical record for river basin or putative new species. Data on number of parasite specimens collected, prevalence, mean intensity of infection, mean abundance and infection/infestation sites for all helminths found are shown in Table III.

Table II. Host and helminth parasites (indicating their respective Figure number) of the eight siluriform fish species from the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo State, Brazil, analyzed for this study. NHR – new host record; NGR – new geographical record for river basin; PNS – putative new species.

Siluriform hosts	Helminth	Figure	NHR	NGH	PNS
Doradidae Bleeker, 1858					
<i>Pterodoras granulosus</i> (Valenciennes, 1821)	Monogenea				
	Dactylogyridae				
	Bychowsky, 1933				
	<i>Cosmetocleithrum bulbocirrus</i> Kritsky, Thatcher and Boeger, 1986	2			
	<i>Vancleaveus januacaensis</i> Kritsky, Thatcher and Boeger, 1986	3			
	Digenea				
	Paramphistomidae				
	Fischoeder, 1901				
	<i>Dadaytrema oxycephala</i> (Diesing, 1836)	4			

Table II. Continued.

Siluriform hosts	Helminth	Figure	NHR	NGH	PNS
	Diplostomidae Poirier, 1886				
	Type 1 metacercariae of Diplostomidae	5	x		
	Nematoda				
	Camallanidae Railliet and Henry, 1915				
	<i>Procamallanus</i> (<i>Spirocamallanus</i>) <i>inopinatus</i> Travassos, Artigas and Pereira, 1928	6			
	Atractidae Railliet, 1917				
	<i>Rondonia rondoni</i> Travassos, 1920	7			
	Cestoda				
	Proteocephalidae La Rue, 1911				
	Cestoda gen. sp.				
<i>Trachydoras paraguayensis</i> (Eigenmann and Ward, 1907)	Monogenea				
	Gyrodactylidae Van Beneden and Hesse, 1863				
	Gyrodactylidae gen. sp.	8	x		
	Dactylogyridae Bychowsky, 1933				
	<i>Ameloblastella</i> sp. 1	9			x
	<i>Pavanelliella pavanellii</i> Kritsky and Boeger, 1998	10	x		
	*Dactylogyridae n. sp. 6	11			x
	Digenea				
	Diplostomidae Poirier, 1886				
	<i>Austrodiplostomum</i> sp. metacercariae	12	x		
	Type 1 metacercariae of Diplostomidae	13	x		
	Type 2 metacercariae of Diplostomidae	12	x		
	Type 3 metacercariae of Diplostomidae	12	x		
	Nematoda				
	Pharyngodonidae Travassos, 1919				

Table II. Continued.

Siluriform hosts	Helminth	Figure	NHR	NGH	PNS
	<i>Ichthyouris laterifilamenta</i> Moravec, Kohn and Fernandes, 1992	14			
	Quimperiidae Gendre, 1928				
	<i>Neoparaseuratum travassosi</i> Moravec, Kohn and Fernandes, 1992	15			
	Camallanidae Railliet and Henry, 1915				
	<i>Procamallanus (Spirocamallanus) inopinatus</i> Travassos, Artigas and Pereira, 1928	16			
	<i>Procamallanus (Spirocamallanus) inopinatus</i> 4th stage larvae Travassos, Artigas and Pereira, 1928	17	x		
	Anisakidae Railliet and Henry, 1912				
	<i>Contracaecum</i> sp. larvae Railliet and Henry, 1912	18	x		
	Cestoda				
	Proteocephalidae La Rue, 1911				
	Cestoda gen. sp.		x		
Heptapteridae Gill, 1861					
<i>Pimelodella avanhandavae</i> Eigenmann, 1917	Monogenea				
	Dactylogyridae Bychowsky, 1933				
	<i>Ameloblastella formatrium</i> Mendoza-Franco, Mendoza-Palmero and Scholz, 2016	19	x	x	
	<i>Aphanoblastella chagresii</i> Mendoza-Franco, Mendoza-Palmero and Vidal-Martínez, 2007	20	x	x	
	Digenea				
	Type 1 digenean	21	x		
	Type 2 digenean		x		
	Unidentified metacercariae		x		

Table II. Continued.

Siluriform hosts	Helminth	Figure	NHR	NGH	PNS
	Derogenidae Nicoll, 1910				
	<i>Genarchella</i> sp.	22	x		
	<i>Phyllodistomum</i> sp.	23	x	x	
	Paramphistomidae				
	Fischoeder, 1901				
	<i>Dadaytrema</i> sp.	24	x		
	Diplostomidae Poirier, 1886				
	<i>Austrodiplostomum</i> sp. metacercariae	25	x		
	Nematoda				
	Pharyngodonidae				
	Travassos, 1919				
	<i>Brasilnema pimellodellae</i> Moravec, Kohn and Fernandes, 1992	26	x		
	Atractidae Railliet, 1917				
	<i>Rondonia rondoni</i> Travassos, 1920	27	x		
	Cystidicolidae Skrjabin, 1946				
	† <i>Spinitectus</i> sp.	28			x
	Anisakidae Railliet and Henry, 1912				
	<i>Contracaecum</i> sp. larvae Railliet and Henry, 1912	29	x		
Loricariidae					
Rafinesque, 1815					
<i>Loricariichthys platymetopon</i>					
Isbrücker and Nijssen, 1979	Monogenea				
	Gyrodactylidae Van Beneden and Hesse, 1863				
	Gyrodactylidae gen. sp.	30	x		
	Dactylogyridae				
	Bychowsky, 1933				
	<i>Demidospermus paranaensis</i> Ferrari-Hoeinghaus, Bellay, Takemoto and Pavanelli, 2010	31			
	Digenea				
	Clinostomidae Lühe, 1901				

Table II. Continued.

Siluriform hosts	Helminth	Figure	NHR	NGH	PNS
	<i>Clinostomum</i> sp. metacercariae	32			
	Diplostomidae Poirier, 1886				
	<i>Austrodiplostomum</i> sp. metacercariae	33	x		
	Type 1 metacercariae of Diplostomidae	34	x		
	Type 4 metacercariae of Diplostomidae	35	x		
	Type 5 metacercariae of Diplostomidae	35	x		
	Nematoda				
	Anisakidae Railliet and Henry, 1912				
	<i>Raphidascaris</i> (<i>Sprentascaris</i>) <i>mahnerti</i> (Petter and Cassone, 1984)	36			
<i>Pterygoplichthys ambrosettii</i> (Holmberg, 1893)	Monogenea				
	Dactylogyridae Bychowsky, 1933				
	<i>Heteropriapulus</i> <i>heterotylus</i> (Jogunoori, Kritsky and Venkatanarasaiah, 2004)	37	x	x	
	<i>Heteropriapulus simplex</i> Li and Huang, 2012	38	x	x	
	‡ <i>Heteropriapulus</i> sp. 1	39			x
	‡ <i>Heteropriapulus</i> sp. 2	40			x
	‡ <i>Heteropriapulus</i> sp. 4	41			x
	‡ <i>Heteropriapulus</i> sp. 5	42			x
	<i>Telethecium nasalis</i> Kritsky, Van Every and Boeger, 1996	43	x	x	
	<i>Trinigyrus</i> sp.	44			x
	<i>Unilatus brittani</i> Mizelle, Kritsky and Crane, 1968	45	x	x	
	<i>Unilatus unilatus</i> Mizelle and Kritsky, 1967	46	x		
	Digenea				
	Diplostomidae Poirier, 1886				
	<i>Austrodiplostomum</i> sp. metacercariae	47	x		

Table II. Continued.

Siluriform hosts	Helminth	Figure	NHR	NGH	PNS
<i>Rhinelepis aspera</i> Spix and Agassiz, 1829	Monogenea				
	Dactylogyridae				
	Bychowsky, 1933				
	* <i>Demidospermus</i> sp.	48			x
	‡ <i>Heteropriapulius</i> n. sp. 3	49			x
	<i>Telethecium nasalis</i>				
	Kritsky, Van Every and Boeger, 1996	50	x	x	
	Digenea				
	Diplostomidae Poirier, 1886				
	<i>Austrodiplostomum</i> sp. metacercariae	51	x		
	Type 1 metacercariae of Diplostomidae	51	x		
	Nematoda				
	Pharyngodonidae				
	Travassos, 1919				
	<i>Parasynodontisia petterae</i>				
	Moravec, Kohn and Fernandes, 1992	52			
Pimelodidae					
Bonaparte, 1835					
<i>Hemisorubim platyrhynchos</i> (Valenciennes, 1840)	Monogenea				
	Dactylogyridae				
	Bychowsky, 1933				
	<i>Ameloblastella formatrium</i> Mendoza-Franco, Mendoza-Palmero and Scholz, 2016	53	x	x	
	<i>Ameloblastella</i> sp. 2	54			x
	<i>Pavanelliella pavanelli</i>				
	Kritsky and Boeger, 1998	55	x		
	Dactylogyridae gen sp. 1	56			x
	Dactylogyridae gen sp. 2	57			x
	Digenea				
	Type 1 metacercariae	58	x		
	Diplostomidae Poirier, 1886				
	Metacercariae of <i>Austrodiplostomum</i> sp.	58	x		
	Type 1 metacercariae of Diplostomidae	58	x		
	Nematoda				
	Nematode larva	59	x		

Table II. Continued.

Siluriform hosts	Helminth	Figure	NHR	NGH	PNS
<i>Sorubim lima</i> (Bloch and Schneider, 1801)	Monogenea				
	Dactylogyridae				
	Bychowsky, 1933				
	Dactylogyridae gen sp. 5	60			x
	Digenea				
	Derogenidae Nicoll, 1910				
	<i>Genarchella</i> sp.	61			
	Diplostomidae Poirier, 1886				
	Metacercariae of <i>Austrodiplostomum</i> sp.	61	x		
	Type 1 metacercariae of Diplostomidae	62	x		
	Nematoda				
	Anisakidae Railliet and Henry, 1912				
	<i>Contracaecum</i> sp. larvae Railliet and Henry, 1912	63	x		
	Cestoda				
	Proteocephalidae La Rue, 1911				
	<i>Manaosia bracodemoca</i> Woodland, 1935	64			
	<i>Spatulifer marigaensis</i> Pavanelli and Rego, 1989	65			

(*) See section 4. for species descriptions.

(†) See section 6. for species description.

(‡) See section 5. for species descriptions.

Table III. (NH) number of hosts analyzed, (N) number of helminths collected, Prevalence (P%), mean intensity of infection (MII), mean abundance (MA), and infestation/infection site (IS) of the helminthes found in the eight fish species from the Aguapeí River, municipality of Castilho, São Paulo State, Brazil, sampled in Aug/13 and Jan, Jun and Nov/14, analyzed for this study. MII and MA are showed as mean $\pm$ standard error (minimum-maximum).

Siluriform hosts and helminths	NH	N	P (%)	MI	MA	IS
Doradidae						
<i>Pterodoras granulosus</i>	59					
Monogenea						
<i>Cosmetocleithrum bulbocirrus</i>		8,820	98	152 $\pm$ 21.5 (9–862)	149.5 $\pm$ 21.3 (0–862)	G
<i>Vancleaveus januacaensis</i>		10,659	98	180 $\pm$ 29.7 (2–1,225)	176.8 $\pm$ 29.4 (0–1,225)	G
Digenea						
<i>Dadaytrema oxycephala</i>		638	66	16.3 $\pm$ 6.3 (1–246)	10.8 $\pm$ 4.2 (0–246)	I
Type 1 metacercariae of Diplostomidae		1	1	-	0.01 $\pm$ 0.01 (0–1)	C
Nematoda						
<i>Procamallanus</i> (<i>Spirocamallanus</i>) <i>inopinatus</i>		2	1	-	0.03 $\pm$ 0.03 (0–2)	I
<i>Rondonia rondoni</i>		464,343	93	8,442.6 $\pm$ 1,340.7 (1–42,560)	7,870.2 $\pm$ 1,279.7 (0–42,560)	I
Cestoda						
Cestoide gen. sp.		3	5	1 (1–1)	0.05 $\pm$ 0.02 (0–1)	I
<i>Trachydoras paraguayensis</i>	70					
Monogenea						
<i>Ameloblastella</i> sp. 1		67	48	2 $\pm$ 0.2 (1–5)	0.9 $\pm$ 0.2 (0–5)	G
<i>Pavanelliella pavanellii</i>		17	11	2.1 $\pm$ 0.5 (1–5)	0.2 $\pm$ 0.09 (0–5)	NC
Dactylogyridae gen. sp. 6		413	70	8.4 $\pm$ 2.8 (1–127)	6 $\pm$ 2 (0–127)	G
Gyrodactylidae gen. sp.		1	1	-	0.04 $\pm$ 0.04 (0–1)	G
Digenea						
<i>Austrodiplostomum</i> sp. metacercariae		3	3	1.5 $\pm$ 0.5 (1–2)	0.04 $\pm$ 0.03 (0–2)	E
Type 1 metacercariae of Diplostomidae		1	1	-	0.02 $\pm$ 0.02 (0–1)	C
Type 2 metacercariae of Diplostomidae		22	7	4.4 $\pm$ 1.8 (1–11)	0.3 $\pm$ 0.2 (0–11)	Go
Type 3 metacercariae of Diplostomidae		32	8	4.8 $\pm$ 2.6 (1–18)	0.4 $\pm$ 0.3 (0–18)	C
Nematoda						
<i>Ichthyouris laterifilamenta</i>		1,051	47	31.9 $\pm$ 6.9 (1–137)	15 $\pm$ 3.8 (0–137)	I

Table III. Continued.

Siluriform hosts and helminths	NH	N	P (%)	MII	MA	IS
<i>Neoparaseuratum travassosi</i>		122	64	2.7±0.2 (1–8)	1.7±0.2 (0–8)	I
<i>Procamallanus</i> (<i>Spirocamallanus</i>) <i>inopinatus</i>		125	94	1.8±0.09 (1–6)	1.7±0.1 (0–6)	I
<i>Procamallanus</i> (<i>Spirocamallanus</i>) <i>inopinatus</i> fourth stage larvae		145	27	7.6±2.9 (1–50)	2±0.8 (0–50)	I
<i>Contracaecum</i> sp. larvae		293	53	7.9±1.7 (1–52)	4.2±1.04 (0–52)	SB
Cestoda						
Cestoda gen. sp.		1	1	-	0.01±0.01 (0–1)	I
Heptapteridae						
<i>Pimelodella avanhandavae</i>	40					
Monogenea						
<i>Ameloblastella formatrium</i>		1	2	-	0.02±0.02 (0–1)	G
<i>Aphanoblastella chagresii</i>		339	87	9.7±1.8 (1–48)	8.5±1.7 (0–48)	G
Digenea						
<i>Austrodiplostomum</i> sp. metacercariae		18	20	2.2±0.5 (1–5)	0.4±0.2 (0–5)	E
<i>Dadaytrema</i> sp.		10	7	3.3±0.3 (3–4)	0.2±0.1 (0–4)	I
<i>Genarchella</i> sp.		23	35	1.6±0.2 (1–3)	0.6±0.1 (0–3)	S
<i>Phyllodistomum</i> sp.		8	10	2±0.7 (1–4)	0.2±0.1 (0–4)	Go
Type 1 digenean		35	5	17.5±16.5 (1–34)	0.8±0.8 (0–34)	I
Type 2 digenean		1	2	-	0.02±0.02 (0–1)	I
Unidentified metacercariae		66	7	22±16.1 (2–54)	1.6±1.3 (0–54)	C
Nematoda						
<i>Brasilnema pimellodellae</i>		313	57	13±3.8 (1–75)	7.8±2.5 (0–75)	I
<i>Rondonia rondoni</i>		2,934	17	419±280 (15–2,014)	73.3±52.6 (0–2,014)	I
<i>Spinitectus</i> sp.		97	50	4.85±1 (1–21)	2.4±0.6 (0–21)	I
<i>Contracaecum</i> sp. larvae		12	15	2±0.8 (1–6)	0.3±0.1 (0–6)	SB
Loricariidae						
<i>Loricariichthys platymetopon</i>	80					
Monogenea						
<i>Demidospermus paranaensis</i>		2,018	86	29.3±5.4 (1–261)	22.2±4.8 (0–261)	G
Gyrodactylidae gen. sp.		8	5	2±0.4 (1–3)	0.1±0.05 (0–3)	G

Table III. Continued.

Siluriform hosts and helminths	NH	N	P (%)	MII	MA	IS
Digenea						
<i>Austrodiplostomum</i> sp. metacercariae		1	1	-	0.01±0.01 (0–1)	E
<i>Clinostomum</i> sp. metacercariae		29	5	7.2±3.0 (1–13)	0.4±0.2 (0–13)	Su, F
Type 1 metacercariae of Diplostomidae		1,034	85	15.2±2.3 (1–90)	13±2 (0–90)	C
Type 4 metacercariae of Diplostomidae		27	1	-	0.3±0.3 (0–27)	L
Type 5 metacercariae of Diplostomidae		16	4	5.3±1.8 (3–9)	0.2±0.1 (0–9)	C
Nematoda						
<i>Raphidascaris</i> (<i>Sprentascaris</i>) <i>mahnerti</i>		5,620	94	74.9±7 (3–358)	70.2±7 (0–358)	I
<i>Pterygoplychthys ambrosettii</i>	80					
Monogenea						
<i>Heteropriapulius heterotylus</i>		653	66	12.3±2.4 (1–83)	8.2±1.7 (0–83)	G
<i>Heteropriapulius simplex</i>		566	62	11.3±1.9 (1–69)	7±1.4 (0–69)	G
<i>Heteropriapulius</i> sp. 1		171	60	3.5±0.5 (1–20)	2.1±0.4 (0–20)	G
<i>Heteropriapulius</i> sp. 2		85	33	3.1±0.6 (1–15)	1±0.2 (0–15)	G
<i>Heteropriapulius</i> sp. 4		2	1	2 (2–2)	0.02±0.02 (0–2)	G
<i>Heteropriapulius</i> sp. 5		95	44	2.7±0.7 (1–20)	1.2±0.3 (0–20)	G
<i>Telethecium nasalis</i>		144	44	4.1±0.7 (1–17)	1.8±0.4 (0–17)	NC
<i>Trinigyryus</i> sp.		294	12	29.4±24 (1–246)	14.7±12.2 (0–246)	G
<i>Unilatus brittani</i>		14	12	1.4±0.1 (1–2)	0.2±0.05 (0–2)	G
<i>Unilatus unilatus</i>		24	10	1.4±0.1 (1–9)	0.3±0.1 (0–9)	G
Digenea						
<i>Austrodiplostomum</i> sp. metacercariae		67	9	9.6±6.2 (1–45)	0.9±0.6 (0–45)	E
<i>Rhinelepis aspera</i>	30					
Monogenea						
<i>Demidospermus</i> sp.		332	100	11.7±3.3 (1–70)	11.7±3.3 (0–70)	G
<i>Heteropriapulius</i> sp. 3		20	23	2.9±1 (1–8)	0.7±0.3 (0–8)	G
<i>Telethecium nasalis</i>		5	13	1.2±0.2 (1–2)	0.2±0.08 (0–2)	NC
Digenea						
<i>Austrodiplostomum</i> sp. metacercariae		11	33	1.1±0.1 (1–2)	0.4±0.1 (0–2)	E

Table III. Continued.

Siluriform hosts and helminths	NH	N	P (%)	MII	MA	IS
Type 1 metacercariae of Diplostomidae		46	13	11.5±10.5 (1–43)	1.53±1.43 (0–43)	C
Nematoda						
<i>Parasynodontisia petterae</i>		11,676	87	449±88.6 (4–1,432)	389.2±81.6 (0–1,432)	I
Pimelodidae						
<i>Hemisorubim platyrhynchos</i>	21					
Monogenea						
<i>Ameloblastella</i> sp. 2		1	3	-	0.04±0.04 (0–1)	G
<i>Ameloblastella formatrium</i>		99	10	49±23 (26–72)	4.7±3.6 (0–72)	G
<i>Pavanelliella pavanellii</i>		1	3	-	0.04±0.04 (0–1)	NC
Dactylogyridae gen. sp. 1		1,031	86	57.3±22.3 (1–390)	49±19.6 (0–390)	G
Dactylogyridae gen. sp. 2		74	47	7.4±1.4 (2–17)	3.5±1 (0–17)	G
Digenea						
<i>Austrodiplostomum</i> sp. metacercariae		16	33	2.3±1 (1–8)	0.8±0.4 (0–8)	E
Type 1 metacercariae of Diplostomidae		3	3	-	0.1±0.1 (0–3)	C
Type 1 metacercariae		29	38	3.6±1.3 (1–12)	1.4±0.6 (0–12)	C
Nematoda						
Nematode larva		1	3	-	0.04±0.04 (0–1)	C
Sorubim lima						
Monogenea						
Dactylogyridae gen. sp. 5		1,102	92	47.9±12.5 (3–223)	44±11.8 (0–223)	G
Digenea						
<i>Austrodiplostomum</i> sp. metacercariae		27	52	2.1±0.6 (1–8)	1.1±0.4 (0–8)	E
<i>Genarchella</i> sp.		2	4	-	0.08±0.08 (0–2)	S
Type 1 metacercariae of Diplostomidae		25	20	5±2.2 (1–13)	1±0.6 (0–13)	C
Nematoda						
<i>Contracaecum</i> sp. larvae		1,113	60	74.2±33.8 (2–460)	44.5±21.3 (0–460)	SB
Cestoda						
<i>Manaosia bracodemoca</i>		24	24	4±0.8 (2–8)	1±0.4 (0–8)	I
<i>Spatulifer maringaensis</i>		655	88	29.8±9.5 (1–194)	26.2±8.6 (0–194)	I

Infection/infestation sites: Su – surface; NC – nasal cavity; G – gills; E – eyes; Go – gonads; SB – swim bladder; L – liver; S – stomach; I – Intestine; F – flesh; C – cavity.

From the total of host specimens analyzed, 400 (99%) were infected by at least one helminth taxon. A total of 501,484 was recovered in this study, with a mean of 1,238.23 parasite/fish. Fifty-four helminth parasite taxa were found: 25 monogeneans (the most diverse taxa), 15 digeneans, 11 nematodes, and three cestodes. With the exception of metacercariae of Diplostomidae, and *Contracaecum* sp. larvae reported by Yamada et al. (2017) all the helminths found are new locality record for the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo State, Brazil. The species accumulation curves (SAC) showed that the sampling effort for *P. granulosus*, *P. avandavae*, *L. platymetopon*, and *R. aspera* was enough to recover all the helminth parasite taxa that these hosts can harbor. However, for *T. paraguayensis*, *P. ambrosettii*, *H. platyrhynchos*, and *S. lima* the SACs indicated that more hosts should be sampled in order to recover all helminth parasite taxa of these hosts (Figure 1).

Metacercariae of *Austrodiplostomum* sp. were the most widely distributed taxon, occurring in seven fish species. Type 1 metacercariae of Diplostomidae also showed wide distribution, occurring in six host species. The most prevalent helminth taxon was the monogenean *Demidospermus* sp. (100%), followed by the monogeneans *Cosmetocleithrum bulbocirrus* and *V. januacaensis* (98%). The helminth taxon that showed the highest values of mean intensity of infection (8,442.6) and mean abundance (7,870.2) was the nematode *Rondonia rondoni* from *P. granulosus*, followed by the nematode *Parasynodontisia petterae* from *R. aspera* (mean intensity of infection 449; mean abundance 389.2). The helminth taxon with the greatest number of specimens recovered was *R. rondoni* from *P. granulosus* (n=464,343) followed by *P. petterae* (n=11,676). Table IV shows the values of Shannon-Wiener index calculated at component community level for each host species. The host species *P. ambrosettii* showed the highest diversity of helminth community while *R. aspera* showed the lowest, interestingly both host species belong to the Loricariidae family.

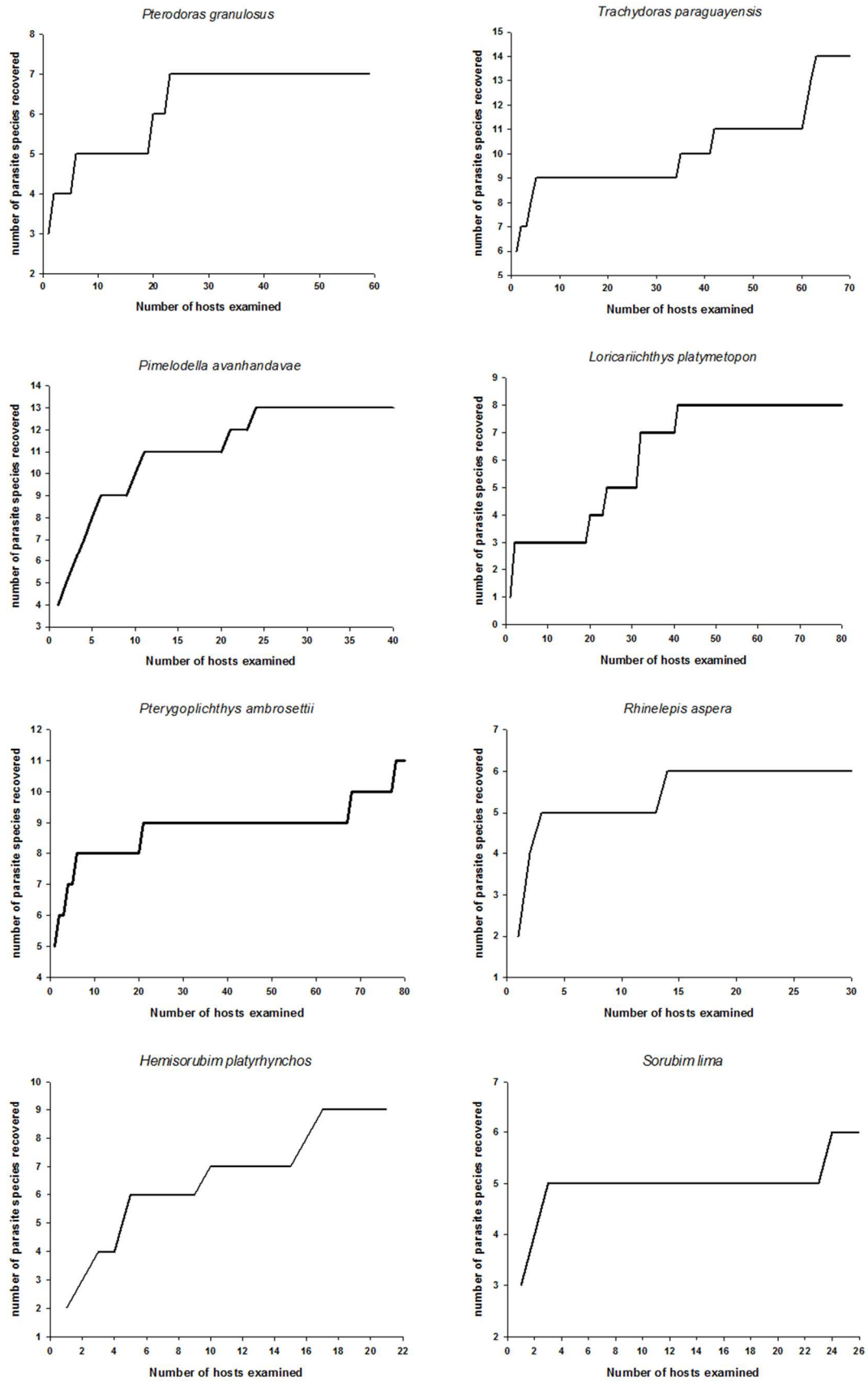


Figure 1. Species accumulation curves of the parasites recovered from the eighth siluriform species surveyed for this study from the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo, State, Brazil.

Table IV. Shannon-Wiener index for the eight host species surveyed for this study from the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo State, Brazil.

Siluriform host	Shannon-Wiener index
<i>Pterygoplichthys ambrosettii</i>	1.84
<i>Trachydoras paraguayensis</i>	1.68
<i>Sorubim lima</i>	1.20
<i>Pimelodella avanhandavae</i>	0.94
<i>Loricariichthys platymetopon</i>	0.93
<i>Hemisorubim platyrhynchos</i>	0.70
<i>Pterodoras granulosus</i>	0.21
<i>Rhinelepis aspera</i>	0.17

From the 54 helminth *taxa* recovered in this study, only nine occurred in more than one host: the monogeneans *A. formatrium*, *P. pavanellii*, and *T. nasalis*, the digeneans *Austrodiplostomum* sp. metacercariae, *Genarchella* sp., and Type 1 metacercariae of Diplostomidae, and the nematodes *P. (Spirocamallanus) inopinatus*, *R. rondoni*, and *Contracaecum* sp. larvae.

For *P. granulosus* (n=59), 100% were parasitized by at least one parasite species. In total 484,466 parasites were found, with a mean of 8,211.28 parasite/fish, representing the highest values among all host species analyzed in this study. Six helminth parasite *taxa* were found (Table III), representing the host with the lowest number of helminth *taxa* recovered, with richness varying from 1 to 4 parasites and the mean richness was 3.67 parasite/host. The helminths found in *P. granulosus* have all been previously recorded in the literature (see Table XII). The only new host record is one Diplostomidae metacercaria (Type 1). The nematode *R. rondoni* showed the highest values of mean intensity of infection (MII) and mean abundance (MA), while the monogeneans *C. bulbocirrus* and *V. januacaensis* showed the highest levels of prevalence, and considerable high values MII and MA (Table III). Simpson index was 0.08 and demonstrated that the diversity of the component community of *P. granulosus* is considerably low. Berger-Parker indexes for each helminth *taxa* (Table V) showed that *R. rondoni* was the dominant species while all the other species showed low values. It was not possible to calculate the discrepancy index for this host species because the maximum intensity was too high.

Table V. Berger-Parker index for the helminth species of *Pterodoras granulosus* from the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo State, Brazil.

Helminths	Berger-Parker
<i>Cosmetocleithrum bulbocirrus</i>	0.018
<i>Vancleaveus januacaensis</i>	0.022
<i>Dadaytrema oxycephala</i>	0.001
Type 1 metacercariae of Diplostomidae	-
<i>Procamallanus (Spirocamallanus) inopinatus</i>	-
<i>Rondonia rondoni</i>	0.958
Cestode gen. sp.	-

The host specimens of *Trachydoras paraguayensis* (n=70) were all parasitized (100%) by at least one parasite species. A total of 2,293 parasites was found, with a mean of 32.75 parasite/fish. Fourteen helminth parasite *taxa* were recovered, which is the highest number of *taxa* per host species found in this study. The richness varied from 1 to 8 parasites and the mean richness was 4.4 parasite/host. All nematode species found in this study were previously recorded in *T. paraguayensis* (see Table XII). The monogeneans Gyrodactylidae gen. sp. and *P. pavanellii*, *Austrodiplostomum* sp. metacercariae, and *Contracaecum* sp. larvae are recorded for the first time in *T. paraguayensis* (Table II). The nematode *P. (Spirocamallanus) inopinatus* had the highest prevalence, while Dactylogyridae gen. sp. 6 showed the highest values of MII and MA. Simpson index was 0.73 demonstrating that the diversity of the component community of *T. paraguayensis* is high. Berger-Parker indexes for each helminth *taxa* (Table VI) showed that *I. laterifilamenta* was the dominant species. The discrepancy index was 0.54.

Table VI. Berger-Parker index for the helminth species of *Trachydoras paraguayensis* from the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo State, Brazil.

Helminths	Berger-Parker
<i>Ameloblastella</i> sp. 1	0.029
<i>Pavanelliella pavanellii</i>	0.007
Dact. gen. sp. 6	0.180
Gyrodactylidae gen. sp.	-
<i>Austrodiplostomum</i> sp. metacercariae	0.001
Type 1 metacercariae of Diplostomidae	-
Type 2 metacercariae of Diplostomidae	0.010
Type 3 metacercariae of Diplostomidae	0.014
<i>Ichthyouris laterifilamenta</i>	0.458
<i>Neoparaseuratum travassosi</i>	0.053
<i>Procamallanus (Spirocamallanus) inopinatus</i>	0.055
<i>Procamallanus (Spirocamallanus) inopinatus</i> 4th stage larvae	0.063
<i>Contracaecum</i> sp. larvae	0.128
Cestoda gen. sp.	-

For *P. avanhandavae* (n=40), 100% were parasitized by at least one parasite species. A total of 3,857 parasites was found, with a mean of 96.4 parasite/fish. Thirteen helminth parasite *taxa* were recovered, accounting for the second highest number of *taxa* per host species found in this study. The richness varied from 1 to 5 parasites and the mean richness was 3.2 parasite/host. All helminths in *P. avanhandavae* found in this study are new host record, since there is not any study on helminthfauna for this host species thus far. The monogenean *A. chagresii* had the highest prevalence, and the nematode *R. rondoni* showed the highest values of MII and MA. Simpson index was 0.41 demonstrating that the component community of *P. avanhandavae* is not very diverse. Berger-Parker indexes for each helminth *taxa* (Table VII) showed that *R. rondoni* was the dominant species. The discrepancy index was 0.78 indicating that the component community of *P. avanhandavae* showed aggregated pattern.

Table VII. Berger-Parker index for the helminth species of *Pimelodella avanhandavae* from the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo State, Brazil.

Helminths	Berger-Parker
<i>Ameloblastella formatrium</i>	-
<i>Aphanoblastella chagresii</i>	0.088
<i>Austrodiplostomum</i> sp. metacercariae	0.005
<i>Dadaytrema</i> sp.	0.003
<i>Genarchella</i> sp.	0.006
<i>Phyllodistomum</i> sp.	0.002
Type 1 digenean	0.009
Type 2 digenean	-
Unidentified metacercariae	0.017
<i>Brasilnema pimellodellae</i>	0.081
<i>Rondonia rondoni</i>	0.761
<i>Spinitectus</i> sp.	0.025
<i>Contracaecum</i> sp. larvae	0.003

The host specimens of *L. platymetopon* (n=80) were all parasitized (100%) by at least one parasite species. A total of 8,753 parasites was found, with a mean of 109.4 parasite/fish. Eight parasite *taxa* were recovered, with richness varying from 1 to 4 parasites and mean richness of 2.8 parasite/host. For *L. platymetopon*, this study is a new host record for *Austrodiplostomum* sp. metacercariae, Type 1, 4 and 5 metacercariae of Diplostomidae and a gyrodactylid monogenean. The monogenean *D. paranaensis* and the nematode *R. (Sprentascaris) mahnerti* were previously described in this host (Table XII). The helminth *taxa* *R. (Sprentascaris) mahnerti* was the most prevalent and had the highest values of MII and AM. Simpson index was 0.52 demonstrating that the component community of *L. platymetopon* is not very diverse. Berger-Parker indexes for each helminth *taxa* (Table VIII) showed that *R. (Sprentascaris) mahnerti* was the dominant species. The discrepancy index was 0.41.

Table VIII. Berger-Parker index for the helminth species of *Loricariichthys platymetopon* from the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo State, Brazil.

Helminths	Berger-Parker
<i>Demidospermus paranaensis</i>	0.231
Gyrodactylidae gen sp.	0.001
<i>Austrodiplostomum</i> sp. metacercariae	-
<i>Clinostomum</i> sp. metacercariae	0.003
Diploptomidae metacercariae Type 1	0.118
Diploptomidae metacercariae Type 4	0.003
Diploptomidae metacercariae Type 5	0.002
<i>Raphidascaris (Sprentascaris) mahnerti</i>	0.642

For *P. ambrosettii*, 96% of the hosts were parasitized by at least one parasite species. A total of 2,115 parasites was found, with a mean of 26.4 parasite/fish. Eleven taxa were recovered, from which 10 are monogenean species, making *P. ambrosettii* the host with the most diverse monogenean community in this study. The richness varied from 1 to 8 parasites and the mean richness was 3.56 parasite/host. All helminths in *P. ambrosettii* found in this study are new host record, since to date there are no studies on helminthfauna for this host species. The monogenean *H. heterotylus* showed the highest prevalence, while the monogenean *Trinigyryus* sp. showed the highest values of MII and MA. Simpson index was 0.8 demonstrating that the component community of *P. ambrosettii* is considerably diverse. Berger-Parker indexes for each helminth taxa (Table IX) showed that *H. heterotylus* was the dominant species. The discrepancy index was 0.6 indicating that the component community of *P. ambrosettii* tends to show aggregated pattern.

Table IX. Berger-Parker index for the helminth species of *Loricariichthys platymetopon* from the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo State, Brazil.

Helminths	Berger-Parker
<i>Heteropriapulus heterotylus</i>	0.309
<i>Heteropriapulus simplex</i>	0.268
<i>Heteropriapulus</i> sp. 1	0.081
<i>Heteropriapulus</i> sp. 2	0.040
<i>Heteropriapulus</i> sp. 4	0.001
<i>Heteropriapulus</i> sp. 5	0.045
<i>Telethecium nasalis</i>	0.068
<i>Trinigyryus</i> sp.	0.139
<i>Unilatus brittani</i>	0.007
<i>Unilatus unilatus</i>	0.011
<i>Austrodiplostomum</i> sp. metacercariae	0.032

The host specimens of *R. aspera* (n=30) had all representatives parasitized by at least two helminth *taxa* (100%). A total of 12,090 parasites was found, with a mean of 403 parasite/fish. Six helminth *taxa* were recovered, which also represents the host with the lowest number of helminth *taxa* found, with richness varying from 2 to 4 parasites and mean richness of 2.53 parasite/host. With the exception of *P. petterae*, whose type host is *R. aspera* (see Table XII), all the other helminths found in this study are new host record. The monogenean *Demidospermus* sp. showed the highest prevalence while *P. petterae* showed the highest values of MII and AM. Simpson index was 0.07 demonstrating that the diversity of the component community of *R. aspera* is very low. Berger-Parker indexes for each helminth *taxa* (Table X) showed that *P. petterae* was the dominant species while all the other species showed low values. The discrepancy index was 0.5.

Table X. Berger-Parker index for the helminth species of *Rhinelepis aspera* from the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo State, Brazil.

Helminths	Berger-Parker
<i>Demidospermus</i> sp.	0.027
<i>Heteropriapulus</i> sp. 3	0.002
<i>Telethecium nasalis</i>	-
<i>Austrodiplostomum</i> sp. metacercariae	0.001
Type 1 metacercariae of Diplostomidae	0.004
<i>Parasynodontisia petterae</i>	0.966

For *H. platyrhynchos* (n=21), 95% of the host specimens were parasitized by at least one helminth species. A total of 1,255 parasites was found, with a mean of 59.7 parasite/fish. Nine helminth *taxa* were recovered, with richness varying from 1 to 4 parasites and mean richness of 2.34 parasite/host. All helminth *taxa* found in *H. platyrhynchos* in this study are new host records. Dactylogyridae gen. sp. 1 had the highest values of prevalence, MII and MA. Simpson index was 0.31 demonstrating that the diversity of the component community of *H. platyrhynchos* is low. Berger-Parker indexes for each helminth *taxa* (Table XI) showed that Dactylogyridae gen. sp. 1 was the dominant species. The discrepancy index was 0.6 indicating that the component community of *P. ambrosetii* tends to show aggregated pattern.

Table XI. Berger-Parker index for the helminth species of *Hemisorubim platyrhynchos* from the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo State, Brazil.

Helminths	Berger-Parker
<i>Ameloblastella</i> sp. 2	0.001
<i>Ameloblastella formatrium</i>	0.079
<i>Pavanelliella pavanellii</i>	0.001
Dactylogyridae gen. sp. 1	0.822
Dactylogyridae gen. sp. 2	0.059
<i>Austrodiplostomum</i> sp. metacercariae	0.013
Type 1 metacercariae of Diplostomidae	0.002
Type 1 metacercariae	0.023
Nematode larva	0.001

All host specimens of *S. lima* (n=25) were parasitized by at least one helminth *taxa* (100%). A total of 2,948 parasites was found, with a mean of 117.9 parasite/fish. Seven helminth *taxa* were recovered, with richness varying from 1 to 5 parasites, and mean richness of 3.3 parasite/host. This study represents new host record for Dactylogyridae gen. sp. 5, *Austrodiplostomum* sp. metacercariae, and *Contracaecum* sp. larvae. For the remaining endoparasites, the records of this study are in accordance with previous records for this host (see Table XII). Dactylogyridae gen. sp. 5 showed the highest values of prevalence, MII and AM. Simpson index was 0.67 demonstrating that the component community of *S. lima* can be considered as diverse. Berger-Parker indexes for each helminth *taxa* (Table XII) showed that *Contracaecum* sp. larvae was the dominant species, however, Dactylogyridae gen. sp. 5 showed similar value. The discrepancy index was 0.52.

Table XI. Berger-Parker index for the helminth species of *Hemisorubim platyrhynchos* from the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo, State, Brazil.

<i>Sorubim lima</i>	Berger-Parker
Dactylogyridae gen. sp. 5	0.374
<i>Austrodiplostomum</i> sp. metacercariae	0.009
<i>Genarchella</i> sp.	0.001
Type 1 metacercariae of Diplostomidae	0.008
<i>Contracaecum</i> sp. larvae	0.378
<i>Manaosia bracodemoca</i>	0.008
<i>Spatulifer maringaensis</i>	0.222

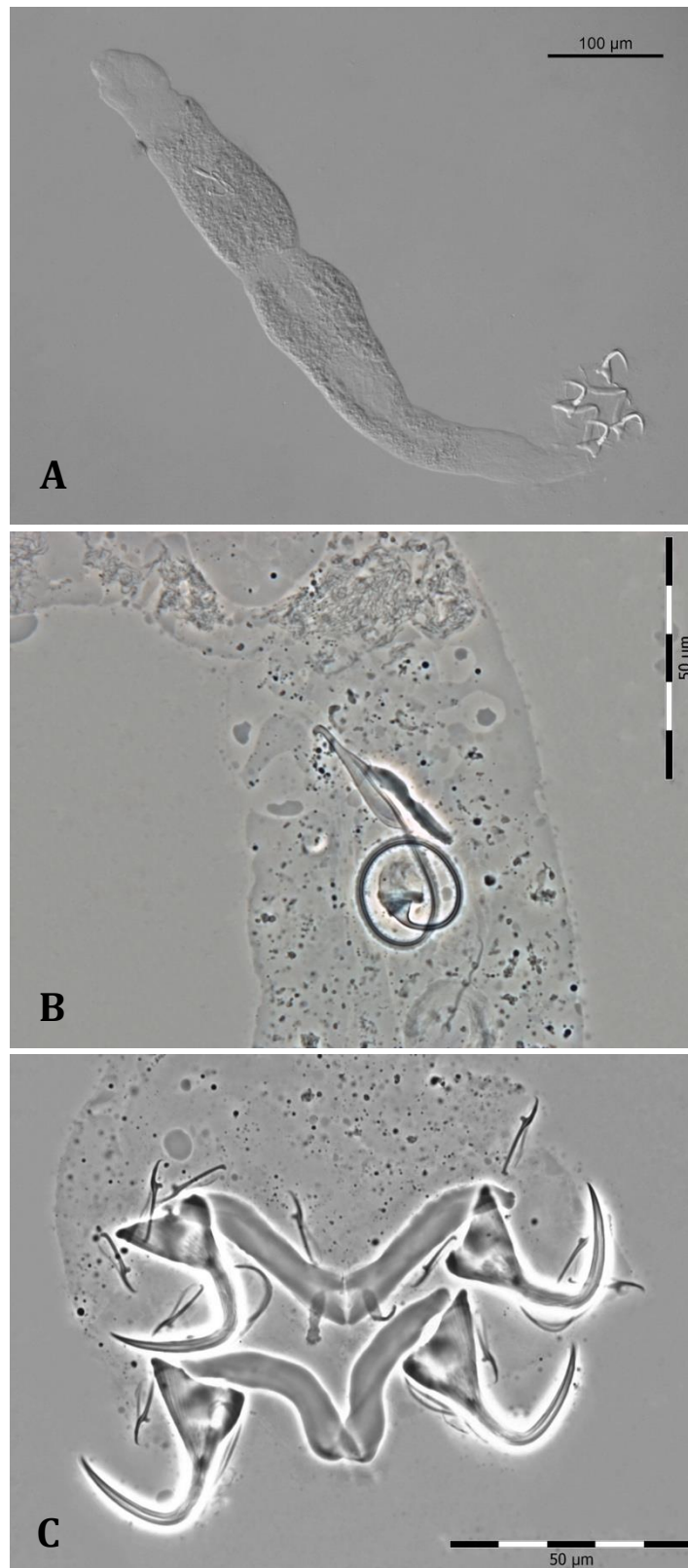


Figure 2. Monogenean found on the gills of *Pterodoras granulosus* from the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo State, Brazil: (A) *Cosmetocleithrum bulbocirrus*; (B) copulatory complex of *Cosmetocleithrum bulbocirrus*; (C) haptor of *Cosmetocleithrum bulbocirrus*.

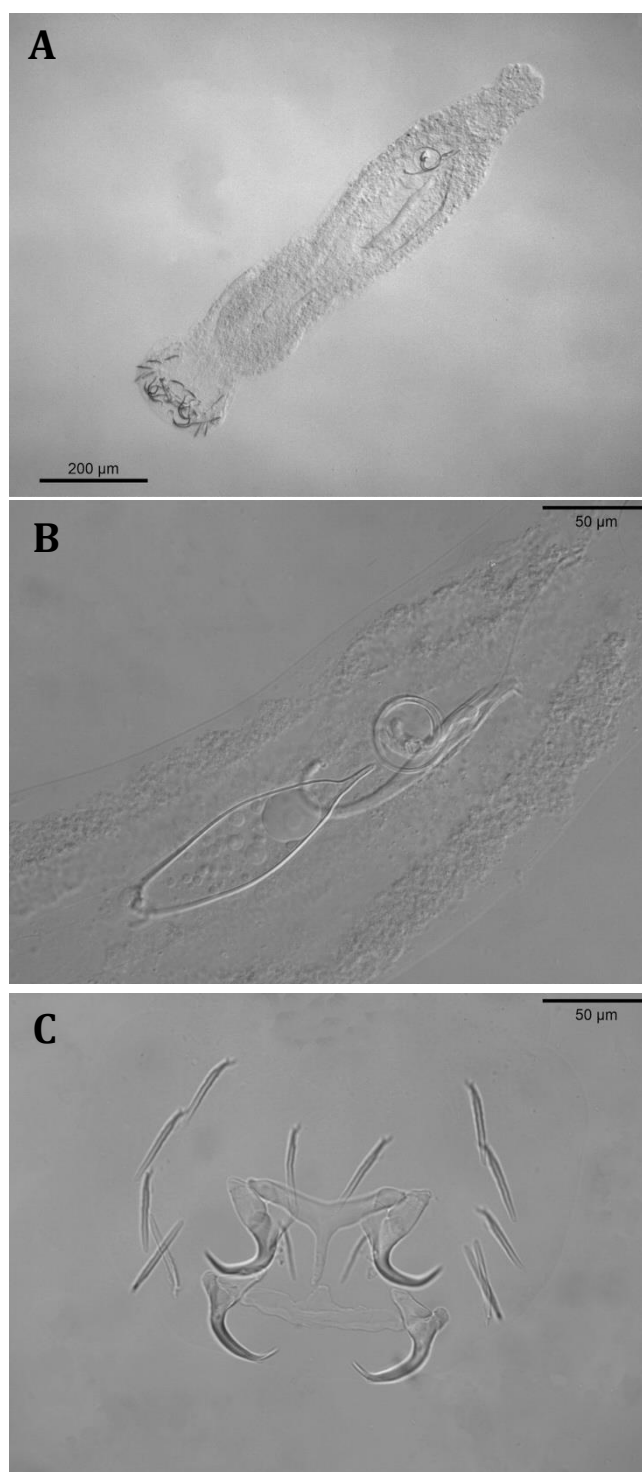


Figure 3. Monogenean found on the gills of *Pterodoras granulatus* from the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo State, Brazil: (A) *Vancleaveus januacaensis*; (B) complexo copulatório de *Vancleaveus januacaensis*; (C) haptor de *Vancleaveus januacaensis*.

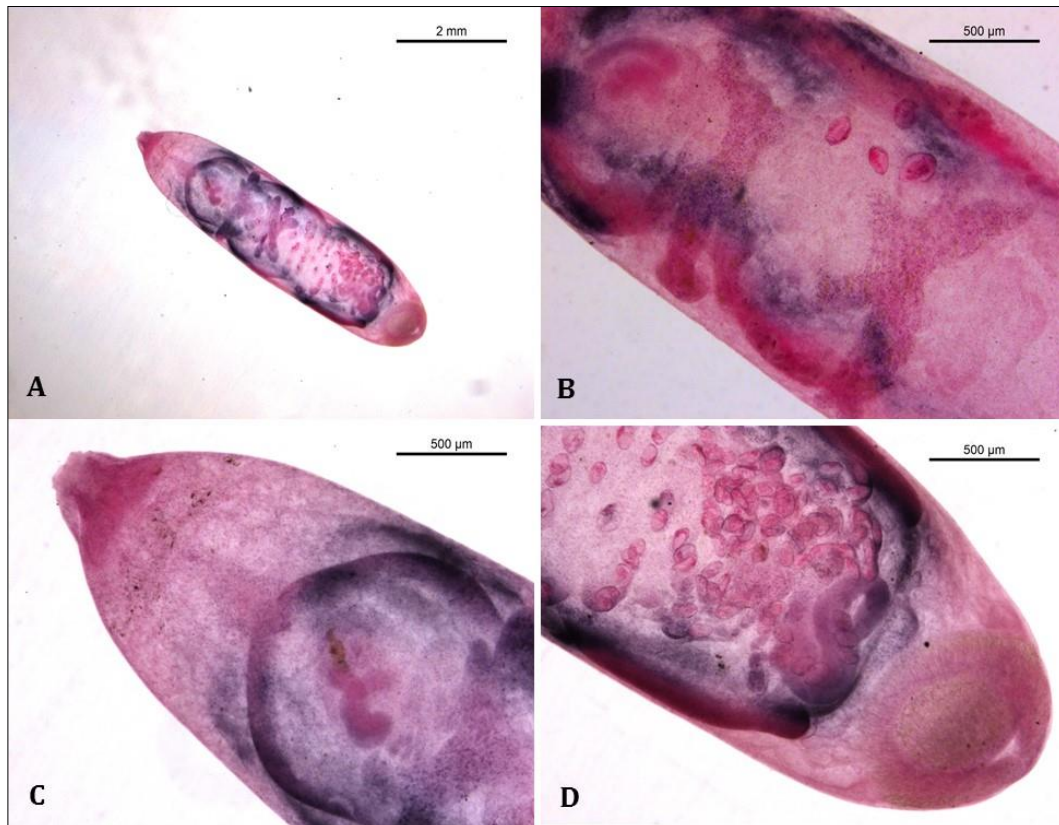


Figure 4. Digenean from *Pterodoras granulosus* from the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo State, Brazil: (A–D) *Dadaytrema oxycephala* – (A) total view; (B) detail of the profoundly lobed testes; (C) anterior extremity; (D) acetabulum.



Figure 5. Digenean from *Pterodoras granulosus* from the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo State, Brazil: (A) Type 1 metacercaria of Diplostomidae found in the cavity.

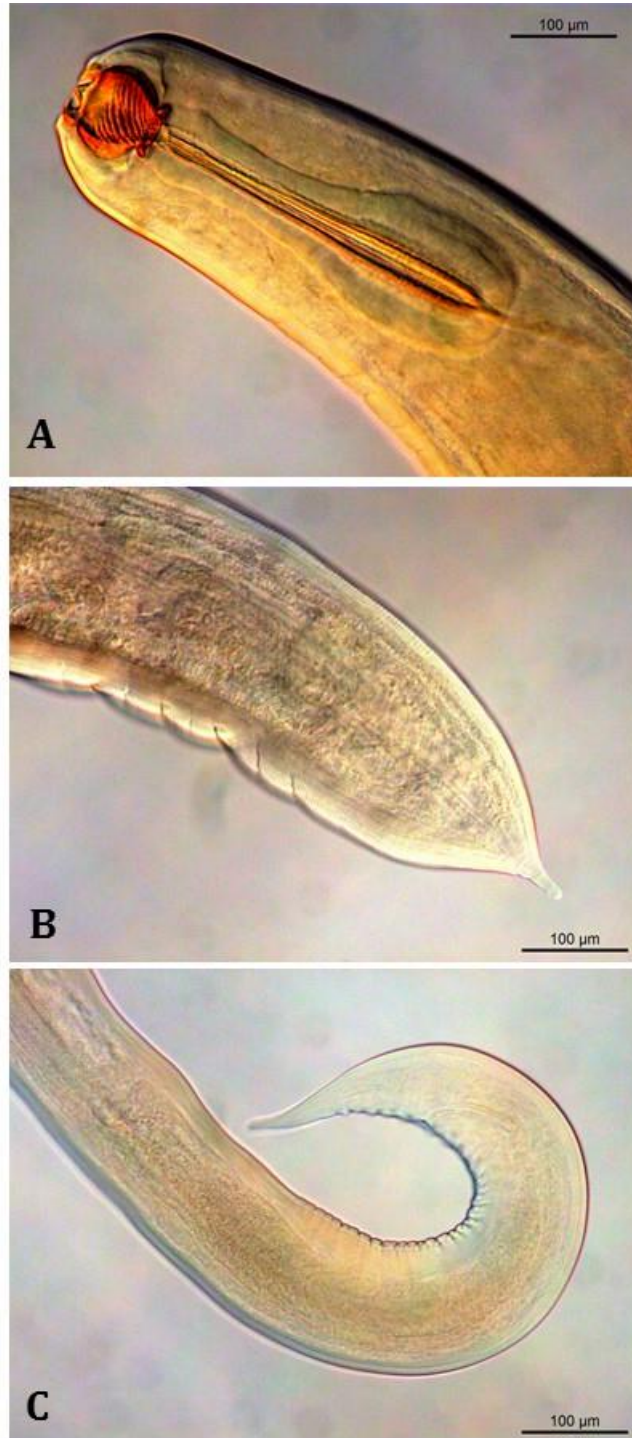


Figure 6. Nematode from the intestine of *Pterodoras granulosus* from the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo State, Brazil: (A–C) *Procamallanus* (*Spirocamallanus*) *inopinatus* – (A) anterior extremity; (B) female posterior extremity; (C) male posterior extremity.

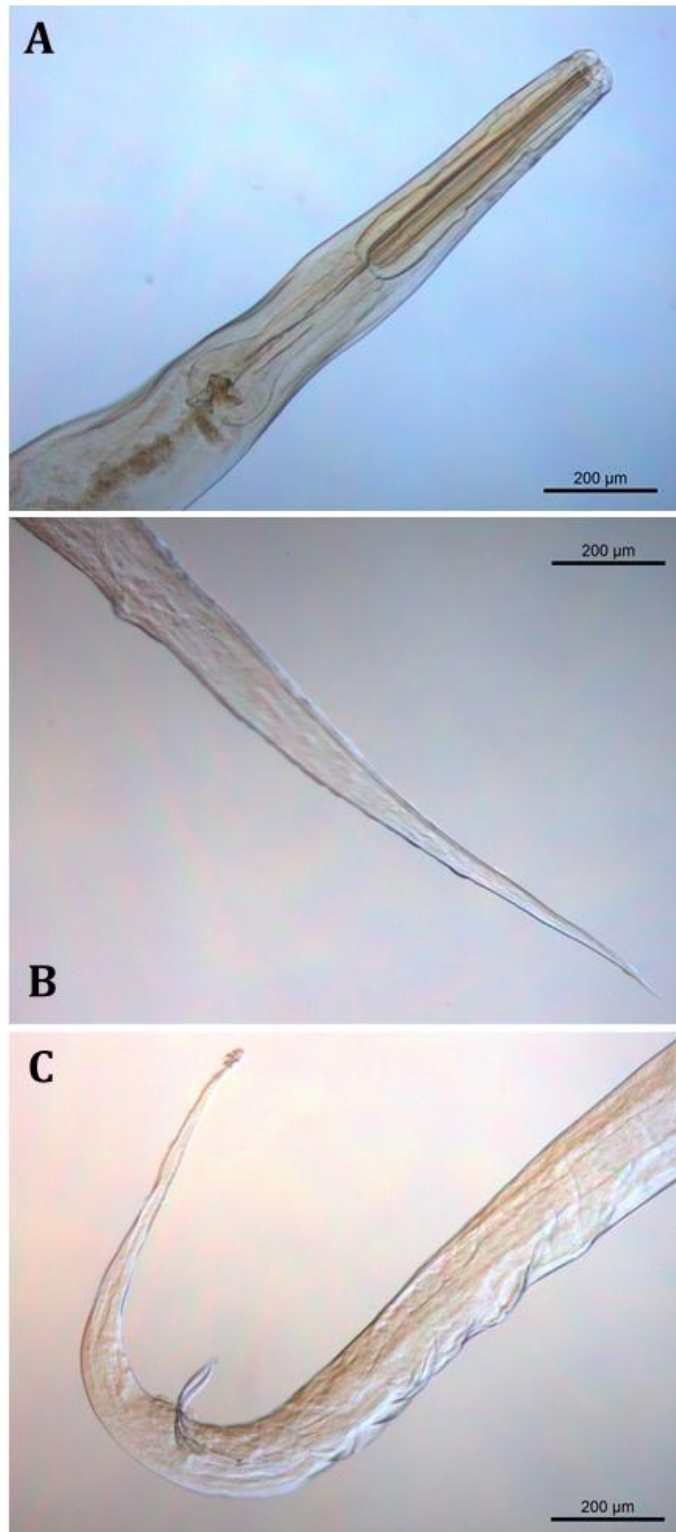


Figure 7. Nematode from the intestine of *Pterodoras granulosus* from the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo State, Brazil: (A–C) *Rondonia rondoni* – (A) anterior extremity; (B) female posterior extremity; (C) male posterior extremity.

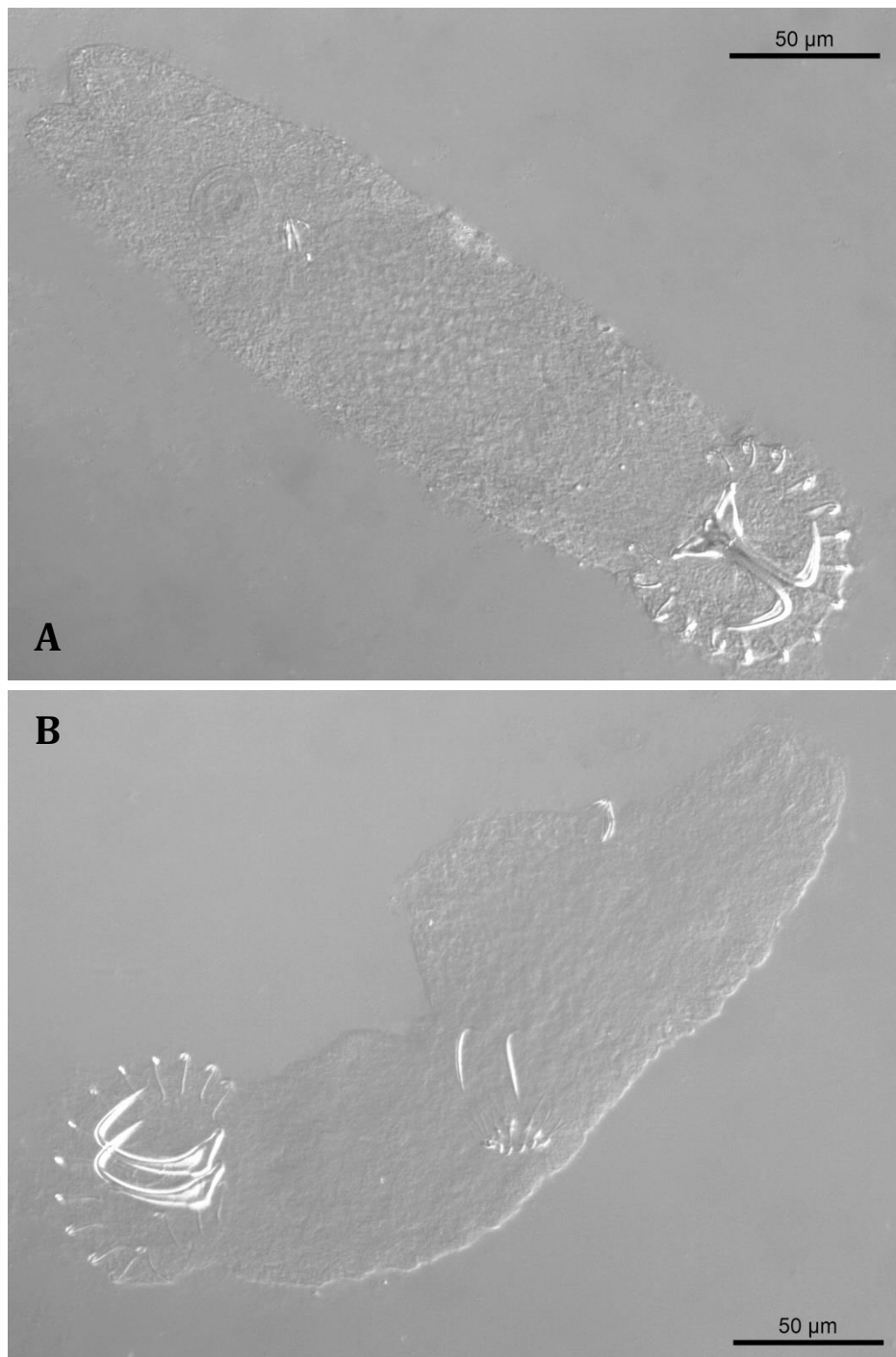


Figure 8. Monogenean from the gills of *Trachydoras paraguayensis* from the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo State, Brazil: (A– B) Gyrodactylidae gen. sp.

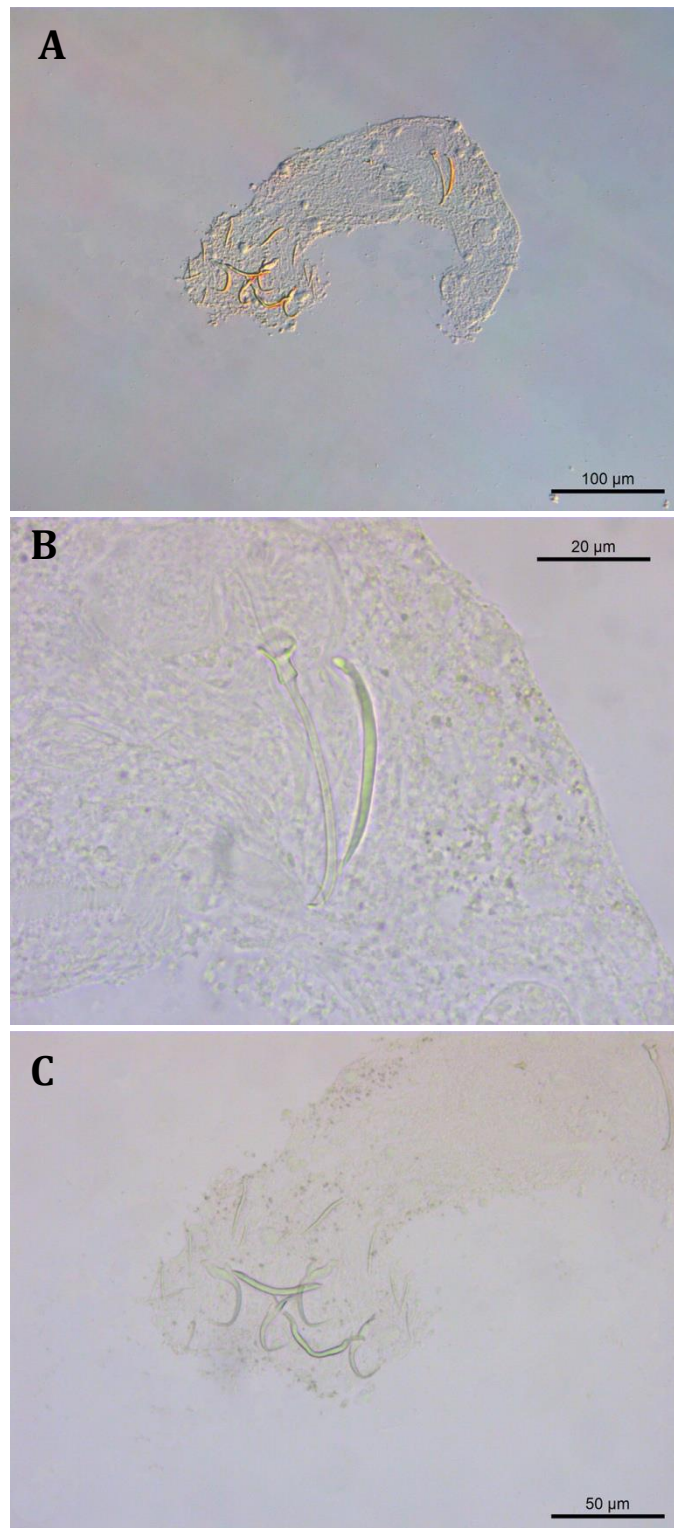


Figure 9. Monogenean from the gills of *Trachydoras paraguayensis* from the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo State, Brazil: (A) *Ameloblastella* sp. 1; (B) copulatory complex of *Ameloblastella* sp. 1; (C) haptor of *Ameloblastella* sp. 1.

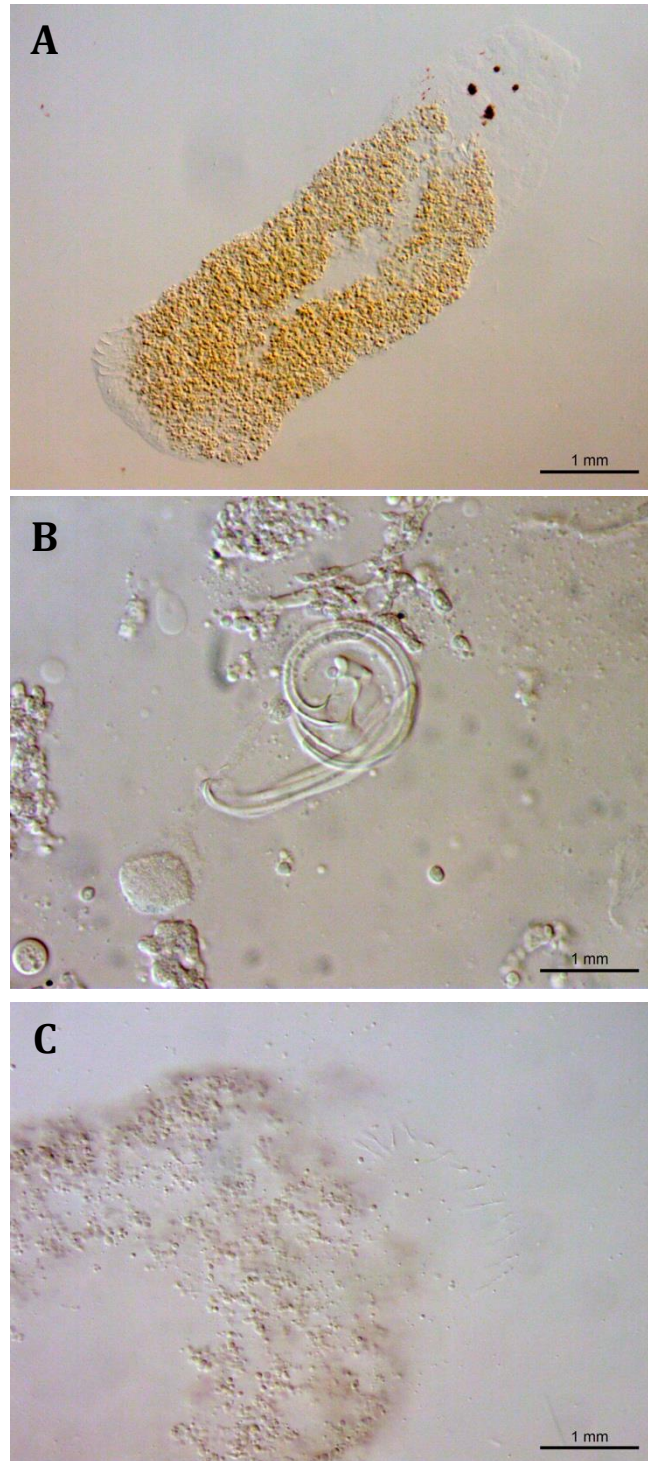


Figure 10. Monogenean from the nasal cavity of *Trachydoras paraguayensis* from the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo State, Brazil: (A) *Pavanelliella pavanellii*; (B) Copulatory complex of *Pavanelliella pavanellii*; (C) haptor of *Pavanelliella pavanellii*.



Figure 11. Monogenean from the gills of *Trachydoras paraguayensis* from the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo State, Brazil: (A) Dactylogyridae gen. sp. 6; (B) copulatory complex of Dactylogyridae gen. sp. 6; (C) haptor of Dactylogyridae gen. sp. 6.

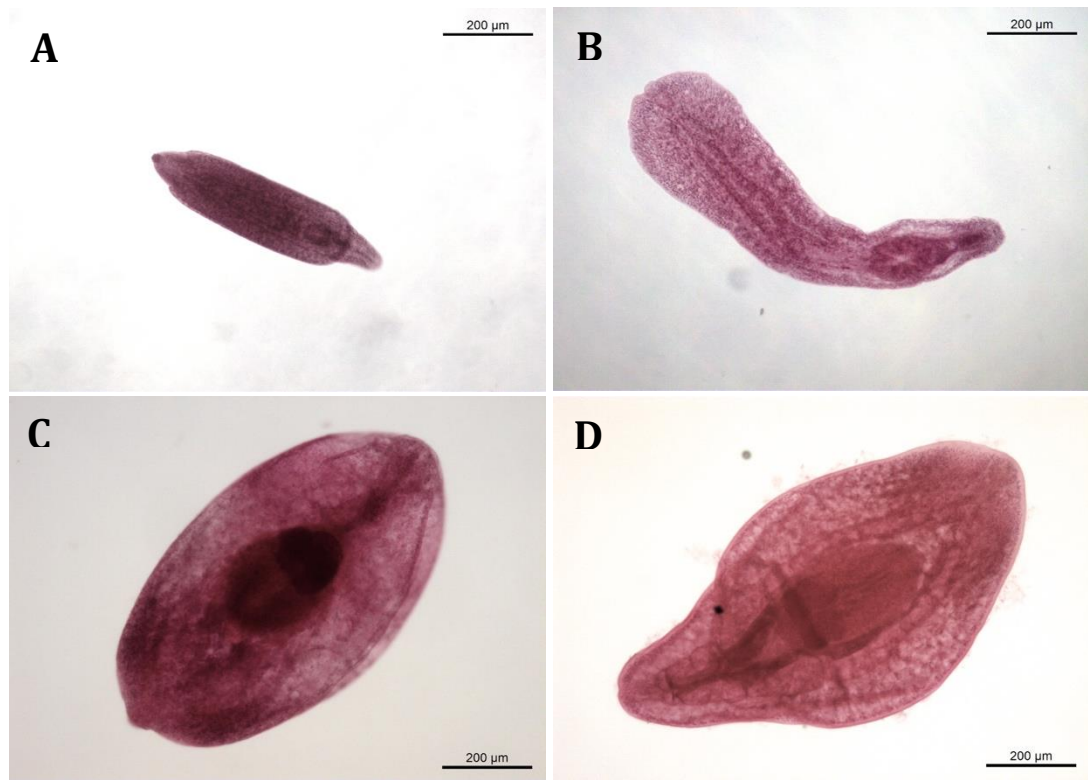


Figure 12. Digenean from *Trachydoras paraguayensis* from the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo State, Brazil: (A) *Austrodiplostomum* sp. metacercaria infecting the eyes; (B) Type 2 metacercariae of Diplostomidae; (C, D) Type 3 metacercariae of Diplostomidae. B, C, and D were recovered from the cavity.

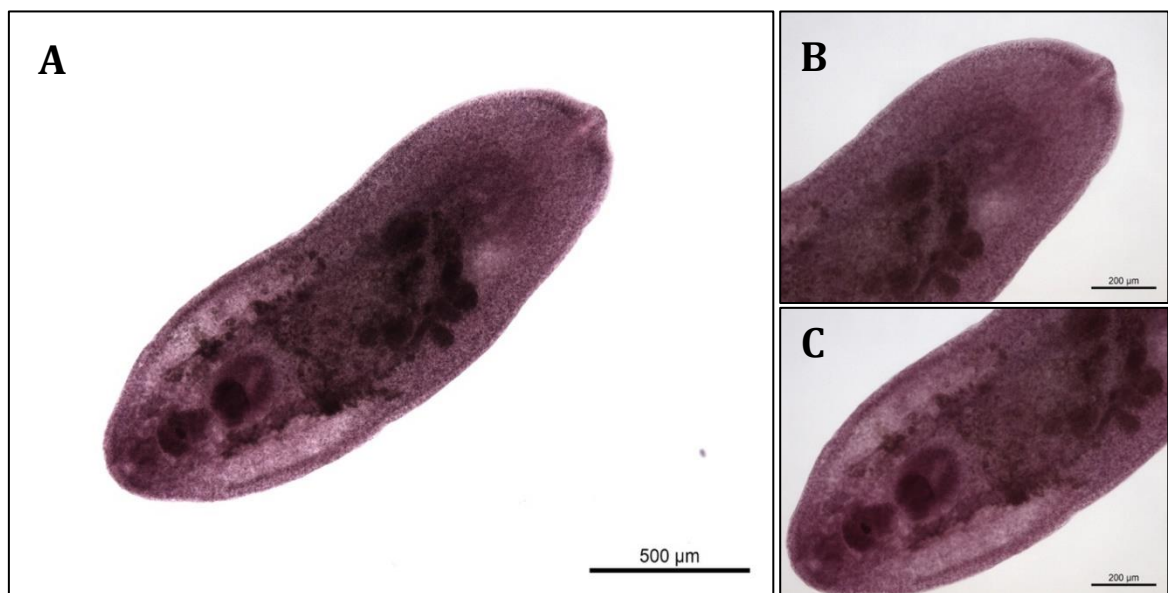


Figure 13. Digenean from *Trachydoras paraguayensis* from the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo State, Brazil: (A–C) Type 1 metacercariae of Diplostomidae in the cavity – (A) total view; (B) anterior extremity; (C) posterior extremity.

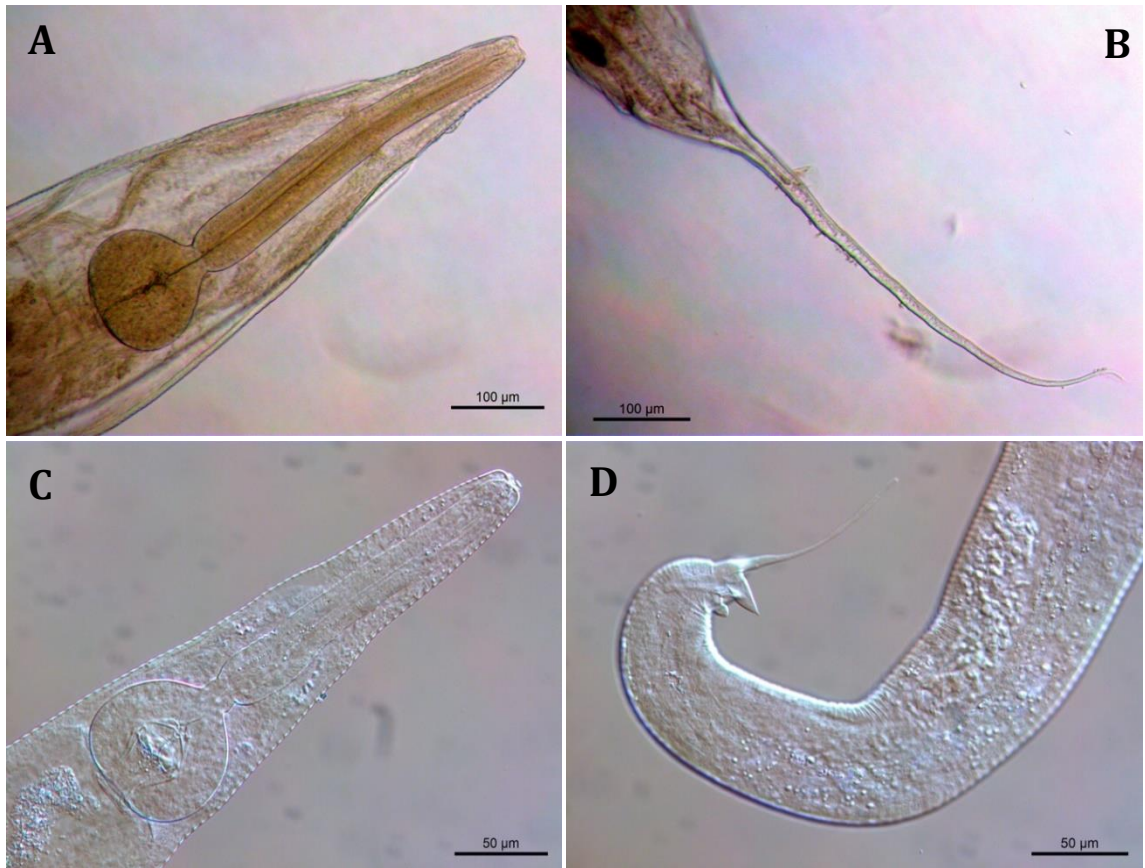


Figure 14. Nematode from the intestine of *Trachydoras paraguayensis* from the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo State, Brazil: (A–D) *Ichthyouris laterifilamenta* – (A) female anterior extremity; (B) female posterior extremity; (C) male anterior extremity; (D) male posterior extremity.

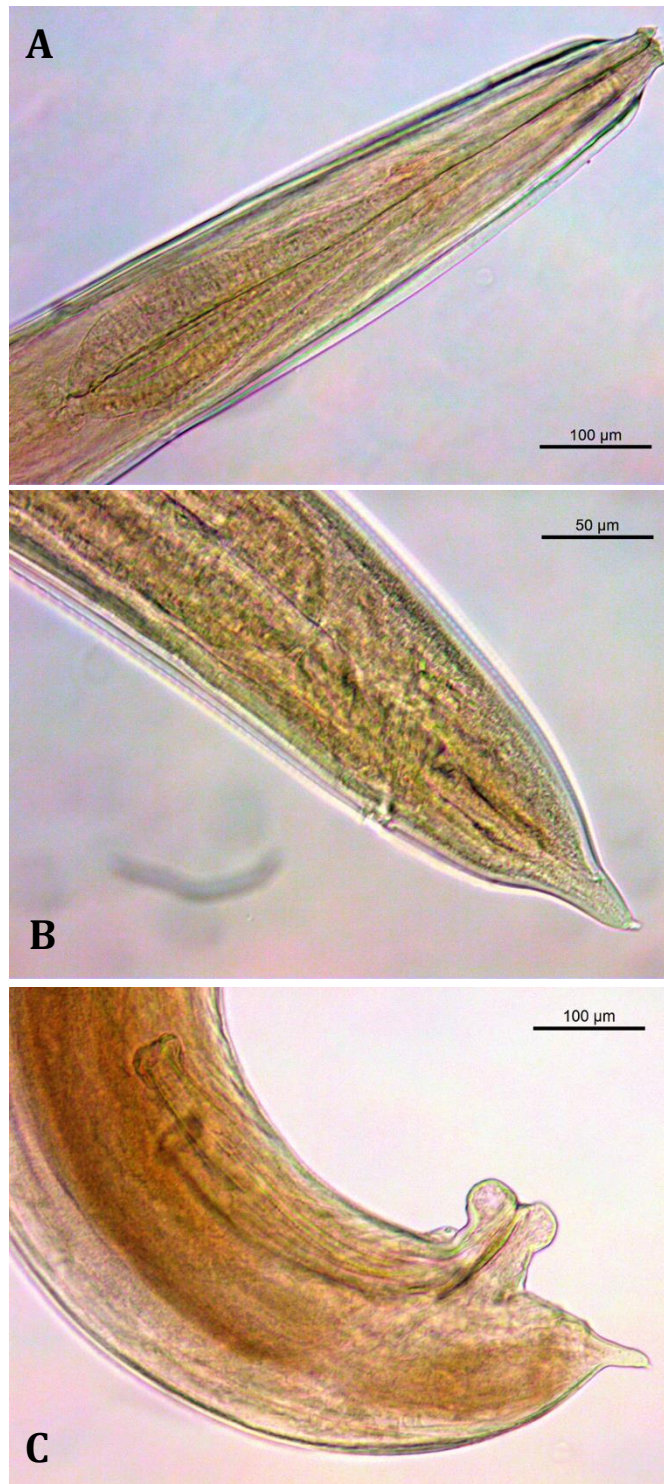


Figure 15. Nematode from the intestine of *Trachydoras paraguayensis* from the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo State, Brazil: (A–C) *Neoparaseuratum travassosi* – (A) anterior extremity; (B) female posterior extremity; (C) male posterior extremity.

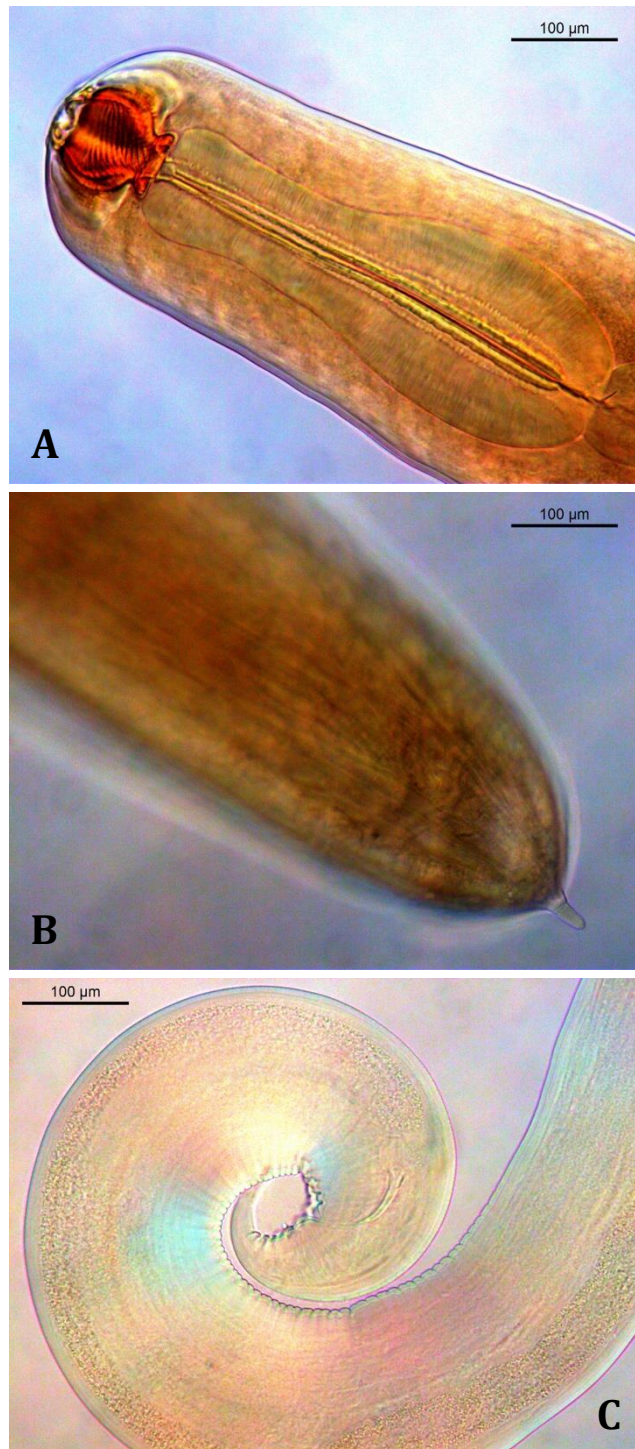


Figura 16. Nematode from the intestine of *Trachydoras paraguayensis* from the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo State, Brazil: (A–C) *Procamallanus* (*Spirocamallanus*) *inopinatus* – (A) anterior extremity; (B) female anterior extremity; (C) male posterior extremity.

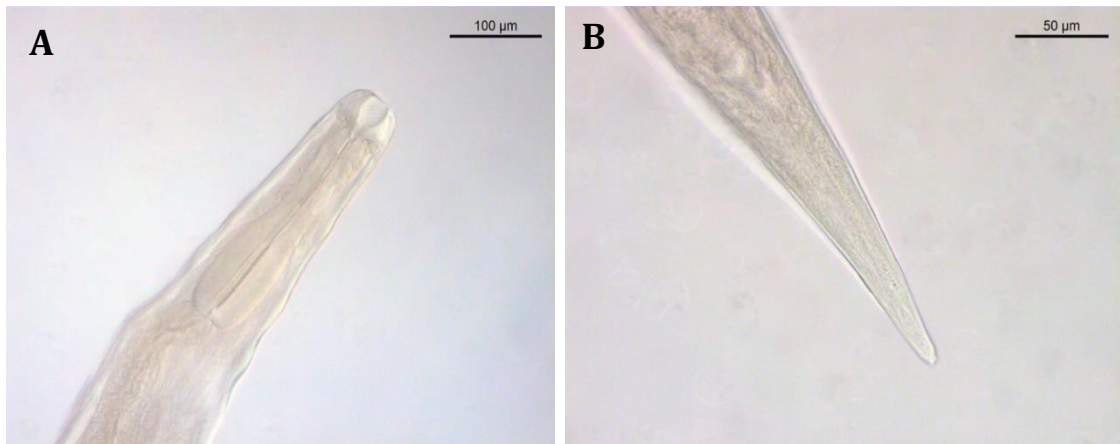


Figure 17. Nematode from the intestine of *Trachydoras paraguayensis* from the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo State, Brazil: (A) anterior extremity of 4th stage larva of *Procamallanus (Spirocamallanus) inopinatus*; (B) posterior extremity of 4th stage larva of *Procamallanus (Spirocamallanus) inopinatus*.

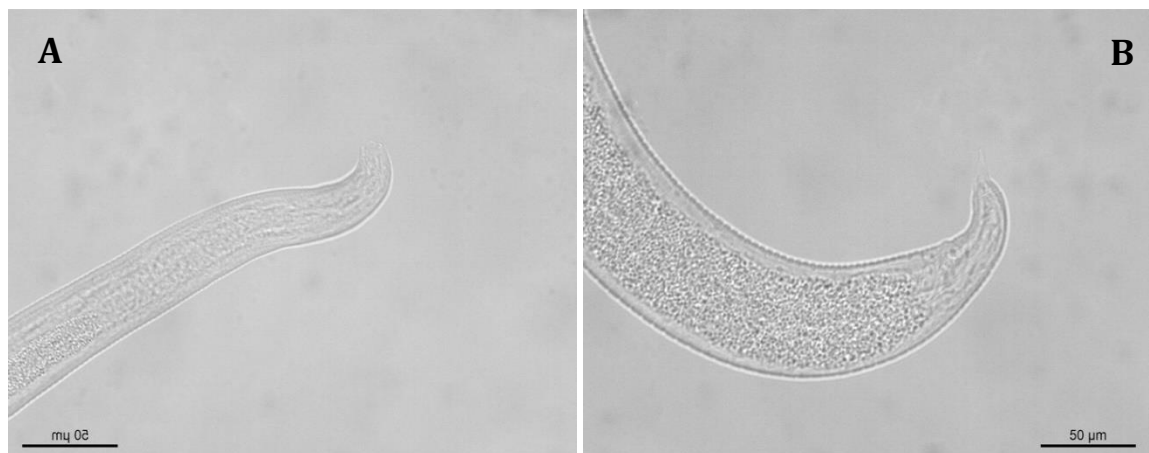


Figure 18. Nematode from the swim bladder of *Trachydoras paraguayensis* from the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo State, Brazil: (A–B) *Contracecum* sp. larva – (A) anterior extremity; (B) posterior extremity.

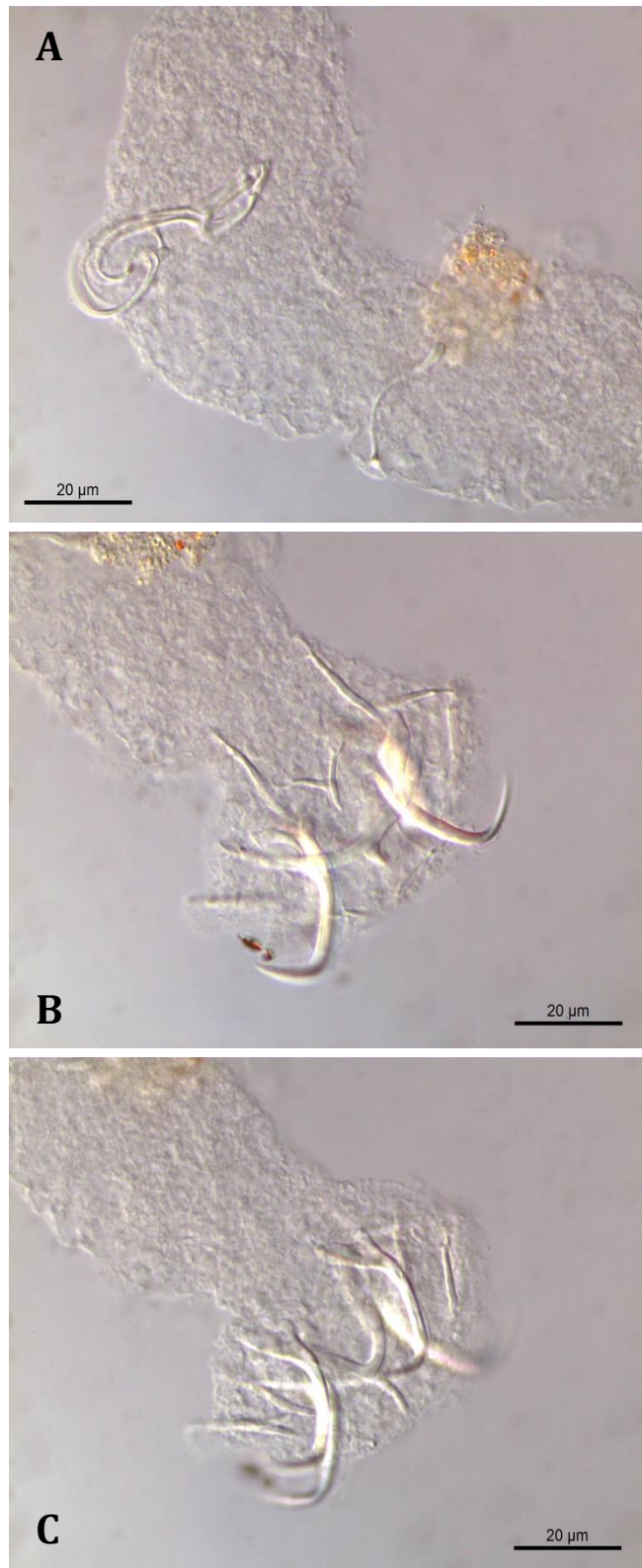


Figure 19. Monogenean from the gills of *Pimelodella avanhandavae* from the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo State, Brazil: (A–C) *Ameloblastella formatrium* – (A) copulatory complex and vagina of *Ameloblastella formatrium*; (B) haptor of *Ameloblastella formatrium* showing some hooks and ventral anchors; (C) haptor of *Ameloblastella formatrium* showing dorsal anchors and bar.

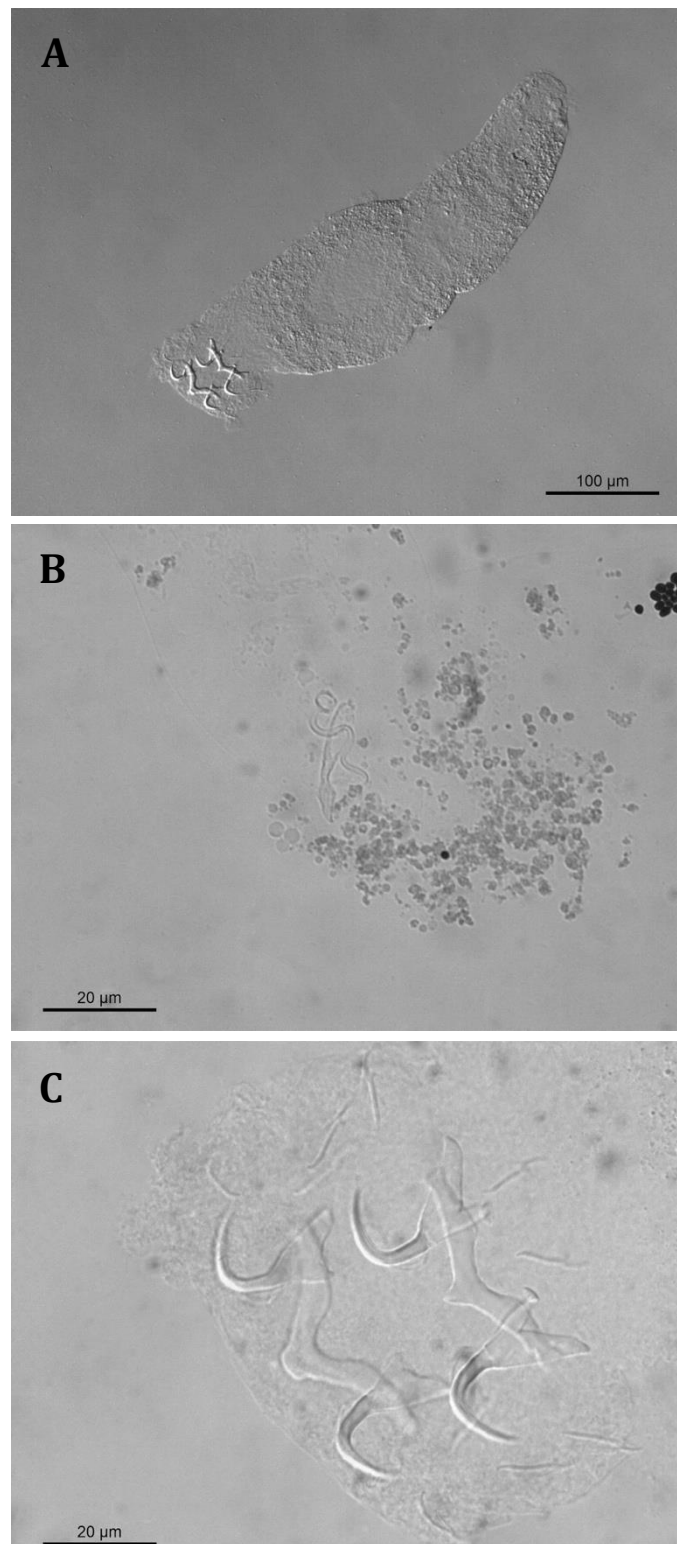


Figure 20. Monogenean from the gills of *Pimelodella avanhandavae* from the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo State, Brazil: (A) *Aphanoblastella chagresii* (B) copulatory complex of *Aphanoblastella chagresii*; (C) haptor of *Aphanoblastella chagresii*.

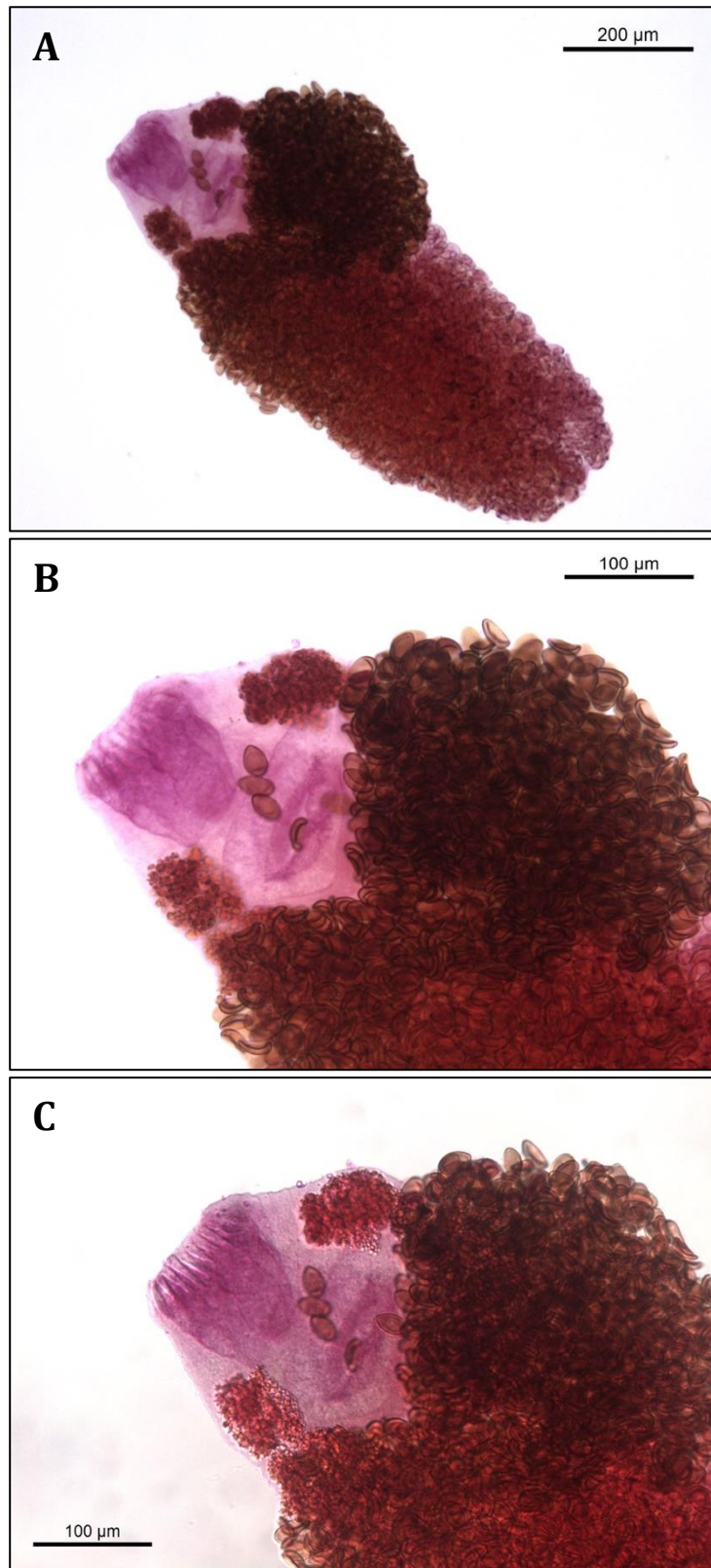


Figure 21. Digenean from *Pimelodella avanhandavae* from the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo State, Brazil: (A–C) Type 1 digenean from the intestine – (A) total view; (B) anterior extremity; (C) detail of spines.

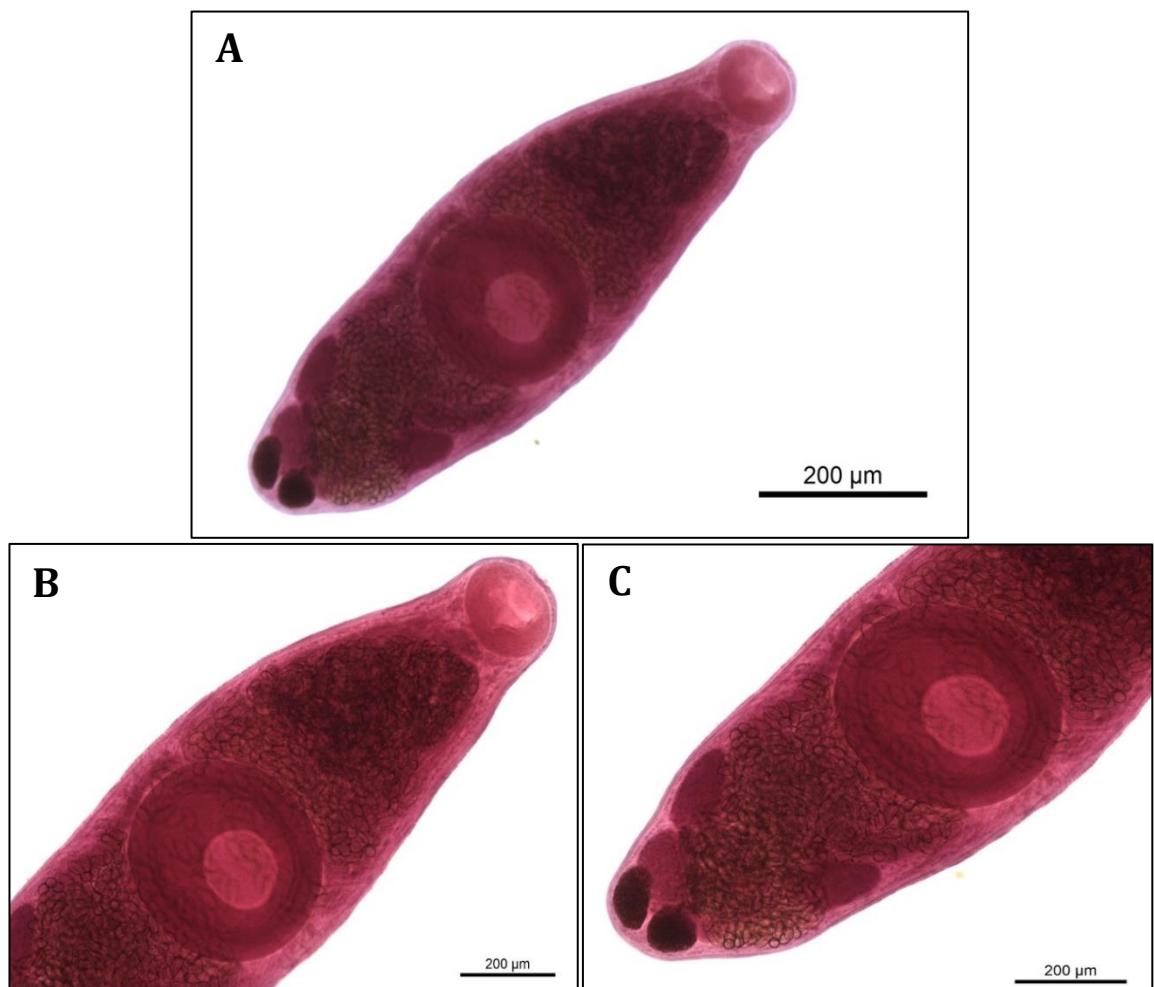


Figure 22. Digenean from *Pimelodella avanhandavae* from the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo State, Brazil: (A–C) *Genarchella* sp. from the stomach – (A) total view; (B) detail of ventral and oral suckers; (C) detail of the vitellaria concentrated in the posterior extremity.

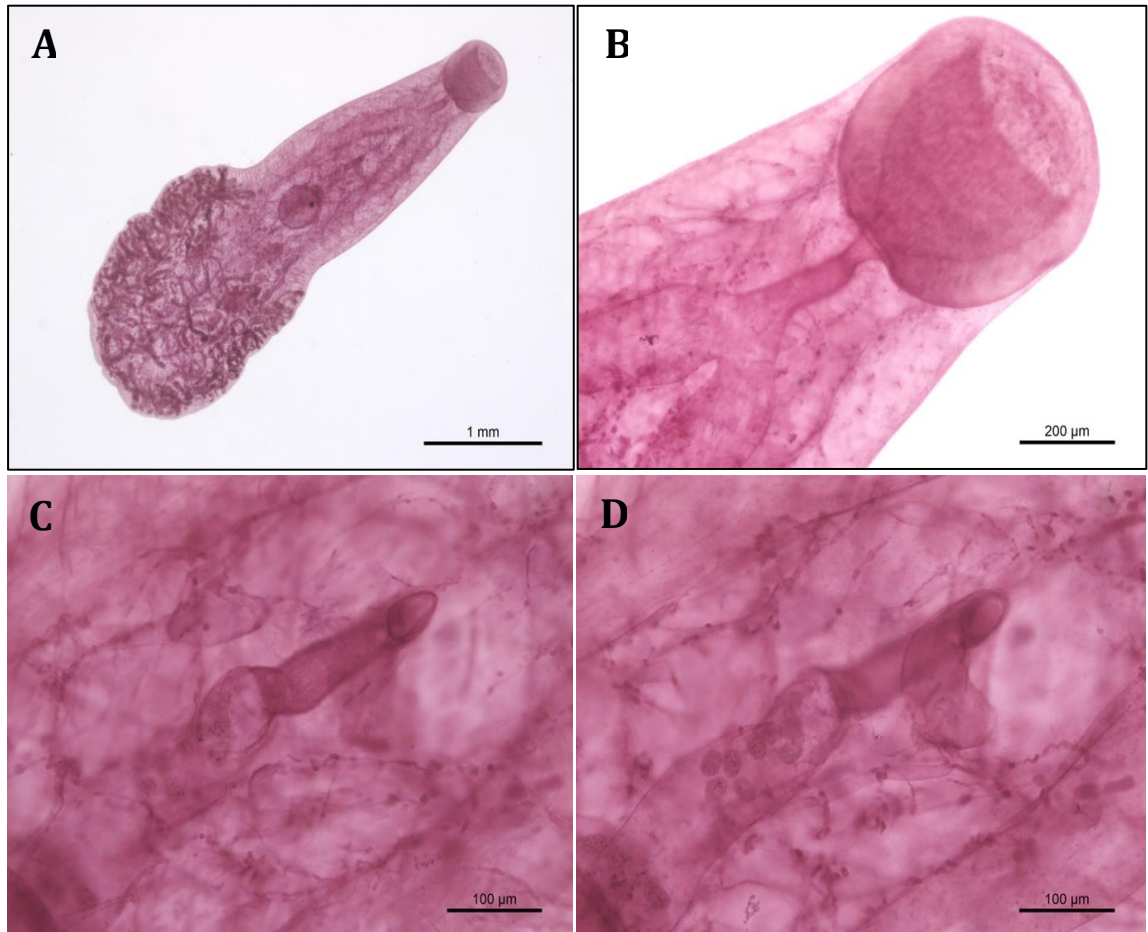


Figure 23. Digenean from *Pimelodella avanhandavae* from the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo State, Brazil: (A–D) *Phyllodistomum* sp. from the gonads – (A) total view; (B) anterior extremity; (C) vagina; (D) cirrus pouch.

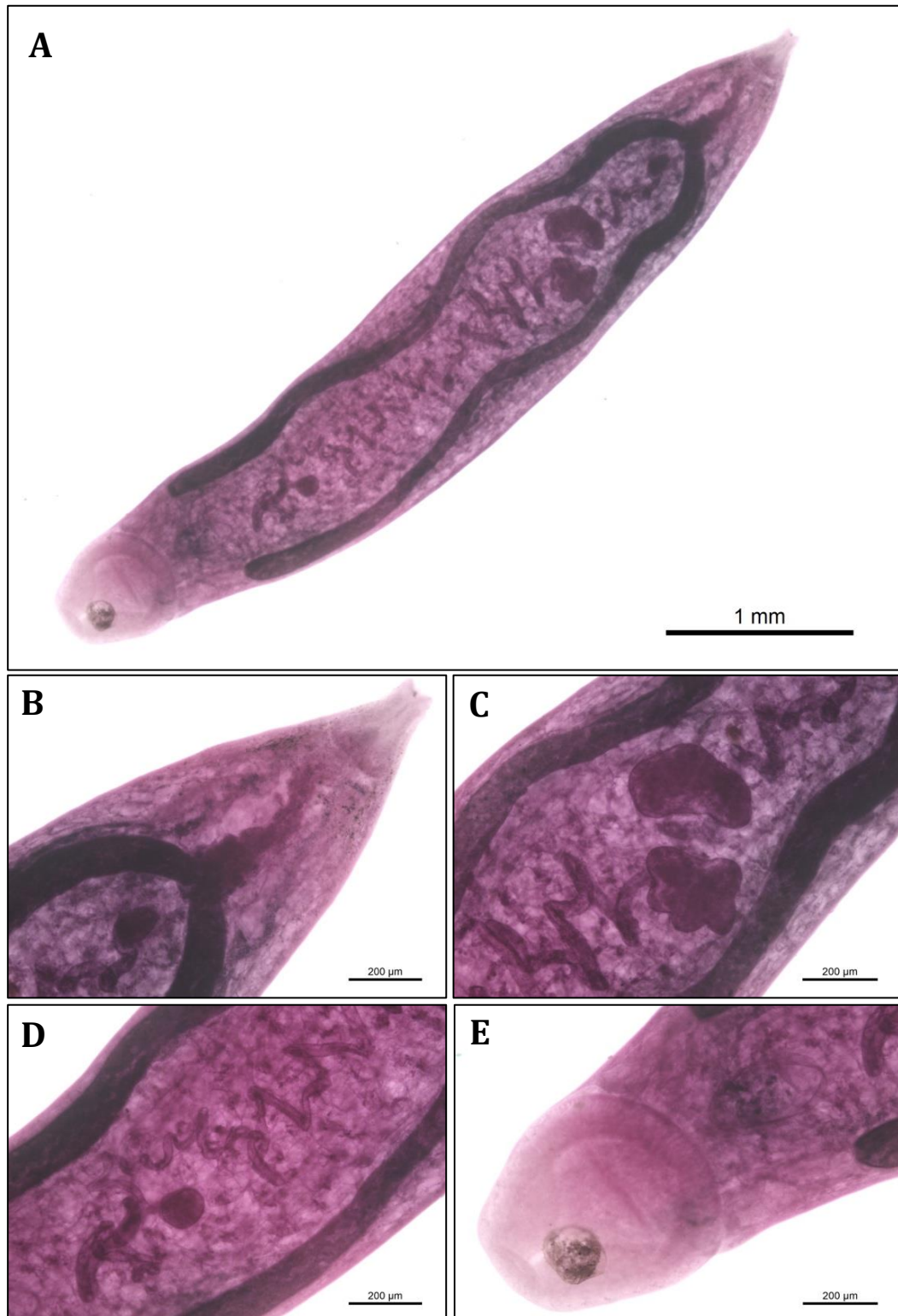


Figure 24. Digenean from *Pimelodella avanhandavae* from the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo State, Brazil: (A–E) *Dadaytrema* sp. from the intestine – (A) total view; (B) anterior extremity; (C) male reproductive organs; (D) female reproductive organs; (E) suckers.



Figure 25. Digeneans from *Pimelodella avanhandavae* from the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo State, Brazil: (A) *Austrodiplostomum* sp. metacercaria from the eyes; (B) *Austrodiplostomum* sp. metacercaria from the cavity.

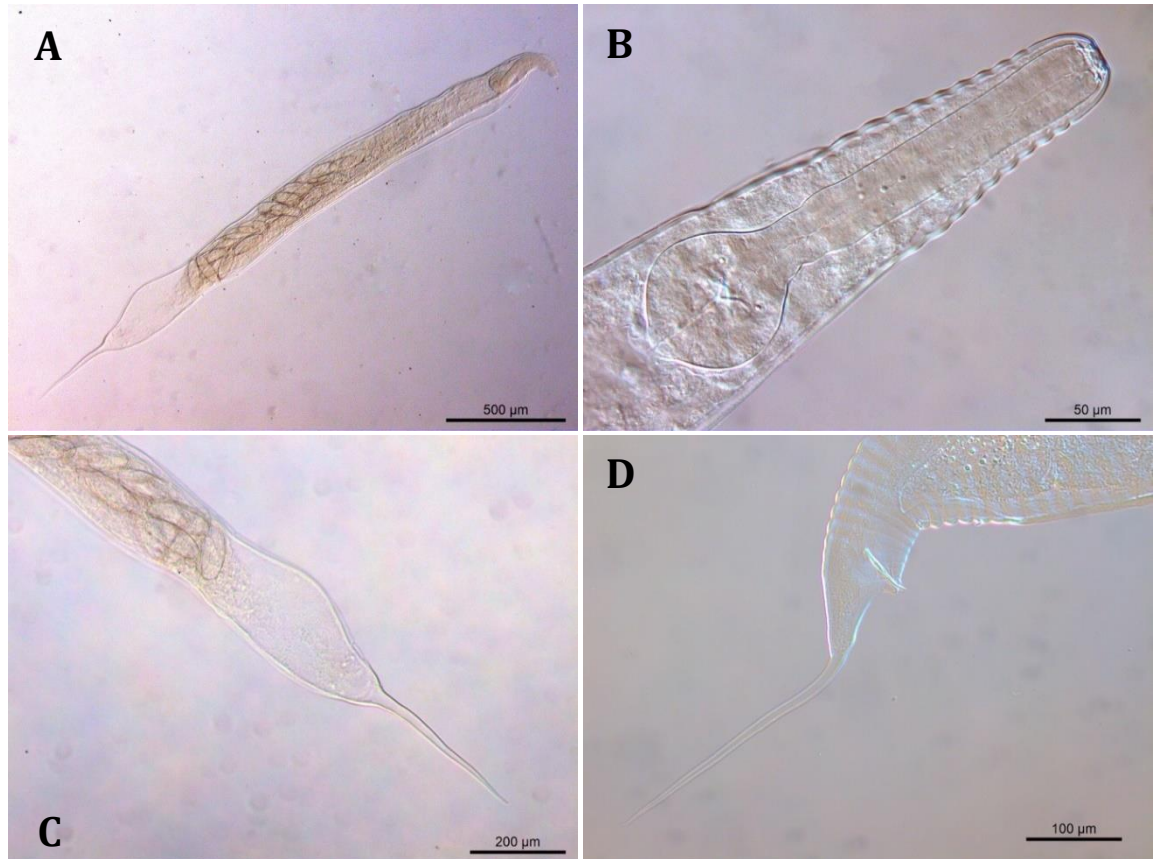


Figure 26. Nematode from the intestine *Pimelodella avanhandavae* from the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo State, Brazil: (A–D) *Brasilnema pimellodellae* – (A) total view; (B) anterior extremity; (C) female posterior extremity; (D) male posterior extremity.

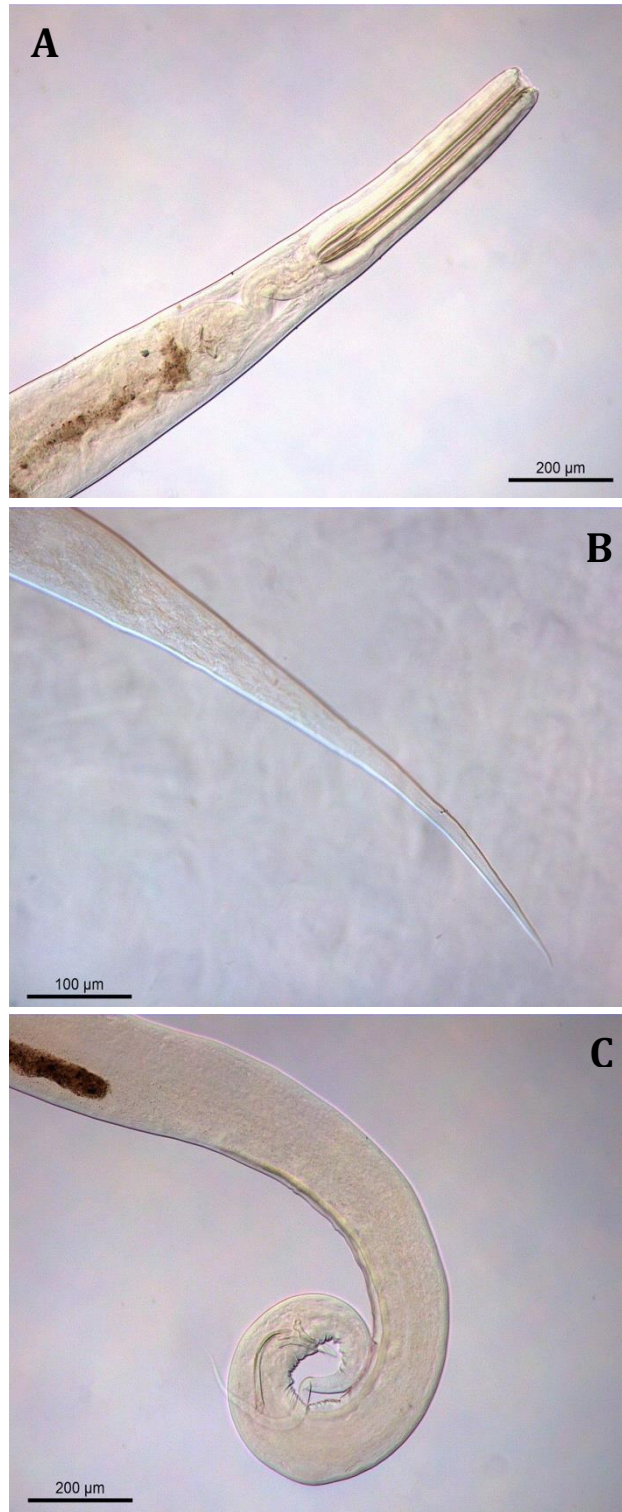


Figure 27. Nematode from the intestine of *Pimelodella avanhandavae* from the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo State, Brazil: (A–C) *Rondonia rondoni* – (A) anterior extremity; (B) female posterior extremity; (C) male posterior extremity.

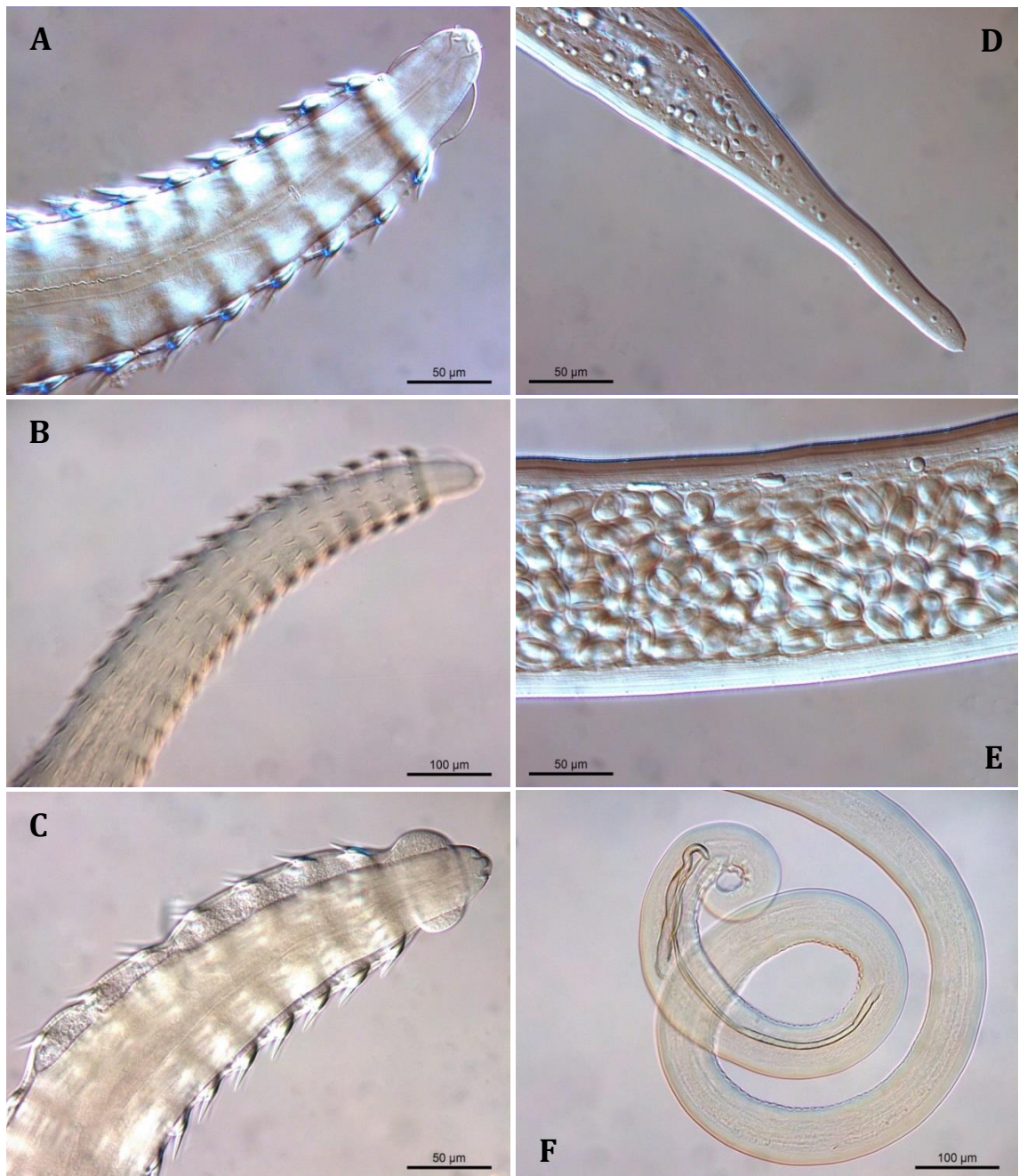


Figure 28. Nematode from the intestine of *Pimelodella avanhandavae*, from the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo State, Brazil: (A–F) *Spinitectus* sp. – (A–C) anterior extremity with details of cuticular spines; (D) posterior extremity of female; (E) female with eggs; (F) male posterior extremity spirally coiled.

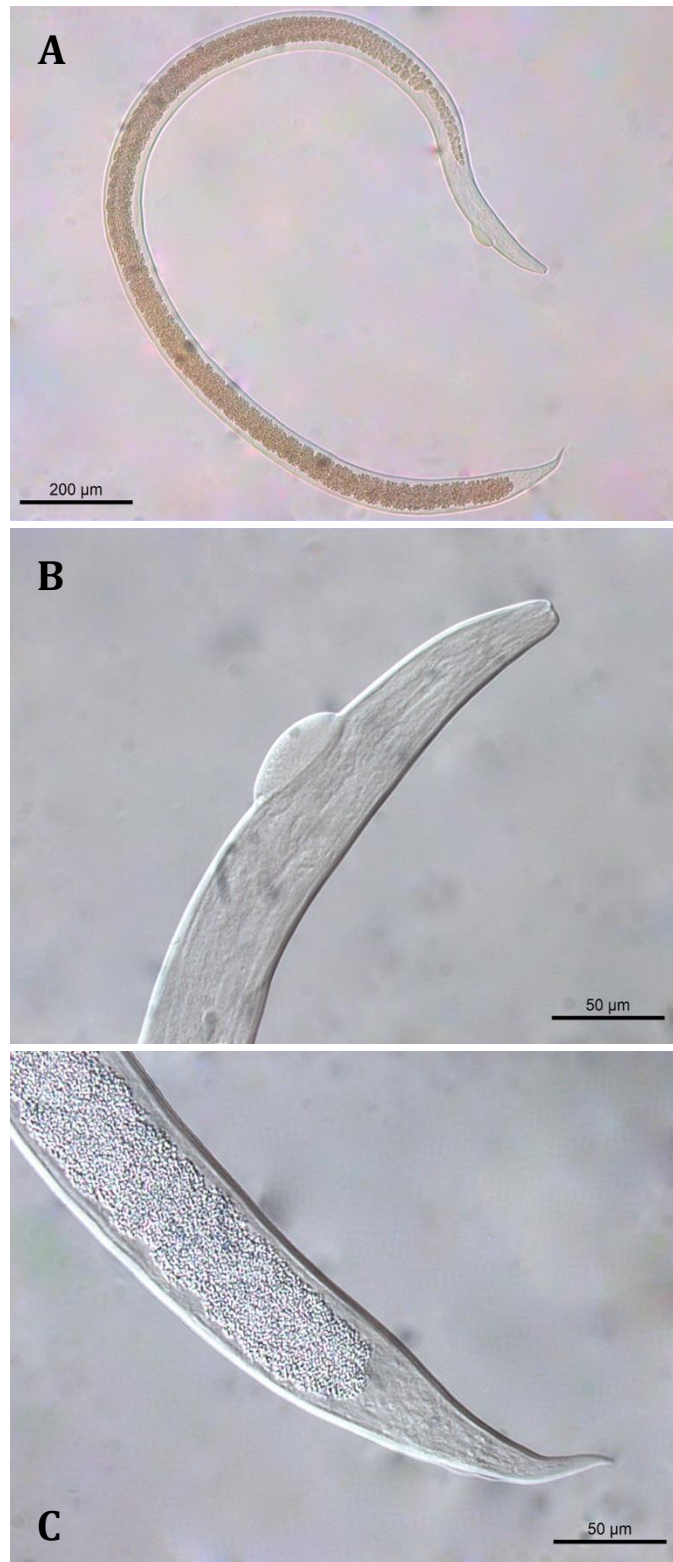


Figure 29. Nematode from *Pimelodella avanhandavae* from the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo State, Brazil: (A–C) *Contracecum* sp. larva from the swim bladder – (A) total view; (B) anterior extremity; (C) posterior extremity.

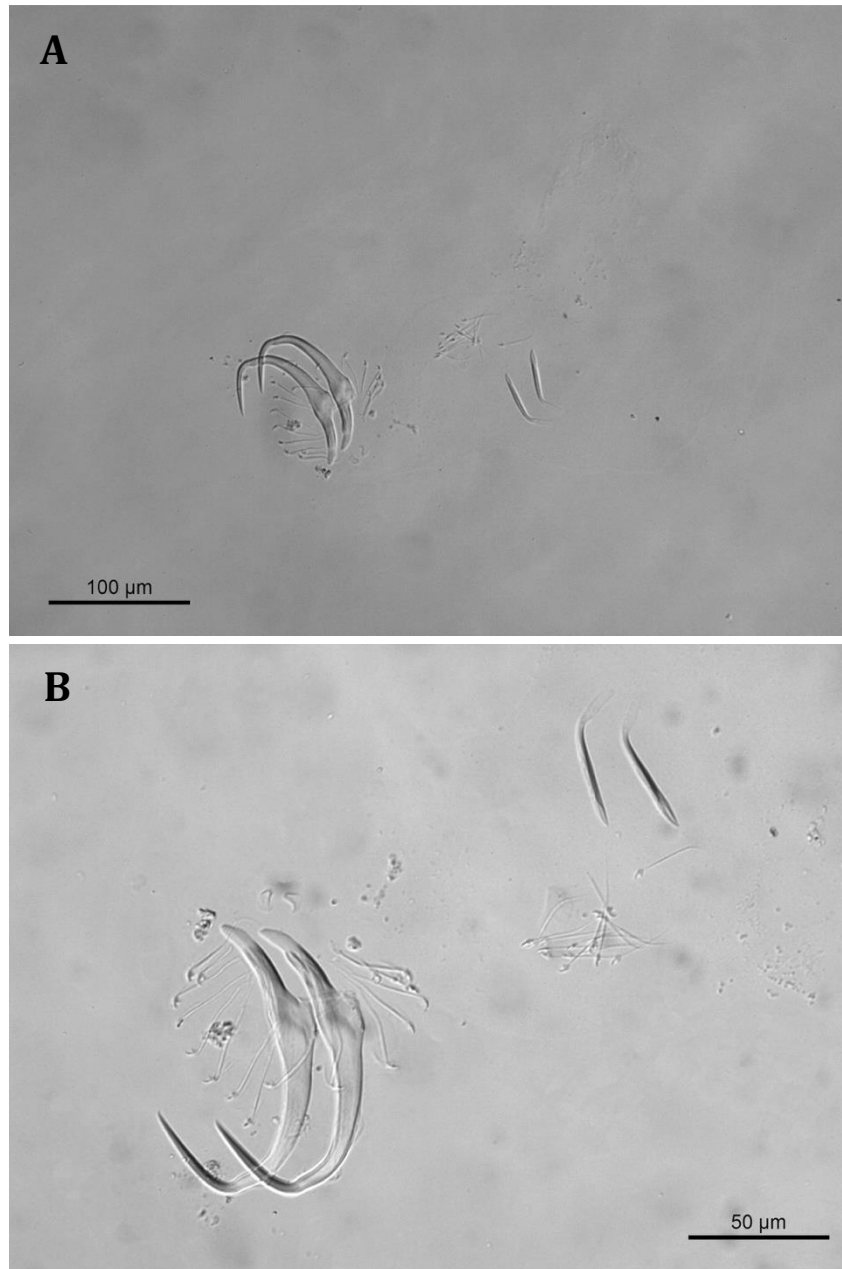


Figure 30. Monogenean from the gills of *Loricariichthys platymetopon*, from the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo State, Brazil: (A–B) Gyrodactylidae gen. sp.

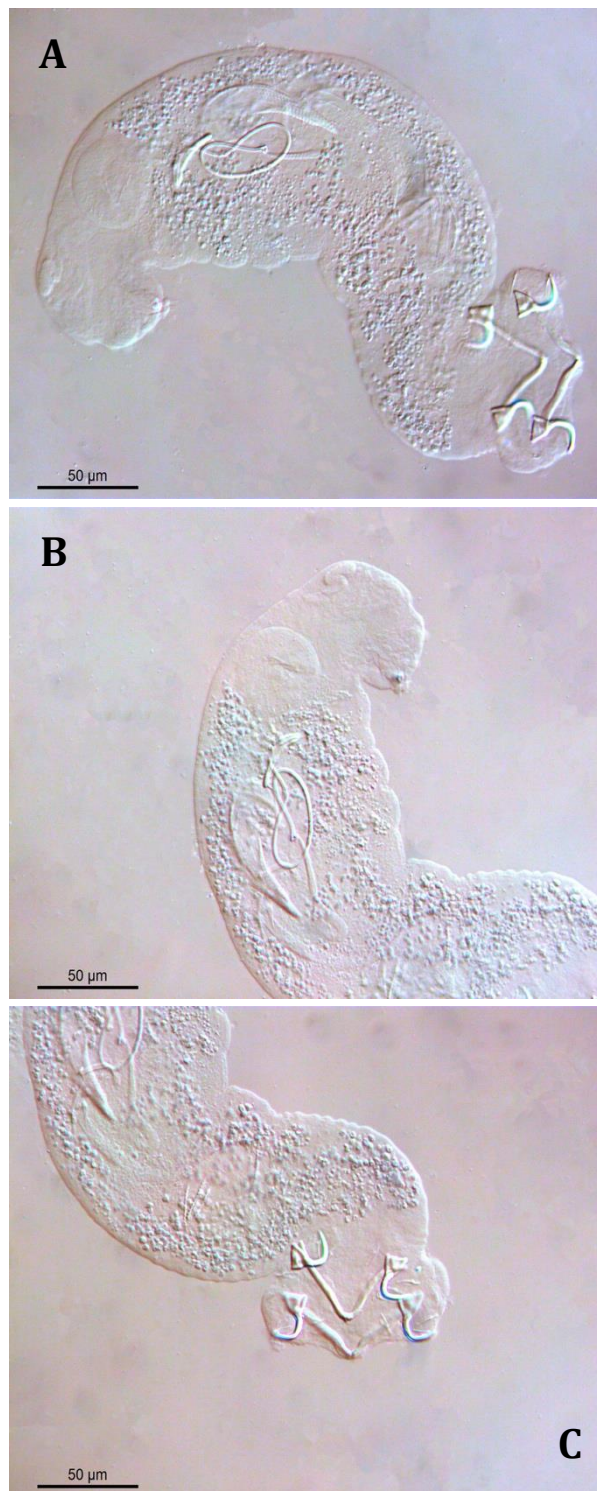


Figure 31. Monogenean from the gills of *Loricariichthys platymetopon* from the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo State, Brazil: (A) *Demidospermus paranaensis*; (B) copulatory complex of *Demidospermus paranaensis*; (C) haptor of *Demidospermus paranaensis*.

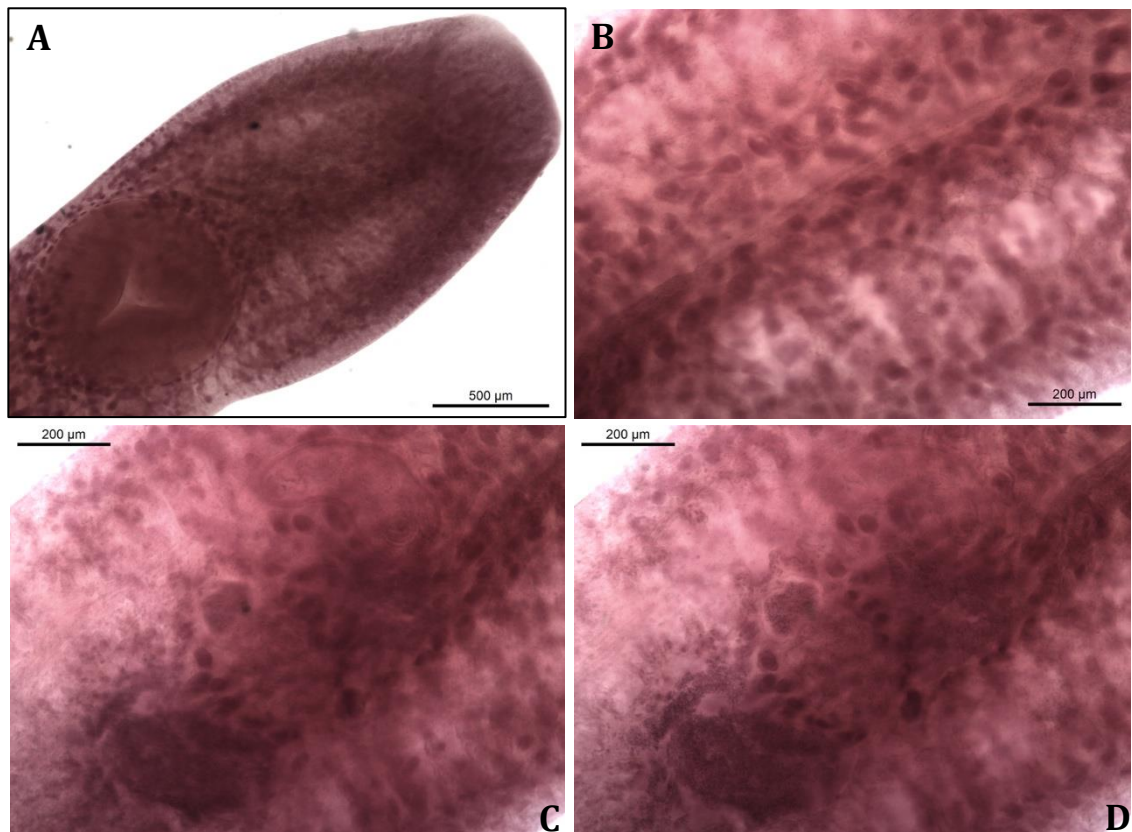


Figure 32. Digenean from *Loricariichthys platymetopon* from the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo State, Brazil: (A–D) metacercaria of *Clinostomum* sp. found in the cavity and flesh – (A) oral and ventral suckers; (B) uterus; (C) cirrus pouch; (D) testes and ovary.



Figure 33. Digenean from *Loricariichthys platymetopon* from the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo State, Brazil: (A) *Austrodiplostomum* sp. metacercaria found in the eyes.

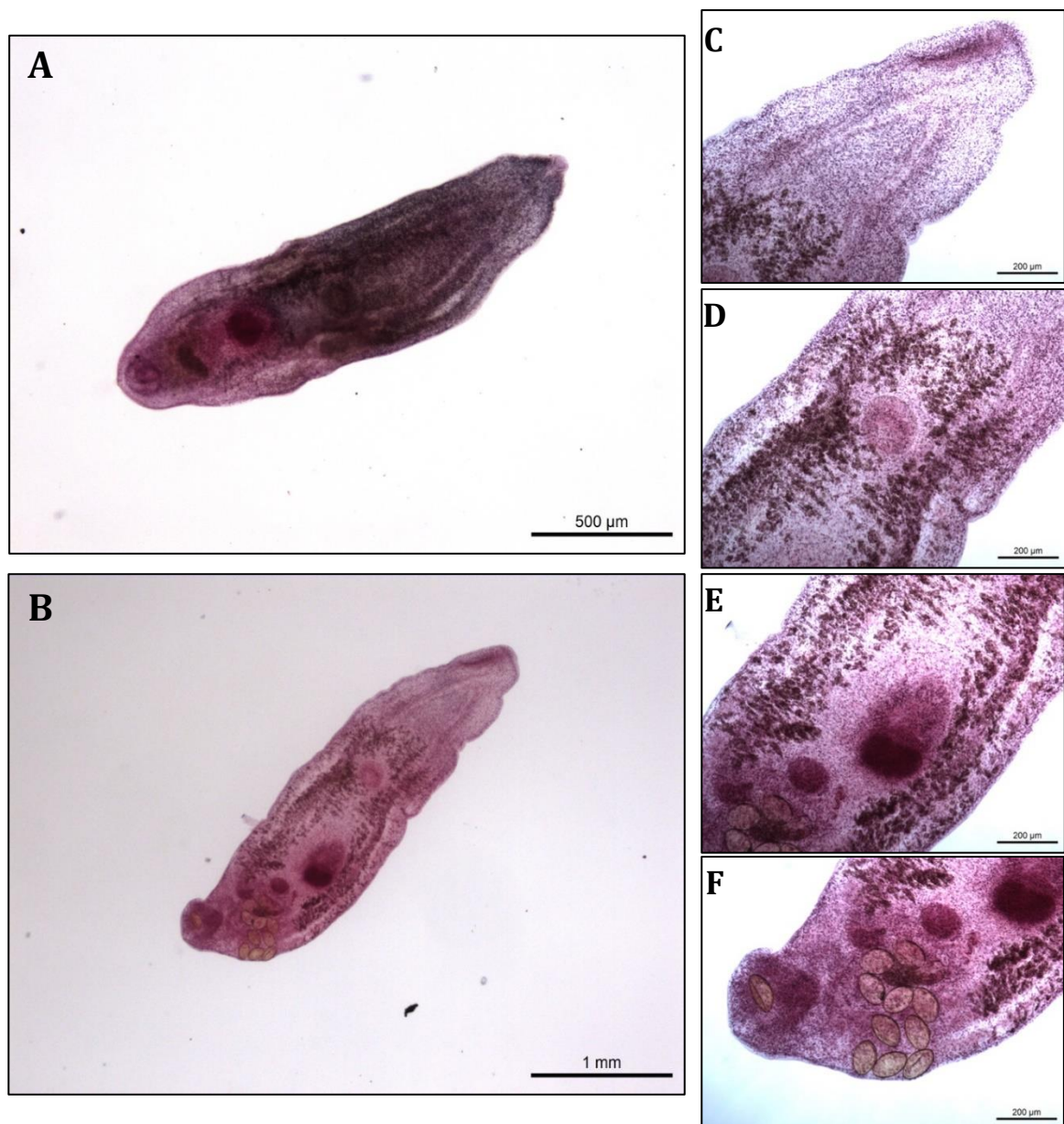


Figure 34. Digeneans from *Loricariichthys platymetopon* from the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo State, Brazil: (A–F) Type 1 metacercarie of Diplostomidae found in the cavity – (A and B) total view; (C) anterior extremity; (D) ventral sucker; (E) copulatory organs; (F) posterior extremity with detail of the eggs.

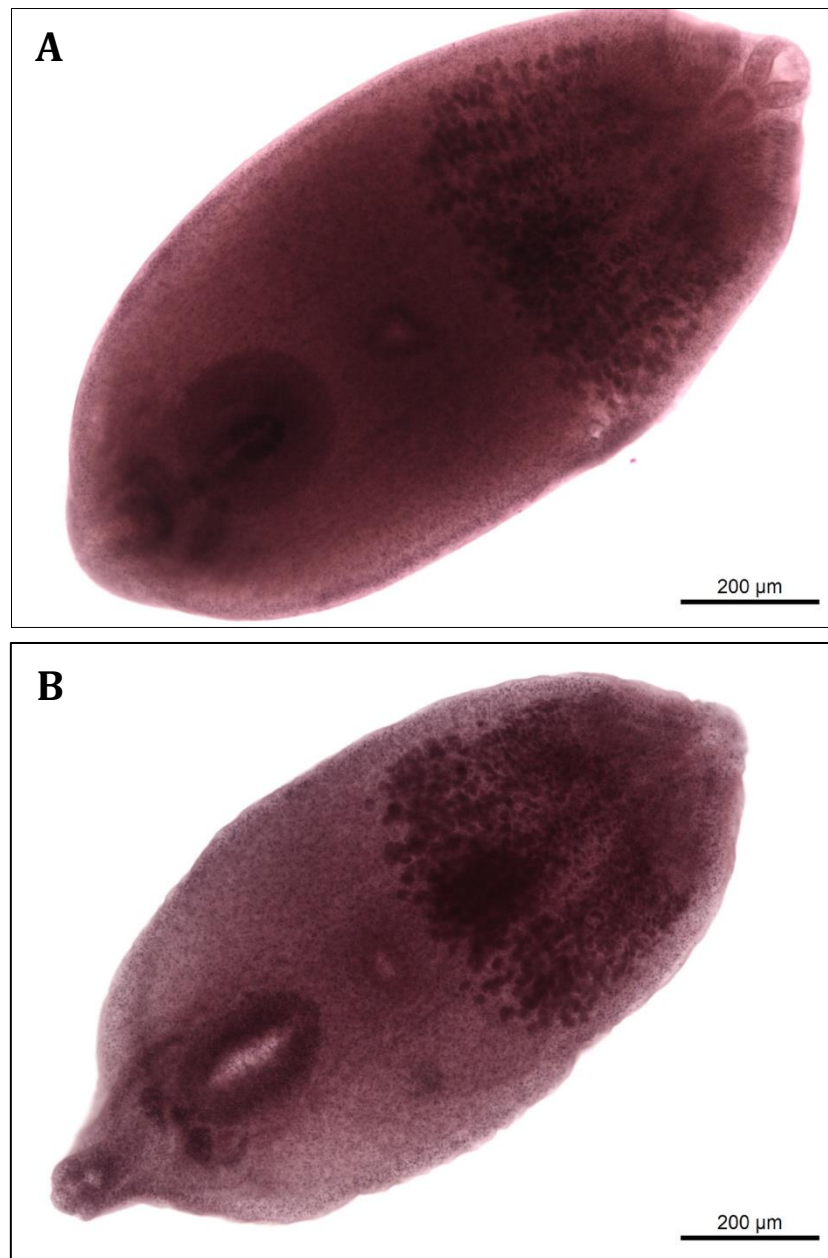


Figure 35. Digenean from *Loricariichthys platymetopon* from the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo State, Brazil: (A) Type 4 metacercaria of Diplostomidae found on the liver; (B) Type 5 metacercaria of Diplostomidae found in the cavity.

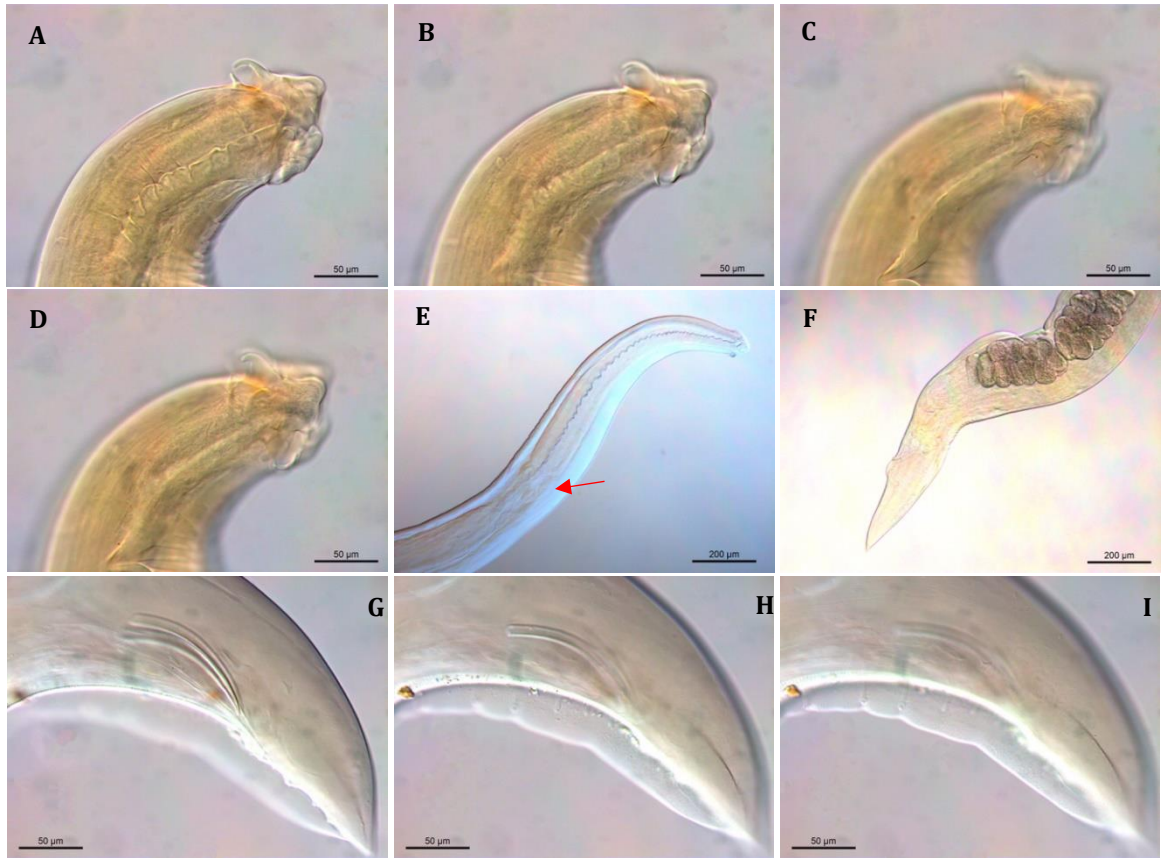


Figure 36. Nematode from the intestine of *Loricariichthys platymetopon* from the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo State, Brazil: (A–I) *Raphidascaris (Sprentascaris) mahnerti* – (A–D) anterior extremity of *Raphidascaris (Sprentascaris) mahnerti* with details of the cuticular elevations at level of base of lips; (E) esôfago de *Raphidascaris (Sprentascaris) mahnerti* with detail of the posteriorly directed ventricular appendix; (F) female posterior extremity; (G–I) male posterior extremity with details of caudal alae and papillae.

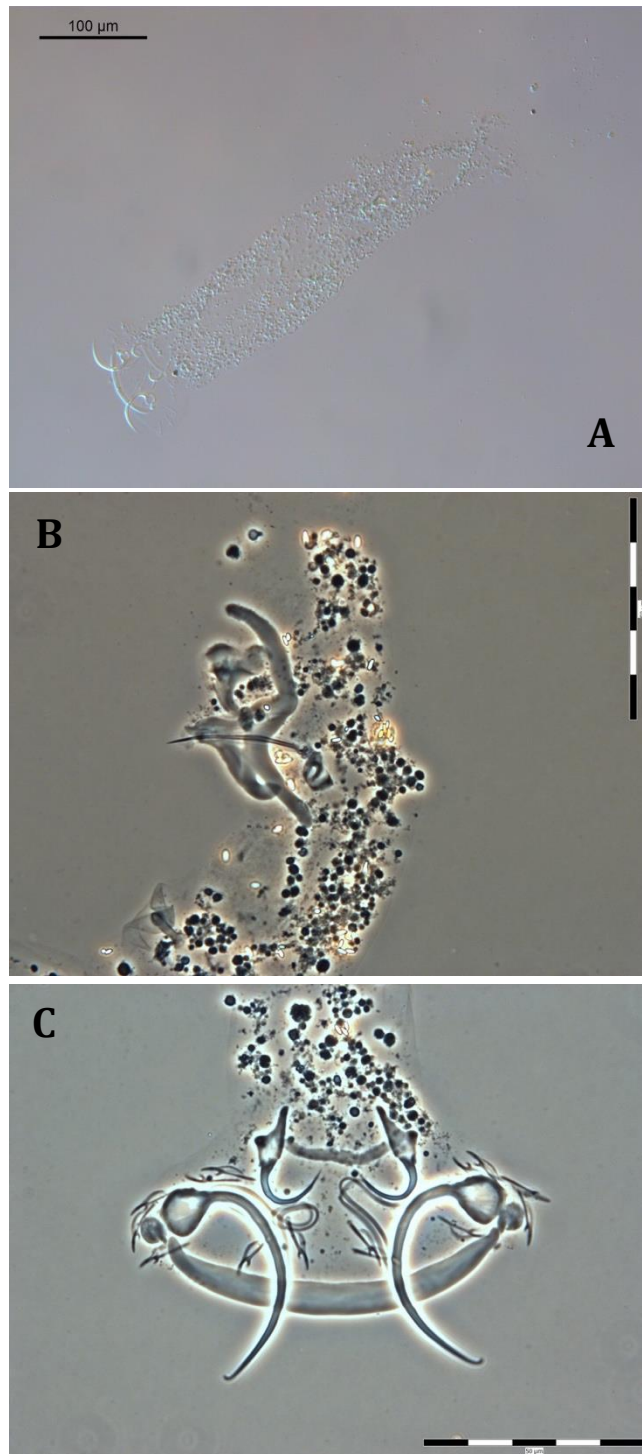


Figure 37. Monogenean from the gills of *Pterygoplichthys ambrosettii* from the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo State, Brazil: (A) *Heteropriapulus heterotylus*; (B) copulatory complex of *Heteropriapulus heterotylus*; (C) haptor of *Heteropriapulus heterotylus*.



Figure 38. Monogenean from the gills of *Pterygoplichthys ambrosettii* from the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo State, Brazil: (A) *Heteropriapulus simplex*; (B) copulatory complex of *Heteropriapulus simplex*; (C) haptor of *Heteropriapulus simplex*.

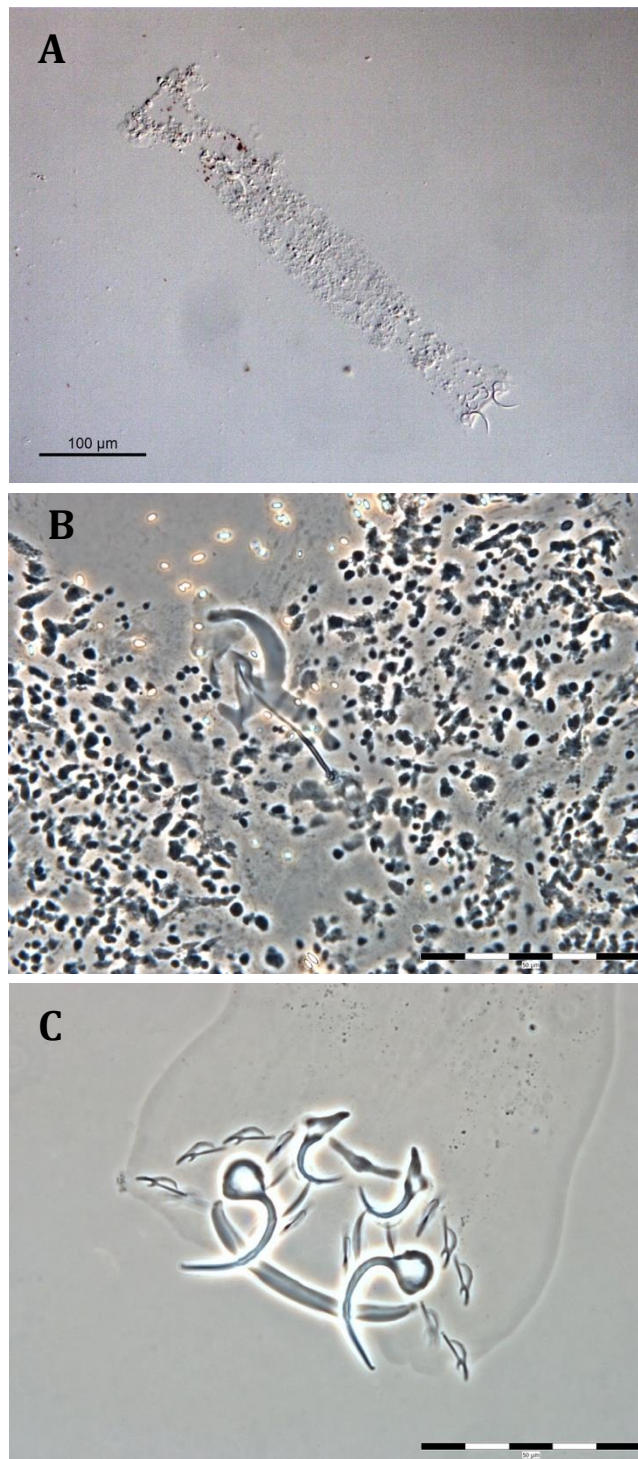


Figure 39. Monogenean from the gills of *Pterygoplichthys ambrosettii* from the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo State, Brazil: (A) *Heteropriapulus* sp. 1; (B) copulatory complex of *Heteropriapulus* sp. 1; (C) haptor of *Heteropriapulus* sp. 1.

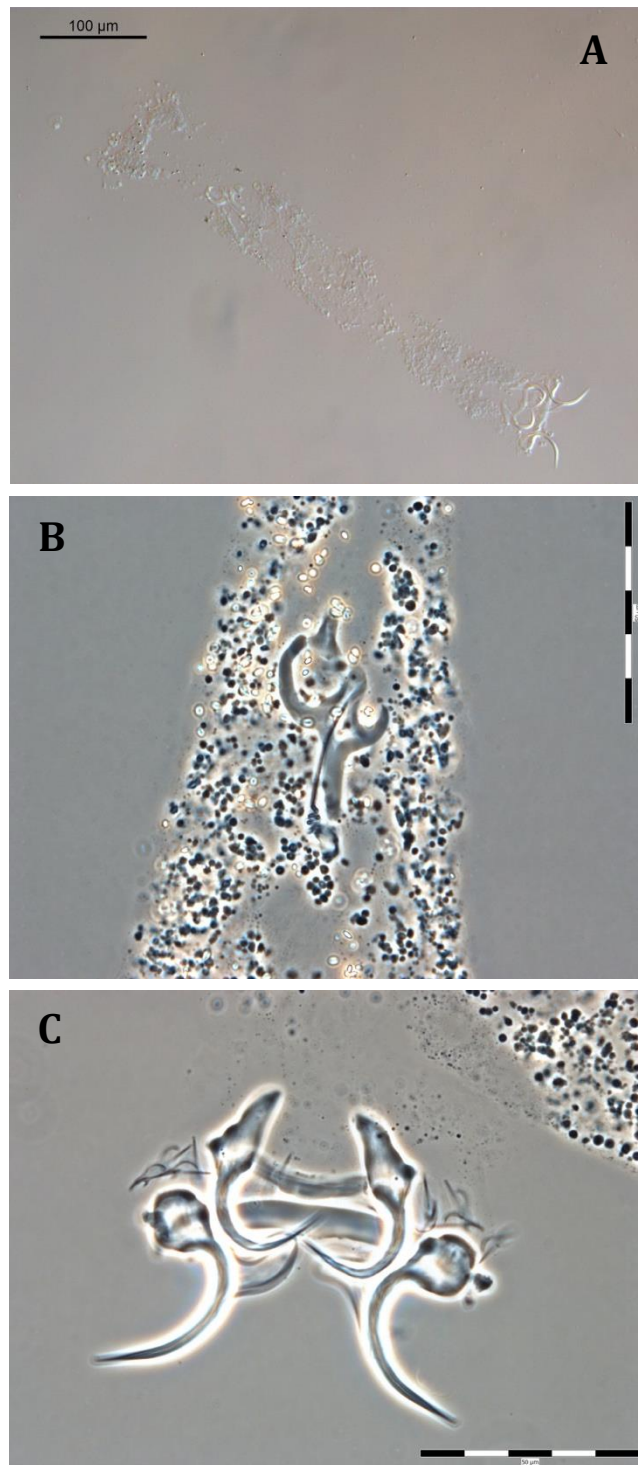


Figure 40. Monogenean from the gills of *Pterygoplichthys ambrosettii* from the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo State, Brazil: (A) *Heteropriapulus* sp. 2; (B) copulatory complex of *Heteropriapulus* sp. 2; (C) haptor of *Heteropriapulus* sp. 2.

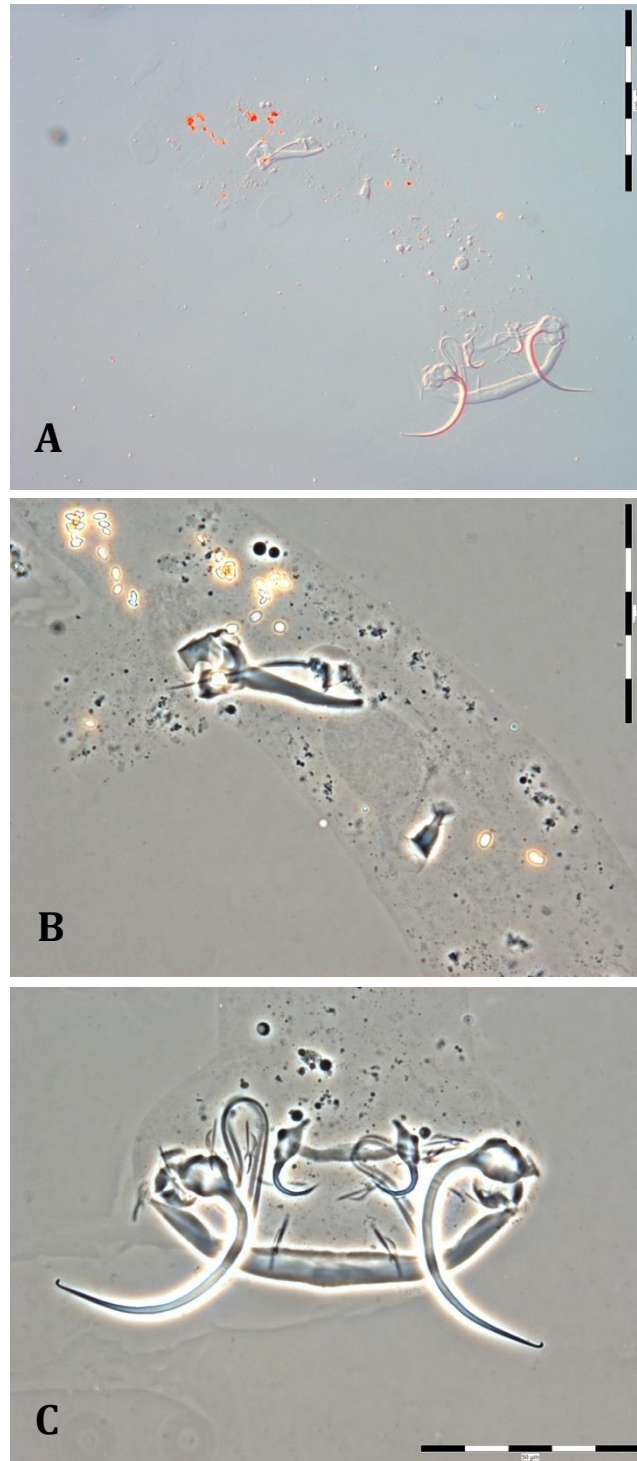


Figure 41. Monogenean from the gills of *Pterygoplichthys ambrosettii* from the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo State, Brazil: (A) *Heteropriapulus* sp. 4; (B) copulatory complex of *Heteropriapulus* sp. 4; (C) haptor of *Heteropriapulus* sp. 4.

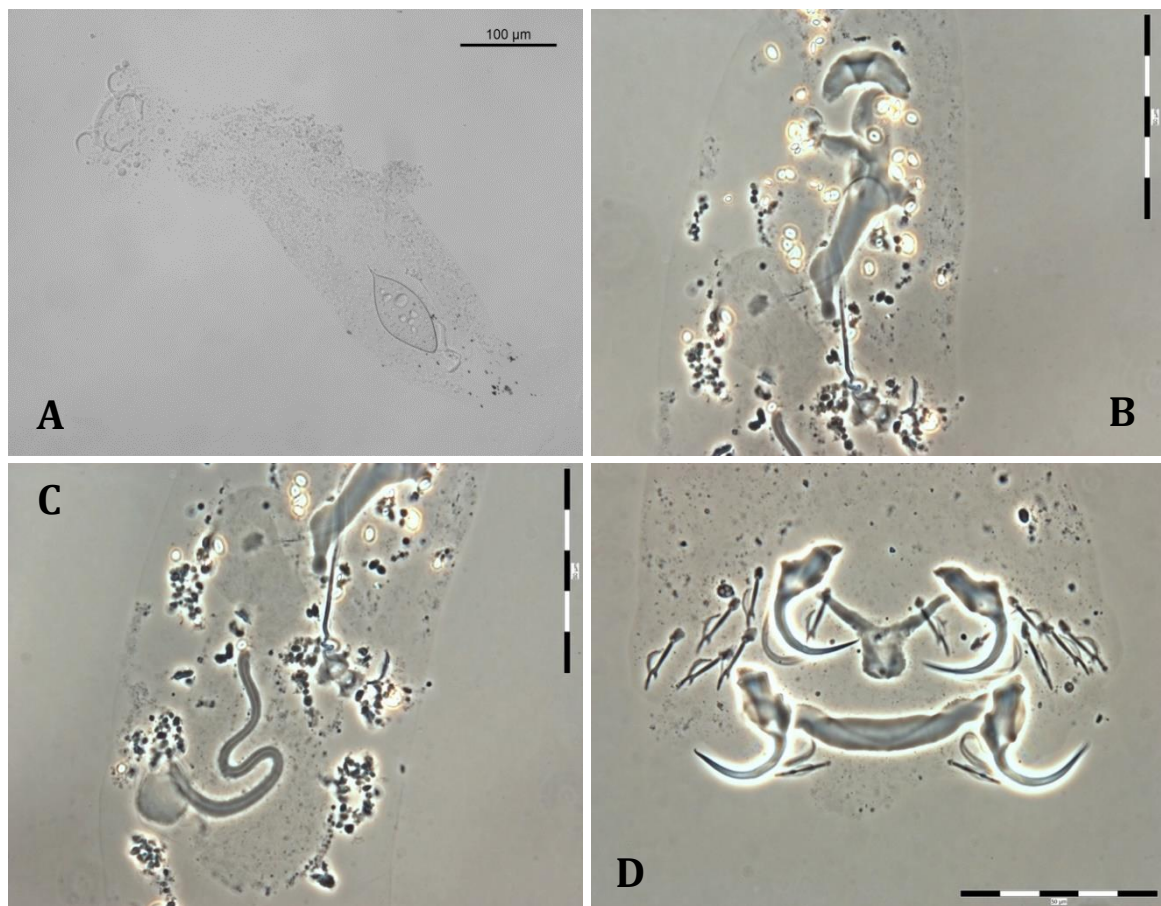


Figure 42. Monogenean from the gills of *Pterygoplichthys ambrosettii* from the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo State, Brazil: (A) *Heteropriapulus* sp. 5; (B) copulatory complex of *Heteropriapulus* sp. 5 with egg; (C) vagina of *Heteropriapulus* sp. 5; (D) haptor of *Heteropriapulus* sp. 5.

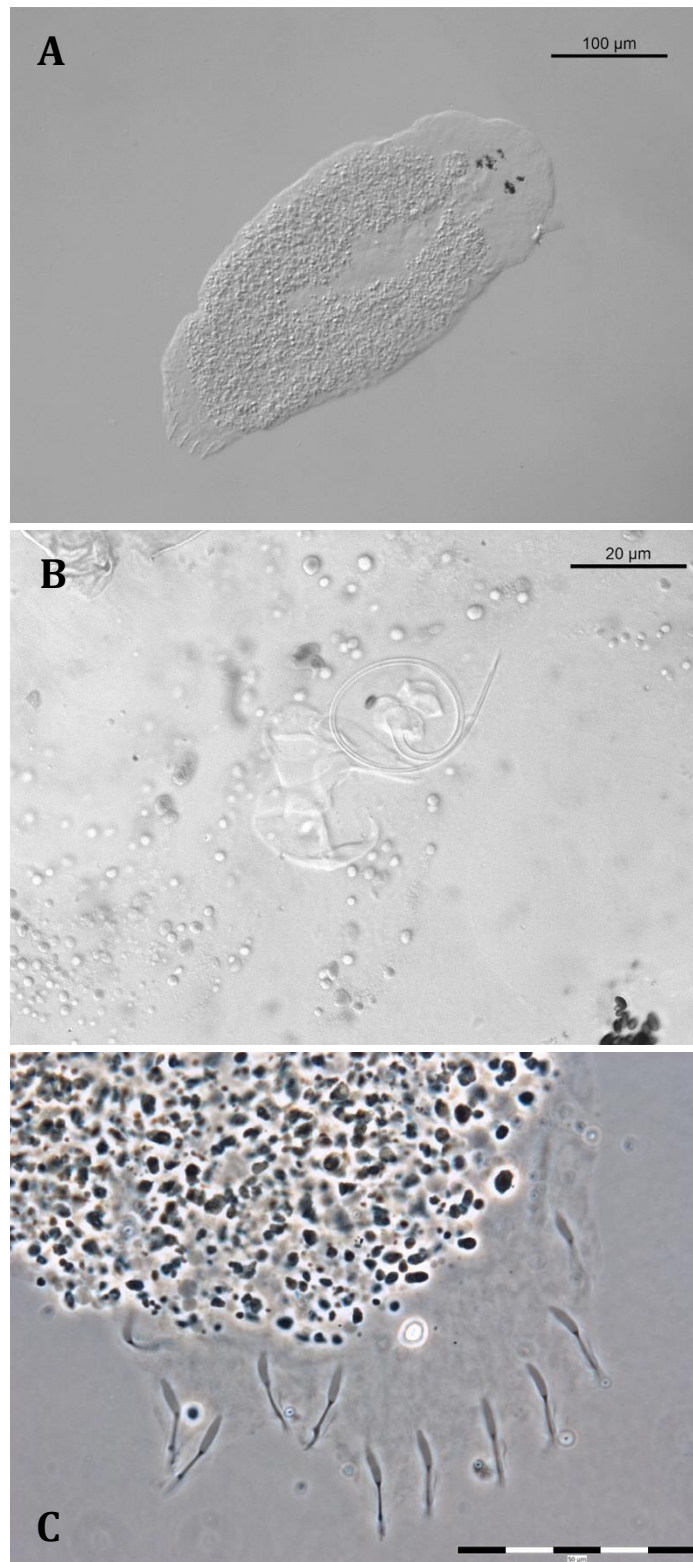


Figure 43. Monogenean from the nasal cavity of *Pterygoplichthys ambrosettii* from the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo State, Brazil: (A) *Telethecium nasalis*; (B) Copulatory complex of *Telethecium nasalis*; (C) haptor of *Telethecium nasalis*.

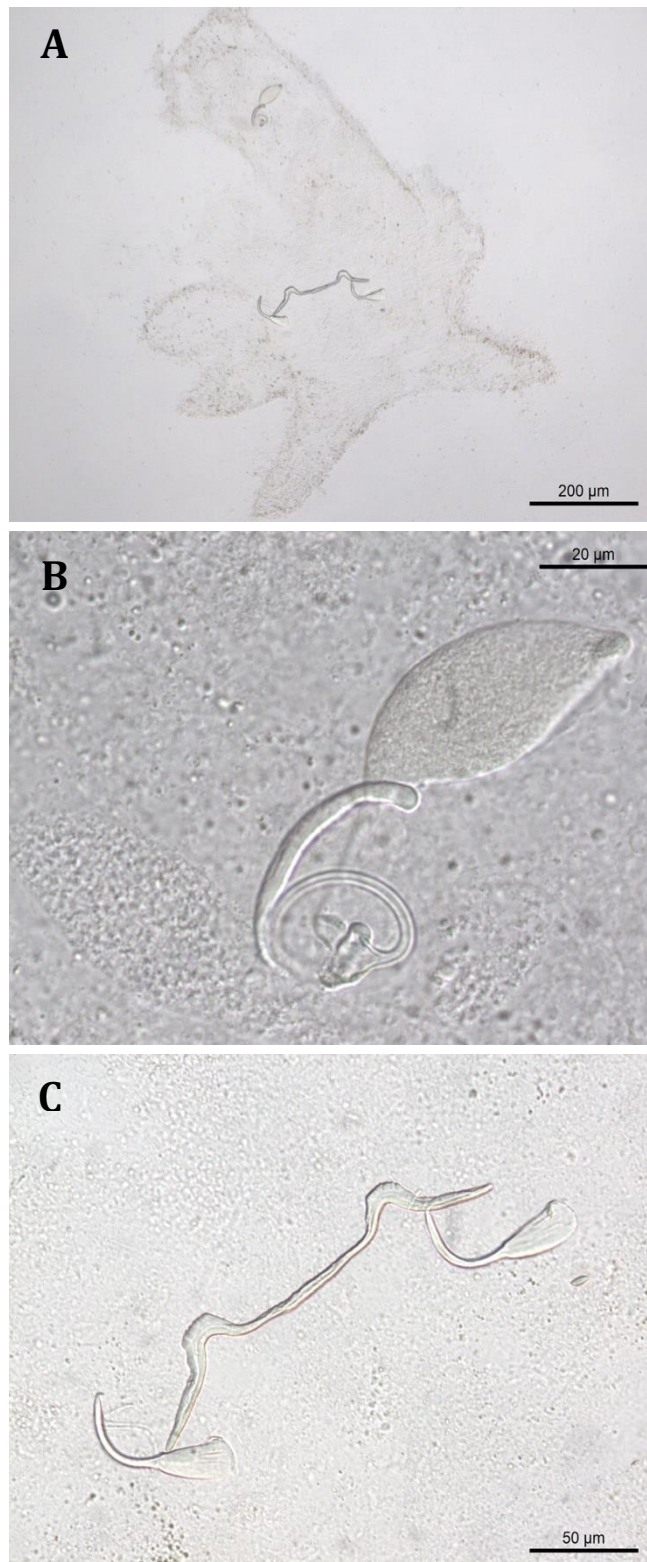


Figure 44. Monogenean from the gills of *Pterygoplichthys ambrosettii* from the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo State, Brazil: (A) *Trinigyryus* sp.; (B) Copulatory complex of *Trinigyryus* sp.; (C) haptor of *Trinigyryus* sp.

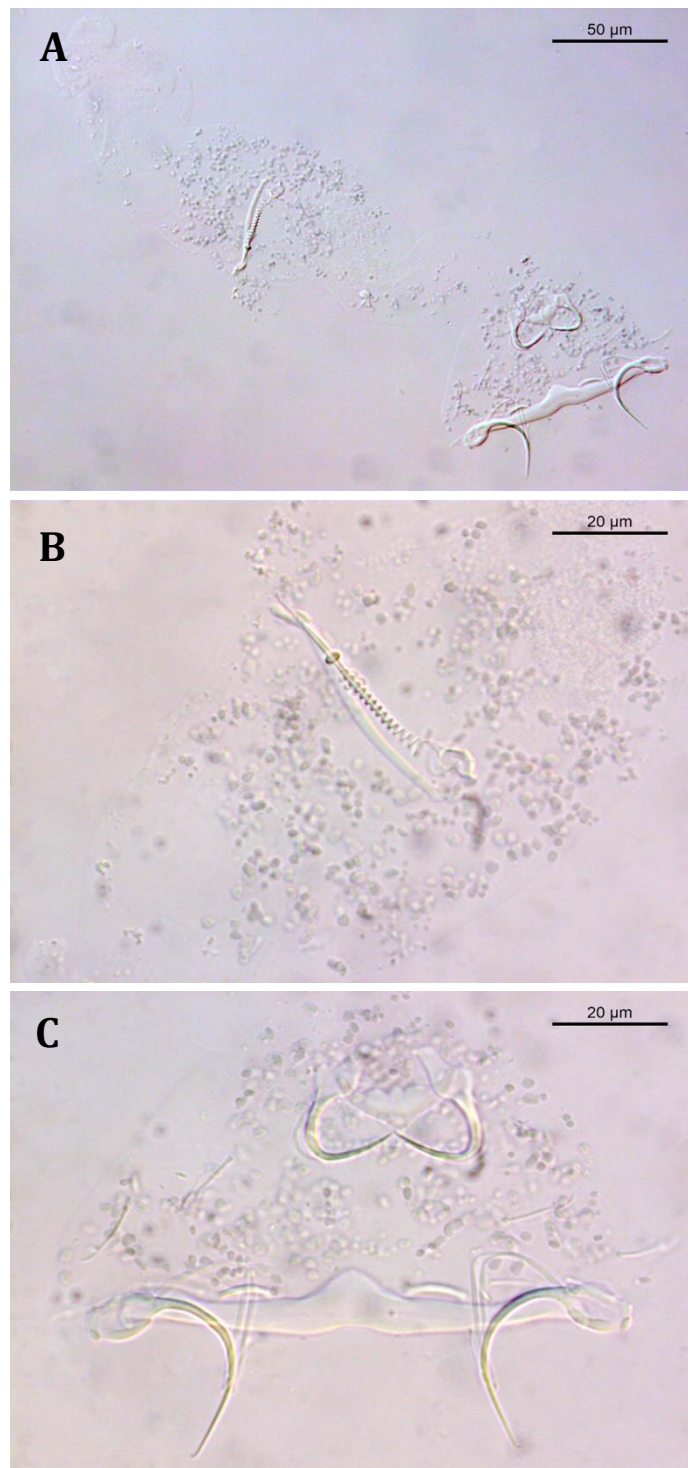


Figure 45. Monogenean from the gills of *Pterygoplichthys ambrosettii* from the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo State, Brazil: (A) *Unilatus brittani*; (B) copulatory complex of *Unilatus brittani*; (C) haptor of *Unilatus brittani*.

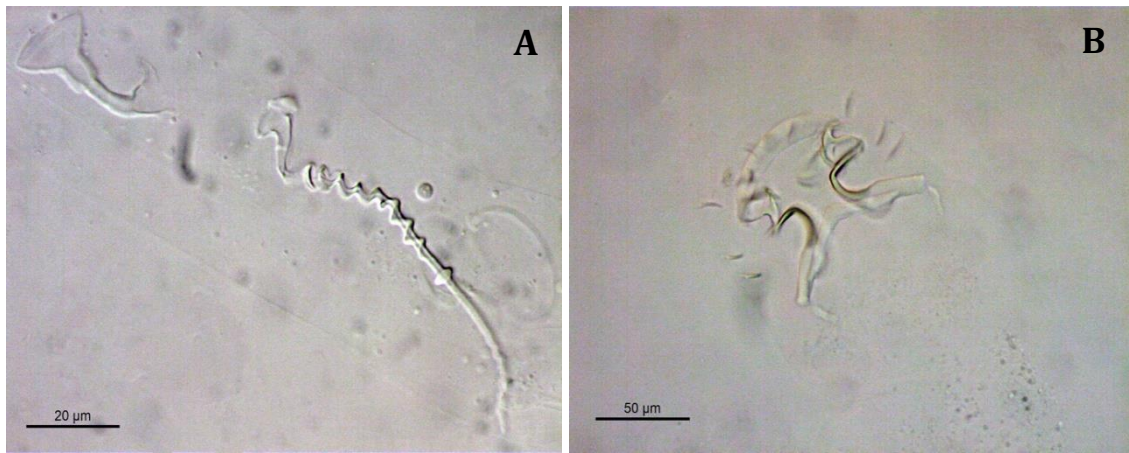


Figure 46. Monogenean from the gills of *Pterygoplichthys ambrosettii* from the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo State, Brazil: (A) Copulatory complex of *Unilatus unilatus*; (B) haptor of *Unilatus unilatus*.



Figure 47. Digenean from *Pterygoplichthys anisitsi* from the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo State, Brazil: (A) *Austrodiplostomum* sp. metacercaria found in the eyes.



Figure 48. Monogenea from the gills of *Rhinelepis aspera* from the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo State, Brazil: (A) *Demidospermus* sp.; (B) Copulatory complex of *Demidospermus* sp.; (C) haptor of *Demidospermus* sp.

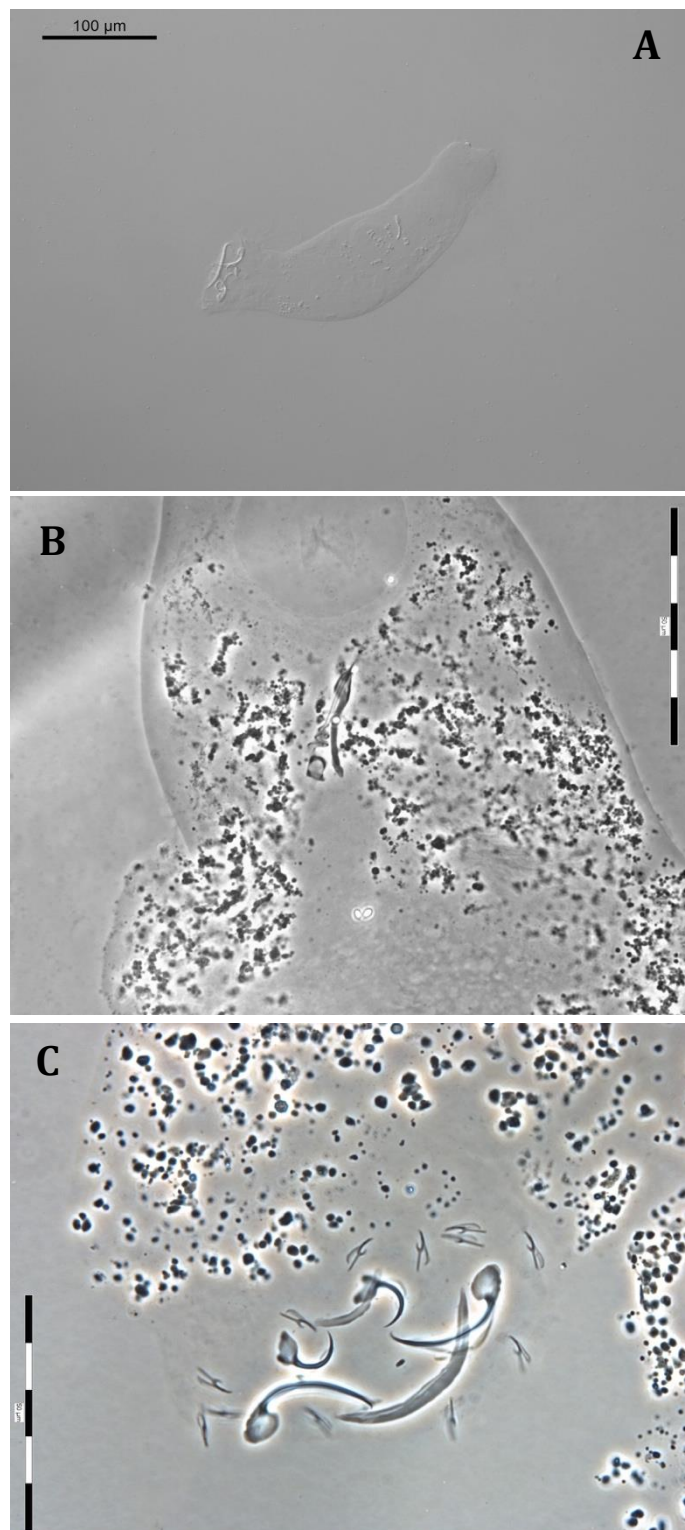


Figura 49. Monogenean from the gills of *Rhinelepis aspera* from the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo State, Brazil: (A) *Heteropriapulus* sp. 3; (B) copulatory complex of *Heteropriapulus* sp. 3; (C) haptor of *Heteropriapulus* sp. 3.

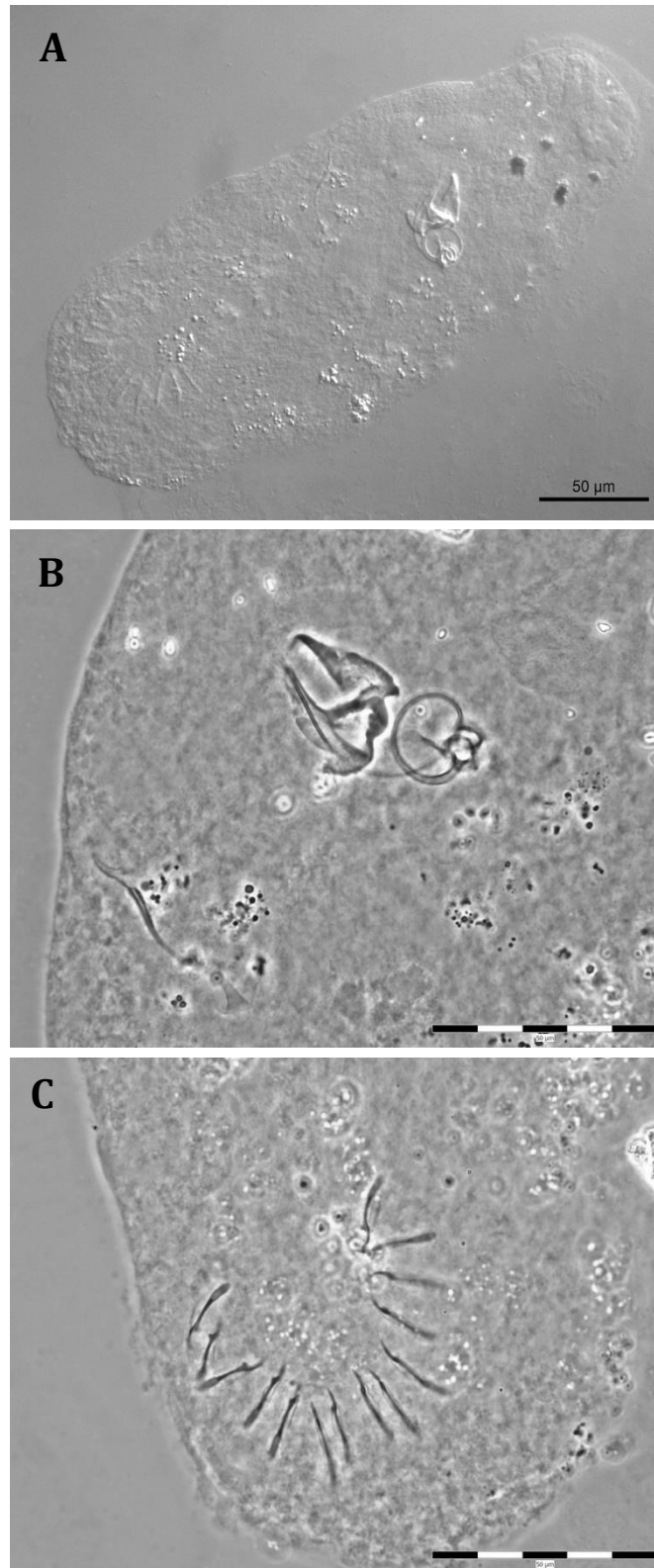


Figure 50. Monogenea from the nasal cavity of *Rhinelepis aspera* from the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo State, Brazil: (A) *Telethecium nasalis*; (B) Copulatory complex of *Telethecium nasalis*; (C) haptor of *Telethecium nasalis*.



Figure 51. Digeneans from *Rhinelepis aspera* from the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo State, Brazil: (A) *Austrodiplostomum* sp. metacercaria found in the eyes; (B) Type 1 metacercariae of Diplostomidae found in the cavity.

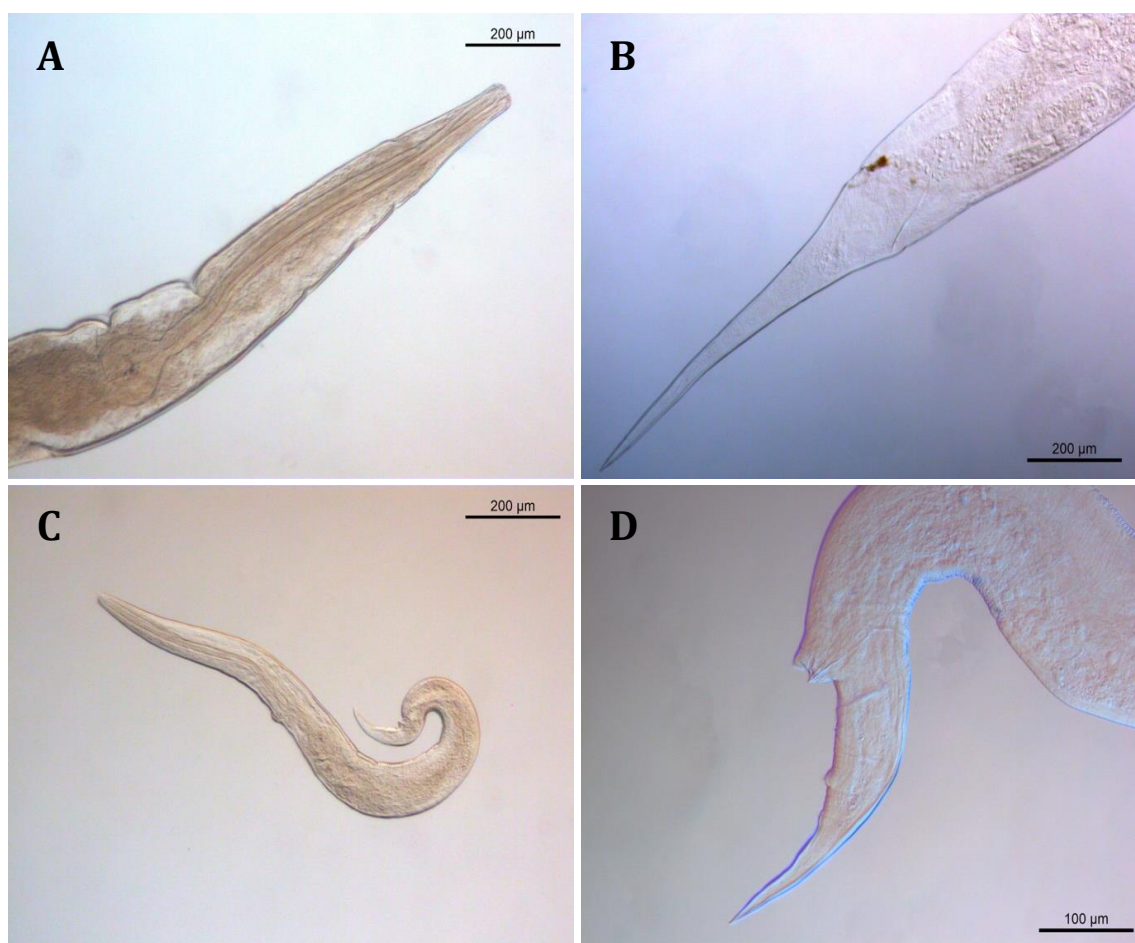


Figure 52. Nematode from the intestine of *Rhinelepis aspera* from the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo State, Brazil: (A–D) *Parasynodontisia petterae* – (A) female anterior extremity; (B) female posterior extremity; (C) male total view; (D) male posterior extremity.

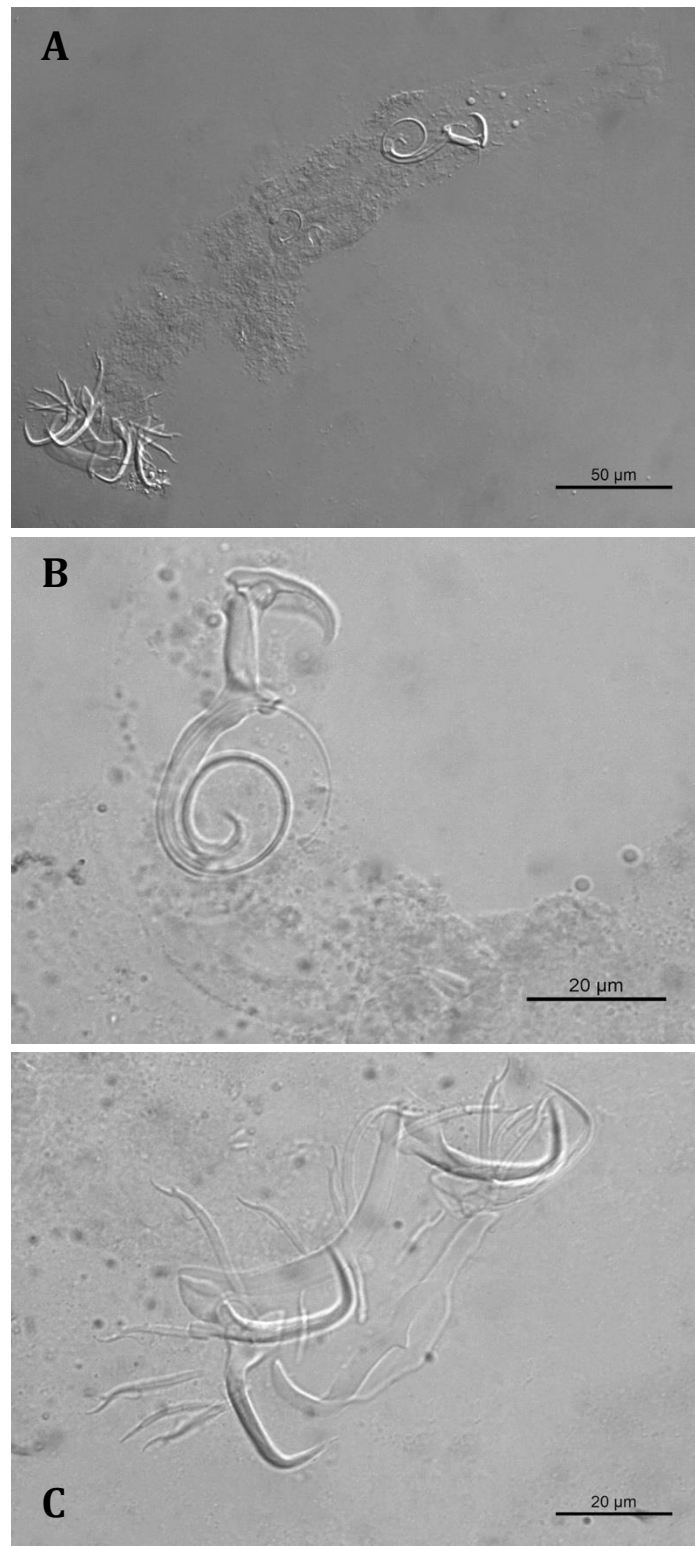


Figure 53. Monogenean from the gills of *Hemisorubim platyrhynchos* from the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo State, Brazil: (A) *Ameloblastella formatrium*; (B) copulatory complex of *Ameloblastella formatrium*; (C) haptor of *Ameloblastella formatrium*.

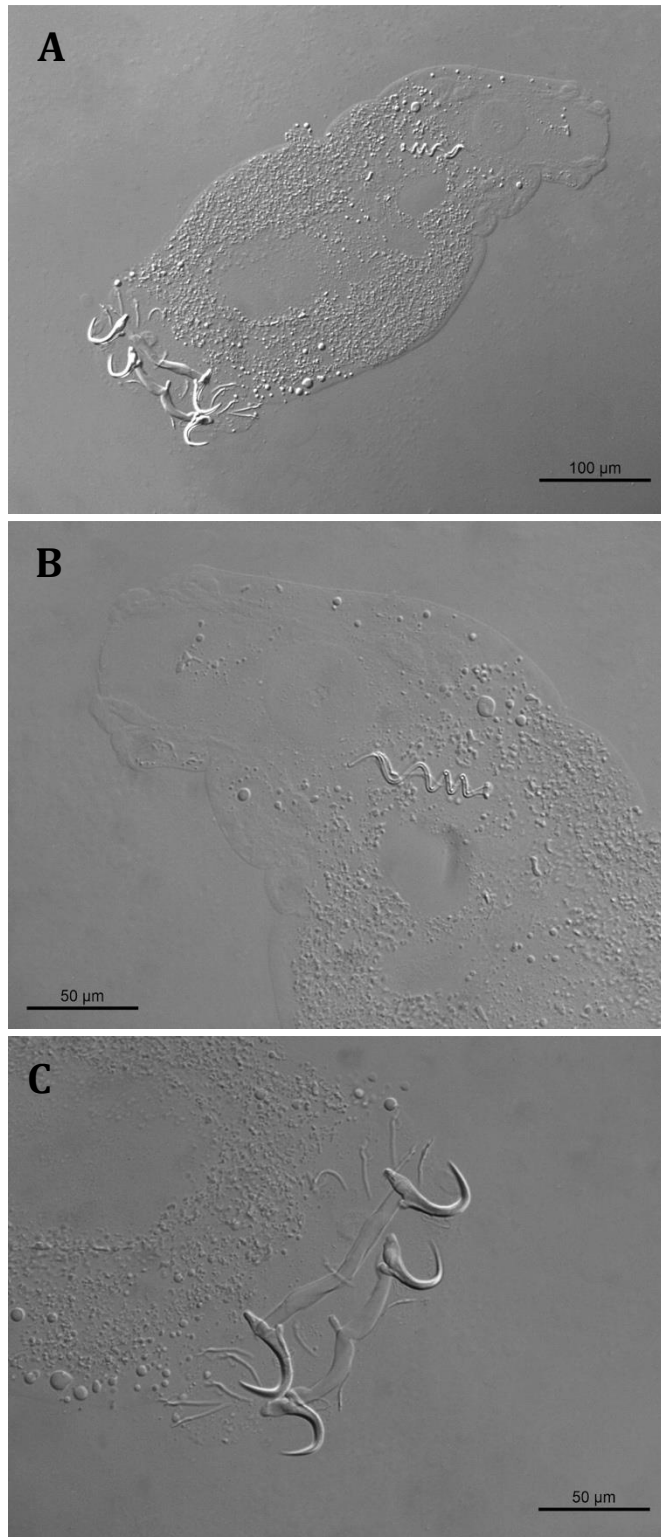


Figure 54. Monogenean from the gills of *Hemisorubim platyrhynchos* from the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo State, Brazil: (A) *Ameloblastella* sp. 2; (B) complexo copulatório de *Ameloblastella* sp. 2; (C) haptor de *Ameloblastella* sp. 2.

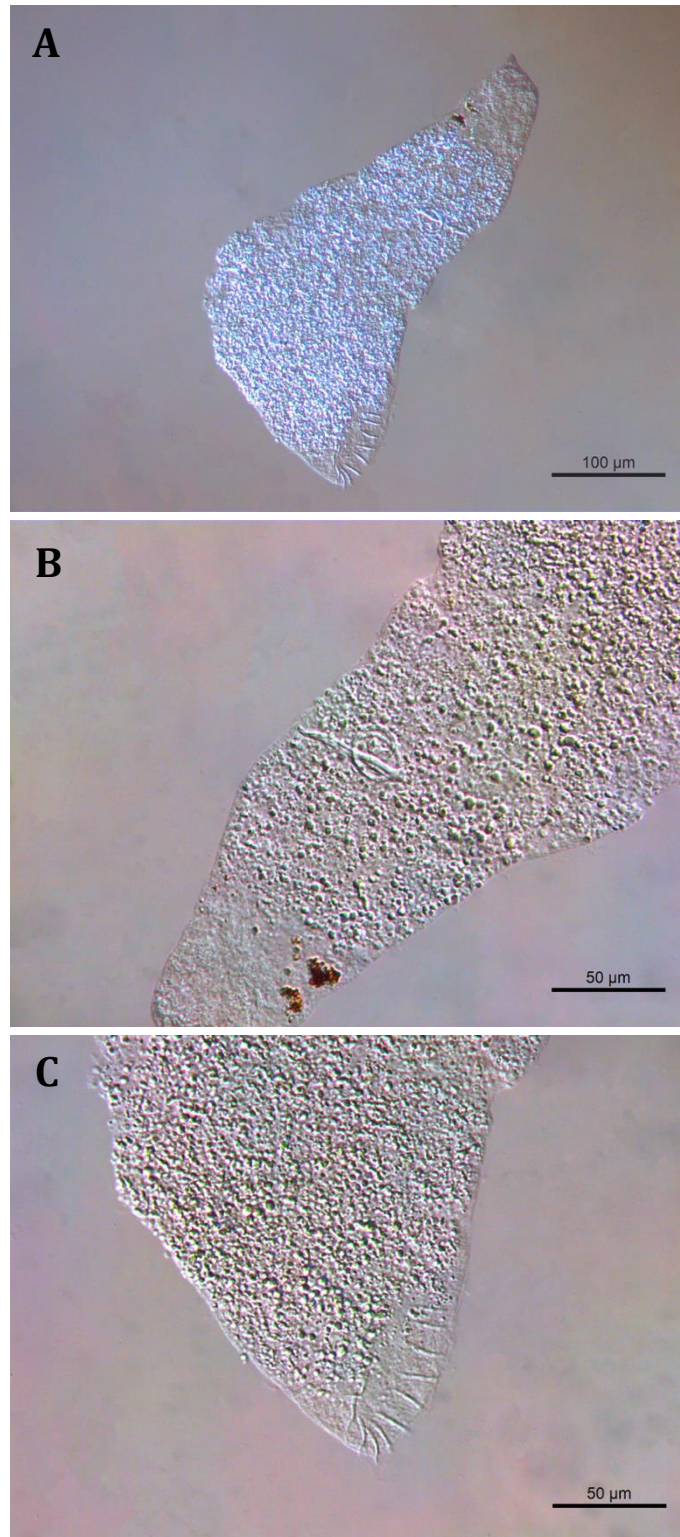


Figure 55. Monogenean from the nasal cavity of *Hemisorubim platyrhynchos* from the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo State, Brazil: (A) *Pavanelliella pavanellii*; (B) Copulatory complex of *Pavanelliella pavanellii*; (C) haptor of *Pavanelliella pavanellii*.

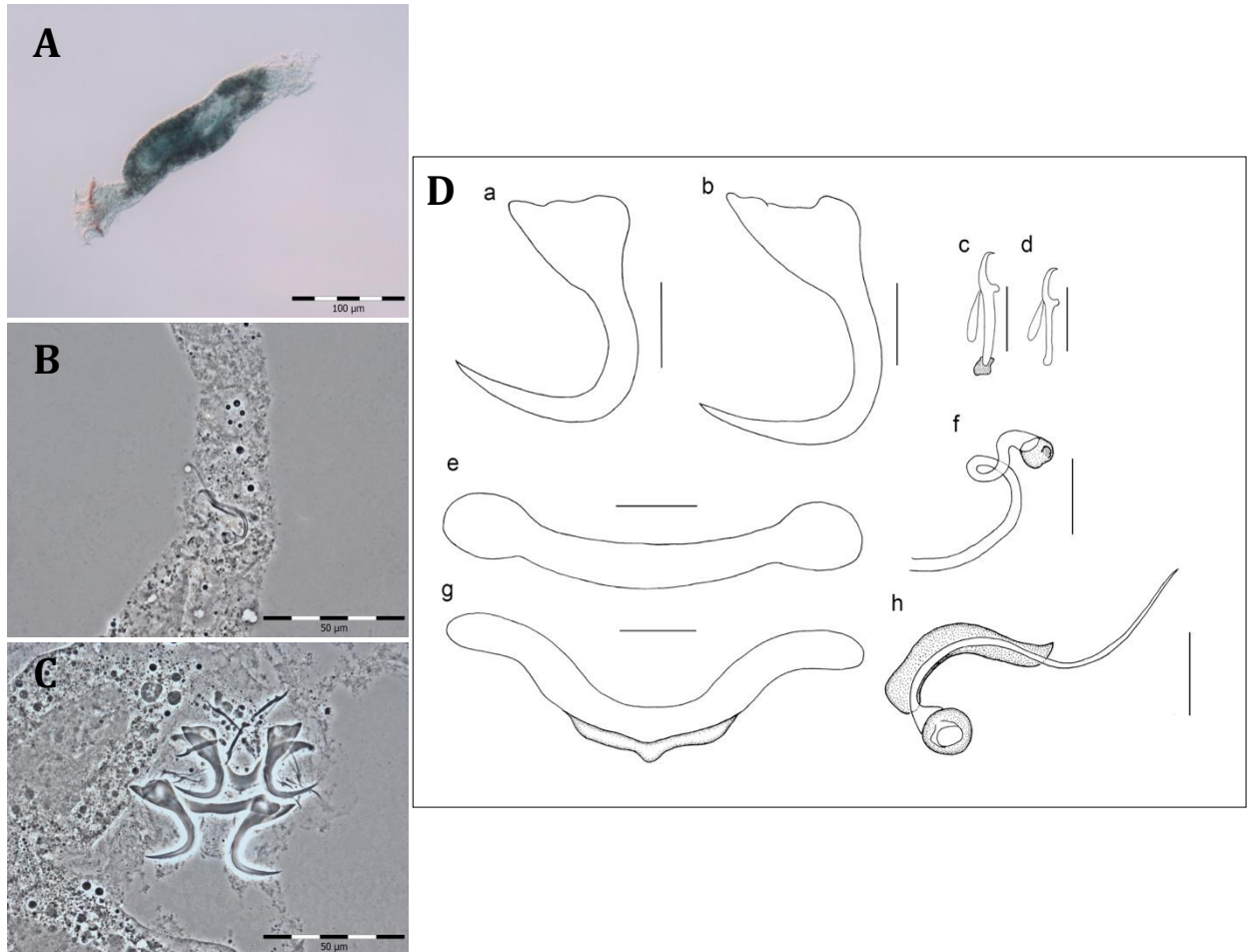


Figure 56. Monogenean from the gills of *Hemisorubim platyrhynchos* from the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo State, Brazil: (A–D) Dactylogyridae gen. sp. 1 – (A) total view; (B) copulatory complex; (C) haptor; (D) line drawing – (a) dorsal anchor; (b) ventral anchor; (c) hook pairs I and II; (d) remaining hook pairs; (e) dorsal bar; (f) vagina; (g) ventral bar; (h) male copulatory complex. Scale bars = 10 µm.

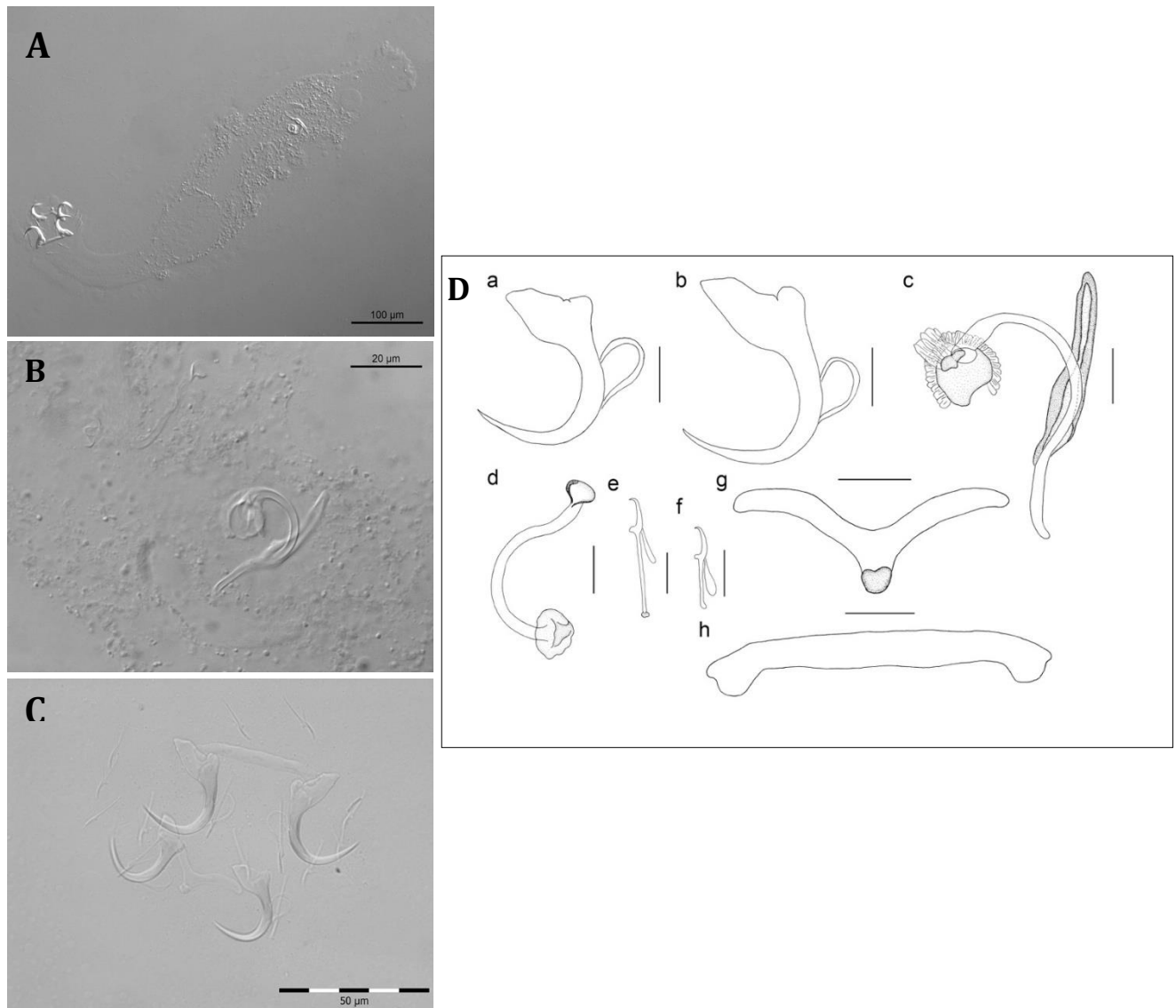


Figure 57. Monogenean from the gills of *Hemisorubim platyrhynchos* from the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo State, Brazil: (A–D) Dactylogyridae gen. sp. 2 – (A) total view; (B) copulatory complex; (C) haptor; (D) line drawing – (a) ventral anchor; (b) dorsal anchor; (c) male copulatory complex; (d) vagina; (e) hook pair I and VII; (f) remaining hook pairs; (g) ventral bar; (h) dorsal bar. Scale bars = 10 µm.

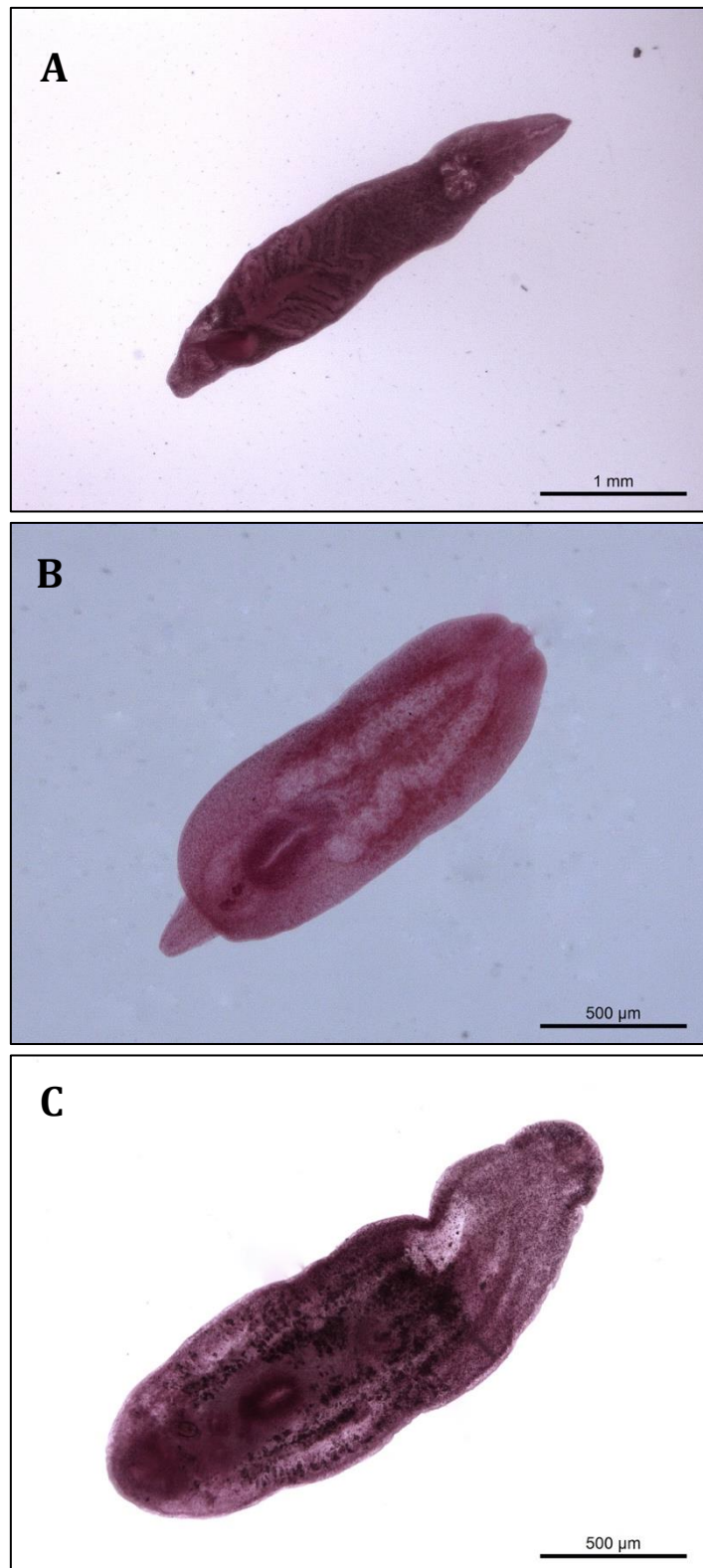


Figure 58. Digeneans from *Hemisorubim platyrhynchos* from the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo State, Brazil: (A) Type 1 metacercariae found in the cavity of *Hemisorubim platyrhynchos*; (B) *Austrodiplostomum* sp. metacercaria found in the eyes; (C) Type 1 metacercariae of Diplostomidae found in the cavity of *Hemisorubim platyrhynchos*.

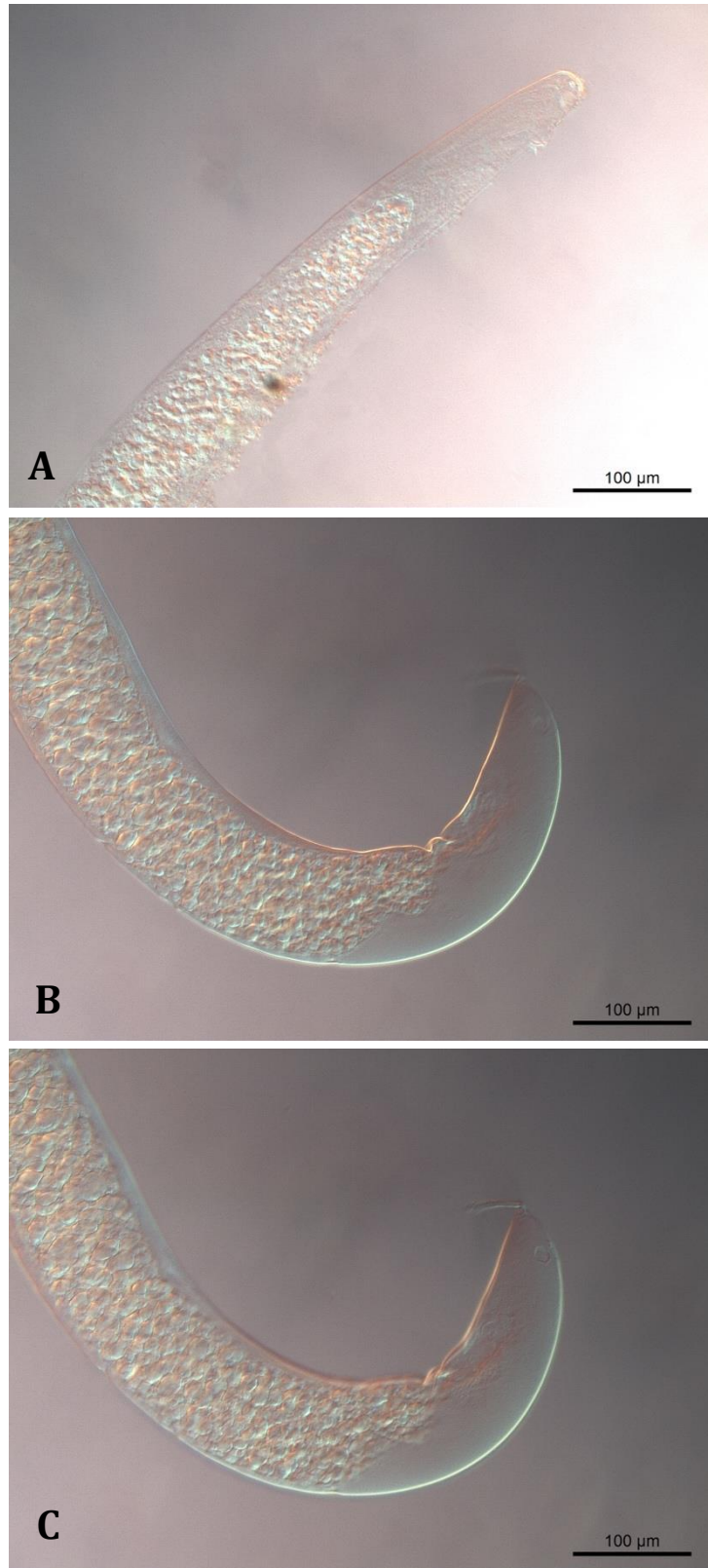


Figure 59. Nematode from *Hemisorubim platyrhynchos* from the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo State, Brazil: (A–C) nematode larvae found in the cavity of *Hemisorubim platyrhynchos* – (A) anterior extremity; (B,C) posterior extremity.

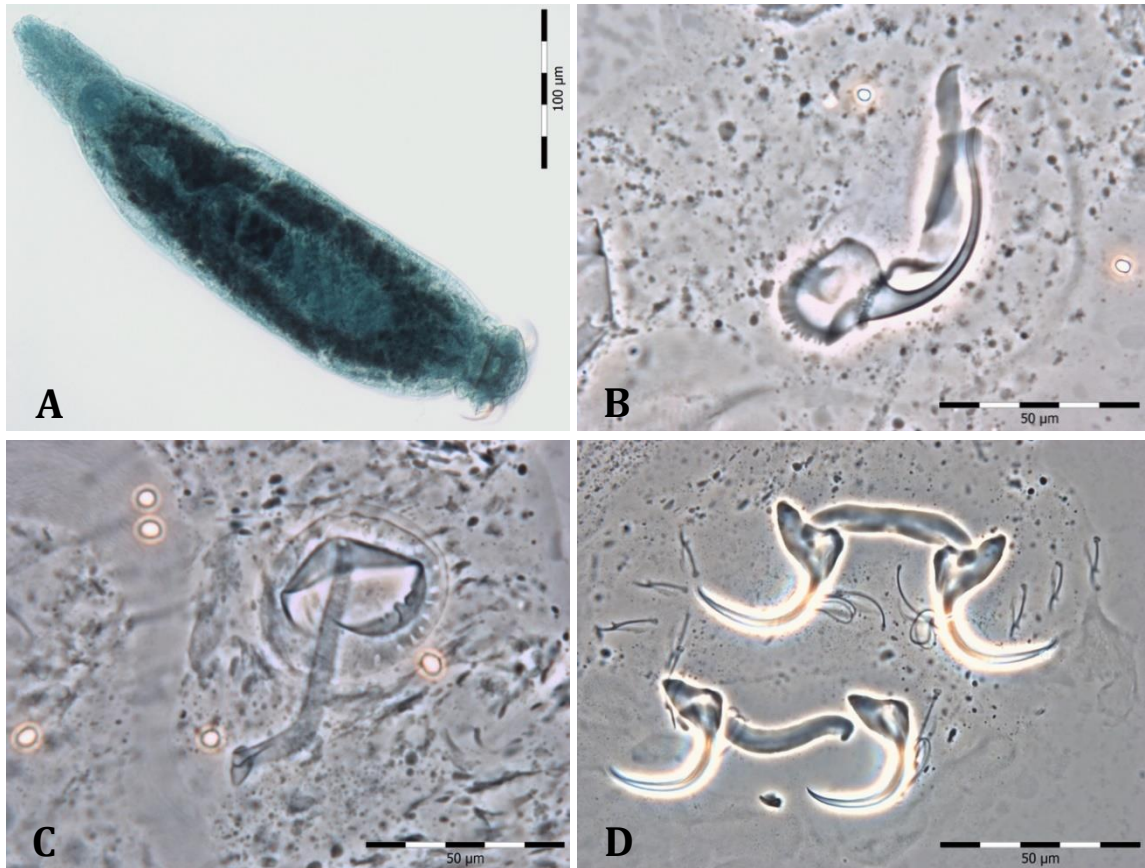


Figure 60. Monogenean from the gills of *Sorubim lima* from the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo State, Brazil: (A) *Dactylogyridade* gen. sp. 5; (B) Copulatory complex of *Dactylogyridade* gen. sp. 5; (C) vagina of *Dactylogyridade* gen. sp. 5; (D) haptor of *Dactylogyridade* gen. sp. 5.



Figure 61. Digenean from *Sorubim lima* from the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo State, Brazil: (A) *Genarchella* sp. found in the stomach; (B) *Austrodiplostomum* sp. metacercaria found in the eyes.

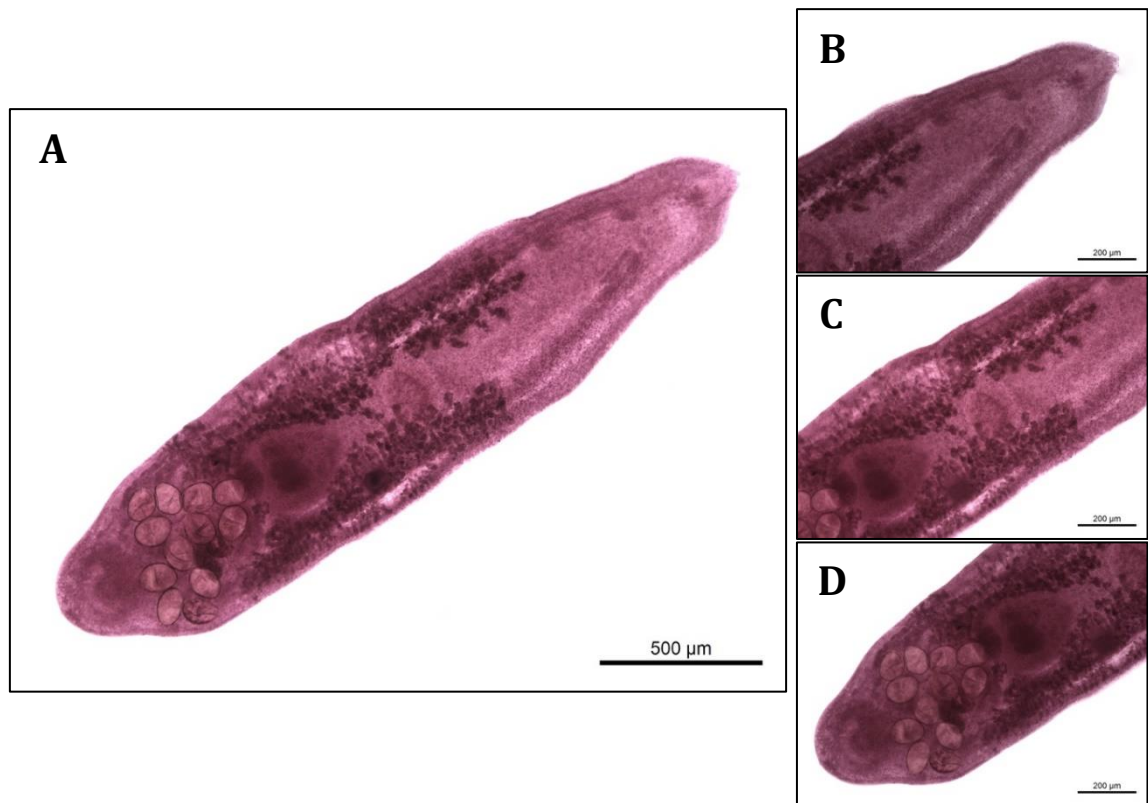


Figura 62. Digenean from *Sorubim lima* from the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo State, Brazil: (A–D) Type 1 metacercariae of Diplostomidae found in the cavity – (A) total view; (B) anterior extremity; (C) tribocytic organ; (D) posterior extremity with eggs.

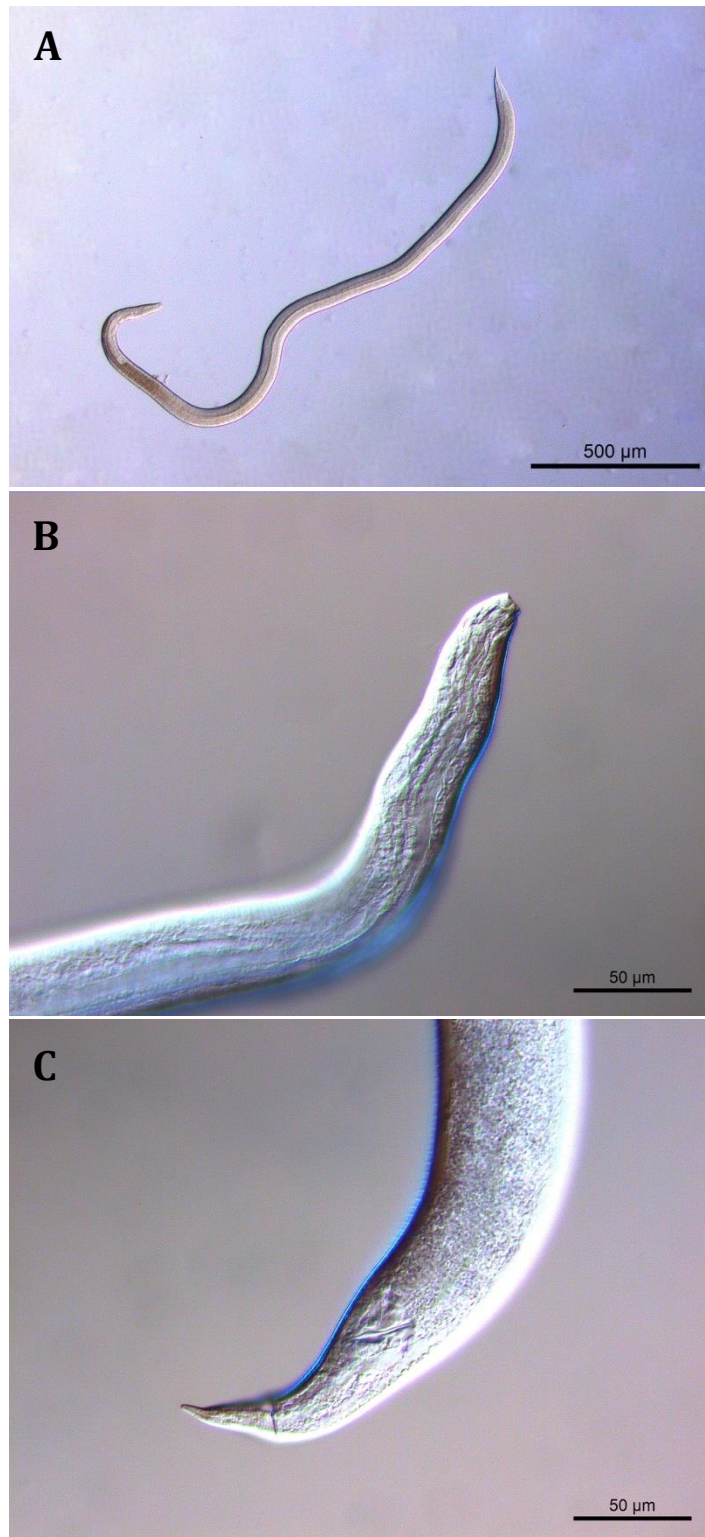


Figure 63. Nematode from *Sorubim lima* from the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo State, Brazil: (A–C) *Contracecum* sp. larva found on the swim bladder – (A) total view; (B) anterior extremity; (C) posterior extremity.

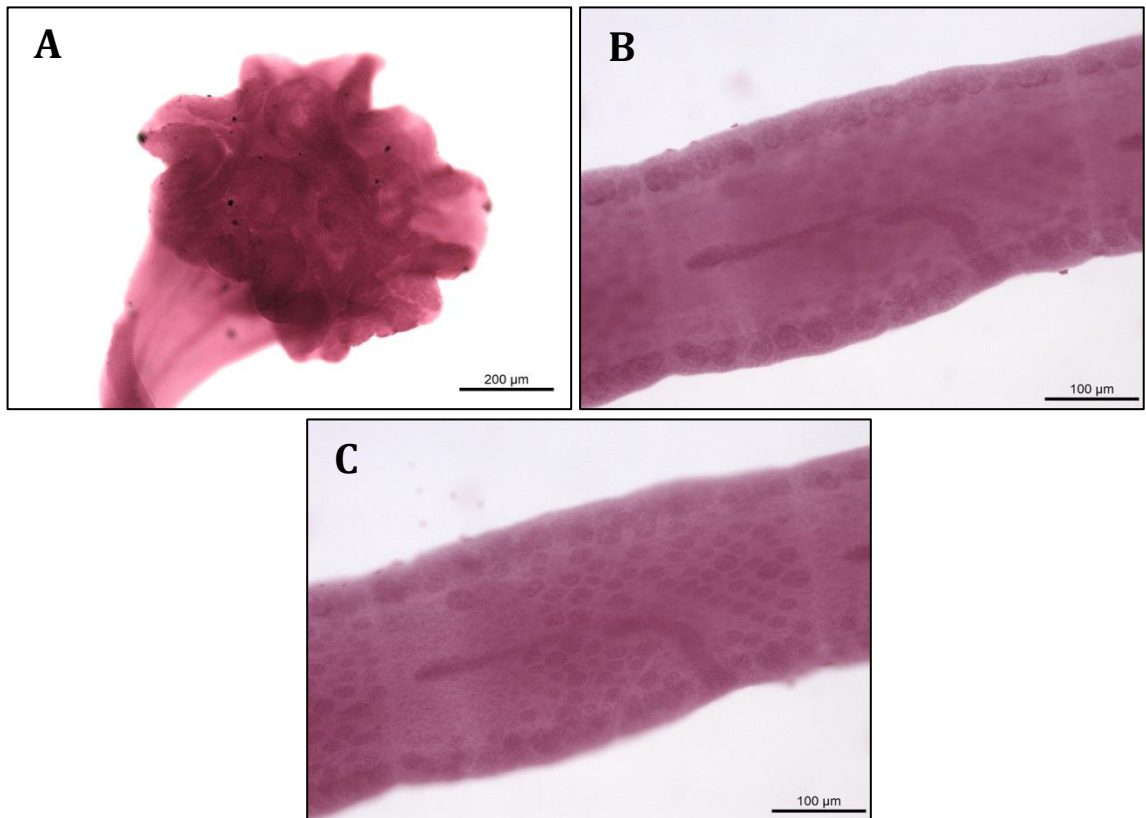


Figure 64. Cestode from the intestine of *Sorubim lima* from the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo State, Brazil: (A–C) *Spatulifer maringaensis* (A) scolex; (B) reproductive organs with mature proglottids with vitellaria concentrated in the edges; (C) eggs.

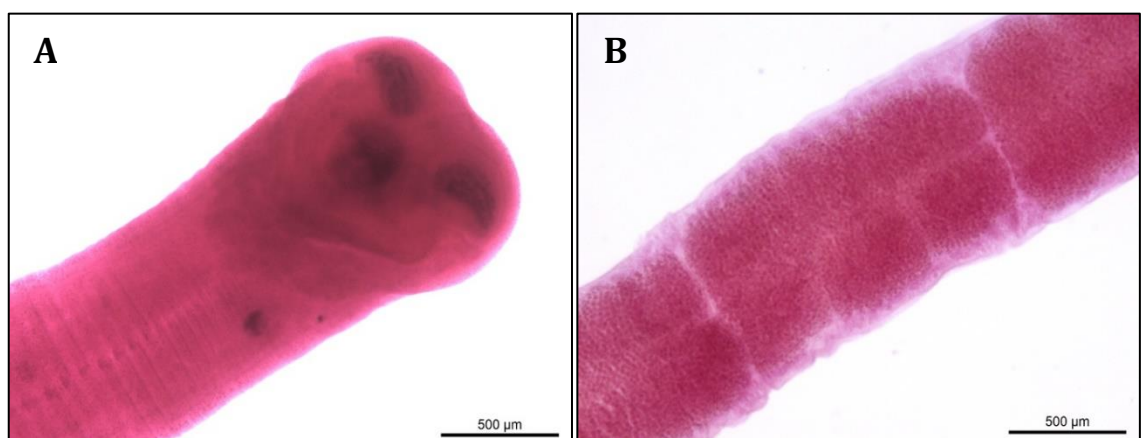


Figure 65. Cestode from the intestine *Sorubim lima* from the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo State, Brazil: (A–B) *Manaosia bracodemoca* (A) scolex; (B) proglottids.

3.4. Discussion

From the 405 specimens belonging to the eight host species analyzed for this study, a total of 54 helminth parasite *taxa* were found: 25 monogeneans, 15 digeneans, 11 nematodes, and three cestodes. Monogenea was the most diverse group, followed by the class Digenea. Mendoza-Palmero *et al.* (2012) listed 56 Dactylogyridae species found in siluriform fishes from the Peruvian Amazon and Mendoza-Franco *et al.* (2016) described six of them. The dactylogyrids found in this study that have not been identified to the species level were compared with those 50 undescribed species from the Peruvian Amazon, and none of them could be considered as cospecific. Takemoto *et al.* (2009) analyzed data of helminth parasites of 72 fish species sampled during seven years in the Upper Parana river basin, and the class Monogenea showed the highest number of species also followed by the class Digenea. Very recently, Luque *et al.* (2016) analyzed data of all records of helminth parasites of fishes in South America and found out that monogeneans represent the most diverse group with 835 species recorded from different countries and 1,133 host-parasite associations, also followed by trematodes with 662 species recorded with 1,127 host-parasite association.

Monogenea and Digenea have long associations of coevolution, host switching and adaptation with fishes, which account for their high success in their respective habitats. The high level diversity of these two groups is interpreted as the result of site specialization, life-cycle adaptation, and dietary specialization (Cribb *et al.*, 2002). The fact that monogeneans are still more diverse than digeneans may be due to the generation time: gyrodactylids and dactylogyrids have generation times of less than a month, while the life cycle of all digeneans is prolonged by the multiple generations that they undergo (Cribb *et al.*, 2002 and references therein). Monogeneans present diversity for three main reasons: they are diverse regarding morphology and number of species, they have a phylogeny well resolved at least to the family level, and they tend to be host specific with most species infecting only one or very few host species (Poulin, 1992; Sasal *et al.*, 1998; Poulin, 2002). This specificity can be understood as the result of strict coespeciation between host and parasite and also from other adaptive and non-adaptive process, like speciation by host switching (Boeger and Kritsky, 1997). Diversity of monogeneans at least at the family-level is mainly associated to morphological adaptation, specially the attachment organ that is directly related to the colonization of new hosts (Cribb *et al.*, 2002; Bueno-Silva *et al.*, 2011). The morphology of the attachment organ (haptor)

supposedly play an important role in parasite specialization and adaptation to host species (Šimková *et al.*, 2006).

The helminths found in *P. granulosus* have all been previously recorded in the literature for this host (see Table XII). The only new host record is for metacercariae of Diplostomidae type 1. The helminth component community of *P. granulosus* presented low diversity, which was showed by the diversity indexes values. The monogeneans *C. bulbocirrus* and *V. januacaensis* showed the highest levels of prevalence and were found in very high quantity (see Table III). These two dactylogyrids were not found in other hosts in this study. *Pterodoras granulosus* is the type host for both *C. bulbocirrus* and *V. januacaensis* (see Kritsky *et al.*, 1986) and since their descriptions these two dactylogyrids have been recorded only in *P. granulosus* by Mendoza-Palmero *et al.* (2012) and Mendoza-Palmero *et al.* (2015) in Peru, but the two studies are the results of the same sampling efforts. Even though these two dactylogyrids are specific to the same host and share the same habitat, the study of Mendoza-Palmero *et al.* (2015) showed that they are not phylogenetically close. They might share the same history of coevolution with *P. granulosus* even belonging to separately distant genera. The amphistome *D. oxycephala* was first described for a characiform, however it is a generalist parasite being reported in several different host species and it is widely distributed in Brazil, but also with records in Argentina and Venezuela (Thatcher, 1999; Kohn *et al.*, 2007). In Amazonia, is a common parasite of *Piaractus brachypomus* (Cuvier, 1818) and some catfishes, but especially *P. granulosus* (Thatcher, 1999). The nematode *R. rondoni* showed high rates of infection, with a range of 1–42,560 parasites. Dias *et al.* (2004) also reported this high rate of infection, and such study also investigated the effect of parasitic loads of *R. rondoni* upon the condition factor of *P. granulosus*, however no influence was detected, and they suggest that this parasite-host association is not recent and apparently there is a balanced relationship between them. *Rondonia rondoni* is a generalist species, being reported in hosts of different orders and families (Moravec, 1998; Eiras *et al.*, 2010). The nematode *P. (S.) inopinatus* was also recovered from *P. granulosus* and it is also a generalist species (Moravec, 1998; Eiras *et al.*, 2010; Luque *et al.*, 2011). The cestode found could not be identified at the species level.

The helminth component community of *T. paraguayensis* was the second most diverse in this study, which was showed by the diversity indexes values. There is only one record of monogenean in the literature for *T. paraguayensis* (Table XII), however,

this study recovered three dactylogyrid *taxa* and one gyrodactylid. The species that is regarded as belonging to *Ameloblastella* (namely *Ameloblastella* sp. 1) underwent only morphological analyses, but it would be interesting to perform molecular analysis of this species to confirm its genus identity, since phylogenetic data of the type species of the genus is available in the literature by Mendoza-Palmero *et al.* (2015). The other species is named as Dactylogyridae gen. sp. 6 and it does not show any resemblance with any dactylogyrid described thus far (data on the morphology and molecular phylogenetic relationships of this species are investigated in section 4.). The monogenean *P. pavanellii* is specific to the nasal cavity described for a siluriform fish (Kritsky and Boeger, 1998) and it has been recorded in other siluriforms (Cohen *et al.*, 2013). Four *taxa* of digeneans were recovered, however, all at the metacercariae level. There is only one report of digenean infecting *T. paraguayensis* (Table XII) but it is also at the metacercariae level. Thus, it can be suggested that *T. paraguayensis* plays an important role as intermediate host of these digeneans and it is used as food resource by their final host. All nematode species found in this study were previously recorded (see Table XII). Nematodes are generally transmitted by feeding and since *T. paraguayensis* uses a wide variety of food resources found at the bottom, they get exposed to a considerable variety of these parasites (Lucas *et al.*, 2011). *Trachydoras paraguayensis* is the type host of *I. laterifilamenta* and this nematode has not been recorded in any other host since, indicating that this nematode may present specificity with *T. paraguayensis*. *Neoparaseuratum travassosi* was described from *P. granulosus* (Moravec *et al.*, 1992a) that is also a doradid fish. This nematode has been recorded only in these two hosts (Moravec, 1998; Eiras *et al.*, 2010; Luque *et al.*, 2011), which may indicate some specificity at family level. The cestode found could not be identified at the species level.

Table XII. Parasites already recorded in the Neotropical region for six of the siluriform species sampled for this study.

Host	Parasite	Reference	Locality
Doradidae			
<i>Pterodoras granulosus</i> (Valenciennes, 1821)	Monogenea		
	<i>Cosmetocleithrum bulbocirrus</i> Kritsky, Thatcher and Boeger, 1986	Kritsky <i>et al.</i> (1986)	Brazil
		Mendoza-Palmero <i>et al.</i> (2012)	Peru
	<i>Vancleaveus januacaensis</i> Kritsky, Thatcher and Boeger, 1986	Kritsky <i>et al.</i> (1986)	Brazil
		Mendoza-Palmero <i>et al.</i> (2012)	Peru
		Mendoza-Palmero <i>et al.</i> (2015)	Peru
		Suriano and Incorvia (1995)	Argentina
	Digenea		
	<i>Dadatyrema oxycephala</i> (Diesing, 1836) Thatcher, 1931	Thatcher (1999)	Peru
		Travassos <i>et al.</i> (1928)	Brazil
	Nematoda		
	<i>Cucullanus (Cucullanus) pinnai pterodorasi</i> Moravec, Kohn and Fernandes, 1997	Moravec <i>et al.</i> (1997a)	Brazil
	<i>Hysterothylacium sp.</i> Moravec, Kohn and Fernandes, 1993 larvae	Moravec (1998)	Brazil
	<i>Klossinemela iheringi</i> (Travassos, Artigas and Pereira, 1928)	Moravec (1998)	Brazil
	<i>Neoparaseuratum travassosi</i> Moravec, Kohn and Fernandes, 1992	Moravec <i>et al.</i> (1992a)	Brazil
	<i>Procamallanus</i> (<i>Spirocamallanus</i>) <i>inopinatus</i> Travassos, Artigas and Pereira, 1928	Moravec <i>et al.</i> (1993)	Brazil
	<i>Raphidascaroides brasiliensis</i> Moravec and Thatcher, 1997	Moravec and Thatcher (1997)	Brazil

Table XII. Continued.

Host	Parasite	Reference	Locality
	<i>Rondonia rondoni</i> Travassos, 1920	Dias <i>et al.</i> (2004)	Brazil
		Moravec (1998)	Brazil
	<i>Spectatus spectatus</i> Travassos, 1923	Moravec (1998)	Brazil
Cestoda			
	<i>Monticellia belavistensis</i> Pavanelli, Machado, Takemoto and dos Santos, 1994	de Chambrier and Vaucher (1999)	Paraguay
		de Chambrier <i>et al.</i> (2015)	Peru
		Gil de Pertierra (2005)	Argentina
	<i>Proteocephalus kuyukuyu</i> Woodland, 1935	de Chambrier <i>et al.</i> (2015)	Peru
	<i>Proteocephalus</i> sp.	de Chambrier <i>et al.</i> (2006), (2015)	Peru
<i>Trachydoras paraguayensis</i> (Eigenmann and Ward, 1907)	Monogenea		
	<i>Vancleaveus</i> sp.	Takemoto <i>et al.</i> (2009)	Brazil
Digenea			
	Strigeoidea (metacercaria)	Takemoto <i>et al.</i> (2009)	Brazil
Nematoda			
	<i>Cosmoxyinemoides</i> sp.	Takemoto <i>et al.</i> (2009)	Brazil
	<i>Ichthyouris laterifilamenta</i> Moravec, Kohn and Fernandes, 1992	Moravec <i>et al.</i> (1992b)	Brazil
	<i>Neoparaseuratum travassosi</i> Moravec, Kohn and Fernandes, 1992	Moravec (1998)	Brazil
	<i>Procamallanus</i> (<i>Spirocamallanus</i>) <i>inopinatus</i>	Moravec (1998)	Brazil
	<i>Rondonia rondoni</i>	Kohn <i>et al.</i> (2011)	Brazil
Loricariidae	<i>Procamallanus</i> sp.	Takemoto <i>et al.</i> (2009)	Brazil

Table XII. Continued.

Host	Parasite	Reference	Locality
<i>Loricariichthys platymetopon</i> Isbrücker and Nijssen, 1979	Monogenea		
	<i>Demidospermus anus</i> Suriano, 1983	Cohen and Kohn (2008)	Brazil
	<i>Demidospermus paranaensis</i> Ferrari-Hoeinghaus, Bellay, Takemoto and Pavanelli, 2010	Ferrari-Hoeinghaus <i>et al.</i> (2010)	Brazil
	Digenea		
	<i>Clinostomum marginatum</i> Rudolphi, 1819	Eiras <i>et al.</i> (1999)	Brazil
	<i>Clinostomum complanatum</i> Rudolphi, 1814	Dias <i>et al.</i> (2006)	Brazil
		Ferrari-Hoeinghaus <i>et al.</i> (2007)	Brazil
	<i>Crocodilicola pseudostoma</i> (Willemoes-Suhm, 1870)	Ferrari-Hoeinghaus <i>et al.</i> (2007)	Brazil
	Diplostomidae gen. sp.	Kohn <i>et al.</i> (2011)	Brazil
	Nematoda		
	<i>Raphidascaris</i> (<i>Sprentascaris</i>) <i>mahnerti</i> (Petter and Cassone, 1984)	Fortes <i>et al.</i> (1999)	Brazil
		Petter and Cassone (1984)	Paraguay
	Cestoda		
	Proteocephalidae gen. sp. (metacestode)	Schaeffer <i>et al.</i> (1992)	Brazil
<i>Rhinelepis aspera</i> Spix and Agassiz, 1829	Monogenea		
	<i>Paranaella luquei</i> Kohn, Baptista-Faria and Cohen, 2000	Kohn <i>et al.</i> (2000)	Brazil
	Nematoda		
	<i>Parasynodontisia petterae</i> Moravec, Kohn and Fernandes, 1992	Moravec <i>et al.</i> (1992a)	Brazil
Pimelodidae			

Table XII. Continued.

Host	Parasite	Reference	Locality
<i>Hemisorubim platyrhynchos</i> (Valenciennes, 1840)	Monogenea		
	<i>Vancleaveus platyrhynchi</i> Kritsky, Thatcher and Boeger, 1986	Kritsky <i>et al.</i> (1986)	Brazil
	Digenea		
	<i>Crocodilicola pseudostoma</i>	Guidelli <i>et al.</i> (2003)	Brazil
	<i>Sanguinicola platyrhynchi</i> Guidelli, Isaac and Pavanelli, 2002	Guidelli <i>et al.</i> (2002)	Brazil
		Guidelli <i>et al.</i> (2003)	Brazil
	<i>Sanguinicola</i> sp.	Fernandes and Kohn (2001)	Brazil
	<i>Sphincterodiplostomum</i> sp.	Guidelli <i>et al.</i> (2003)	Brazil
	Nematoda		
	<i>Brevimulticaecum</i> sp.	Luque <i>et al.</i> (2011)	Brazil
	<i>Contracaecum</i> larvae	Guidelli <i>et al.</i> (2003)	Brazil
	<i>Cucullanus</i> (<i>Cucullanus</i>) <i>zungaro</i> Vaz and Pereira, 1934	Guidelli <i>et al.</i> (2003)	Brazil
	<i>Eustrongylides ignotus</i> Jägerkiöld, 1909	Guidelli <i>et al.</i> (2003)	Brazil
	<i>Goezia</i> sp.	Guidelli <i>et al.</i> (2003)	Brazil
	Cestoda		
	<i>Chambriella paranaensis</i> (Pavanelli and Rego, 1989)	de Chambrier and Vaucher (1999)	Paraguay
		de Chambrier <i>et al.</i> (2006)	Peru
		de Chambrier <i>et al.</i> (2015)	Peru
		Guidelli <i>et al.</i> (2003)	Brazil
		Pavanelli and Machado (1991)	Brazil
		Pavanelli and Rego (1989)	Brazil

Table XII. Continued.

Host	Parasite	Reference	Locality
		Rego <i>et al.</i> (1999)	Brazil
	<i>Manaosia bracademoca</i> Woodland, 1935	Pavanelli and Machado (1991)	Brazil
		Rego <i>et al.</i> (1999a)	Brazil
	<i>Mariauxiella piscatorum</i> de Chambrier and Vaucher, 1999	de Chambrier and Vaucher (1999)	Paraguay
		de Chambrier <i>et al.</i> (2006a)	Peru
		de Chambrier <i>et al.</i> (2015a)	Peru
		Guidelli <i>et al.</i> (2003)	Brazil
	<i>Spatulifer maringaensis</i> Pavanelli and Rego, 1989	de Chambrier and Vaucher (1999)	Paraguay
		de Chambrier <i>et al.</i> (2006)	Peru
		de Chambrier <i>et al.</i> (2015)	Peru
		Guidelli <i>et al.</i> (2003)	Brazil
		Pavanelli and Machado (1991)	Brazil
		Pavanelli and Rego (1989)	Brazil
Acanthocephala			
	<i>Quadrigyrus machadoi</i> Fabio, 1983	Guidelli <i>et al.</i> (2003)	Brazil
<i>Sorubim lima</i> (Bloch and Schneider, 1801)	Monogenea		
	<i>Ameloblastella unapioides</i> Mendoza-Franco, Mendoza- Palmero and Scholz, 2016	Mendoza-Franco <i>et al.</i> (2016)	Peru
	<i>Urocleidoides megorchis</i> Mizelle and Kritsky, 1969	Mizelle and Kritsky (1969)	Argentina
	Dact. gen. sp. 12	Mendoza-Palmero <i>et al.</i> (2015)	Peru
Digenea			
	<i>Genarchella genarchella</i> Travassos, Artigas and Pereira, 1928	Hamann (1989)	Argentina
Cestoda			

Table XII. Continued.

Host	Parasite	Reference	Locality
	<i>Manaosia bracodemoca</i>	de Chambrier and Vaucher (1999)	Brazil
		de Chambrier <i>et al.</i> (2015a,b)	Peru
		Kohn <i>et al.</i> (2011)	Brazil
		Pavanelli and Machado (1991)	Brazil
		Pavanelli and Takemoto (2000)	Brazil
		Pavanelli and Rego (1991)	Brazil
		Takemoto and Pavanelli (2000)	Brazil
		Woodland (1935)	Brazil
	<i>Nupelia portoricensis</i> Pavanelli and Rego, 1991	de Chambrier and Vaucher (1999)	Paraguay
		de Chambrier <i>et al.</i> (2015)	Peru
		Pavanelli and Machado (1991)	Brazil
		Pavanelli and Rego (1991)	Brazil
		Pavanelli and Takemoto (2000)	Brazil
		Takemoto and Pavanelli (2000)	Brazil
	<i>Spasskyellina spinulifera</i> (Woodland, 1935)	Pavanelli and Takemoto (2000)	Brazil
		Takemoto and Pavanelli (2000)	Brazil
	<i>Spatulifer maringaensis</i>	Arrendondo and Gil de Pertierra (2008)	Argentina
		de Chambrier and Vaucher (1999)	Paraguay
		de Chambrier <i>et al.</i> (2015)	Peru
		Hypša <i>et al.</i> (2005)	Paraguay
		Mariaux, 1998	Brazil
		Pavanelli and Machado (1991)	Brazil

Table XII. Continued.

Host	Parasite	Reference	Locality
		Pavanelli and Rego (1989)	Brazil
		Pavanelli and Takemoto (2000)	Brazil
		Takemoto and Pavanelli (2000)	Brazil
		Zehnder and Mariaux (1999)	Paraguay

For *P. avanhandavae*, the diversity indexes showed that helminth component community is not very diverse, and the discrepancy index indicated that the component community of *P. avanhandavae* showed aggregated pattern. All helminths of this study are new host record, since there is not any study on helminthfauna for this host thus far. However, several other fish species of *Pimelodella* have had their helminthfauna surveyed and this study have recorded similar findings. Table XIII provides a list of the parasites recorded for *Pimelodella* spp. in the Neotropical region. For monogeneans, the species *Ameloblastella formatrium* is recorded for the first time in a member of Heptapteridae, since it is only known record in the literature is in the type-host *Hypophthalmus edentatus* Spix and Agassiz, 1829 (Mendoza-Franco *et al.*, 2016) and also in this study in *H. platyrhynchos* but both belong to Pimelodidae, however, only one specimen of *A. formatrium* was found in *P. avanhandavae* in this study, which might be the case of an accidental infection. *Aphanoblastella chagresii*, described in *Pimelodella chagresii* (Steindachner, 1876) (Mendoza-Franco *et al.*, 2007), showed high prevalence in this study (see Table III). *Dadaytrema gracilis*, *Genarchella parva*, and *Phyllodistomum spatula* (see Table XIII for parasites descriptors) were recorded in *Pimelodella gracilis* (Valenciennes, 1835), *Pimelodella howesi* Fowler, 1940, and *Pimelodella laticeps* Eigenmann, 1917, showing similarity with the findings of this study in which *Dadaytrema* sp., *Genarchella* sp., and *Phyllodistomum* sp. was recorded (Lunaschi, 1989; Hamman, 1989; Lunaschi and Martorelli, 1990). Likewise, it was possible to observe similarity among the nematode fauna of this study and the other records for *Pimelodella* species. *Brasilnema pimelodellae*, *Spinitectus rodolphiheringi*, *Spinitectus yorkei*, *Spinitectus multipapillatus*, and *R. rondoni* (see Table XIII for parasites descriptors) were recorded in *Pimelodella lateristriga* (Lichtenstein, 1823),

Pimelodella hasemanni Eigenmann, 1917, and *Pimelodella gracilis*, respectively (Travassos *et al.*, 1928; Petter, 1984, 1987; Moravec *et al.*, 1992b; Kohn *et al.*, 2011) and in this study *B. pimelodellae*, *R. rondoni*, and *Spinitectus* sp. were recorded. *Brasilnema pimelodellae* has only been reported in *Pimelodella lateristriga*, which may suggest host-specificity of this parasite with the genus *Pimelodella*. The species *Spinitectus* sp. found in this study is regarded as a new species to science (see section 6. for description).

Table XIII. Parasites recorded for *Pimelodella* spp. (Siluriformes: Heptapteridae) in the Neotropical region.

Parasite	Host	Reference	Locality
Monogenea			
Heptapteridae			
Gyrodactylidae			
<i>Gyrodactylus pimelodellus</i> An, Jara and Cone, 1991	<i>Pimelodella yuncensis</i> Steindachner, 1902	An <i>et al.</i> (1991)	Peru
<i>Scleroductus yuncensi</i> Jara, An and Cone, 1989	<i>Pimelodella yuncensis</i>	Jara and Cone (1989)	Peru
Dactylogyridae			
<i>Aphanoblastella travassosi</i> (Price, 1938)	<i>Pimelodella laticeps</i> Eigenmann, 1917	Kritsky <i>et al.</i> (2000)	Argentina
<i>Aphanoblastella chagresii</i> Mendoza-Franco, Aguirre- Macedo and Vidal-Martínez, 2007	<i>Pimelodella chagresii</i> (Steindachner, 1876)	Mendoza-Franco <i>et al.</i> (2007)	Panamá
<i>Demidospermus cornicinus</i> Kritsky and Gutiérrez, 1998	<i>Pimelodella</i> sp.	Cohen and Kohn (2008)	Brazil
<i>Demidospermus leptosynophallus</i> Kritsky and Gutiérrez, 1998	<i>Pimelodella</i> sp.	Cohen and Kohn (2008)	Brazil
Digenea			
<i>Dadaytrema gracilis</i> Lunaschi, 1989	<i>Pimelodella gracilis</i> (Valenciennes, 1835)	Lunaschi (1989)	Argentina
<i>Genarchella parva</i> Travassos, Artigas and Pereira, 1928	<i>Pimelodella gracilis</i>	Hamann (1989)	Argentina
<i>Genarchella parva</i>	<i>Pimelodella howesi</i> Fowler, 1940	Hamann (1989)	Argentina
<i>Parspina argentinensis</i> (Szidat, 1954)	<i>Pimelodella lateristriga</i> (Lichtenstein, 1823)	Fernandes and Kohn (2001)	Brazil
	<i>Pimelodella gracilis</i>	Ostrowski de Núñez <i>et al.</i> (2011)	Argentina
<i>Acanthostomum gnerii</i> Szidat, 1954	<i>Pimelodella laticeps</i>	Lunaschi (1986)	Argentina

Table XIII. Continued.

Parasite	Host	Reference	Locality
		Ostrowski de Núñez and de Pertierra (1991)	Argentina
<i>Parspina bagre</i> Pearse, 1920	<i>Pimelodella metae</i> Eigenmann, 1917	Pearse (1920)	Venezuela
<i>Parspina pimelodellae</i> Arredondo and de Núñez, 2013	<i>Pimelodella gracilis</i>	Arredondo and de Núñez (2013)	Argentina
<i>Phyllodistomum spatula</i> Odhner, 1902	<i>Pimelodella laticeps</i>	Lunaschi and Martorelli (1990)	Argentina
Nematoda			
<i>Spinitectus multipapillatus</i> Petter, 1987	<i>Pimelodella</i> sp.	Petter (1987)	Equator
	<i>Pimelodella hasemanni</i> Eigenmann, 1917	Petter (1987)	Equator
<i>Spinitectus rodolphiheringi</i> Vaz and Pereira, 1934	<i>Pimelodella lateristriga</i>	Vaz and Pereira (1934)	Paraguay
<i>Spinitectus yorkei</i> Travassos, Artigas and Pereira, 1928	<i>Pimelodella lateristriga</i>	Travassos <i>et al.</i> (1928)	Brazil
<i>Cucullanus (Cucullanus) pimelodellae</i> Moravec, Kohn and Fernandes, 1993	<i>Pimelodella lateristriga</i>	Moravec <i>et al.</i> (1993)	Brazil
<i>Cucullanus (Cucullanus) pinnai pinnai</i> Travassos, Artigas and Pereira, 1928	<i>Pimelodella gracilis</i>	Moravec <i>et al.</i> (1997a)	Brazil
<i>Brasilnema pimelodellae</i> Moravec, Kohn and Fernandes, 1992	<i>Pimelodella lateristriga</i>	Moravec <i>et al.</i> (1992a,b)	Brazil
<i>Procamallanus (Spirocamallanus) pimelodus</i> Pinto, Fábio, Noronha and Rolas, 1974	<i>Pimelodella lateristriga</i>	Moravec <i>et al.</i> (1993)	Brazil
<i>Procamallanus (Spirocamallanus) rarus</i> Travassos, Artigas and Pereira, 1928	<i>Pimelodella lateristriga</i>	Travassos <i>et al.</i> (1928)	Brazil
<i>Rhabdochona (Rhabdochona) acuminata</i> Molin, 1860	<i>Pimelodella lateristriga</i>	Vaz and Pereira (1934)	Brazil
Cestoda			
Cestoda gen. sp.	<i>Pimelodella lateristriga</i>	Kohn <i>et al.</i> (2011)	Brazil

The helminth component community of *L. platymetopon* is not diverse, which was showed by the diversity indexes values. This study is a new host record for *Austrodiplostomum* sp. metacercariae and a gyrodactylid monogenean. The other helminths were previously described in this host (see Table XII). *Demidospermus paranaensis* and *R. (Sprentascaris) mahnerti* occurred in high prevalence (see Table III) and they were both described in *L. platymetopon* (Ferrari-Hoeinghaus *et al.*, 2010; Petter and Cassone, 1984). This study recorded five *taxa* of digeneans, but all at the metacercariae level, suggesting that this host plays an important role as intermediate host of these trematodes and it is used as food resource by their final host. Some studies also reported metacercariae of *Clinostomum* sp. in *L. platymetopon* and they discuss about the important role of this fish species as intermediate host of these parasites (Eiras *et al.*, 1999; Dias *et al.*, 2006; Ferrari-Hoeinghaus *et al.*, 2007). *Raphidascaris (Sprentascaris) mahnerti* has been reported in other hosts, but the majority belong to Loricariidae family, with only one record in a serrasalmid (Luque *et al.*, 2011).

The host species *P. ambrosettii* presented the most diverse helminth component community of this study, which was showed by the diversity indexes values, besides, the helminth component community of *P. ambrosettii* tends to show aggregated pattern. All helminths found in this study are new host record given that there are no studies on helminthfauna for this fish host (see Table II). However, there are several studies on helminth parasites of other *Pterygoplichthys* spp., which are present in Table XIV. For dactylogyrids, the host species *P. ambrosettii* presented an unexpected diversity harboring 10 different species on the gills and one in the nasal cavity (see Table III). Furthermore, a remarkable diversity of *Heteropriapulus* spp. were found in this host: two of the already described species *H. heterotylus* and *H. simplex* and four other morphotypes regarded as putative new species in this study. Data on the morphology and molecular phylogenetic relationships of *Heteropriapulus* spp. are presented in section 5. Congeneric monogeneans exploring the same host can be a case of intrahost (or sympatric) speciation (Poulin, 2002). Kennedy and Bush (1992) also suggest that the existence of multiple congeneric parasite species in a host population may be the case of species flocks, resulting from small-scale radiation event. *Heteropriapulus* spp., *Trinigyrus* spp., and *Unilatus* spp. are restricted to loricariid fishes, however, *Telethecium nasalis* was described from the nasal cavity of *Osteoglossum bicirrhosum* (Cuvier, 1829) from the Amazon in Brazil (Kritsky *et al.*, 1996) and was reported only once more by

Lemos *et al.* (2012) in the same host in the Brazilian Amazon. As for digeneans, only metacercariae of *Austrodiplostomum* sp. were found, thus this host might be important as intermediate host for this trematode and it might be used as food resource by their final host.

Table XIV. Parasites recorded for *Pterygoplichthys* spp. (Siluriformes: Loricariidae) in the Neotropical region.

Parasite	Host	Reference	Locality
Monogenea	Loricariidae		
<i>Heteropriapulus heterotylus</i> (Jogunoori, Kritsky and Venkatanarasaiah, 2004)	<i>Pterygoplichthys disjunctivus</i> (Weber, 1991)	Nitta and Nagasawa (2013)	Japan
		Rodriguez-Santiago <i>et al.</i> (2015), (2016)	Mexico
	<i>Pterygoplichthys pardalis</i> (Castelnau, 1855)	Rodriguez-Santiago <i>et al.</i> (2015), (2016)	Mexico
		Rodriguez-Santiago <i>et al.</i> (2016)	Mexico
<i>Heteropriapulus</i> sp.	<i>Pterygoplichthys pardalis</i>	Porto <i>et al.</i> (2012)	Brazil
<i>Unilatus brittani</i> Mizelle, Kritsky and Crane, 1968	<i>Pterygoplichthys anisitsi</i> Eigenmann and Kennedy, 1903	Mendoza-Palmero <i>et al.</i> (2012)	Peru
	<i>Pterygoplichthys multiradiatus</i> (Hancock, 1828)	Suriano (1985)	Brazil
<i>Unilatus unilatus</i> Mizelle and Kritsky, 1967	<i>Pterygoplichthys anisitsi</i>	Mendoza-Palmero <i>et al.</i> (2012)	Peru
	<i>Pterygoplichthys multiradiatus</i>	Suriano (1985)	Brazil
<i>Unilatus</i> sp.	<i>Pterygoplichthys pardalis</i>	Porto <i>et al.</i> (2012)	Brazil
Digenea			
<i>Austrodiplostomum compactum</i> (Lutz, 1928)	<i>Pterygoplichthys pardalis</i>	Porto <i>et al.</i> (2012)	Brazil
<i>Dadaytrema</i> sp.	<i>Pterygoplichthys aculeatus</i> (Perugia, 1891)	Kohn <i>et al.</i> (2011)	Brazil
<i>Dendrorchis</i> sp.	<i>Pterygoplichthys</i> sp.	Kohn <i>et al.</i> (2011)	Brazil
<i>Genarchella tropica</i> (Manter, 1936)	<i>Pterygoplichthys aculeatus</i>	Kohn <i>et al.</i> (2011)	Brazil

Table XIV. Continued.

Parasite	Host	Reference	Locality
<i>Megacoelium spinicavum</i> Thatcher and Varella, 1981	<i>Pterygoplichthys pardalis</i>	Thatcher and Varella (1981)	Brazil
	<i>Pterygoplichthys pardalis</i>	Porto <i>et al.</i> (2012)	Brazil
<i>Megacoelium spinispecum</i> Thatcher and Varella, 1981	<i>Pterygoplichthys</i> sp.	Thatcher and Varella (1981)	Brazil
<i>Saccocoelioides magnus</i> Szidat, 1954	<i>Pterygoplichthys aculeatus</i>	Kohn <i>et al.</i> (2011)	Brazil
Nematoda			
<i>Ichthyouris brasiliensis</i> Moravec, Kohn and Fernandes, 1992	<i>Pterygoplichthys aculeatus</i>	Moravec <i>et al.</i> (1992)	Brazil
<i>Guyanema</i> sp.	<i>Pterygoplichthys multiradiatus</i>	Moravec <i>et al.</i> (1997b)	Venezuela
<i>Procamallanus (Procamallanus) annipetterae</i> Kohn and Fernandes, 1988	<i>Pterygoplichthys aculeatus</i>	Kohn <i>et al.</i> (2011)	Brazil
Acanthocephala			
<i>Gorytocephalus</i> sp.	<i>Pterygoplichthys pardalis</i>	Porto <i>et al.</i> (2012)	Brazil
<i>Gracilisentis variabilis</i> (Diesing, 1856)	<i>Pterygoplichthys lituratus</i> (Kner, 1854)	Santos <i>et al.</i> (2008)	Brazil

The diversity of the helminth component community of *R. aspera* was the lowest among all host species analyzed in this study, which was showed by the diversity indexes values. The only species already recorded for *R. aspera* are the monogenean *Paranaella luquei* (a microcotylid) (Kohn *et al.*, 2000) and the nematode *P. petterae* (Moravec *et al.*, 1992a) (Table XII) and both were described in this host. This study recovered one dactylogyrid species in the nasal cavity and two in the gills of *R. aspera*. The dactylogyrid *T. nasalis* was recovered from the nasal cavity and it was also found in this study in *P. ambrosettii* that is also a loricariid. The type host of *T. nasalis* is an osteoglossid fish from the Amazon, this fish and those two loricariids might be providing similar habitat conditions for this parasite. Some monogenean genera may have a wider host range including distantly related host species (Braga *et al.*, 2014). A putative new species of *Demidospermus* was found in this host, and data on the morphology and molecular phylogenetic relationships of this dactylogyrid is provided in section 4. Another morphotype of *Heteropriapulius* sp. was found in *R. aspera* and is regarded as a new

species – data on its morphology is presented in section 5. This study is a new host record for *Austrodiplostomum* sp. metacercariae in *R. aspera* and it can be suggested that this parasite uses this fish as intermediate host that will be ingested by the final host of the trematode so that it can complete its life cycle. *Rhinelepis aspera* showed in this study high rates of infection by the nematode *P. petterae*. This nematode was described in *R. aspera* (Moravec *et al.*, 1992a) and ever since there are no reports of this nematode in other host species, thus indicating host-specificity.

The helminth component community of *H. platyrhynchos* is not very diverse, as showed by the diversity indexes values, but it tends to show aggregated pattern. The monogeneans *P. pavanellii*, *Ameloblastella* sp. 2, *A. formatrium* are new host record. Two other monogeneans species (namely Dactylogyridae gen. sp. 1 and 2) do not show any resemblance with none of the dactylogyrids species described in the literature, being considered in this study as new species, or even new genus, to science. Section 4. provides data on molecular phylogenetic relationships of these two dactylogyrids, however these will be formally described in a separated publication. There is only one monogenean species recorded for *H. platyrhynchos*, which is *Vancleaveus platyrhynchi* (Kritsky *et al.*, 1986; Mendoza-Palmero *et al.*, 2012). This way, this study shows that the monogenean fauna of *H. platyrhynchos* has been neglected considering that only this survey was able to find four different species (one already described and three putative new species). Metacercariae of *Austrodiplostomum* sp. is a new host record in *H. platyrhynchos*, which suggest that this parasite uses this fish as intermediate host that will be consumed by the final host of the parasite so it can complete its life cycle. For endoparasites, there are several records of digeneans, nematodes, cestodes, and acanthocephalans, however any of these species were observed in this study (see Table XII for all heminths previously recorded in *H. platyrhynchos*).

For *S. lima*, the helminth component community can be considered as diverse, which was showed by the diversity indexes. There are three records of dactylogyrids in *S. lima* (Table XII): *Urocleidoides megorchis* that was described in *S. lima* by Mizelle and Kritsky (1969), however there is not any other record of that monogenean even since; *Ameloblastella unapioides* (Mendoza-Franco *et al.*, 2016) that was recently described in *S. lima* from the Peruvian Amazon; and a morphotype named Dactylogyridae gen. sp. 12 by Mendoza-Palmero *et al.* (2015), in which only molecular data is provided. As for this study, only one monogenean species was recorded (Dactylogyridae gen. sp. 5) that does not

present any resemblance with dactylogyrids described thus far, therefore regarded as a putative new species or even new genus. Section 4. provides data on molecular phylogenetic relationships of this parasite but its formal description will be part of a separated publication. For endoparasites, this study represents new host record for *Austrodiplostomum* sp. metacercariae, suggesting that this parasite uses this fish as intermediate host that will be ingested by the final host of and the parasite can complete its life cycle. The endoparasites found in this study are in accordance with previous records for *S. lima*, as showed in Table XII. This study is also a new host record for *Contracaecum* sp. larvae in *S. lima*. The presence of these larvae indicate that this host is acting as intermediate host for this parasite. Larvae of *Contracaecum* sp. has been largely reported in several fish host species belonging to distinct families and genera (Moravec, 1998; Luque *et al.*, 2011). Larvae of anisakid nematodes are a problem of human health due to their zoonotic potential as causative agents anisakiasis and food-borne allergens (Daschner and Pascual, 2005). *Sorubim lima* is used for human consumption, this way the population that consumes this fish should be aware of proper cooking of this fish flesh, since larvae of *Contracaecum* sp. can be encysted in the musculature of the fish.

From the 25 monogeneans *taxa* found, only *A. formatrium*, *P. pavanellii*, and *T. nasalis* occurred in more than one host. The gyrodactylids were not identified at species level. Monogenea is considered the most host-specific group of parasites (Kearn, 1998; Whittington *et al.*, 2000). Some species are highly host-specific, which are restricted to one single host or a few related host species, and some can be even niche-specific species, which are restricted to special microhabitats within the same host species (Šimková *et al.*, 2006). The high host-specificity of monogeneans indicate that they probably have coevolved closely with their hosts (Barker, 1991). However, this host-specificity may not necessarily reflect a historical association between host and parasites, but can also be the result of host switching, parasite adaptation, and speciation (Brooks, 1979; Brooks and McLennan, 1993; Norton and Carpenter, 1998). Host specificity can also be a function of host range, which is the number of host species that could be colonized by a certain parasite species (Lymbery, 1989; Poulin, 1998; Šimková *et al.*, 2006).

Metacercariae of *Austrodiplostomum* sp. were the most widely distributed *taxon*, occurring in seven fish species. Type 1 metacercariae of Diplostomidae also showed wide distribution, occurring in six host species. Metacercariae of *Austrodiplostomum* spp. have been reported in a wide range of fish hosts (Eiras *et al.*, 2010; Locke *et al.*, 2015a), which

allows to consider as a genus with generalist species. *Dadaytrema oxycephala* as well as other members of the genera have been recorded in several host species of different genera (Kohn *et al.*, 2007; Eiras *et al.*, 2010). The same occurs for member of the genera *Genarchella* and *Phyllodistomum* (Kohn *et al.*, 2007; Eiras *et al.*, 2010). Metacercarie of *Clinostomum* spp. have been reported in many different fish species in Brazil and all over the world (Locke *et al.*, 2015b; Acosta *et al.*, 2016). Therefore, all the digenean species found in this study can be regarded as generalist species. Parasites that have indirect life cycles show less specificity than parasites with direct cycle (Noble *et al.*, 1989). Notes on host specificity for the nematodes found in this study are mentioned in the above paragraphs. The cestodes *M. bracademoca* and *S. maringaensis* have been recorded only in the pimelodids *H. platyrhynchos* and *S. lima* (Alves *et al.*, 2017 and references therein).

Brazil is considered a megadiverse country and holds the highest biodiversity of freshwater fishes in the Neotropical region (Heywood, 1995; Luque and Poulin, 2007). The Upper Paraná river basin harbor approximately 310 species of fishes, distributed in 11 orders and 38 families and from this total, 41% belong to Siluriformes order (Langeani *et al.*, 2007). For the Aguapeí River, siluriforms represent 45.2% of the whole ichthyofauna (Vilela *et al.*, 2015). Population density of different host species could be the determinant of parasite species richness, since the number of parasite species is a reflex of the number of host species in a certain locality and the parasites' capacity of transmission and infection of intermediate and definitive hosts (Kennedy, 1993; Takemoto *et al.*, 2009). However, it is still impossible to make an inventory of all groups of parasites because of taxonomic constraints and the existence of cryptic species (Poulin, 2014). Therefore, it is necessary a good knowledge of species composition to better understand the role of the parasite communities in an ecosystem, this way, continuing efforts for taxonomic surveys and species inventories are of great importance (Takemoto *et al.*, 2009).

This study recorded 54 helminth *taxa* in eight siluriform species that have not been thoroughly surveyed for helminth parasites, specially for monogeneans. From this total, 25 are monogenean with 13 species that are putatively new to science and some other represent first host and or geographical record; 15 digeneans from which some are new host or geographical record; 11 nematodes with one species new to science, and three cestodes. Therefore, this study has considerably contributed to increase the knowledge on the vast and far to be known diversity of helminth parasites of Neotropical

siluriform, specially regarding the Aguapeí River. However, more studies on helminth diversity are necessary for the Aguapeí River, since this study represented only a very small portion of the actual diversity of parasites, including putatively new species, that this ecosystem might be holding.

3.5. References

- Acosta A.A., Caffara M., Fioravanti M.L., Utsunomia R., Zago A.C., Franceschini L., Silva R.J. 2016. Morphological and Molecular Characterization of *Clinostomum detruncatum* (Trematoda: Clinostomidae) Metacercariae Infecting *Synbranchus marmoratus*. *Journal of Parasitology*, 102(1):151–156. DOI: 10.1645/15-773
- Aguiar K.D. 2008. Influência de uma barragem sobre atributos ecológicos da comunidade e biologia reprodutiva de peixes do rio Paraíba do Sul, UHE Ilha dos Pombos, Rio de Janeiro, Brasil. Master Dissertation, Mestrado em Ecologia e Conservação, Universidade Federal do Paraná (UFPR), Paraná, Brazil.
- Albert J.S., Reis R.E. 2011. Introduction to Neotropical Freshwater. In: (Eds. Albert, J.S., Reis, R. E.) *Historical Biogeography of Neotropical Freshwater Fishes*. California: University of California Press. pp. 3–19.
- Alves P.V., de Chambrier A., Scholz T., Luque J.L. 2017. Annotated checklist of fish cestodes from South America. *Zookeys* [in press].
- An L., Jara C.A., Cone D.K. 1991. Five species of *Gyrodactylus* Nordmann, 1832 (Monogenea) from freshwater fishes of Peru. *Canadian Journal of Zoology*, 69, 1199–1201.
- Arredondo N.J., Gil De Pertierra A.A. 2008. The taxonomic status of *Spatulifer* cf. *maringaensis* Pavanelli & Rego, 1989 (Eucestoda: Proteocephalidea) from *Sorubim lima* (Bloch & Schneider) (Pisces: Siluriformes), and the use of the microthrix pattern in the discrimination of *Spatulifer* spp. *Systematic Parasitology*, 70, 223–236. DOI: 10.1007/s11230-008-9142-x
- Arredondo N.J., Ostrowski De Núñez M.C. 2013. A new species of *Paraspina* Pearse, 1920 (Digenea: Cryptogonimidae) from *Pimelodella gracilis* (Valenciennes) (Siluriformes: Heptapteridae) in the Paraná River basin, Argentina, and a key to the genus. *Systematic Parasitology*, 84, 81–87. DOI: 10.1007/s11230-012-9394-3
- Barker S.C. 1991. Evolution of host-parasite associations among species of lice and rock-wallabies: coevolution? *International Journal for Parasitology* 21, 497–501.

- Begon M., Townsend C.R., Harper J.L. 2007. *Ecologia de Indivíduos a Ecossistemas*. 4th ed. Artmed, Porto Alegre, 750 pp.
- Bizerril C.R.S.F., Primo P.B.S. (Eds.). 2001. *Peixes de águas interiores do Estado do Rio de Janeiro*. Fundação de Estudos do Mar, Rio de Janeiro, 417 pp.
- Boeger W.A., Kritsky D.C. 1997. Coevolution of the Monogenoidea (Platyhelminthes) based on a revised hypothesis of parasite phylogeny. *International Journal for Parasitology*, 27, 1495–1511.
- Braga M.P., Araújo S.B.L., Boeger W.A. 2014. Patterns of interaction between Neotropical freshwater fishes and their gill Monogenoidea (Platyhelminthes). *Parasitology Research*, 113, 481–490. DOI: 10.1007/s00436-013-3677-8
- Brabec J., Scholz T., Králová-Hromadová I., Bazsalovicsová E., Olson P.D. 2012. Substitution saturation and nuclear paralogs of commonly employed phylogenetic markers in the Caryophyllidea, an unusual group of non-segmented tapeworms (Platyhelminthes). *International Journal of Parasitology*, 42, 259–267. DOI: 10.1016/j.ijpara.2012.01.005
- Brooks D.R. 1979. Testing the context and extent of host-parasite coevolution. *Systematic Zoology*, 26, 277–289.
- Brooks D.R., McLennan D.A. 1993. Comparative study of adaptive radiations with an example using parasitic flatworms (Platyhelminthes: Cercomeria). *The American Naturalist*, 142, 755–778.
- Brooks D.R., Hoberg E.P. 2001. Parasite systematics in the 21st century: opportunities and obstacles. *Trends in Parasitology*, 17, 273–275.
- Bueno-Silva M., Boeger W.A., Pie M.R. 2011. Choice matters: incipient speciation in *Gyrodactylus corydori* (Monogenoidea: Gyrodactylidae). *International Journal for Parasitology*, 41, 657–667. DOI: 10.1016/j.ijpara.2011.01.002
- Bush A.O., Holmes J.C. 1986. Intestinal helminths of lesser scaup ducks: an interactive community. *Canadian Journal of Zoology*, 64(1), 142–152. DOI: 10.1139/z86-023
- Bush A.O., Lafferty K.D., Lotz J.M., Shostak A.W. 1997. Parasitology meets ecology on its own terms: Margolis et al. revisited. *The Journal of Parasitology*, 83(4), 575–583.
- Cohen S.C., Kohn A. 2008. New data on species of *Demidospermus* (Dactylogyridae: Monogenea) parasitizing fishes from the reservoir of the Itaipu Hydroelectric

- Power Station, Paraná State, Brazil, with new synonymies. *Revista Brasileira de Parasitologia Veterinária*, 17, 167–170.
- Cohen S.C., Justo M.C.N., Kohn A. (Eds.). 2013. South American Monogeneoidea Parasites of Fishes, Amphibians and Reptiles. Fundação Oswaldo Cruz – FIOCRUZ and Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq, Oficina de Livros, Rio de Janeiro, 663 pp.
- Colwell R.K., Coddington J.A. 1994. Estimating terrestrial biodiversity through extrapolation. *Philosophical Transactions of the Royal Society B*, 345, 101–118.
- Cribb T.H., Chisholm L.A., Bray R.A. 2002. Diversity in the Monogenea and Digenea: does lifestyle matter? *International Journal for Parasitology*, 32, 321–328. DOI: 10.1016/S0020-7519(01)00333-2
- Crispim J.Q., Stevaux J.C. 2003. Alterações na hidrologia do canal do Rio Paraná após a construção do reservatório de Porto Primavera. In: Universidade Estadual De Maringá. Nupélia/PELD. A planície de inundação do alto rio Paraná: Site 6 PELD/CNPq – Relatório anual 2002. Maringá, 4 p.
- Daschner A., Pascual, C.Y. 2005. Anisakis simplex: Sensitization and clinical allergy. *Current Opinion in Allergy and Clinical Immunology* 5, 281–285.
- De Chambrier A., Vaucher, C. 1999. Proteocephalidae et Monticelliidae (Eucestoda: Proteocephalidea) parasites de poissons d'eau douce au Paraguay, avec descriptions d'un genre nouveau et de dix espèces nouvelles. *Revue suisse de Zoologie*, 106, 165–240.
- De Chambrier A., Scholz T., Kuchta, R., Posel P., Mortenthaler M., Guardia C.C. 2006. Tapeworms (Cestoda: Proteocephalidea) of fishes from the Amazon River in Peru. *Comparative parasitology*, 73, 111–120. DOI: 10.1654/4182.1
- De Chambrier A., Kuchta R., Scholz T. 2015a. Tapeworms (Cestoda: Proteocephalidea) of teleost fishes from the Amazon River in Peru: additional records as an evidence of unexplored species diversity. *Revue Suisse de Zoologie*, 122, 149–163. DOI: 10.5281/zenodo.14580
- De Chambrier A., Waeschenbach A., Fisseha M., Scholz T., Mariaux J. 2015b. A large 28S rDNA-based phylogeny confirms the limitations of established morphological characters for classification of proteocephalidean tapeworms (Platyhelminthes, Cestoda). *ZooKeys*, 500, 25–59. DOI:10.3897/zookeys.500.9360

- Dias P.G., Furuya W.M., Pavanelli G.C., Machado M.H., Takemoto R.M. 2004. Carga parasitária de *Rondonia rondoni*, Travassos, 1920 (Nematoda, Atractidae) e fator de condição do armado, *Pterodoras granulosus*, Valenciennes, 1833 (Pisces, Doradidae). *Acta Scientiarum Biological Sciences*, 26(2), 151–156.
- Dias M.L.G.G., Minte-Vera C.V., Eiras J.C., Machado M.H., Souza G.T.R., Pavanelli, G.C. 2006. Ecology of *Clinostomum complanatum* Rudolphi, 1814 (Trematoda: Clinostomidae) infecting fish from the floodplain of the high Paraná River, Brazil. *Parasitology Research*, 99, 675–681. DOI: 10.1007/s00436-006-0205-0
- Eiras J.C., Dias M.L.G.G., Pavanelli G.C., Machado M.H. 1999. Histological studies on the effects of *Clinostomum marginatum* (Digenea: Clinostomidae) in its second intermediate host *Loricariichthys platymetopon* (Osteichthyes, Loricariidae) of upper Paraná River, Brazil. *Acta Scientiarum Biological Sciences*, 21(2), 237–241.
- Eiras J.C., Takemoto R.M., Pavanelli G.C. (Eds.). 2006. Métodos de estudo e técnicas laboratoriais em parasitologia de peixes. 2nd ed. Eduem, Maringá, 199 pp.
- Eiras J.C., Takemoto R.M., Pavanelli G.C. (Eds.). 2010. Diversidade de peixes de água doce do Brasil. Clichetec, Maringá, 333 pp.
- Ergens R. 1969. The suitability of ammonium picrate-glycerin in preparing slides of lower Monogenea. *Folia Parasitologica*, 16, 320.
- Fernandes B.M.M., Kohn A. 2001. On some trematode parasites of fishes from Paraná River. *Brazilian Journal of Biology*, 61(3), 461–466.
- Ferrari-Hoeninghaus A.P., Takemoto R.M., Pavanelli G.C. 2007. Digenetic trematode parasites of *Loricariichthys platymetopon* (Loricariidae: Siluriformes) of the upper Paraná river floodplain, Brazil. *Acta Scientiarum Biological Sciences*, 29(3), 327–329.
- Ferrari-Hoeninghaus A.P., Bellay S., Takemoto R.M., Pavanelli G.C. 2010. A new species *Demidospermus* Suriano, 1983 (Monogenea, Dactylogyridae) parasitic on *Loricariichthys platymetopon* Isbrücker et Nijssen (Loricariidae, Siluriformes) from the Upper Paraná River floodplain, Brazil. *Acta Parasitologica*, 55, 16–19. DOI: 10.2478/s11686-010-0007-5
- Fortes E., Hoffmann R.P., Querol M.V.M. 1999. Presença de *Raphidascaris* (*Sprentascaris*) *mahnerti* (Petter et Cassone, 1984) Nematoda, Anisakidae em *Loricariichthys platymetopon* (cascudo viola) da bacia do Rio Uruguai medio,

- Uruguaiana, Rio Grande do Sul, Brasil. *Revista Brasileira de Medicina Veterinaria*, 21, 13–14.
- Gibson D.I., Jones A., Bray R.A. 2002, 2005, 2008. Keys to the Trematoda. Vols. 1, 2, 3. CABI Publishing and The Natural History Museum, London, UK.
- Gil De Pertierra, A.A. 2005. Comparative study of the microtriches of adult Cestodes (Proteocephalidea: Monticelliidae) and some comments on their systematic value. *Zoologischer Anzeiger*, 243, 295–304.
- Guidelli G.M., Isaac A., Pavanelli G.C. 2002. *Sanguinicola platyrhynchi* n. sp. (Digenea: Sanguinicolidae) parasite of visceral cavity of *Hemisorubim platyrhynchos* (Valenciennes, 1840) (Pisces: Pimelodidae) from the floodplain of the Upper Paraná River, Brazil. *Brazilian Journal of Biology*, 62(4B), 801–806.
- Guidelli G.M., Isaac A., Takemoto R.M., Pavanelli, G.C. 2003. Endoparasites infracommunities of *Hemisorubim platyrhynchos* (Valenciennes, 1840) (Pisces: Pimelodidae) of the Baía River, Upper Paraná River Floodplain, Brazil: specific composition and ecological aspects. *Brazilian Journal of Biology*, 63(2), 261–268.
- Hammann M.I. 1989. *Genarchella* Travassos, Artigas & Pereira, 1928 (Digenea: Hemiuridae) parasitos de peces de agua dulce del rio Paraná, provincia de Corrientes, Republica Argentina. 1: Anatomia y posicion sistemática. 2: Contribuciones ecologicas. *Physis (Buenos Aires)*, 47, 15–30.
- Heywood V.H. 1995. Global Biodiversity Assessment. United Nations Environment Programme. Cambridge University Press, Cambridge.
- Holmes J.C., Price P.W. 1986. Communities of parasites. In: (Eds. D.J. Anderson and J. Kikkawa) *Community ecology: Patterns and process*. Blackwell Scientific Publications, Oxford, U.K, 187–213.
- Humason G.L. 1979. Animal tissue techniques, W.H. Freeman Co., San Francisco, California, 661 pp.
- Hypša V., Škeríková A., Scholz, T. 2005. Phylogeny, evolution and host–parasite relationships of the order Proteocephalidea (Eucestoda) as revealed by combined analysis and secondary structure characters. *Parasitology*, 130, 359–371. DOI: 10.1017/S0031182004006638
- Jara C.A., Cone D.K. 1989. *Scleroductus yuncensi* gen. et sp. n. (Monogenea) from *Pimelodella yuncensis* (Siluriformes: Pimelodidae) in Peru. *Proceedings of the Helminthological Society of Washington*, 56: 125–127.

- Kearn G.C. 1998. Parasitism and the Platyhelminths. Chapman and Hall, London.
- Kearse M., Moir R., Wilson A., Stones-Havas S., Cheung M., Sturrock S., Buxton S., Cooper A., Markowitz S., Duran C., Thierer T., Ashton B., Mentjies P., Drummond A. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, 28, 1647–1649. DOI: 10.1093/bioinformatics/bts199
- Kennedy C.R. 1993. The dynamics of intestinal helminth communities in eels *Anguilla anguilla* in a small stream: long-term changes in richness and structure. *Parasitology*, 10(1), 71–78.
- Kennedy C.R., Bush A.O. 1992. Species richness in helminth communities: the importance of multiple congeners. *Parasitology*, 104, 189–197.
- Kohn A., Fernandes B.M.M., Cohen S.C. (Eds.). 2007. South American Trematodes Parasites of Fishes. Editora Imprinta, Rio de Janeiro, 318 pp.
- Kohn A., Baptista-Farias M.D., Cohen S.C. 2000. *Paranaella luquei* gen. et sp. n. (Monogenea: Microcotylidae), a new parasite of Brazilian catfishes. *Folia Parasitologica*, 47, 279–83. DOI: 10.14411/fp.2000.048
- Kohn A., Moravec F., Cohen S.C., Canzi C., Takemoto R.M., Fernandes M.M. 2011. Helminths of freshwater fishes in the reservoir of the Hydroelectric Power Station of Itaipu, Paraná, Brazil. *Checklist*, 7(5), 681–690.
- Krebs C.J. 1989. Ecological methodology. Harper & Row, New York.
- Kritsky D.C., Thatcher V.E., Boeger W.A. 1986. Neotropical Monogenea. 8. Revision of *Urocleidoides* (Dactylogyridae, Ancyrocephalinae). *Proceedings of the Helminthological Society of Washington*, 53, 1–37.
- Kritsky D.C., Van Every L.R., Boeger W.A. 1996. Neotropical Monogenoidea. 27. Two new Species of *Telethecium* gen. sp. from the Nasal Cavities of Central Amazonian Fishes and a Redescription of *Kritskyia moravecii* Kohn, 1990 (Dactylogyridae, Ancyrocephalinae). *Journal of the Helminthological Society of Washington*, 63(1), 35–41.
- Kritsky D.C., Mendoza-Franco E.F., Scholz, T. 2000. Neotropical Monogenoidea. 36. Dactylogyrids from the Gills of *Rhamdia guatemalensis* (Siluriformes: Pimelodidae) from Cenotes of the Yucatan Peninsula, Mexico, with Proposal of *Ameloblastella* gen. n. and *Aphanoblastella* gen. n. (Dactylogyridae: Ancyrocephalinae). *Comparative Parasitology*, 67(1), 76–84.

- Lafferty K. D. 1997. Environmental parasitology: what can parasites tell us about human impacts on the environment? *Parasitology Today*, 13, 251–255.
- Lafferty K. D., Kuris, A. M. 2005. Parasitism and environmental disturbances. In: (Eds. Thomas F., Renaud F., Guégan J. F.) *Parasitism & Ecosystems*. Oxford University Press, p. 113–123.
- Langeani F., Castro R.M.C., Oyakawa O.T., Shibatta O.A., Pavanelli C.S., Casatti L. 2007. Diversidade da ictiofauna do Alto Rio Paraná: composição atual e perspectivas futuras. *Biota Neotropica*, 7(3), 181–197.
- Locke S.A., Al-Nasiri F.S., Caffara M., Drago F., Kalbe M., Angela Rose Lapierre A.R., McLaughlin J.D., Nie P., Overstreet R.M., Souza G.T.R., Takemoto R.M., Marcogliese D.J. 2015a. Diversity, specificity and speciation in larval Diplostomidae (Platyhelminthes: Digenea) in the eyes of freshwater fish, as revealed by DNA barcodes. *International Journal for Parasitology*, 45, 841–855. DOI: 10.1016/j.ijpara.2015.07.001
- Locke S.A., Caffara M., Marcogliese D.J., Fioravanti M.L. 2015b. A large-scale molecular survey of Clinostomum (Digenea, Clinostomidae). *Zoologica Scripta* 44, 203–217. DOI: 10.1111/zsc.12096
- Lucas A.P.C., Fernandes E.S., Tarifa D.S., Lizama M.A.P., Takemoto, R.M. 2011. Relação entre fator de condição relativo (Kn) e abundância de endoparasitos de *Trachydoras paraguayensis* (Eigenmann & Ward, 1907) (Osteichthyes: Doradidae) na Planície de inundação do Alto Rio Paraná, Paraná – Brasil. Anais Eletrônicos VII EPCC - Encontro Internacional de Produção Científica Cesumar, Anais Eletrônicos VII EPCC Maringá, Paraná, Brasil.
- Lunaschi L.I. 1986. Helmintos parasitos de peces de agua dulce de la Argentina V. Redescription de *Acanthostomum (Atrophecaecum) gnerii* Szidat, 1954 (Trematoda-Acanthostomidae). *Neotropica*, 32, 35–42.
- Lunaschi L.I. 1989. Helmintos parasitos de peces de agua dulce de la Argentina VII. Acerca de dos nuevas especies de paranfistomidos (Trematoda-Paramphistomidae). *Neotropica*, 35, 35–42.
- Lunaschi L.I., Martorelli S.R. 1990. Presencia de *Phyllodistomum spatula* Odhner (Trematoda-Gorgoderidae) en dos especies de pimelodidos capturados en la provincia de Buenos Aires, Argentina. Aportes al conocimiento de su ciclo biologico. *Neotropica*, 36, 55–63.

- Luque J. L., Poulin, R. 2007. Metazoan parasite species richness in Neotropical fishes: hotspots and the geography of biodiversity. *Parasitology*, 134, 865–878. DOI: 10.1017/S0031182007002272.
- Luque J.L., Aguiar J.C., Vieira F.M., Gibson D.I., Santos, C.P. 2011. Checklist of Nematoda associated with the fishes of Brazil. *Zootaxa*, 3082, 1–88.
- Luque J.L., Pereira F.B., Alves P.V., Oliva M.E., Timi J.T. 2016. Helminth parasites of South American fishes: current status and characterization as a model for studies of biodiversity. *Journal of Helminthology*, 1–15. DOI: 10.1017/S0022149X16000717
- Lymbery A.J. 1989. Host specificity, host range and host preference. *Parasitology Today*, 9, 298.
- Macarthur R.H., Macarthur, J.W. 1961. On bird species diversity. *Ecology*, 42, 594–598.
- Mariaux J. 1998. A molecular phylogeny of the Eucestoda. *Journal of Parasitology*, 84, 114–124. DOI:10.2307/3284540
- Mendoza-Franco E.F., Aguirre-Macedo M.L., Vidal-Martinez, V.M. 2007. New and previously described species of Dactylogyridae (Monogeneida) from the gills of Panamanian freshwater fishes (Teleostei). *The Journal of Parasitology*, 93(4), 761–771. DOI: 10.1645/GE-1068R.1
- Mendoza-Franco E.F., Mendoza-Palmero C.A., Scholz, T. 2016. New species of *Ameloblastella* Kritsky, Mendoza-Franco & Scholz, 2000 and *Cosmetocleithrum* Kritsky, Thatcher & Boeger, 1986 (Monogenea: Dactylogyridae) infecting the gills of catfishes (Siluriformes) from the Peruvian Amazonia. *Systematic Parasitology*, 93, 847–862. DOI: 10.1007/s11230-016-9671-7
- Mendoza-Palmeiro C.A., Scholz T., Mendonza-Franco E.F., Kuchta R. 2012. New species and geographical records of Dactylogyrids (Monogenea) of catfish (Siluriformes) from the Peruvian Amazonia. *Journal of Parasitology*, 98(3), 484–497. DOI: 10.1645/GE-2941.1
- Mendoza-Palmero C. A., Blasco-Costa I., Scholz T. 2015. Molecular phylogeny of Neotropical monogeneans (Platyhelminthes: Monogenea) from catfishes (Siluriformes). *Parasites and Vectors*, 8, 164. DOI: 10.1186/s13071-015-0767-8
- Mizelle J.D., Kritsky, D.C. 1969. Studies on monogenetic trematodes. XL. New species from marine and freshwater fishes. *American Midland Naturalist*, 82, 417–428.

- Moravec F. (Ed.). 1998. Nematodes of freshwater fishes of the Neotropical Region. Academia Praha, Czech Republic, 464 pp.
- Moravec F., Thatcher V.E. 1997. *Raphidascaroides brasiliensis* n. sp. (Nematoda: Anisakidae), an intestinal parasite of the thorny catfish *Pterodoras granulosus* from Amazonia, Brazil. *Systematic Parasitology*, 38, 65–71.
- Moravec F., Kohn A., Fernandes, B.M.M. 1992a. *Neoparaseuratum travassosi* n. g. sp. (Nematoda: Quimperiidae), a new parasite from thorny catfish *Pterodoras granulosus* in Brazil. *Memórias do Instituto Oswaldo Cruz*, 87(1), 145–150.
- Moravec F., Kohn A., Fernandes B.M.M. 1992b. Nematode parasites of fishes of the Parana River, Brazil. Part 1. Trichuroidea, Oxyuroidea and Cosmocercidae. *Folia Parasitologica*, 39: 327–353.
- Moravec F., Kohn A., Fernandes, B.M.M. 1993. Nematode parasites of fishes of the Paraná River, Brazil. Part 3. Camallanidae and Dracunculoidea. *Folia Parasitologica*, 40, 211–229.
- Moravec F., Kohn A., Fernandes B.M.M. 1997a. New observations on seuratoid nematodes parasitic in fishes of Paraná River, Brazil. *Folia Parasitologica*, 44, 209–223.
- Moravec F., Prouza A., Royero R. 1997b. Some nematodes of freshwater fishes in Venezuela. *Folia Parasitologica*, 44, 33–47.
- Nelson J.S. (Ed.). 2006. Fishes of the world. John Wiley & Sons, Mexico, 601 pp.
- Nitta M., Nagasawa K. 2013. First Japanese Record of *Heteropriapulius heterotylus* (Monogenea: Dactylogyridae), from the Alien Catfish *Pterygoplichthys disjunctivus* (Siluriformes: Loricariidae) in Okinawa. *Species Diversity*, 18, 281–284. DOI: 10.12782/sd.18.2.281
- Noble E.R., Noble G.A., Schad G.A., MacInnes A.J. 1989. Parasitology: The Biology of Animal Parasites, 6th Ed. Lea & Febiger, Philadelphia.
- Norton D.A., Carpenter M.A. 1998. Mistletoes as parasites: host specificity and speciation. *Trends in Ecology and Evolution*, 13, 101–105.
- Olson P.D., Cribb T.H., Tkach V.V., Bray R.A., Littlewood D.T.J. 2003 Phylogeny and classification of the Digenea (Platyhelminthes: Trematoda). *International Journal for Parasitology*, 33, 733–755. DOI: 10.1016/S0020-7519(03)00049-3

- Ostrowski de Núñez M., Gil De Pertierra A. 1991. The life history of *Acanthostomum gnerii* Szidat, 1954 (Trematoda: Acanthostomidae), from the catfish *Rhamdia sapo* in Argentina. *Zoologischer Anzeiger*, 227, 58–71.
- Ostrowski de Núñez M.C., Arredondo N.J., Doma I.L., Gil De Pertierra A.A. 2011. Redescription of *Paraspina argentinensis* (Szidat, 1954) (Digenea: Cryptogonimidae) from freshwater fishes (Pimelodidae) in the basins of the Paraná and La Plata Rivers, Argentina, with comments on *P. bagre* Pearse, 1920. *Systematic Parasitology*, 78: 27–40. DOI: 10.1007/s11230-010-9274-7
- Pavanelli G.C., Machado M.H. 1991. Proteocefalídeos parasitos de peixes, em especial pimelodídeos do rio Paraná, Paraná. *Revista Unimar*, 13, 163–175.
- Pavanelli G.C., Rego, A.A. 1989. Novas espécies de proteocefalídeos (Cestoda) de *Hemisorubim platyrhynchos* (Pisces: Pimelodidae) do Estado do Paraná. *Revista Brasileira de Biologia*, 49, 381–386.
- Pavanelli G.C., Rego A.A. 1991. Cestóides proteocefalídeos de *Sorubim lima* (Schneider, 1801) (Pisces: Pimelodidae) do rio Paraná e reservatório de Itaipu. *Revista Brasileira de Biologia*, 51, 7–12.
- Pavanelli G.C., Takemoto R.M. 2000. Aspects of the ecology of proteocephalid cestodes, parasites of *Sorubim lima* (Pimelodidae), of the Upper Paraná River, Brazil: II. interspecific associations and distribution of gastrointestinal parasites. *Brazilian Journal of Biology*, 60(4), 585–590.
- Pearse A.S. 1920. The fishes of Lake Valencia, Venezuela. *Univ. Wisconsin Stud.*, 12, 51.
- Petter A.J. 1984. Nematodes de poissons du Paraguay II. Habronematoidea (Spirurida). Description de 4 especes nouvelles de la famille des Cystidicolidae. *Revue Suisse Zoologie*, 91, 935–952.
- Petter A.J. 1987. Nematodes de Poissons de l'Equateur. *Revue Suisse Zoologie*, 94, 61–76.
- Petter A.J., Cassone J. 1984. Nématodes de Poisson du Paraguay; I. Ascaridoidea: *Sprentascaris*, n. gen. *Revue Suisse Zoologie*, 91, 617–634.
- Porto D.B., Vital J.F., Santos A.K.S., Morais A.M., Varella M.B., Malta, J.C.O. 2012. Metazoários parasitos de *Pterygoplichthys pardalis* (Castelnau, 1855) (Siluriformes: Loricariidae) da Amazônia central, Brasil. *Revista Brasileira de Zoociências*, 14(1, 2, 3), 35–40.

- Poulin R. 1992. Determinants of host-specificity in parasites of freshwater fishes. *International Journal for Parasitology*, 22, 753–758.
- Poulin R. 1993. The disparity between observed and uniform distributions – A new look at parasite aggregation. *International Journal of Parasitology*, 23(7), 937–944. DOI: 10.1016/0020-7519(93)90060-C
- Poulin R. 2002. The evolution of monogenean diversity. *International Journal for Parasitology*, 32, 245–254. DOI: 10.1016/S0020-7519(01)00329-0
- Poulin R. 2014. Parasite biodiversity revisited: frontiers and constraints. *International Journal for Parasitology*, 44, 581–589. DOI: 10.1016/j.ijpara.2014.02.003
- Poulin R., Morand S. 2004. Parasite Biodiversity. Smithsonian Books, Washington, 216 pp.
- Rego A.A., Chubb J.C., Pavanelli G.C. 1999. Cestodes in South American freshwater teleost fishes: keys to genera and brief description of species. *Revista Brasileira de Zoologia*, 16, 299–367.
- Rodríguez-Santiago M.A., Grano-Maldonado M., Ávila E., Gómez, S. 2015. Occurrence of *Heteropriapulus heterotylus* (Monogeneoidea: Dactylogyridae), ectoparasite of two invasive sailfin catfishes (Siluriformes: Loricariidae) from the Southeastern Mexico. *Neotropical Helminthology*, 9(1), 55–64.
- Rodríguez-Santiago M.A., García-Prieto L., Mendoza-Garfias B., González-Solís D., Grano-Maldonado M.I. 2016. Parasites of two coexisting invasive sailfin catfishes (Siluriformes: Loricariidae) in a tropical region of Mexico. *Neotropical Ichthyology*, 14, 3, e160021. DOI: 10.1590/1982-0224-20160021
- Rózsa L, Reiczigel J, Majoros G. 2000. Quantifying parasites in samples of hosts. *Journal of Parasitology*, 86(2), 228–232. DOI: 10.1645/0022-3395(2000)086[0228:QPISOH]2.0.CO;2
- Santos C.P., Gibson D.I., Tavares L.E.R., Luque, J.L. 2008. Checklist of Acanthocephala associated with the fishes of Brazil. *Zootaxa*, 1938: 1–22.
- Sasal P., Desdevise Y., Morand, S. 1998. Host-specialization and species diversity in fish parasites: phylogenetic conservatism? *Ecography*, 21, 639–643.
- Schäffer G.V., Rego A.A., Pavanelli G.C. 1992. Peritoneal and visceral cestode larvae in Brazilian freshwater fishes. *Memórias do Instituto Oswaldo Cruz*, 87, 257–258.
- Secretaria do Meio Ambiente do Estado de São Paulo – Sma. Plano de Manejo – Parque Estadual do Aguapeí (Encarte 3). Disponível em:

http://observatorio.wwf.org.br/site_media/upload/gestao/planoManejo/PM_PEAguapei_parte_002.pdf. Accessed in: Dez. 2016.

- Šimková A., Verneuvau O., Gelnar M., Morand S. 2006. Specificity and specialization of congeneric monogeneans parasitizing cyprinid fish. *Evolution*, 60(5), 1023–1037. DOI: 10.1554/05-521.1
- Suriano D.M. 1985. El genero *Unilatus* Mizelle y Kritsky, 1967 (Monogenea: Ancyrocephalidae) del Rio Negro, Manaus, Brasil. *Neotropica*, 31, 163–175.
- Suriano D.M., Incorvaia, I.S. 1995. Ancyrocephalid (Monogenea) parasites from siluriform fishes from the ParaneanPlatean ichthyogeographical province in Argentina. *Acta Parasitologica*, 40, 113–124.
- Takemoto R.M., Pavanelli, G.C. 2000. Aspects of the ecology of Proteocephalid cestodes parasites of *Sorubim lima* (Pimelodidae) of the Upper Paraná River, Brazil. I. Structure and influence of host's size and sex. *Brazilian Journal of Biology*, 60(4), 577–584.
- Takemoto R.M., Pavanelli G.C., Lizama M.A.P., Lacerda A.C.F., Yamada F.H., Moreira L.H.A., Ceschini T.L., Bellay S. 2009. Diversity of parasites of fish from the Upper Paraná River floodplain, Brazil. *Brazilian Journal of Biology*, 69(2, Suppl.), 691–705.
- Thatcher V.E. 1999. Surface morphology of some amphistomes (Trematoda) of Amazonas fishes and the description of a new genus and species. *Acta Amazonica*, 29, 237–240.
- Thatcher V.E. 2006. Amazon fish parasites. 2nd Ed. Pensoft, Sofia-Moscow, 508 pp.
- Thatcher V.E., Varella, A.B. 1981. Duas novas espécies de *Megacoelium* Szidat, 1954 (Trematoda: Haploporidae) parasitas estomacais de peixes da Amazônia Brasileira, com uma redefinição do gênero. *Acta Amazonica*, 11, 285–289.
- Thomas F., Bonsall M.B., Dobson A. 2005. Parasitism, biodiversity and conservation. In: (Eds. Thomas, F., Renaud, F., Guégan, J. F.) *Parasitism & Ecosystems*. Oxford University Press, pp. 124–139.
- Travassos L., Artigas P., Pereira C. 1928. Fauna helminthologica dos peixes de água doce do Brasil. *Arquivos do Instituto Biológico de São Paulo*, 1, 5–68.
- Vaz Z., Pereira, C. 1934. Contribuição ao conhecimento dos nematoides de peixes fluviais do Brasil. *Arquivos do Instituto Biológico de São Paulo*, 5, 87–103.

- Vilela M.J., Dutra M.A., Marques H., Dias J.H.P., Langeani F., Carvalho F.R., Martins F.O. 2013. Resultados parciais do inventário da ictiofauna do rio Aguapeí, na área da RPPN Foz do Aguapeí, São Paulo, Bacia do Alto Paraná. XX Encontro Brasileiro de Ictiologia, Maringá.
- Whittington I.D., Cribb B.W., Hamwood T.E., Halliday J.A. 2000. Host-specificity of monogenean (platyhelminth) parasites: a role for anterior adhesive areas? *International Journal for Parasitology*, 30, 305–320.
- Woodland W.N.F. 1935. Some more remarkable cestodes from Amazon siluroid fish. *Parasitology*, 27, 207–225.
- Yamada P.O.F., Yamada F.H., Silva R.J., Anjos L.A. 2017. Ecological implications of floods on the parasite communities of two freshwater catfish in a Neotropical floodplain. *Acta Parasitologica*, 62(2). DOI: 10.1515/ap-2017-00
- Zehnder M.P., Mariaux, J. 1999. Molecular systematic analysis of the order Proteocephalidea (Eucestoda) based on mitochondrial and nuclear rDNA sequences. *International Journal for Parasitology*, 29, 1841–1852.

***Added resolution to circumscription of some
gill monogeneans species (Dactylogyridae) of
Neotropical catfishes including description of a new
Demidospermus species and erection of a new genus***

4. Added resolution to circumscription of some gill monogeneans species (Dactylogyridae) of Neotropical catfishes including description of a new *Demidospermus* species and erection of a new genus¹

Abstract

The present study aims at adding resolution to the assessment of interrelations of Neotropical dactylogyrids from catfishes by providing new 28S rDNA sequences of representatives from these poorly represented host groups. Based on new molecular and morphological data, a diagnosis amendment of the dactylogyrid genus *Demidospermus* (with a new species description) is proposed, a new dactylogyrid genus is erected, and generic assignment of unidentified taxa for which molecular data are available is briefly discussed. Thirteen new sequences of the 28S rDNA fragment were generated (1526–1575 bp) and aligned with 74 published sequences from Genbank. Phylogenetic analyses were run under Maximum likelihood (ML) and Bayesian inference (BI) criteria, applying the model of nucleotide evolution GTR + Γ + I for the three datasets. *Demidospermus rhinelepus* n. sp. is described from the gills of *Rhinelepis aspera* and the erection of the monotypic genus *Paracosmetocleithrum* n. gen. is proposed with the description of *Paracosmetocleithrum trachydorus* n. sp. from the gills of *Trachydoras paraguayensis* is presented. Future studies addressing morphology and molecular phylogeny are still necessary to help circumscription of monophyletic clades within the Dactylogyridae.

Key-words: dactylogyrids, Monogenea, taxonomy, phylogeny, 28S rDNA.

¹ Manuscript to be submitted to the journal Parasitology International.

4.1. Introduction

Neotropical freshwater fishes host a rich fauna of gill parasites, especially monogeneans (Dactylogyridae: Ancyrocephalinae) [1, 2]. However, the true diversity of these ectoparasites is still poorly known and very little is known about their interrelations [3, 4]. Whereas circumscription of individual species does not make generally serious problems, delimitation of genera and their differentiation remain problematic in many cases, especially because of shortage of synapomorphies that would define unequivocally individual genera. Some genera are defined by unique combination of non-unique characteristics including those of internal structures (e.g. mutual position of genital organs – see [5]). Species composition of some genera such as *Urocleidoides* Mizelle & Price, 1964 has changed considerably, depending on the generic concept accepted by given authors. Whereas some genera, e.g. *Heteropriapulus* Kritsky, 2007 and *Aphanoblastella* Kritsky, Mendoza-Franco and Scholz, 2000, are well-defined, the others, e.g. *Demidospermus* Suriano, 1983, include species with a wide range of morphological differences that occur on different groups of fish hosts [3, 6]. Some of the morphological characters that have been used for circumscription of genera are probably homoplastic and unsuitable for generic diagnoses. It is thus necessary to use molecular tools to unravel interrelations of individual taxa and to reveal possible patterns in host use.

Unfortunately, shortage of molecular data represents another obstacle in a better characterization of individual taxa that should represent monophyletic lineages of closely related species. Mendoza-Palmero et al. [4] provided first comprehensive molecular data on Neotropical gill monogeneans of catfishes (Siluriformes) and revealed that they do not form a monophyletic lineage. In addition, non-monophyly of some genera such as *Demidospermus* and *Ancyrocephalus* Creplin, 1839 was detected. However, the study of Mendoza-Palmero et al. [4] included mainly monogeneans from pimelodid catfishes and taxa from other siluriform hosts, especially loricariids and doradids, were under-represented.

Therefore, the present study aims at adding resolution to the assessment of interrelations of Neotropical dactylogyrids from catfishes by providing new 28S rDNA sequences of representatives from these poorly represented host groups. Based on new molecular and morphological data, a diagnosis amendment of the dactylogyrid genus *Demidospermus* Suriano, 1983 (with a new species description) is proposed, a new

dactylogyrid genus is erected, and generic assignment of unidentified taxa for which molecular data are available [see 4] is briefly discussed.

4.2. Materials and Methods

4.2.1. Study area

Refer back to section 1.4.1.

4.2.2. Hosts and parasites sampling

Refer back to section 3.2.2.

4.2.3. Morphological analyses of the monogeneans

The monogeneans were taken from the gills and some specimens collected were mounted on slides with Hoyer's or Grey and Wess' mediums, and a mixture of glycerin-ammonium picrate (GAP) to study sclerotized structures [7, 5, 8], while some specimens were stained with Gomori's trichrome to study internal organs. The parasites were studied using V3 Leica Application Suite computerized system for image analysis with differential interference contrast and an Olympus BX51 microscope equipped with phase contrast optics. Illustrations were made with the aid of a drawing tube (camera lucida) mounted on a Leica DMLS microscope.

Measurements were taken according to Mizelle & Klucka [9] and Kritsky et al. [10] represented by the measurement of a straight line between two points expressed in micrometers as the mean followed by range and number of measurements in parentheses. The terminology for the components of the haptor follows that of Mizelle and Kritsky [11] and Kritsky and Mizelle [12]; hooks are numbered according to Mizelle [13].

Type and voucher specimens will be deposited in the Helminthological Collection of the Institute Oswaldo Cruz, Rio de Janeiro, Brazil (CHIOC), the Helminthological Collection of the Instituto de Biociências, Universidade Estadual Paulista, Botucatu, Brazil (CHIBB), and the Helminthological Collection of the Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, České Budějovice (IPCAS).

4.2.4. DNA extraction, amplification and sequencing

The specimens used for molecular analyses were placed on a slide with a drop of water, covered with a coverslip, identified under the microscope based on their

morphology and then placed into 96% molecular-grade ethanol. Some allegedly conspecific specimens (paragenophores – see [14] for terminology) were fixed with glycerin ammonium picrate (GAP) and mounted as permanent preparations with Canada's balsam following Ergens [8] and deposited at IPCAS. Genomic DNA was extracted using 200 µl of a 5% suspension of Chelex in deionized water and 2 µl of proteinase K, followed by overnight incubation at 56°C, boiling at 90°C for 8 minutes and centrifugation at 14,000 rpm for 10 minutes.

Polymerase chain reactions (PCR) were made in 20 µl of reaction mixture with 3 µl of extraction supernatant, 10 µl of 2× MyFi™ Mix (Bioline, CITY, USA), and 1.6 µl of each PCR primer. Partial 28S rDNA (D1–D3 region) was amplified using the primers U178 (5'-GCA CCC GCT GAA YTT AAG-3') and L1642 (5'-CCA GCG CCA TCC ATT TTC A-3') [15]. The thermocycling profile used was: denaturation of DNA (95°C for 3 min); 35 cycles of amplification (94°C for 30 s, 56°C for 30 s and 72°C for 1.5 min), and 4 min extension hold at 72°C. PCR products were run on 1% agarose gel using gel red and loading buffer. PCR products were purified prior to sequencing using exonuclease I and shrimp alkaline phosphatase enzymes [16]. Amplicons were Sanger sequenced at GATC Biotech (Konstanz, Germany) using the PCR primers and the internal primers 900F (5'-CCGTCTTGAAACACGGACCAAG-3') [17], 300R (5'-CAACTTTCCCTCACGGTACTTG-3') [15]; L1200R (5'-GCATAGTTCACCATCTTTCGG-3') and ECD2 (5'-CCTTGGTCCGTGTTTCAAGACGGG) [18]. Contiguous sequences were assembled using Geneious version R8 (<http://www.geneious.com/>; [19]).

4.2.5. Alignment and phylogenetic analyses

Thirteen new sequences of the 28S rDNA fragment were generated (1526–1575 bp) and aligned with 74 published sequences from Genbank of dactylogyrid species belonging to the subfamilies Ancylo-discoidinae, Ancyrocephalinae, Dactylogyrinae, and Pseudodactylogyrinae of the Dactylogyridae (Table 1) and nine sequences of species of the Monocotylidae, Diplectanidae, Pseudomurraytrema-tidae, and Tetraonchidae were used as outgroups. The sequences were chosen following the results of Mendoza-Palmero et al. [4]. Sequences were aligned using default parameters of MAFFT implemented in Guidance [20] with the extremes of the alignment trimmed to match the shortest sequence. Three datasets of partial 28S rDNA sequences were obtained for phylogenetic

analyses: dataset 1 – all the sequences and nucleotide positions (823 bp); dataset 2 – a stringent alignment (626 bp) excluding nucleotide positions with alignment score below 0.5 [21]; dataset 3 – only long sequences of Neotropical dactylogyrids infecting siluriform and perciform fishes, and three sequences of species belonging to the Ancylo-discoidinae (1400 bp).

Phylogenetic analyses were run under Maximum likelihood (ML) and Bayesian inference (BI) criteria, applying the model of nucleotide evolution GTR + Γ + I for the three datasets. The model was selected using jModelTest 2.1.1 [22, 23]. ML analyses were carried out using the program RAxML v. 8 [24]. The model parameters and bootstrap support values (1,000 repetitions) were estimated using RAxML. BI trees were created using MrBayes v. 3.2 [25] running two independent MCMC runs of four chains for 10^7 generations and sampling tree topologies every 10^3 generations. Burn-in periods were set to the first 25,000 generations. MrBayes and RAxML analyses were carried out on the computational resource CIPRES [26].

Table 1. List of monogeneans included in the phylogenetic analyses. New sequences obtained during the present study are in bold.

Parasite species	Host	Host family	Locality	Genbank ID	Reference
Dactylogyridae					
<i>Actinocleidus recurvatus</i>	<i>Lepomis gibbosus</i>	Centrarchidae	River Danube, Slovak Republic	AJ969951	[27]
<i>Aliatrema cribbi</i>	<i>Chaetodon citrinellus</i>	Chaetodontidae	French Polynesia	AY820612	[28]
<i>Ameloblastella chavarriai</i>	<i>Rhamdia quelen</i>	Heptapteridae	Catemaco Lake, Mexico	KP056251	[4]
<i>Ameloblastella edentensis</i>	<i>Hypophthalmus edentatus</i>	Pimelodidae	Nanay River, Peru	KP056255	[4]
<i>Ameloblastella unapinoides</i>	<i>Sorubim lima</i>	Pimelodidae	Iquitos, Peru	KP056254	[4]
<i>Ameloblastella</i> sp.	<i>Hassar</i> sp.	Doradidae	Aquarium Río Momón, Iquitos, Peru	KP056253	[4]
<i>Ameloblastella</i> sp. 23	<i>Hypophthalmus edentatus</i>	Pimelodidae	Nanay River, Peru	KP056233	[4]
<i>Ancyrocephalus paradoxus</i>	<i>Sander lucioperca</i>	Percidae	Morava River, Czech Republic	AJ969952	[27]
<i>Ancyrocephalus percae</i>	<i>Perca fluviatus</i>	Percidae	Constance Lake, Germany	KF499080	[29]
<i>Ancyrocephalus morgundae</i>	<i>Siniperca chuatsi</i>	Percichthyidae	Wuhan, China	AY841871	[30]
<i>Ancyrocephalus morgundae</i>	<i>Siniperca chuatsi</i>	Percichthyidae	Fuzhou, Fujian Province, China	DQ157667	[31]
<i>Aphanoblastella aurorae</i>	<i>Goeldiella eques</i>	Heptapteridae	Santa Clara, Peru	KP056239	[4]
<i>Aphanoblastella chagresii</i>	<i>Pimelodella avanhandavae</i>	Heptapteridae	Upper Paraná, River, Brazil		Present study
<i>Aphanoblastella</i> sp. 3	<i>Goeldiella eques</i>	Heptapteridae	Nanay River, Peru	KP056238	[4]
<i>Bravohollisia rosetta</i>	<i>Pomadasys maculatus</i>	Haemulidae	Guangdong, China	DQ537364	[32]

Table 1. Continued.

Parasite species	Host	Host family	Locality	Genbank ID	Reference
<i>Bychowskyella pseudobagri</i>	<i>Tachysurus fulvidraco</i>	Bagridae	Shaoguan, China	EF100541	[33]
<i>Chauhanellus boegeri</i>	<i>Genidens genidens</i>	Ariidae	Antonina Bay, Brazil	KP056241	[4]
<i>Cichlidogyrus sclerosus</i>	<i>Oreochromis niloticus</i>	Cichlidae	Panyu, Guangdong Province, China	DQ157660	[31]
<i>Cichlidogyrus tilapiae</i>	<i>Hemichromis fasciatus</i>	Cichlidae	Senegal, Africa	HQ010029	[34]
<i>Cosmetocleithrum bifurcum</i>	<i>Hassar orestis</i>	Doradidae	Aquarium Momón River, Peru	KP056216	[4]
<i>Cosmetocleithrum bulbocirrus</i>	<i>Pterodoras granulosus</i>	Doradidae	Upper Paraná River basin, Brazil		Present study
<i>Dactylogyrus nanus</i>	<i>Rutilus rutilus</i>	Cyprinidae	River Morava, Czech Republic	AJ969942	[27]
<i>Dactylogyrus petruschewskyi</i>	<i>Megalobrama amblycephala</i>	Cyprinidae	China	AY548927	Ding & Liao (unpublished)
<i>Demidospermus mortenthaleri</i>	<i>Brachyplatystoma juruense</i>	Pimelodidae	Santa Clara, Peru	KP056245	[4]
<i>Demidospermus cf. paranaensis</i>	<i>Loricariichthys platymetopon</i>	Loricariidae	Upper Paraná River, basin, Brazil		Present study
<i>Demidospermus rhinelepis</i> n. sp.	<i>Rhinelepis aspera</i>	Loricariidae	Upper Paraná River, basin, Brazil		Present study
<i>Demidospermus</i> sp. 11	<i>Brachyplatystoma vaillantii</i>	Pimelodidae	Nanay River, Peru	KP056235	[4]
<i>Demidospermus</i> sp. 23	<i>Brachyplatystoma vaillantii</i>	Pimelodidae	Nanay River, Peru	KP056236	[4]

Table 1. Continued.

Parasite species	Host	Host family	Locality	Genbank ID	Reference
<i>Ergenstrema mugilis</i>	<i>Liza ramada</i>	Mugilidae	Ebro Delta, Spain	JN996800	[35]
<i>Euryhaliotrema perezponcei</i>	<i>Lutjanus guttatus</i>	Lutjanidae	Bay Cerritos, Mazatlan, Mexico	HQ615996	Soler-Jiménez <i>et al.</i> (unpublished)
<i>Euryhaliotrematoides pirulum</i>	<i>Chaetodon lunula</i>	Chaetodontidae	French Polynesia	AY820618	[29]
<i>Haliotrema cromileptis</i>	<i>Epinephelus bleekeri</i> , <i>E. coioides</i>	Serranidae	Nha Trang Bay, Vietnam	EU523146	[36]
<i>Haliotrema platycephali</i>	<i>Platycephalus indicus</i>	Platycephalidae	Weihai, Shangdong Province, China	DQ157662	[31]
<i>Haliotrematoides guttati</i>	<i>Lutjanus guttatus</i>	Lutjanidae	Bay Cerritos, Mazatlan, Mexico	HQ615993	Soler-Jiménez <i>et al.</i> (unpublished)
<i>Haliotrematoides spinatus</i>	<i>Lutjanus guttatus</i>	Lutjanidae	Pacific Coast, Mexico	KC663679	García-Vázquez <i>et al.</i> (unpublished)
<i>Heteropriapulus anchoradiatus</i>	<i>Pterygoplichthys ambrosettii</i>	Loricariidae	Upper Paraná River basin, Brazil		Present study
<i>Heteropriapulus heterotylus</i>	<i>Pterygoplichthys ambrosettii</i>	Loricariidae	Upper Paraná River basin, Brazil		Present study
<i>Heteropriapulus simplex</i>	<i>Pterygoplichthys ambrosettii</i>	Loricariidae	Upper Paraná River basin, Brazil		Present study
<i>Ligictaluridus pricei</i>	<i>Ameiurus nebulosus</i>	Ictaluridae	River Moldau, Czech Republic	AJ969939	[27]
<i>Ligophorus vanbenedenii</i>	<i>Liza aurata</i>	Mugilidae	Ebro Delta, Spain	JN996802	[35]
<i>Metahaliotrema mizellei</i>	<i>Scatophagus argus</i>	Scatophagidae	Panyu, Guangdong Province, China	DQ157647	[31]
<i>Onchocleidus similis</i>	<i>Lepomis gibbosus</i>	Centrarchidae	River Danube, Slovak Republic	AJ969938	[27]

Table 1. Continued.

Parasite species	Host	Host family	Locality	Genbank ID	Reference
<i>Onchocleidus</i> sp.	<i>Lepomis macrochirus</i>	Centrarchidae	Guangzhou, China	AY841873	[30]
<i>Paracosmetocleithrum trachydorus</i> n. gen., n. sp.	<i>Trachydoras paraguayensis</i>	Doradidae	Upper Paraná river basin, Brazil		present study
<i>Parasciadiclethrum octofasciatum</i>	<i>Rocio octofasciata</i>	Cichlidae	Creek in Ejido, Chiapas, Mexico		Mendoza-Palmero <i>et al.</i> (unpublished)
<i>Pseudodactylogyrus anguillae</i>	<i>Anguilla anguilla</i>	Anguillidae	River Danube, Slovak Republic	AJ969950	[27]
<i>Pseudodactylogyrus bini</i>	<i>Anguilla anguilla</i>	Anguillidae	Neusiedler Lake, Austria	AJ969949	[27]
<i>Pseudohaliotrema sphincteroporus</i>	<i>Siganus doliatus</i>	Siganidae	Green Island, Australia	AF382058	[37]
<i>Protogyrodactylus alienus</i>	<i>Gerres filamentosus</i>	Gerreidae	Dayawan, Guangdong Prvince, China	DQ157650	[31]
<i>Protogyrodactylus haiananensis</i>	<i>Therapon jarbua</i>	Tetrapontidae	Yangjiang, Guangdong Province, China	DQ157653	[31]
<i>Quadriacanthus kobeensis</i>	<i>Clarias batrachus</i>	Clariidae	Guangzhou, China	AY841874	[30]
<i>Schilbetrema</i> sp.	<i>Pareutropius debauwi</i>	Schilbeidae	Aquarium from Czech Republic, origin West Africa	KP056243	[4]
<i>Sciadicleithrum bravolollisae</i>	<i>Paraneetrophus fenestratus</i>	Cichlidae	Lago de Catemaco, VeraCruz, Mexico		Mendoza-Palmero <i>et al.</i> (unpublished)
<i>Sciadicleithrum meekii</i>	<i>Thorichthys meeki</i>	Cichlidae	Creek in Ejido, Chiapas, Mexico		Mendoza-Palmero <i>et al.</i> (unpublished)

Table 1. Continued.

Parasite species	Host	Host family	Locality	Genbank ID	Reference
<i>Sciadicleithrum splendidae</i>	<i>Parachromis friedrichsthalii</i>	Cichlidae	Las Piñas, Campeche, Mexico		Mendoza-Palmero <i>et al.</i> (unpublished)
<i>Scutogyrus longicornis</i>	<i>Oreochromis niloticus</i>	Cichlidae	Panyu, Guangdong Province, China	DQ157659	[31]
<i>Tetrancistrum</i> sp.	<i>Siganus fuscescens</i>	Siganidae	Heron Island, Queensland, Australia	AF026114	[38]
<i>Thaparocleidus asoti</i>	<i>Silurus asotus</i>	Siluridae	Rongchang, China	DQ157669	[31]
<i>Thaparocleidus campyloptercirrus</i>	<i>Pangasianodon hypophthalmus</i>	Pangasidae	Guangzhou, China	AY841872	[30]
<i>Thaparocleidus siluri</i>	<i>Silurus ganis</i>	Siluridae	Morava River, Czech Republic	AJ969940	[27]
<i>Thaparocleidus vistulensis</i>	<i>Silurus ganis</i>	Siluridae	Morava River, Czech Republic	AJ969941	[27]
<i>Thaparocleidus</i> sp.	<i>Pangasius</i> sp.	Pangasidae	Aquarium from Czech Republic, origin Asia	KP056250	[4]
<i>Unibarra paranoplatensis</i>	<i>Aguarunichthys torosus</i>	Pimelodidae	Santa Clara, Peru	KP056219	[4]
<i>Unilatus unilatus</i>	<i>Pterygoplichthys ambrosettii</i>	Loricariidae	Upper Paraná River basin, Brazil		Present study
<i>Vancleaveus januacaensis</i>	<i>Pterodoras granulosus</i>	Doradidae	Itaya River, Peru	KP056247	[4]
<i>Vancleaveus januacaensis</i>	<i>Pterodoras granulosus</i>	Doradidae	Upper Paraná River basin, Brazil		Present study

Table 1. Continued.

Parasite species	Host	Host family	Locality	Genbank ID	Reference
Dactylogyridae gen. sp. 1	<i>Hemisorubim platyrhynchos</i>	Pimelodidae	Upper Paraná River basin, Brazil		Present study
Dactylogyridae gen. sp. 2	<i>Hemisorubim platyrhynchos</i>	Pimelodidae	Upper Paraná River basin, Brazil		Present study
Dactylogyridae gen. sp. 4	<i>Ageneiosus vittatus</i>	Auchenipteridae	Nanay River, Peru	KP056218	[4]
Dactylogyridae gen. sp. 5	<i>Sorubim lima</i>	Pimelodidae	Upper Paraná River basin, Brazil		Present study
Dactylogyridae gen. sp. 9	<i>Platynemichthys notatus</i>	Pimelodidae	Santa Clara, Peru	KP056224	[4]
Dactylogyridae gen. sp. 10	<i>Platynemichthys notatus</i>	Pimelodidae	Santa Clara, Peru	KP056227	[4]
Dactylogyridae gen. sp. 12	<i>Sorubim lima</i>	Pimelodidae	Iquitos-Belém, Peru	KP056228	[4]
Dactylogyridae gen. sp. 13	<i>Hypophtalmus edentatus</i>	Pimelodidae	Nanay River, Peru	KP056230	[4]
Dactylogyridae gen. sp. 18	<i>Pseudoplatystoma fasciatum</i>	Pimelodidae	Santa Clara, Peru	KP056231	[4]
Dactylogyridae gen. sp. 23	<i>Platysilurus mucosus</i>	Pimelodidae	Santa Clara, Peru	KP056232	[4]
Dactylogyridae gen. sp. 26	<i>Platynemichthys notatus</i>	Pimelodidae	Santa Clara, Peru	KP056234	[4]
Diplectanidae					
<i>Murraytrema pricei</i> *	<i>Nibeia albiflora</i>	Scianidae	Panyu, Guangdong Province, China	DQ157672	[31]
<i>Pseudorhabdosynochus epinepheli</i> *	<i>Epinephelus bruneus</i>	Serranidae	Huidong, China	AY553622	[31]

Table 1. Continued.

Parasite species	Host	Host family	Locality	Genbank ID	Reference
<i>Pseudorhabdosynochus lantauensis</i> *	<i>Epinephelus bruneus</i>	Serranidae	Huidong, China	AY553624	[31]
<i>Sinodiplectanotrema argyromus</i> *	<i>Nibea albiflora</i> , <i>Pennahia anea</i>	Scianidae	Panyu, Guangdong Province, China	DQ157673	[31]
Pseudomurraytrematidae					
<i>Pseudomurraytrema</i> sp.*	<i>Catostomus ardens</i>	Catostomidae	Snake River, Idaho, USA	AF382059	[39]
Monocotylidae					
<i>Clemacotyle australis</i> *	<i>Aetobatus narinari</i>	Myliobatidae	Heron Island, Australia	AF348350	[39]
<i>Decacotyle lymmae</i> *	<i>Aetobatus narinari</i>	Myliobatidae	Heron Island, Australia	AF348359	[39]
<i>Dendromonocotyle octodiscus</i> *	<i>Dasyatis americana</i>	Dasyatidae	Gulf of Mexico, Mexico	AF348352	[39]
Tetraonchidae					
<i>Tetraonchus monenteron</i> *	<i>Esox lucius</i>	Esocidae	River Moldau, Czech Republic	AJ969953	[27]

4.3. Results

4.3.1. Amended diagnosis of *Demidospermus* Suriano, 1983 based on morphological features and phylogenetic relationships

***Demidospermus* (Suriano, 1983) Kritsky and Gutiérrez, 1998**

Amended diagnosis: Body fusiform, comprising cephalic region, trunk, peduncle, haptor. Tegument thin, smooth. Two terminal, cephalic lobes; head organs present; cephalic glands unicellular, lateral or posterolateral to pharynx. Eyes present or absent; granules subspherical. Mouth subterminal, midventral; pharynx muscular, glandular; esophagus present; intestinal caeca 2, confluent posterior to gonads, lacking diverticula. Genital pore midventral near level of intestinal bifurcation. Gonads in tandem, intercaecal, testis post ovary. Vas deferens looping left intestinal caecum; seminal vesicle a dilation of vas deferens. Copulatory complex comprising tubular male copulatory organ with one or more counterclockwise rings, and accessory piece. Vagina weakly sclerotized or non-sclerotized. Haptor subhexagonal with dorsal and ventral anchor/bar complexes. Seven pairs of hook with ancerocephalinae distribution, of similar size and shape. Bars V shaped presenting or not constriction at mid point. Parasites of the gills of Neotropical loricariid fishes.

Type species: *Demidospermus anus* Suriano, 1983 from *Loricariichthys anus* (Valenciennes, 1835).

Other species: *Demidospermus paranaensis* Ferrari-Hoeinghaus, Bellay, Takemoto and Pavanelli, 2010 from *Loricariichthys platymetopon* Isbrücker and Nijssen, 1979;

Demidospermus rhinelepus n. sp. from *Rhinelepis aspera* Spix & Agassiz, 1829.

Remarks: Currently, there are 27 dactylogyrids from the gills of Neotropical siluriforms assigned to *Demidospermus*. The hosts belong to the families Auchenipteridae, Loricariidae, and Pimelodidae [6]. *Demidospermus* was described by Suriano [40] for a dactylogyrid found in the gills of *L. anus* from Argentina. Suriano [40] considered as the main diagnosis feature of the genus the presence of encapsulated sperm within the testis. Gutiérrez and Suriano [41] added other characteristics to the genus, as well as more species, and named the encapsulated sperm as sperm packets. Kritsky and Gutiérrez [42] amended the diagnosis of *Demidospermus* to include dactylogyrids from the gills of

siluriforms that possess: tandem gonads (testis posterior to ovary), counterclockwise coiled male copulatory organ (MCO), vagina sinistral, haptoral bars U, W or V shaped, subspherical eyes granules, and a sheatlike accessory piece serving as guide for the MCO. Kritsky and Gutiérrez [42] also disregarded the presence of sperm packets as a characteristic to define the genus because they are not always present or visible. Besides, these authors considered *Omothecium* Kritsky, Thatcher and Boeger, 1987 and *Paramphocleithrum* Suriano and Incorvaia, 1995 as junior synonyms of *Demidospermus*. The second species described for a loricariid is *D. paranaensis* in *L. platymetopon* from the Upper Paraná river basin [43]. Mendoza-Palmero et al. [4] is the first study that assessed phylogenetic relationships of dactylogyrids from Neotropical siluriforms, and included *Demidospermus mortenthaleri* Mendoza-Palmero, Scholz, Mendoza-Franco, Kuchta, 2012 and two allegedly *Demidospermus* species named as *Demidospermus* sp. 11 and sp. 23, however, this study included dactylogyrids only from pimelodid hosts. This current study included into the phylogenetic analyses all the species used by Mendoza-Palmero et al. [4] and two sequences of *Demidospermus* species from loricariids (see Table 1). The phylogenetic tree (Fig. 3) showed with good support that the *Demidospermus* spp. from loricariid fishes cluster together, whereas the *Demidospermus* spp. from pimelodid cluster together, indicating that this genus is not natural due to this paraphyly. However, given that the type species was described in a loricariid host, the species sequenced for this study from loricariids share synapomorphies with the type species, and the fact they clustered together, enable to propose that the true *Demidospermus* species should be restricted to dactylogyrids that present the above mentioned morphological characteristics (see amended diagnosis) and belong to loricariid hosts.

4.3.2. Morphological descriptions

***Demidospermus rhinelepus* n. sp. (Fig. 1)**

Description (based on 7 specimens fixed in Hoyer's medium and 8 stained with Gomori's trichrome): Body fusiform 624 (443–742; n=8) long, greatest width 117 (70–141; n=8) at mid-length. Cephalic lobes poorly developed; 4 bilateral head organs; eyes absent. Pharynx spherical, 41 (37–45; n=8) long, 42 (36–47; n=8) wide; esophagus short; intestinal caeca confluent posterior to testis. Peduncle broad; haptor subhexagonal, 66 (54–79; n=8) long, 82 (72–93; n=8) wide. Anchors similar in shape, each with poorly

developed roots, presenting some fissures at the base, shaft and point evenly curved at level of superficial root; ventral anchor 28 (27–30; n=7) long, base 19 (18–20; n=7) wide; dorsal anchor 29 (28–30; n=7) long, base 20 (17–22; n=7) wide. Ventral bar V shaped, constricted in the middle with a sclerotized patch attached to the middle portion, becomes broader in its extension, rounded ends, 62 (45–70; n=7) total length, distance between ends 48 (32–56; n=7). Dorsal bar V shaped, articulated in the middle in which the two pieces are broader, tapering along its extension, rounded ends, 61 (49–68; n=7) total length, distance between ends 57 (38–62; n=7). Hooks similar, 14 (13–14; n=7) long, recurved point, erect thumb, slender shank, with round, weakly sclerotized subunit at base, FH loop about $\frac{3}{4}$ of shank length. Male copulatory organ (MCO) slender, forming about 1 $\frac{1}{2}$ counterclockwise rings, 38 (32–47; n=7) long, proximal ring diameter 26 (19–32; n=7). Accessory piece 49 (46–52; n=7) long, with broad ends and tapering in the middle, flat rounded proximal end, distal end folds in both sides, with folds extending to the beginning of proximal end. Gonads in tandem; ovary pretesticular. Testis 67 (n=1) long, 40 (n=1) wide; seminal vesicle a dilation of vas deferens, with two conspicuous dilations; prostatic reservoir not observed. Vas deferens looping left intestinal caecum. Ovary 134 (n=1) long, 43 (n=1) wide; oviduct, ootype, and uterus not observed. Vaginal aperture sinistral, weakly sclerotized, spherical shaped, with striations. Vitellaria densely scattered throughout trunk, except around reproductive organs.

Taxonomic summary

Type host: *Rhinelepis aspera* Spix & Agassiz, 1829 (Siluriformes: Loricariidae)

Site of infestation: Gills.

Type locality: Aguapeí River, municipality of Castilho (Paraná River basin), São Paulo State, Brazil (21°3'36.20"S, 51°45'38.58"W).

Representative DNA sequence: 1,542 bp long sequence of the D1–D3 region of the *lsrDNA* of one specimen collected on Jan, 22nd, 2016 (Access. No. XXXX) [paragenophore No. XXXX IPCAS].

Type specimens: Holotype to be deposited at CHIOC; 8 paratypes to be deposited at CHIBB; 3 to be deposited at CHIOC; 5 paratypes to be deposited at IPCAS.

Etymology: The specific name refers to the generic name of its host species.

Remarks

This species is the third species described in a loricariid, and differs from its congeners by the presence of a sclerotized patch attached to the middle portion of the ventral bar, whereas in *D. anus* and *D. paranaensis* this is absent, and in *D. paranaensis* the ventral bar is articulated and constricted in its middle portion. The dorsal bar of the species described herein is articulated (non articulated in *D. paranaensis*) and is broader next to the articulation, whereas in *D. anus* the dorsal bar is constricted at the middle. The cirrus of *D. anus* and *D. paranaensis* presents only one coil, and the accessory piece in both species is relatively small compared to the total length of the cirrus. The cirrus of the species described herein presents 1½ coils, and the accessory piece is bigger in length than the cirrus and also present a very distinct shape compared to the congeners (broad ends, and folded at sides). *Demidospermus anus* and *D. paranaensis* do not present sclerotized vagina, whereas the described species presents conspicuous, although weakly sclerotized, sinistral vagina.

***Paracosmetocleithrum* n. gen.**

Diagnosis: Dactylogyridae, Ancyrocephalinae. Body divisible into cephalic region, trunk, peduncle, and haptor. Tegument thin, smooth. Head organs, cephalic lobes present; cephalic glands unicellular, comprising two bilateral groups, posterolateral to pharynx. Eyes absent. Mouth subterminal, midventral; pharynx muscular, glandular; esophagus present; intestinal caeca 2, confluent posterior to testis, lacking diverticula. Gonads in tandem, intercaecal; testis posterior to ovary. Vas deferens looping left intestinal caecum; seminal vesicle a dilation of vas deferens; prostatic reservoir present. Copulatory complex comprising non-articulated copulatory organ and accessory piece; copulatory organ counterclockwise coiled; accessory piece rod shaped with bifurcated distal end. Common genital pore midventral, at level of intestinal bifurcation. Seminal receptacle at level of anterior margin of ovary; vagina dextral, non-sclerotized. Vitellaria present throughout trunk, except in regions of reproductive organs. Haptor armed with two pairs of anchors (dorsal and ventral), 14 hooks with ancyrocephalinae distribution, ventral and dorsal bars. Ventral bar presents a heavily sclerotized ornamentation in its middle portion; dorsal presents a heavily sclerotized patch on surface of bar with inconspicuous medial process and with the two submedial projections arising from the tapered ends of this patch. Parasite of the gills of Neotropical doradid.

Etymology: The generic name is a combination of the Greek suffix *para*=resembling, similar to and the Dactylogyridae genus name *Cosmetocleithrum* Kritsky, Thatcher and Boeger, 1986, and it is due to its morphological resemblance and close phylogenetic relationship with members of *Cosmetocleithrum* genus.

Type and only species: *Pseudocosmetocleithrum trachydorus* n. sp. from *Trachydoras paraguayensis* (Eigemann & Ward, 1907) (Siluriformes: Doradidae) collected at the Aguapeí River, municipality of Castilho (Paraná River basin), São Paulo State, Brazil (21°3'36.20"S, 51°45'38.58"W).

Remarks

Paracosmetocleithrum n. gen. resembles *Cosmetocleithrum* regarding the internal anatomy and the presence of two submedial projections on the dorsal bar. There are 10 dactylogyrids placed in *Cosmetocleithrum* genus, from which eight are parasites of doradids. Based on morphological features, it is possible to distinguish the monotypic new genus from *Cosmetocleithrum* spp. by the presence of a heavily sclerotized ornamentation in the middle portion of the ventral bar, and a heavily sclerotized patch on the surface of the dorsal bar with inconspicuous medial process and with the two submedial projections arising from the tapered ends of this patch. *Paracosmetocleithrum* n. gen. presents dextral vagina, which is described as sinistral in the generic diagnosis of *Cosmetocleithrum* by Kritsky et al. [5]. However, Mendoza-Franco et al. [44] reported the presence of a dextral vagina in the species *Cosmetocleithrum tortum* Mendoza-Franco, Mendoza-Palmero, Scholz, 2016 from the doradid *Nemadoras hemipeltis* (Eigenmann, 1925) from Peru. The results of the phylogenetic analyses gave support to the erection of this new genus (Fig. 3) because the species sequenced regarded herein as *Paracosmetocleithrum trachydorus* n. sp. from the doradid *Trachydoras paraguayensis* did not cluster together with any of the genera used in the analyses. This new genus still presents an unclear relationship with the genera *Cosmetocleithrum* from doradids and *Demidospermus* from loricariids. The morphological resemblance of *Paracosmetocleithrum* n. gen. with members of *Cosmetocleithrum* spp. can be the case of a convergent evolution. Therefore, the presence of the two submedial projection on the dorsal bar is no longer a sole characteristic of *Cosmetocleithrum*.

***Paracosmetocleithrum trachydorus* n. sp. (Fig. 2)**

Description (based on 3 specimens fixed in GAP, 6 specimens fixed in Hoyer's medium and 8 stained with Gomori's trichrome): Body fusiform 322 (209–399; n=6) long, greatest width 78 (44–99; n=6) at mid-length. Cephalic lobes poorly developed; 4 bilateral head organs; eyes absent. Pharynx spherical, 23 (19–26; n=5) long, 22 (17–26; n=5) wide; esophagus short; intestinal caeca confluent posterior to testis. Peduncle inconspicuous; haptor subhexagonal, 45 (29–63; n=4) long, 56 (44–65; n=4) wide. Anchors similar in shape, shaft and point evenly curved extending beyond anchor base, base with groove; ventral anchor 26 (25–28; n=8) long, base 18 (17–19; n=8) wide; dorsal anchor 27 (25–28; n=8) long, base 17 (16–17; n=8) wide, with superficial root more developed than ventral anchor. Ventral bar bowed, broad at ends, with heavily sclerotized ornamentation in its middle portion (Fig. 2G), 40 (31–48; n=8) long. Dorsal bar bowed, broad at ends, with a heavily sclerotized patch on surface of bar with inconspicuous medial process and the two submedial projections arise from the tapered ends of this patch (Fig. 2F), 40 (33–45; n=8) long. Hooks similar, 11 (10–11; n=5) long, recurved point, erect thumb, slender shank, FH loop about less than $\frac{3}{4}$ of shank length. MCO slender, forming 1 incomplete counterclockwise ring, broad base, 63 (58–67; n=9) long, proximal ring diameter 28 (22–32; n=9). Accessory piece 53 (47–61; n=8) long, rod shaped with bifurcated distal end. Gonads in tandem; ovary pretesticular. Testis 32 (n=1) long, 18 (n=1) wide; seminal vesicle a dilation of vas deferens; prostatic reservoir present. Vas deferens looping left intestinal caecum. Ovary 39 (n=1) long, 23 (n=1) wide; oviduct, ootype, and uterus not observed. Seminal receptacle at level of anterior margin of ovary; vagina dextral, non-sclerotized. Vitellaria present throughout trunk, except in regions of reproductive organs.

Taxonomic summary

Type host: *Trachydoras paraguayensis* (Eigemann & Ward, 1907) (Siluriformes: Doradidae)

Site of infestation: Gills.

Type locality: Aguapeí River, municipality of Castilho (Paraná River basin), São Paulo State, Brazil (21°3'36.20"S, 51°45'38.58"W).

Representative DNA sequence: 1,547 bp long sequence of the D1–D3 region of the *lsr*DNA of one specimen collected on Jan, 23rd, 2016 (Access. No. XXXX) [paragenophore No. XXXX IPCAS].

Type specimens: Holotype to be deposited at CHIOC; 7 paratypes to be deposited at CHIBB; 3 to be deposited at CHIOC; 4 paratypes to be deposited at IPCAS.

Etymology: The specific name refers to the generic name of its host species.

Remarks

Paracosmetocleithrum trachydorus n. sp. is the type and only known species of *Paracosmetocleithrum* n. gen.

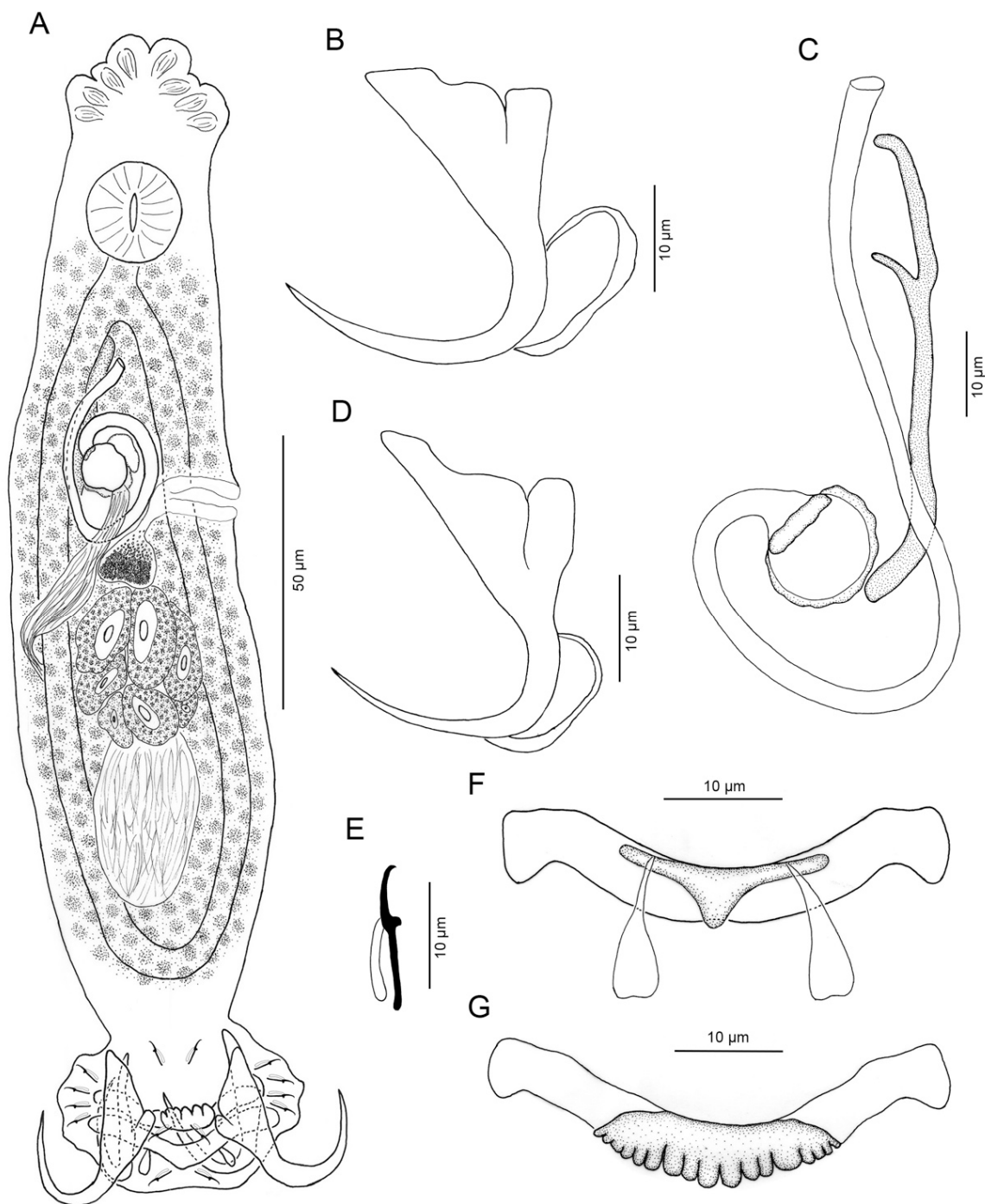


Fig. 2. (A–G) *Paracosmetocleithrum trachydorus* n. sp. from *Trachydoras paraguayensis*. A – whole mount (dorsal view). B – ventral anchor. C – male copulatory organ (ventral view). D – dorsal anchor. E – hook. F – dorsal bar. G – ventral bar.

4.3.3. Phylogenetic relationships

Thirteen new sequences of the 28S rDNA fragment of 13 species of gill monogeneans from Neotropical catfishes were generated (1526–1575 bp) (Table 1). Analyses of datasets 1 and 2 (see Materials and Methods above) yielded similar topologies and for each of the dataset the BI and ML analyses produced phylogenetic trees with mostly congruent branching topology and support values. Figure 3 shows the phylogenetic tree obtained using dataset 1. The phylogenetic trees depicted three main clades (A, B and C) with strong support, comprising species of the Dactylogyridae.

Clade A (Fig. 3) includes species of Neotropical dactylogyrids of the genera *Ameloblastella* Kritsky, Mendoza-Franco, Scholz, 2000, *Heteropriapulius* Kritsky, 2007, *Unibarra* Suriano and Incorvaia, 1995, *Unilatus* Mizelle & Kritsky, 1967, and *Vancleaveus* Kritsky, Thatcher and Boeger, 1986, species of the Nearctic genera *Actinocleidus* Mueller, 1937, *Ligictaluridus* Beverly-Burton, 1984, and *Onchocleidus* Mueller, 1936, and two species of *Ancyrocephalus* from the Palaearctic Region. Clade B comprises only monogeneans from siluriform fishes with species of the genera of the Ancylo-discoidinae, namely *Bychowskyella* Achmerow, 1952, *Chauhanellus* Bychowsky and Nagibina, 1969, *Quadriacanthus* Paperna, 1961, *Schilbetrema* Paperna and Thurston, 1968, *Thaparocleidus* Jain, 1952 and the unidentified species Dactylogyridae gen. sp. 4, and Neotropical dactylogyrids of *Aphanoblastella*, *Cosmetocleithrum*, *Demidospermus*, *Paracosmetocleithrum* n. gen., unidentified species Dactylogyridae gen. sp. 9, 10, 12, 13, 18, and 26 of Mendoza-Palmero et al. [4], and newly sequenced specimens of Dactylogyridae gen. sp. 1, 2, and 5. Clade C contains monogeneans of marine and freshwater anguilliform, cypriniform, mugiliform, scorpaeniform, and perciform fishes with cosmopolitan distribution belonging to *Aliatrema* Plaisance and Kritsky, 2004, *Ancyrocephalus*, *Bravohollisia* Bychowsky and Nagibina, 1970, *Cichidogyrus* Paperna, 1960, *Dactylogyrus* Diesing, 1850, *Ergenstrema* Paperna, 1964, *Euryhaliotrema* Kritsky and Boeger, 2002, *Euryhaliotrematoides* Plaisance and Kritsky, 2004, *Haliotrema* Johnston and Tiegs, 1922, *Haliotrematoides* Kritsky, Yang and Sun, 2009, *Ligophorus* Euzet and Suriano, 1977, *Metahaliotrema* Yamaguti, 1953, *Parasciadicleithrum*, *Protogyrodactylus* Johnston and Tiegs, 1922, *Pseudodactylogyrus* Gusev, 1965, *Pseudohaliotrema* Yamaguti, 1953, *Sciadicleithrum* Kritsky, Thatcher and Boeger, 1989, *Scutogyrus* Pariselle and Euzet, 1995, and *Tetrancistrum* Goto and Kikuchi, 1917.

4.3.4. Phylogenetic relationships among dactylogyrids from Neotropical siluriforms

The phylogenetic tree resulting from the analysis of the most inclusive dataset (3, not shown) was congruent with that inferred from analyses of dataset 1 and 2, revealing the relationships described above. The dataset with longer sequences (1400 bp) did not improve the number of resolved nodes in the tree.

The newly sequenced species from Neotropical siluriform fishes appeared in clades A and B (Fig. 3). Clade A is composed from two subclades, one exclusively with Neotropical species in which species of *Ameloblastella* are sister to *Vancleaveus januacaensis* Kritsky, Thatcher and Boeger, 1986 and *Unibarra paranoplatensis* Suriano and Incorvaia, 1995. These dactylogyrids occur on doradid, heptapterid, and pimelodid catfishes. The other subclade within clade A comprises Neotropical monogeneans from siluriforms and Nearctic and Palearctic species of dactylogyrids from siluriform and perciform fishes. *Unilatus unilatus* Mizelle and Kritsky, 1967 appeared as a sister taxon to all three species of monophyletic *Heteropriapulius*, all species parasitizing Neotropical loricariids. Within Clade B, *Aphanoblastella* (3 species, 1 newly sequenced), *Cosmetocleithrum* (2/1), and *Thaparocleidus* (5/0) appeared monophyletic. Two species of *Demidospermus*, *Demidospermus mortenthaleri*, and *Demidospermus* cf. *paranaensis*, did not cluster together, the latter species being sister taxa to *Demidospermus* spp. (both found on loricariid fishes) in a large clade including *Paracosmetocleithrum* n. gen., n. sp. from a doradid catfish and *Cosmetocleithrum* spp. also from doradids. In contrast, *D. mortenthaleri* appears within a clade formed by Dactylogyridae gen. spp., sister group to a clade of unidentified *Demidospermus* spp., all from pimelodid catfishes. The species identified as *Demidospermus* sp. 11 and 23 by Mendoza-Palmero et al. [4] from the same host – *Brachyplatystoma vaillantii* (Valenciennes, 1840), appeared as sister species and clustered together with Dactylogyridae gen. sp. 9 and 26 (sister species) that are also from the same host – *Platynemichthys notatus* (Jardine, 1841), all with strong support. *Demidospermus mortenthaleri* and Dactylogyridae gen. sp. 23 appear as sister species, but with low support. The following pairs of unidentified taxa for well supported lineages are: Dactylogyridae gen. sp. 10 from *Platynemichthys notatus* and 13 from *Hypophthalmus edentatus* Spix and Agassiz, 1829, Dactylogyridae gen. sp. 5 and 12 from *Sorubim lima* (Bloch and Schneider, 1801), and Dactylogyridae gen. sp. 1 and 2 from *Hemisorubim platyrhynchos* (Valenciennes, 1840) (all Pimelodidae).

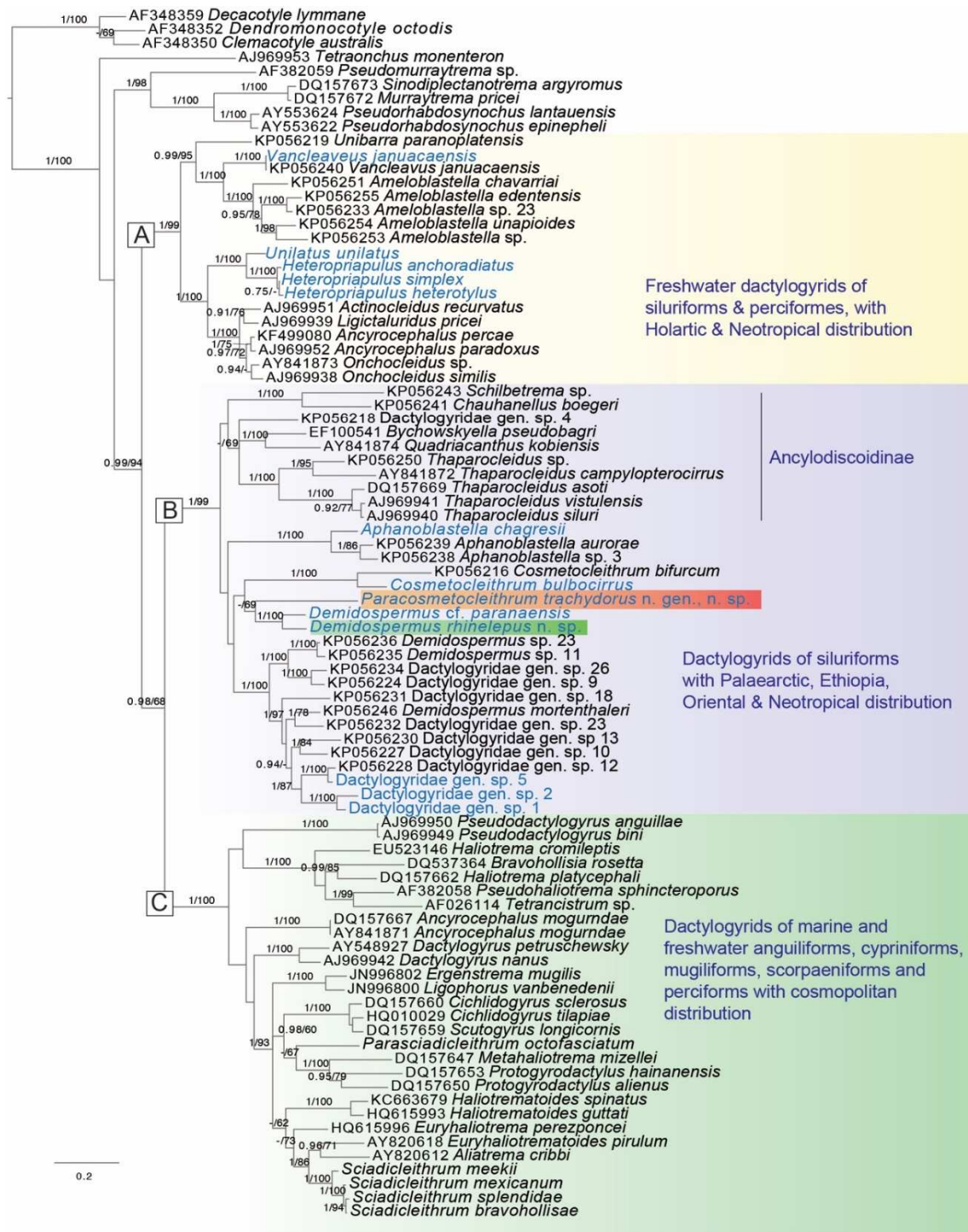


Fig. 3. Molecular phylogeny of the Dactylogyridae using partial sequences of the 28S rDNA gene. Species newly sequenced for this study are in blue. Species belonging to Monocotylidae, Tetraonchidae, Pseudomurraytrematidae, and Diplectanidae were used as outgroups. GenBank sequence ID precedes species name. Posterior probabilities and Maximum Likelihood bootstrap supports are given above the branches (posterior probability <0.90 and bootstrap values <60 are not shown).

4.3.5. Genetic divergence

Genetic divergence between species ranged from 1.4 to 30.7%, whereas intergeneric divergence was 13.6–34.9%. Genetic divergence at the intergeneric level was 23.3–23.5% between *Unibarra* and *Vancleaveus*; 24.1–26.8% between *Ameloblastella* and *Unibarra* and 22.5–27.5% between *Ameloblastella* and *Vancleaveus*. Between *Unilatus* and *Heteropriapulus* the divergence was from 13.6 to 14.2%. The divergence between the two species of *Cosmetocleithrum* and *Paracosmetocleithrum* n. gen. ranged from 33.7 to 34.9%. *Demidospermus* and *Paracosmetocleithrum* n. gen. ranged from 27.2 to 29.3%. *Demidospermus* and *Cosmetocleithrum* ranged from 27.9 to 33.9%. Interspecific divergence in *Ameloblastella* ranged from 6.1 to 22.3%, the lowest between *Ameloblastella* sp. 23 and *A. edentensis*, while the highest between *Ameloblastella* sp. and *A. chavarriai*. Among species of *Heteropriapulus* genetic divergence was 1.4 to 2.9%. *Thaparocleidus* spp. showed a wide divergence range from 1.6 to 30.2%, the lowest between *Thaparocleidus siluri* (Zandt, 1924) and *Thaparocleidus vistulensis* (Siwak, 1932) while *Thaparocleidus asoti* (Yamaguti, 1937) and *Thaparocleidus campylopterocirrus* (Zeng, 1988) showed the highest. Among *T. asoti*, *T. vistulensis*, and *T. siluri* divergence ranged between 6.1 to 6.5%, typical for interspecific level, while *Thaparocleidus* sp. and *T. campylopterocirrus* were most divergent (20.4–30.2%), these values are comparable to the differences found among genera. Such range of divergence values can be noticed in the length of their branches in the phylogenetic trees (Fig. 3). *Aphanoblastella* spp. showed genetic divergence among them from 8.4 to 15.9%, the lowest value between *A. aurorae* and *Aphanoblastella* sp. 3, while the highest was between *A. chagresii* and *Aphanoblastella* sp. 3. *Demidospermus* cf. *paranaensis*, and *Demidospermus rhinelepus* n. sp. diverged 12.1% while *Demidospermus* sp. 11 and 23 showed a divergence of 3.2%. *Demidospermus mortenthaleri* and *Dactylogyridae* gen. sp. 23 diverged 13.5%. However, the alleged *Demidospermus* species that did not cluster together (Fig. 3), as if they belonged to different genera, showed divergence ranging from 19.8 to 28.2%, the lowest divergence was found between *D. mortenthaleri* and *Demidospermus* sp. 23, while the highest was between *Demidospermus* sp. 11 and *D. cf. paranaensis*. The other pairs of sister taxa in the same clade showed genetic distances of: *Dactylogyridae* gen. sp. 10 and 13 of 16.1%; *Dactylogyridae* gen. sp. 5 and 12 of 3.5% and *Dactylogyridae* 1 and 2 of 8.3%. Genetic divergence between the sequences of *V. januacaensis* from the Peruvian Amazon and from the Upper Paraná River was 0.1%, a transition from T to C in one out of 823 bp compared.

4.4. Discussion

Mendoza-Palmero et al. [4] showed that monogeneans parasitizing phylogenetically closely related catfishes were recovered as phylogenetically distant in their analyses. The same could be observed in this study, in which two other genera from Neotropical catfishes, *Unilatus* and *Heteropriapulius* (exclusively to loricariids), clustered in clade A (Fig. 3) with dactylogyrids from perciforms with Holarctic distribution.

This study also recovered members of Ancylo-discoidinae among members of Ancyrocephalinae (clade B, Fig. 3), and showed a clear separation between monogeneans infecting siluriforms and monogeneans from anguiliforms, cypriniforms, mugiliforms, scorpaeniforms, and perciforms (clade C, Fig. 3), as in Mendoza-Palmero et al. [4]. However, this current study could recover support for the two clades (B and C, Fig. 3), which did not occur in Mendoza-Palmero et al. [4]. It has been suggested in the past by Gussev [45] and Lim et al. [46] that parasites of catfishes could represent an independent group within the Dactylogyridae, but the fact that more genera of Neotropical siluriforms grouped with dactylogyrids from perciforms with Holarctic distribution raises the need of more studies to better explore that suggestion.

As mentioned above, delimitation of genera and their differentiation is problematic, especially because of shortage of synapomorphies that would define unequivocally individual genera and lack of molecular data to characterize individual taxa that should represent monophyletic lineages of closely related species. The phylogenetic analyses of this study showed that Clade B (Fig. 3) separated the dactylogyrids belonging to the Ancylo-discoidinae and dactylogyrids from Neotropical siluriforms. For this last clade, the monophyly of *Aphanoblastella* from heptapterids is confirmed, as evidenced by Mendoza-Palmero et al. [4]. *Cosmetocleithrum* is recovered as monophyletic, however, more species of the genus should be sequenced to give more support to its monophyly.

The phylogenetic position of *Paracosmetocleithrum* n. gen., n. sp. shows a close relationship with *Cosmetocleithrum* and *Demidospermus*. Kritsky and Gutiérrez [42] address the morphological similarity between *Cosmetocleithrum* and *Demidospermus* pointing that the only difference is the lack of the two submedial projections of the dorsal bar in *Demidospermus*. The morphological synapomorphies shared by *Cosmetocleithrum*, *Demidospermus*, and *Paracosmetocleithrum* n. gen. might represent a case of convergent evolution. The presence of the two submedial projections of the

dorsal bar in *Cosmetocleithrum* and *Paracosmetocleithrum* n. gen. can be considered a homoplastic character. Morphological evolution of the haptor should exhibit a homoplastic pattern, suggesting that monogeneans with the same haptor type are phylogenetically related and tend to use phylogenetically and ecologically related hosts [47].

A diagnosis amendment of the dactylogyrid genus *Demidospermus* (with a new species description) is proposed based on the relations of its members showed by the phylogenetic analyses (Fig. 3). Wide host range for a genus is not common for monogeneans [48]. Species of *Demidospermus* have been recorded in auchenipterids, loricariids, and pimelodids [6] and former phylogenetic analyses of species of this genus have already showed that its classification is not natural [4]. Therefore, we propose in this study that *Demidospermus* shall be limited to the species infecting loricariid fishes based on analogy with type species and phylogenetic results (refer back to the remarks of the genus above).

The last subclade of clade B clustered only dactylogyrids from pimelodid hosts (Fig. 3). Based on this result, on the phylogenetic relationships among these species, and on the fact that this subclade included *D. mortenthaleri* and *Demidospermus* sp. 11 and 23 (the three of them not clustering together), it is necessary further studies to investigate the generic assignment of these unidentified taxa (Dactylogyridae gen. spp.) and a morphological revision (and phylogenetic, if possible) of *Demidospermus* spp. from pimelodid hosts.

The addition of 13 new 28S rDNA sequences of dactylogyrids from poorly represented host groups of Neotropical siluriforms into a big phylogeny of many members of dactylogyrids with wide distribution, combined with morphological analyses of the putatively new species, enabled to add some resolution to the assessment of their interrelationships, to amend the diagnosis of *Demidospermus*, to describe a new species of *Demidospermus*, and to erect a new dactylogyrid genus from a doradid catfish. However, future studies addressing morphology and molecular phylogeny are still necessary to help circumscription of monophyletic clades within the Dactylogyridae.

4.5. References

- [1] Thatcher VE, Amazon Fish Parasites, second ed, Pensoft, Sofia, Bulgaria, 2006.
- [2] Cohen SC, Kohn A. South American Monogenea – list of species, hosts and geographical distribution from 1997 to 2008. *Zootaxa* 2008; 924: 1–42.
- [3] Mendoza-Palmeiro CA, Scholz T, Mendonza-Franco EF, Kuchta R. New species and geographical records of Dactylogyrids (Monogenea) of catfish (Siluriformes) from the Peruvian Amazonia. *Journal of Parasitology* 2012; 98(3): 484–497.
- [4] Mendoza-Palmero CA, Blasco-Costa I, Scholz T. Molecular phylogeny of Neotropical monogeneans (Platyhelminthes: Monogenea) from catfishes (Siluriformes). *Parasites and Vectors* 2015; 8: 164.
- [5] Kritsky DC, Thatcher VE, Boeger WA. Neotropical Monogenea. 8. Revision of *Urocleidoides* (Dactylogyridae, Ancyrocephalinae). *Proceedings of the Helminthological Society of Washington* 1986; 53: 1–37.
- [6] Braga MP, Araújo SBL, Boeger WA. Patterns of interaction between Neotropical freshwater fishes and their gill Monogenoidea (Platyhelminthes). *Parasitology Research* 2014; 113: 481–490.
- [7] Humason GL. Animal tissue techniques, W.H. Freeman Co., San Francisco, California, 1979.
- [8] Ergens R. 1969. The suitability of ammonium picrate-glycerin in preparing slides of lower Monogenoidea. *Folia Parasitologica* 1969; 16: 320.
- [9] Mizelle JD, Klucka AR. (1953) Studies on monogenetic trematodes. XIV. Dactylogyridae from Wisconsin fishes. *American Midland Naturalist* 1953; 49: 720–733.
- [10] Kritsky DC, Boeger WA, Thatcher VE. Neotropical Monogenea. 7. Parasites of pirarucu, *Arapaima gigas* (Cuvier), with descriptions of two new species and redescription of *Dawestrema cycloancistrum* Price and Nowlin, 1967 (Dactylogyridae: Ancyrocephalinae). *Proceedings of the Biological Society of Washington* 1985; 98: 321–331.
- [11] Mizelle JD, Kritsky DC. Studies on monogenetic trematodes. XXX. Five new species of *Gyrodactylus* from the Pacific tomcod, *Microgadus proximus* (Girard). *Journal of Parasitology* 1967; 53: 263–269.

- [12] Kritsky DC, Mizelle JD. Studies on monogenetic trematodes. XXXV. Some new and previously described North American species of *Gyrodactylus*. American Midland Naturalist 1968; 79: 205–215.
- [13] Mizelle JD. 1936. New species of trematodes from the gills of Illinois fishes. American Midland Naturalist 1936; 17: 785–806.
- [14] Pleijel F, Jondelius U, Norlinder E, Nygren A, Oxelman B, Schander C, Sundberg P, Thollesson M. Phylogenies without roots? A plea for the use of vouchers in molecular phylogenetic studies. Molecular Phylogenetics and Evolution 2008; 48: 369–371.
- [15] Lockyer AE, Olson PD, Littlewood DTJ. Utility of complete large and small subunit rRNA genes in resolving the phylogeny of the Neodermata (Platyhelminthes): implications and a review of the cercomer theory. Biological Journal of the Linnean Society 2003; 78: 155–171.
- [16] Werle EC, Schneider C, Renner M, Völker M, Fiehn W. (1994) Convenient single-step, one tube purification of PCR products for direct sequencing. Nucleic Acids Research 1994; 22: 4354–4355.
- [17] Olson PD, Cribb TH, Tkach VV, Bray RA, Littlewood DTJ. (2003) Phylogeny and classification of the Digenea (Platyhelminthes: Trematoda). International Journal for Parasitology 2003; 33: 733–755.
- [18] Littlewood DTJ, Curini-Galletti M, Herniou EA. The interrelationships of Proseriata (Platyhelminthes: Seriata) tested with molecules and morphology. Molecular Phylogenetics and Evolution 2000; 16: 449–466.
- [19] Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Mentjies P, Drummond A. (2012) Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 2012; 28: 1647–1649.
- [20] Sela I, Ashkenazy H, Katoh K, Pupko T. 2015. GUIDANCE2: accurate detection of unreliable alignment regions accounting for the uncertainty of multiple parameters. Nucleic Acids Research 2015; 43: W7–W14.
- [21] Penn O, Privman E, Landan G, Graur D, Pupko T. 2010. An alignment confidence score capturing robustness to guide-tree uncertainty. Molecular Biology and Evolution, 2010; 27: 1759–1767.

- [22] Guindon S, Gascuel O. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology* 2003; 52: 696–704.
- [23] Darriba D, Taboada GL, Doallo R, Posada D. jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* 2012; 9: 772.
- [24] Stamatakis A. RaxML Version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 2014; 30: 1312–1013.
- [25] Ronquist F, Teslenko M, Van Der Mark P, Ayres DL, Darling A, Höhna S. *et al.* MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 2012; 61: 539–542.
- [26] Miller MA, Pfeiffer W, Schwartz T. Creating the CIPRES Science Gateway Computing Environments Workshop (GCE): 14 November 2010; New Orleans, 1–8, 2010.
- [27] Šimková A, Matějusková I, Cunningham CO. A molecular phylogeny of the Dactylogyridae sensu Kritsky & Boeger (1989) based on the D1–D3 domains of large subunit rDNA. *Parasitology* 2006; 133: 43–53.
- [28] Plaisance L, Littlewood DTJ, Olson PD, Morand S. Molecular phylogeny of gill monogeneans (Platyhelminthes, Monogenea, Dactylogyridae) and colonization of Indo-West Pacific butterflyfish hosts (Perciformes, Chaetodontidae). *Zoologica Scripta* 2005; 34: 425–436.
- [29] Berhrmann-Godel J, Roch S, Brinker A. Gill worm *Ancyrocephalus percae* (Ergens 1966) outbreak negatively impacts the Eurasian perch *Perca fluviatilis* L. stock of Lake Constance, Germany. *Journal of Fish Diseases* 2013; 37: 925–30.
- [30] Ding X-J, Liao X-H. Phylogenetic position of the monogeneans *Pseudodactylogyrus*, *Heteronchocleidus* and *Trianchoratus* inferred from the 5'terminal sequences of 28S rDNA. *Acta Zootaxonomica Sinica* 2005; 30: 244–251. In Chinese
- [31] Wu XY, Zhu XQ, Xie MQ, Li AX. 2006. The radiation of *Haliotrema* (Monogenea: Dactylogyridae, Ancyrocephalinae): molecular evidence and explanation inferred from LSU rDNA sequences. *Parasitology* 2006; 132: 659–668.
- [32] Wu XY, Zhu XQ, Xie MQ, Li AX. The evaluation for generic-level monophyly of Ancyrocephalinae (Monogenea, Dactylogyridae) using ribosomal DNA sequence data. *Molecular Phylogenetic and Evolution* 2007; 44: 530–544.

- [33] Wu XY, Zhu XQ, Xie MQ, Li AX. The radiation of *Thaparocleidus* (Monogeneoidea: Dactylogyridae: Ancyrodiscoidinae): phylogenetic analyses and taxonomic implications inferred from ribosomal DNA sequences. *Parasitology Research* 2008; 102: 283–288.
- [34] Mendlová M, Pariselle A, Vyskočilová M, Šimková A. Molecular phylogeny of monogeneans parasitising African freshwater Cichlidae inferred from LSU rDNA sequences. *Parasitol Res.* 2010; 107: 1405–1413.
- [35] Blasco-Costa, I, Míguez-Lozano R, Sarabeev V, Balbuena JA. Molecular phylogeny of species of *Ligophorus* (Monogenea, Dactylogyridae) and their affinities within Dactylogyridae. *Parasitology International* 2012; 61: 619–627.
- [36] Dang BT, Levsen A, Schander A, Bristow GA. Some *Haliotrema* (Monogenea: Dactylogyridae) from cultured grouper (*Epinephelus* spp.) with emphasis on the phylogenetic position of *Haliotrema cromileptis*. *Journal of Parasitology* 2010; 96: 30–39.
- [37] Olson PD, Littlewood DTJ. Phylogenetics of the Monogenea – evidence from a medley of molecules. *International Journal for Parasitology* 2002; 32: 233–244.
- [38] Mollaret I, Jamieson GMB, Adlard RD, Hugall A, Lecointre G, Chombard C, et al. Phylogenetic analysis of the Monogenea and their relationships with Digenea and Eucestoda inferred from 28S rDNA sequences. *Molecular and Biochemical Parasitology* 1997; 90: 433–8.
- [39] Chisholm LA, Morgan JAT, Adlard RD, Whittington ID. 2001. Phylogenetic analysis of the Monocotylidae (Monogenea) inferred from 28S rDNA sequences. *International Journal for Parasitology* 2001; 31: 1537–1547.
- [40] Suriano DM. *Demidospermus anus* gen. nov. sp. nov. (Monogenea: Ancyrocephalinae) parasite branquial de *Loricaria* (L.) *anus* Valenciennes, 1840 (Pisces: Loricariidae) de la Laguna de Chascomus-Provincia de Buenos Aires-Republica Argentina. *Neotropica* 1983; 29: 111–119.
- [41] Gutiérrez PA, Suriano DM. Ancyrocephalids of the genus *Demidospermus* Suriano, 1983 (Monogenea) parasites from siluriform fishes in Argentina, with descriptions of three new species. *Acta Parasitologica* 1992; 37(4): 169–172.
- [42] Kritsky DC, Gutiérrez PA. Neotropical Monogeneoidea. 34. Species of *Demidospermus* (Dactylogyridae, Ancyrocephalinae) from the gills of pimelodids (Teleostei, Siluriformes) in Argentina. *Journal of the Helminthological Society of Washington* 1998; 65: 147–159.

- [43] Ferrari-Hoeinghaus AP, Bellay S, Takemoto RM, Pavanelli GC. (2010) A new species of *Demidospermus* Suriano, 1983 (Monogenea, Dactylogyridae) parasitic on *Loricariichthys platymetopon* Isbrücker et Nijssen (Loricariidae, Siluriformes) from the Upper Paraná River floodplain, Brazil. *Acta Parasitologica* 2010; 55: 16–19.
- [44] Mendoza-Franco EF, Mendoza-Palmero CA, Scholz T. New species of *Ameloblastella* Kritsky, Mendoza-Franco & Scholz, 2000 and *Cosmetocleithrum* Kritsky, Thatcher & Boeger, 1986 (Monogenea: Dactylogyridae) infecting the gills of catfishes (Siluriformes) from the Peruvian Amazonia. *Systematic Parasitology* 2016; 93: 847–862.
- [45] Gusev AV. New subfamily of monogeneans (Monogenoidea). *Dokl Akad Nauk SSSR*. 1961; 139: 1480–82 (in Russian).
- [46] Lim LHS, Timofeeva TA, Gibson DI. Dactylogyridean monogeneans of the siluriform fishes of the Old World. *Systematic Parasitology* 2001; 50: 159–97.
- [47] Morand S, Šimková A, Matejusova I, Plaisance L, Verneua O, Desdevises Y. Investigating patterns may reveal processes: evolutionary ecology of ectoparasitic monogeneans. *International Journal for Parasitology* 2002; 32: 111–119.
- [48] Kritsky DC, Stephens F, 2001. *Haliotrema abaddon* n. sp. (Monogenoidea: Dactylogyridae) from the gills of wild and maricultured West Australian dhufish *Glaucosoma hebraicum* (Teleostei: Glaucosomatidae), in Australia. *Journal of Parasitologia* 2001; 87: 749–754.

***A synoptic review of Heteropriapulus spp.
(Monogenea: Dactylogyridae) from loricariid
catfishes in South America including description
of five new species and first molecular phylogeny***

5. A synoptic review of *Heteropriapulus* spp. (Monogenea: Dactylogyridae) from loricariid catfishes in South America including description of five new species and first molecular phylogeny¹

Abstract

The genus *Heteropriapulus* Kritsky, 2007 which originally included only two species of gill parasites of loricariid catfishes, is reviewed and five newly described species from loricariids in the Paraná River basin in Brazil are added. Diagnosis of the genus is amended and a key to the species identification is provided. *Heteropriapulus anchoradiatus* **n. sp.** from *Pterygoplichthys ambrosettii* (Holmberg) differs from its congeners by having a long sclerotized vagina, ventral anchors with short shaft and conspicuous superficial root, and a conspicuous and robust medial process on the dorsal bar; *H. bitomus* **n. sp.** from the same fish host differs by the presence of two pairs of sclerotized basal patch associated with the ventral anchors; *H. microcleithrus* **n. sp.** from *P. ambrosettii* differs by the absence of a sclerotized patch associated with the ventral anchor and small length of the dorsal bar; *H. pterygoplichthyi* **n. sp.** from the same host presents a unique shape of the longer subunit of the accessory piece of the male copulatory organ, which is represented by ‘two sickles’ jointed by the base; and *H. semitortus* **n. sp.** from *Rhinelepis aspera* Spix & Agassiz can be distinguished by the accessory piece composed of a single straight unit and a cirrus tube with the highest number of spiral rings at the proximal end (2½). First molecular data (partial sequences of the 28S rRNA gene) are provided for three taxa including the type species *H. heterotylus* (Jogunoori, Kritsky & Venkatanarasaiah, 2004). *Unilatus unilatus* Mizelle & Kritsky, 1967 from loricariid catfishes in the Neotropical Region is a sister taxon to the clade with all 3 species of *Heteropriapulus*, thus indicating relatedness of monogeneans of these 2 genera specific to the Loricariidae.

Key-words: *Heteropriapulus heterotylus*, *Heteropriapulus simplex*, *Heteropriapulus anchoradiatus* **n. sp.**, *Heteropriapulus bitomus* **n. sp.**, *Heteropriapulus microcleithrus* **n. sp.**, *Heteropriapulus pterygoplichthyi* **n. sp.**, *Heteropriapulus semitortus* **n. sp.**, Upper Paraná River, Neotropical region

¹ Manuscript submitted to the journal Zootaxa.

5.1. Introduction

Suckermouth armored catfishes are the most diverse (approximately 800 known species) and widespread members of siluriform fishes, distributed throughout South America, with the northernmost reach up to Costa Rica (Lujan *et al.*, 2015). Most of the species are freshwater, with only a few marine and estuarine taxa. Even though some loricariids are largely distributed in South America, many species are endemic with restricted geographic ranges, being known only from the original site in which they were discovered (Burguess, 1989). The limited knowledge of the biological aspects and biogeographic patterns of distribution, and high morphological variability of loricariid catfishes make their studies difficult (Reis *et al.*, 1990; Zawadzki *et al.*, 2002), which concerns also their parasites such as gill monogeneans (Dactylogyridae) (Cohen & Kohn, 2008).

To date, only 16 species of four dactylogyrid genera have been reported from loricariid catfishes in the Neotropical region, namely *Demidospermus* Suriano, 1983 (2 spp.), *Unilatus* Mizelle & Kritsky, 1967 (7 spp.), *Trinigyrus* Hanek, Molnar & Fernando, 1974 (4 spp.), and *Heteropriapulus* Kritsky, 2007 (2 spp.) (Table 1). The latter genus was erected by Kritsky (2007) to replace *Heterotylus* Jogunoori, Kritsky & Venkatanarasaiah, 2004, which appeared to be a homonym of *Heterotylus* Kirsch in Reitter, 1913 (Coleoptera: Curculionidae). In addition to the type species, *Heteropriapulus heterotylus* (Jogunoori, Kritsky & Venkatanarasaiah, 2004), from the gills of *Hypostomus* sp. from aquaria in Hyderabad and Secunderabad, India (Jogunoori *et al.*, 2004), the genus contains only *Heteropriapulus simplex* Li & Huang, 2012 from *Hypostomus plecostomus* (Linnaeus) introduced to China from South America (Li & Huang, 2012).

As part of a long-term study on the fish parasites of siluriform fishes in the Aguapeí River, Upper Paraná River in southern Brazil, dactylogyrid monogeneans resembling those of *Heteropriapulus* were found on the gills of two loricariid catfishes. These specimens are described as five new species of this genus in the present paper and the diagnosis of *Heteropriapulus* is amended. In addition, first molecular data on species of *Heteropriapulus* are provided and a key to the identification of all seven nominal species of the genus is presented.

TABLE 1. List of monogeneans found on loricariid catfishes in the Neotropical Region.

Species	Host	Reference	Country
Dactylogyridae Bychowsky, 1933			
<i>Demidospermus anus</i> Suriano, 1983	<i>*Loricariichthys anus</i> (Valenciennes)	[1]	Argentina
	<i>Loricariichthys platymetopon</i> Isbrücker & Nijssen	[2]	Brazil
<i>Demidospermus paranaensis</i> Ferrari-Hoeinghaus, Bellay, Takemoto & Pavanelli, 2010	<i>*Loricariichthys platymetopon</i> Isbrücker & Nijssen	[3]	Brazil
<i>Heteropriapulus heterotylus</i> (Jogunoori, Kritsky & Venkatanarasiah, 2004)	<i>Pterygoplichthys disjunctivus</i> (Weber)	[4], [5], [6]	Japan, Mexico
	<i>Pterygoplichthys pardalis</i> (Castelnau)	[5], [6]	Mexico
	<i>Hypostomus plecostomus</i> (Linnaeus)	[7]	China
	<i>*Hypostomus</i> sp.	[8]	India
<i>Heteropriapulus simplex</i> Li & Huang, 2012	<i>*Hypostomus plecostomus</i> (Linnaeus)	[7]	China
<i>Heteropriapulus</i> sp.	<i>Pterygoplichthys pardalis</i>	[9, 10]	Brazil
<i>Trinigyrrus acuminatus</i> Kritsky, Boeger & Thatcher, 1986	<i>*Acanthicus hystrix</i> Agassiz	[11]	Brazil
<i>Trinigyrrus hypostomatis</i> Hanek, Molnar & Fernando, 1974	<i>Hypostomus affinis</i> (Steindachner)	[12]	Brazil
	<i>*Hypostomus robinii</i> Valenciennes	[13]	Trinidad
<i>Trinigyrrus mourei</i> Boeger & Belmont-Jégu, 1994	<i>*Squaliforma emarginata</i> (Valenciennes)	[14], [15]	Brazil
<i>Trinigyrrus tentaculoides</i> Kritsky, Boeger & Thatcher, 1986	<i>*Hypoptopoma thoracathum</i> Günther	[11]	Brazil
<i>Unilatus annoculus</i> (Price, 1968)	<i>*Hypostomus bolivianus</i> (Pearson)	[16]	Bolivia
<i>Unilatus brittani</i> Mizelle, Kritsky & Crane, 1968	<i>Hypostomus</i> sp.	[17]	Brazil
	<i>*Plecostomus</i> sp.	[17]	Brazil
	<i>Pterygoplichthys anisitsi</i> Eigmann & Kennedy	[18]	Peru
	<i>Pterygoplichthys multiradiatus</i> (Hancock)	[19]	Brazil
<i>Unilatus dissimilis</i> Suriano, 1985	<i>*Hemiancistrus</i> sp.	[19]	Brazil

TABLE 1. Continued.

Species	Host	Reference	Country
<i>Unilatus irae</i> Branches & Domingues, 2014	* <i>Leporacanthicus galaxias</i> Isbrücker & Nijssen	[20]	Brazil
<i>Unilatus scaphirhynchoe</i> Suriano, 1985	* <i>Dekeyseria scaphirhynchoe</i> (Kner)	[21]	Brazil
<i>Unilatus unilatus</i> Mizelle & Kritsky, 1967	<i>Hypostomus iheringii</i> (Regan)	[21]	Brazil
	<i>Hypostomus regani</i> (Ihering)	[21]	Brazil
	<i>Hypostomus robinii</i> Valenciennes	[22]	Trinidad
	<i>Hypostomus strigaticeps</i> (Regan)	[21]	Brazil
	* <i>Plecostomus</i> sp.	[23]	Brazil
	<i>Pterygoplichthys anisitsi</i>	[18]	Peru
	<i>Pterygoplichthys multiradiatus</i>	[19]	Brazil
<i>Unilatus</i> sp.	<i>Pterygoplichthys pardalis</i>	[9]	Brazil

(*) type host. [1] Suriano (1983); [2] Cohen & Kohn (2008); [3] Ferrari-Hoeinghaus *et al.* (2010); [4] Nitta & Nagasawa (2013); [5] Rodríguez-Santiago *et al.* (2015); [6] Rodríguez-Santiago *et al.* (2016); [7] Li & Huang (2012); [8] Jogunoori *et al.* (2004); [9] Porto *et al.* (2012); [10] Mendoza-Franco *et al.* (2012); [11] Kritsky *et al.* (1986a); [12] Azevedo *et al.* (2010); [13] Hanek *et al.* (1974); [14] Boeger & Belmont-Jégu (1994); [15] Gonçalves *et al.* (2014); [16] Price (1968); [17] Mizelle *et al.* (1968); [18] Mendoza-Palmero *et al.* (2012); [19] Suriano (1985); [20] Branches and Domingues (2014); [21] Zica *et al.* (2012); [22] Molnar *et al.* (1974); [23] Mizelle and Kritsky (1967b).

5.2. Materials and Methods

5.2.1. Sampling

Pterygoplichthys ambrosettii (Holmberg) and *Rhinelepis aspera* Spix & Agassiz were collected in the mouth of the Aguapeí River, a tributary of the Paraná River, from August 2013 to November 2014. Fishes were captured using gill nets and multifilament cast nets, frozen after capture and taken to the laboratory to perform parasitological survey. The gills were removed and checked for monogeneans under stereomicroscope. Monogeneans were isolated from the gills and some specimens found were mounted on slides with Hoyer's and Grey and Wess' medium (Humason, 1979; Kritsky *et al.*, 1986b) to study sclerotized structures; some specimens were stained with Gomori's trichrome to study internal organs. The parasites were studied using V3 Leica

Application Suite computerized system for image analysis with differential interference contrast and an Olympus BX51 microscope equipped with phase contrast optics. Illustrations were made with the aid of a drawing tube (camera lucida) mounted on a Leica DMLS microscope.

Measurements were taken according to Gussev (1985) and Řehulková *et al.* (2013) for descriptions (see Fig. 1), and Mizelle & Klucka (1953) and Kritsky *et al.* (1985) for comparisons (see Table 2); they are in micrometers as the range followed by mean and number of measurements in parentheses. The terminology for the components of the haptor follows that of Mizelle & Kritsky (1967a) and Kritsky & Mizelle (1968); hooks are numbered according to Mizelle (1936).

Type and voucher specimens are deposited in the Helminthological Collection of the Instituto Oswaldo Cruz, Rio de Janeiro, Brazil (CHIOC), the Helminthological Collection of the Instituto de Biociências, Universidade Estadual Paulista, Botucatu, Brazil (CHIBB), and the Helminthological Collection of the Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, České Budějovice (IPCAS), as indicated in the following descriptions. The holotype deposited in the U. S. National Parasite Collection (USNPC 93651; currently at the Smithsonian's National Museum of Natural History, Washington, D.C., USA) and four paratypes of *H. heterotylus* deposited at the Natural History Museum, London, UK (BMNH 2003.9.4.4-12: 19, 23, 24 and 26) were examined. Scientific names of the hosts follow Froese & Pauly (2016).

5.2.2. DNA extraction, amplification and sequencing

The specimens used for molecular analyses were placed on a slide with a drop of water, covered with a coverslip, identified under the microscope based on their morphology and then placed into 96% molecular-grade ethanol. Some allegedly conspecific specimens (paragenophores – see Pleijel *et al.*, 2008 for terminology) were fixed with glycerin ammonium picrate (GAP) and mounted as permanent preparations with Canada's balsam following Ergens (1969) and deposited at IPCAS. Genomic DNA was extracted using 200 µl of a 5% suspension of Chelex in deionized water and 2 µl of proteinase K, followed by overnight incubation at 56°C, boiling at 90°C for 8 minutes and centrifugation at 14,000 rpm for 10 minutes.

Polymerase chain reactions (PCR) were made in 20 µl of reaction mixture with 3 µl of extraction supernatant, 10 µl of 2× MyFi™ Mix (Bioline, CITY, USA), and 1.6

µl of each PCR primer. Partial 28S rDNA (D1–D3 region) was amplified using the primers U178 (5'-GCA CCC GCT GAA YTT AAG-3') and L1642 (5'-CCA GCG CCA TCC ATT TTC A-3') (Lockyer *et al.*, 2003). The thermocycling profile used was: denaturation of DNA (95°C for 3 min); 35 cycles of amplification (94°C for 30 s, 56°C for 30 s and 72°C for 1.5 min) and 4 min extension hold at 72°C. PCR products were run on 1% agarose gel using gel red and loading buffer. PCR products were purified prior to sequencing using exonuclease I and shrimp alkaline phosphatase enzymes (Werle *et al.*, 1994). Amplicons were Sanger sequenced at GATC Biotech (Konstanz, Germany) using the PCR primers and the internal primers 900F (5'-CCGTCTTGAAACACGGACCAAG-3') (Olson *et al.*, 2003), 300R (5'-CAACTTTCCCTCACGGTACTTG-3') (Lockyer *et al.*, 2003); L1200R (5'-GCATAGTTCACCATCTTTCGG-3') and ECD2 (5'-CCTTGGTCCGTGTTTCAAGACGGG) (Littlewood *et al.*, 2000). Contiguous sequences were assembled using Geneious version R8 (<http://www.geneious.com/>; Kearse *et al.*, 2012).

5.2.3. Alignment and phylogenetic analyses

Four newly generated sequences of partial 28S rDNA (D1–D3 region) were aligned with eight sequences of species belonging to the family Dactylogyridae (six from the Neotropical region and two from the Palaearctic region), and sequences of three species of the families Pseudomurraytremaidae and Diplectanidae retrieved from Genbank, which served as outgroups based on the topology of the phylogenetic tree of Neotropical dactylogyrids from catfishes in Mendoza-Palmero *et al.* (2015) (see Table 3 for accession numbers, host species and origin of sequenced taxa); the E-INS-i algorithm of the program MAFFT (Kato & Standley, 2013) implemented in Geneious was used. A preliminary analysis of new sequences with those of the three clades of Mendoza-Palmero *et al.* (2015) showed that the sequences of the species of *Heteropriapulius* and *U. unilatus* clustered together with those of taxa belonging to Clade A, which includes freshwater ancyrocephalines of siluriform and perciform hosts with Holarctic and Neotropical distribution (analysis A). Thereafter, a more specific phylogenetic analysis was performed only with some species belonging to that clade (analysis B). The alignment was trimmed and refined by eye prior to phylogenetic analyses, resulting in 1,181 nucleotide positions.

Phylogenetic reconstructions were performed with Bayesian inference (BI) and Maximum likelihood (ML) criteria, based on GTR+G model of sequence evolution, using MrBayes ver. 3.2.6 (Huelsenbeck & Ronquist, 2001) and PhyML (Guindon & Gascuel, 2003) plugins in Geneious. The model of evolution was chosen using the Akaike Information Criterion (AIC) in PAUP* ver.4a147 (Swofford, 2002). ML nodes were estimated by 100 bootstrap replicates. BI analysis was run for 5,000,000 generations and sampled every 1,000 generations. The burn-in was defined as the point at which the average standard deviation of split frequencies was < 0.01. Phylogenetic trees were edited in FigTree v1.3.1 (Rambaut 2009).

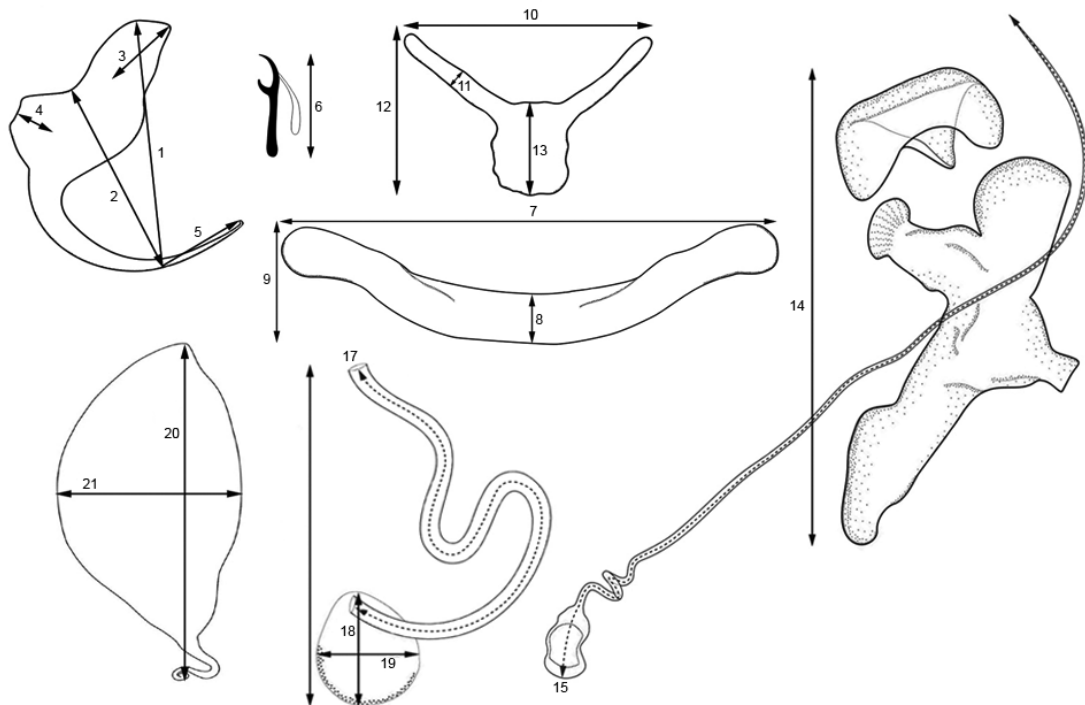


FIGURE 1. Scheme of measurements for the sclerotized structures of the haptor and reproductive organs of *Heteropriapulus* spp.: 1 – anchor total length; 2 – anchor length to notch; 3 – superficial root length (in ventral anchors of *Heteropriapulus* spp. without basal roots; this measurement represents the ventral anchor base width); 4 – deep root length; 5 – point length; 6 – hook length; 7 – ventral bar length; 8 – ventral bar width; 9 – ventral bar height; 10 – dorsal bar length; 11 – dorsal bar width; 12 – dorsal bar height; 13 – width of medial process of dorsal bar; 14 – accessory piece length; 15 – total curve length of MCO; 16 – vagina length; 17 – vagina total curve length; 18 – vagina opening length; 19 – vagina opening width; 20 – egg length; 21 – egg width.

5.3. Results

5.3.1. A synoptic review of species of *Heteropriapulus* Kritsky, 2007

Heteropriapulus heterotylus (Jogunoori, Kritsky & Venkatanarasaiah, 2004) Kritsky, 2007

Syn. *Heterotylus heterotylus* Jogunoori, Kritsky & Venkatanarasaiah, 2004
(Fig. 2)

Material studied: holotype (USNPC 93651) and 4 paratypes (BMNH 2003.9.4.4-12, 19, 23, 24 and 26); 10 specimens from *Pterygoplichthys ambrosettii* from the Aguapeí River (hosts numbers 9, 11, and 15 collected in August 2013; 3, 7, 8, 11, and 15 collected in January 2014 and 11 collected in November 2014); 6 vouchers in CHIBB (Coll. No. XXXX) and 4 vouchers in IPCAS (M-XXXX).

Type host: *Hypostomus* sp. (Siluriformes: Loricariidae).

Type locality: Aquaria in Hyderabad and Secunderabad, India (fish hosts were imported via the aquarium trade).

Additional hosts: *Hypostomus plecostomus*, *Pterygoplichthys ambrosettii* (new host record), *P. disjunctivus* (Weber) and *P. pardalis* (Castelnau) (all Siluriformes: Loricariidae).

Site of infection: Gills.

Distribution: India (introduced), Brazil (new geographical record; most probably the original distribution area), China (introduced), Mexico (introduced).

Representative DNA sequence: 1,575 bp long sequence of the D1–D3 region of the *lsrDNA* of specimen from *P. ambrosettii* collected on 23 November 2015 (GenBank Accession No. XXXX).

Remarks. *Heteropriapulus heterotylus* (Jogunoori, Kritsky & Venkatanarasaiah, 2004) was described (as *Heterotylus heterotylus* – see Kritsky, 2007) from the gills of *Hypostomus* sp. imported to the aquaria in India (Jogunoori *et al.*, 2004). The species was also found on *H. plecostomus* imported to China (Li & Huang, 2012), *P. disjunctivus* imported to Japan (Nitta & Nagasawa, 2013), and invasive *P. pardalis* and *P. disjunctivus* in Mexico (Rodríguez-Santiago *et al.*, 2015, 2016). Specimens found on *P. ambrosettii* from Brazil were identified as *H. heterotylus* and their identification was confirmed by comparison with holotype and paratypes of *H.*

heterotylus. Nevertheless, slight difference was found between some of the measurements of the new material from Brazil and those in the original description, especially in greater size of the present specimens (see Table 2). The cirrus of the Brazilian specimens present 1½ counterclockwise spiral rings next to the rounded base, whereas the male copulatory organ of specimens from type host present inconspicuous proximal bend, according to Jogunoori *et al.* (2004).

In the present study, the first molecular data on *H. heterotylus* are provided (Fig. 9), a new fish host is added (third species of *Pterygoplichthys* Gill and the distribution area of *H. heterotylus* is enlarged to include the original distribution area of the species (all previous records were from introduced fish hosts).

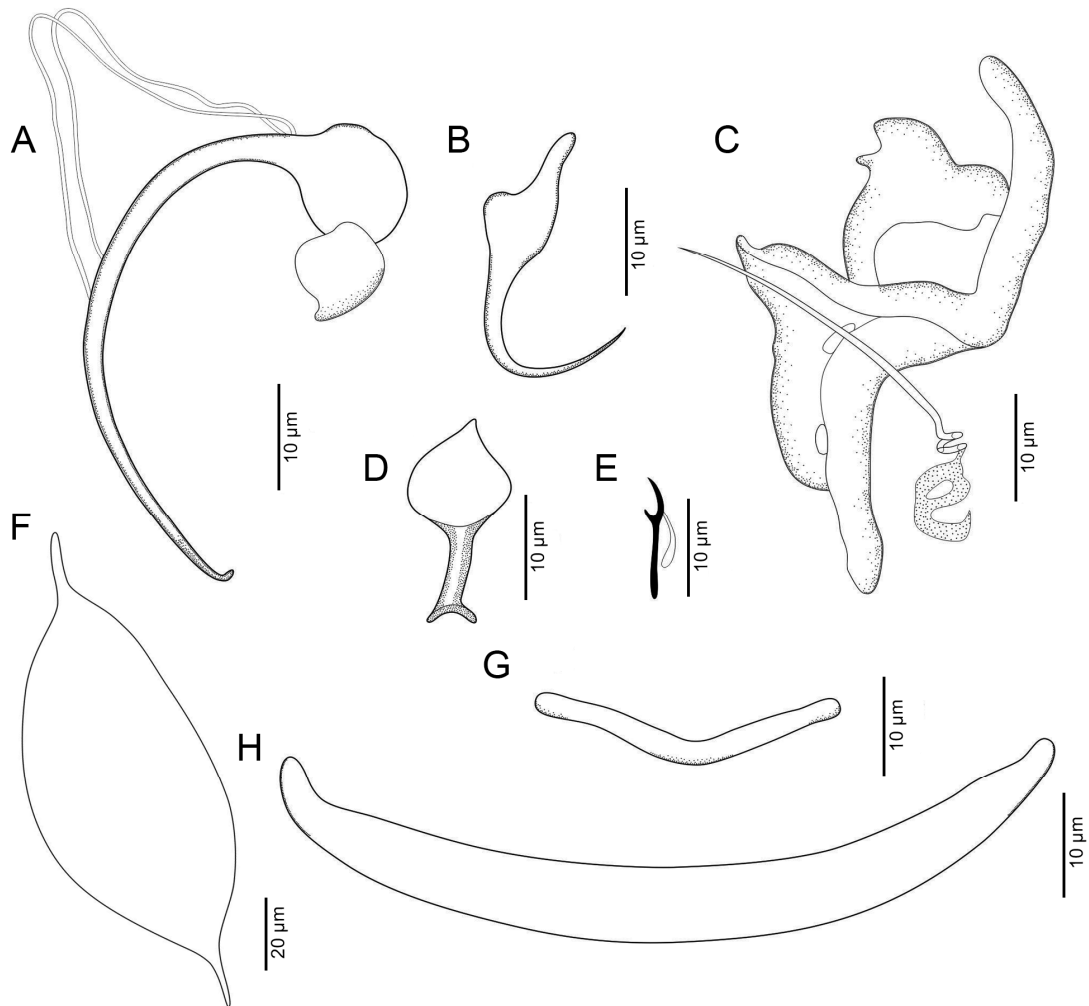


FIGURE 2. (A–H) Sclerotized structures of *Heteropriapulus heterotylus* (Jogunoori, Kritsky & Venkatanarasaiah, 2004) from *Pterygoplichthys ambrosettii* (Holmberg) from the Upper Paraná River, Brazil. A – ventral anchor. B – dorsal anchor. C – male copulatory complex. D – vagina. E – hook. F – egg. G – dorsal bar. H – ventral bar.

***Heteropriapulus simplex* Li & Huang, 2012**

(Fig. 3)

Specimens studied: 10 specimens from *Pterygoplichthys ambrosettii* from the Aguapeí River (hosts numbers 3, 8, and 7 collected in January 2014 and 17 and 20 collected in November 2014); 5 vouchers in CHIBB (XXXX) and 5 vouchers in IPCAS (M- XXXX).

Type host: *Hypostomus plecostomus* (Linnaeus) (Siluriformes: Loricariidae).

Additional host: *Pterygoplichthys ambrosettii* (Holmberg) (Siluriformes: Loricariidae) (new host record).

Site of infection: Gills.

Type locality: Guangzhou, China.

Distribution: China (imported), Brazil (new geographical record; probably original distribution area).

Representative DNA sequence: 1,575 bp long sequence of the D1–D3 region of the *lsrDNA* of specimen from *P. ambrosettii* collected on 22 January 2016 (GenBank Accession No. XXXX).

Remarks. *Heteropriapulus simplex* was described from *H. plecostomus* introduced to China from South America (Li & Huang, 2012). Specimens found in *P. ambrosettii* differ only slightly, especially in their somewhat greater size and a thicker dorsal connecting bar, from specimens described by Li & Huang (2012) from type host (Table 2). The present study provides a new host and geographical record for *H. simplex*, which is reported from the probable original distribution area, i.e. Neotropical Region, for the first time.

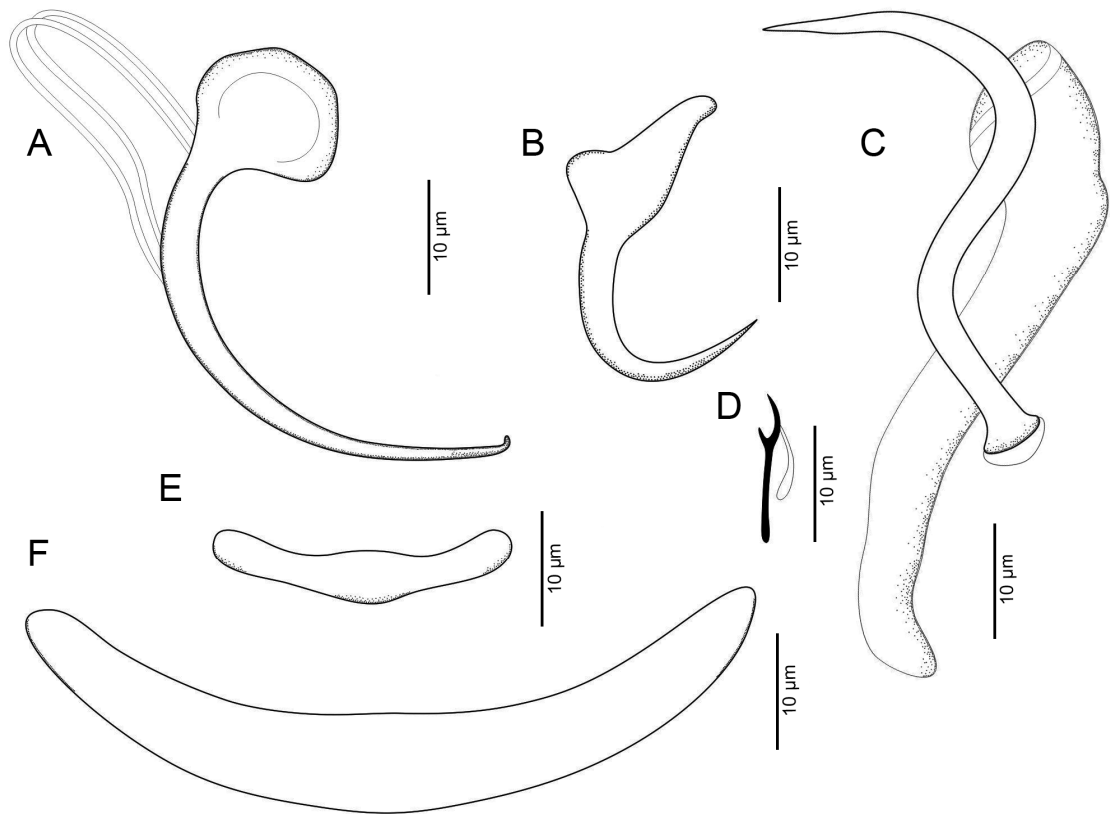


FIGURE 3. (A–F) Sclerotized structures of *Heteropriapulus simplex* Li & Huang, 2012 from *Pterygoplichthys ambrosettii* (Holmberg) from the Upper Paraná River, Brazil. A – ventral anchor. B – dorsal anchor. C – male copulatory complex. D – hook. E – dorsal bar. F – ventral bar.

TABLE 2. Measurements (in micrometers; expressed as range followed by mean and number of specimens in parentheses) of the already described species of *Heteropriapulus* from this study and from Jogunoori *et al.* (2004) and Li & Huang (2012).

Parasite	<i>H. heterotylus</i>	<i>H. heterotylus</i> *	<i>H. simplex</i>	<i>H. simplex</i> ‡
Host	<i>P. ambrosettii</i>	<i>Hypostomus</i> sp.	<i>P. ambrosettii</i>	<i>H. plecostomus</i>
Body length	302–416 (355; n=12)	143–230 (181; n=13)	178–584 (344; n=10)	132–221
Body width	47–106 (67; n=12)	43–77 (58; n=17)	60–128 (79; n=10)	29–103
Haptor length	38–68 (52; n=12)	47–69 (57; n=9)	32–85 (57; n=10)	–
Haptor width	88–111 (99; n=12)	58–71 (64; n=12)	54–118 (91; n=10)	–
Ventral anchor length	25–34 (29; n=12)	34–41 (39; n=11)	24–31 (26; n=10)	27–37
Ventral anchor width	11–14 (12; n=12)	9–12 (10; n=11)	12–15 (13; n=10)	–
Dorsal anchor length	21–28 (23; n=12)	18–21 (20; n=9)	21–25 (23; n=10)	15–20
Dorsal anchor width	8–12 (10; n=12)	6–9 (8; n=9)	12–16 (13; n=10)	12–16
Ventral bar length	68–84 (74; n=11)	40–47 (44; n=10)	50–74 (63; n=10)	48–58
Dorsal bar length	27–35 (30; n=11)	18–23 (21; n=7)	23–26 (25; n=10)	17–23
Hook length	13–14 (13; n=12)	13–15 (14; n=11)	13–14 (13; n=10)	10–13
MCO length	36–50 (41; n=12)	32–38 (35; n=4)	41–51 (45; n=10)	31–39
Accessory piece length	35–56 (45; n=12)	26–32 (29; n=6)	37–58 (52; n=10)	32–39
Vagina length	16–19 (18; n=3)	–	–	–
Vagina width	10–13 (12; n=3)	–	–	–
Egg length	138 (n=1)	–	–	–
Egg width	49 (n=1)	–	–	–

Numbers in bold indicate differences in the present measurements and those in the original descriptions.

(*) Data from from Jogunoori *et al.* (2004).

(‡) Data from from Li & Huang (2012).

***Heteropriapulus anchoradiatus* n. sp.**

(Fig. 4)

Type and only known host: *Pterygoplichthys ambrosettii* (Holmberg) (Siluriformes: Loricariidae).

Site of infection: Gills.

Type locality: Aguapeí River, municipality of Castilho (Paraná River basin), São Paulo State, Brazil (21°3'36.20"S, 51°45'38.58"W).

Representative DNA sequence: 1,575 bp long sequence of the D1–D3 region of the *lsrDNA* of one specimen collected on Jun, 14th, 2014 (Access. No. XXXX) [paragenophore No. XXXX IPCAS].

Type specimens: holotype – 1 whole-mounted stained specimen from *P. ambrosettii* from the Aguapeí River (host number 11 collected in June 2014) examined on 20 April 2015 (CHIOC XXX); 14 paratypes from *P. ambrosettii* from the Aguapeí River (hosts numbers 5, 8, 9 and 14 collected in August 2013; 15 and 18 collected in January 2014; 5, 11, 16 collected in June 2014): 11 paratypes in CHIBB (XXXX) and 3 paratypes in IPCAS (M- XXXX).

Etymology: The name is from Latin and refers to the presence of roots in the base of the ventral anchors (*anchor* = anchor + *radiatus* = roots).

Description [based on 14 specimens mounted in Hoyer's medium]. Body 388–592 (493; n = 12) long, greatest width 75–174 (112; n=12), usually near mid-length. Accessory granules extending from level of gonads to cephalic lobes. Copulatory complex comprising non-articulated copulatory organ and accessory piece. Copulatory tube delicate, total length 86–141 (123; n=13), straight in anterior portion, showing 1½ counterclockwise spiral rings next to the rounded base. Accessory piece 51–71 (61; n=13) long, composed by 2 subunits strongly sclerotized: 1 small and another long. Vagina long tube, sclerotized, 35–60 (50; n=13) long, 63–95 (82; n=13) curved length; vaginal aperture 13–19 (16; n=13) long, 12–18 (13; n=13) wide, sigmoid, with thickened margin near vaginal aperture. Eggs 66–93 (83; n=3) long, 41–51 (47; n=3) wide, operculate, short polar filament opposed to opercular end. Haptor 51–108 (76; n=12) long, 80–147 (106; n=12) wide, globose to slightly subtrapezoidal. Ventral anchors 28–33 (31) long, length to notch 23–27 (24), shaft short, superficial root 12–14 (14) long, deep root 6–8 (7) long, point 14–16 (15) long with discrete recurved tip;

anchor patch absent, anchor filament double and moderately developed. Dorsal anchors 28–34 (32) long, length to notch 22–27 (24), superficial root 13–17 (16) long, deep root 3–5 (4) long, straight point 14–16 (15) long. Ventral bar 62–77 (69) long, 7–11 (8) wide, 14–22 (18) high, bent, rounded ends. Dorsal bar 30–37 (33) long, 3–5 (4) wide, 21–27 (23) high, with robust medial process 12–16 (14) wide. Seven pairs of similar hooks 15–17 (16) long, with round, weakly sclerotized subunit at base, FH loop about ½ of shank length.

Remarks. *Heteropriapulus anchoradiatus* **n. sp.** differs from *H. heterotylus* and *H. simplex* (and also all other species of the genus described as new in the present paper – see below) in the possession of a long, sclerotized vagina, sigmoid in shape. Furthermore, *H. anchoradiatus* **n. sp.** possesses ventral anchors with a short shaft and a conspicuous superficial root, whereas all other species of *Heteropriapulus* have ventral anchors with an elongate shaft and a flattened base. The new species is also characterized by having a conspicuous and robust medial process on the dorsal bar, which is inconspicuous, short or even absent in congeners, and a round, weakly sclerotized subunit at the base of the hooks.

***Heteropriapulus bitomus* n. sp.**

(Fig. 5)

Type and only known host: *Pterygoplichthys ambrosettii* (Holmberg) (Siluriformes: Loricariidae).

Site of infection: Gills.

Type locality: Aguapeí River, municipality of Castilho (Paraná River basin), São Paulo State, Brazil (21°3'36.20"S, 51°45'38.58"W).

Type specimens: holotype – 1 specimen mounted in Hoyer's medium from *P. ambrosettii* from the Aguapeí River (host number 18 collected in November 2014) examined on 3 May 2016 (CHIOC XXX); 1 paratype (host number 18 collected in November 2014) in CHIBB (XXXX).

Etymology: The name is from Latin and refers to the presence of two pairs of sclerotized patch (*bi* = two + *tomus* = patch, piece).

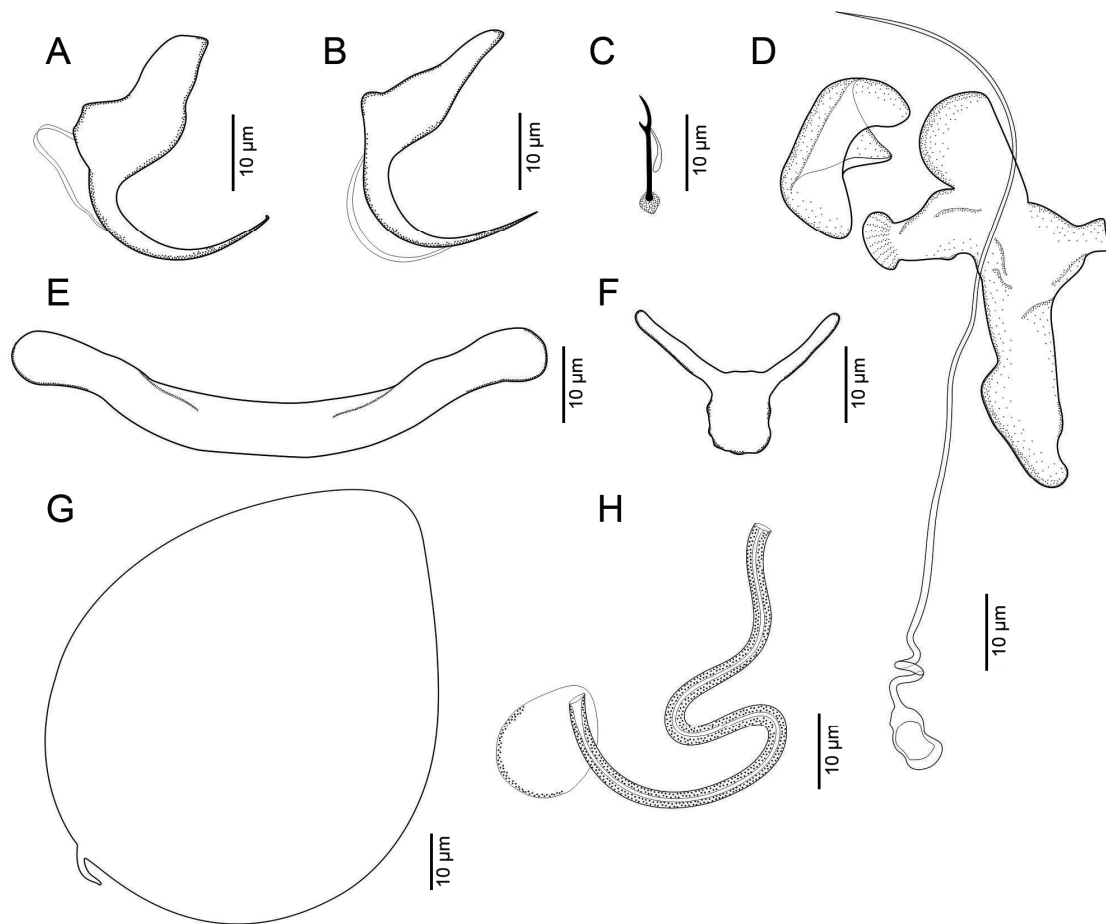


FIGURE 4. (A–F) Sclerotized structures of *Heteropriapulus anchoradiatus* **n. sp.** from *Pterygoplichthys ambrosetti* (Holmberg) from the Upper Paraná River, Brazil. A – ventral anchor. B – dorsal anchor. C – hook. D – male copulatory complex. E – ventral bar. F – dorsal bar. G – egg. H – vagina.

Description [based on two specimens mounted in Hoyer’s medium]. Body 258–453 (356) long, greatest width 54, usually near mid-length. Accessory granules extending from level of gonads to cephalic lobes. Copulatory complex comprising non-articulated copulatory organ and accessory piece. Copulatory tube delicate, total curved length 41–52 (47), straight in anterior portion showing 2 counterclockwise spiral rings next to the rounded base. Accessory piece 42–45 (44) long, composed of single unit, robust, conspicuous and strongly sclerotized. Vagina short and sclerotized, shaped as goblet, 8–12 (10) long, 6–9 (8) curved length, 4 wide, having thickened posterior margins. Haptor 45–53 (49) long, 89–101 (95) wide, subtrapezoidal. Ventral anchors with flattened base, 26–28 (27) long, length to notch 31–35 (33), base 9–11 (10),

elongate shaft and point 28–32 (30) long, recurved tip extending posteroventrally from haptor, anchor filament double and well-developed; 2 pairs of sclerotized anchor patches present: one relatively small and another conspicuous, robust and rounded shape. Dorsal anchors 16–17 (16) long, length to notch 13–15 (14), superficial root 5 long, deep root 1 long, straight point 7–9 (8) long. Ventral bar bowed, rounded ends, 91 long, 8 wide, 19–22 (22) high. Dorsal bar small, bent, 27 long, 4 wide, 5–6 (5) high. Seven pairs of similar hooks, 12 long, FH loop about ½ of shank length.

Remarks. This species can be easily distinguished from congeners by the following characteristics: two pairs of sclerotized patch associated with the ventral anchors, a sclerotized, short, goblet-shaped vagina, and a long, robust, conspicuous and strongly sclerotized accessory piece composed of a single unit.

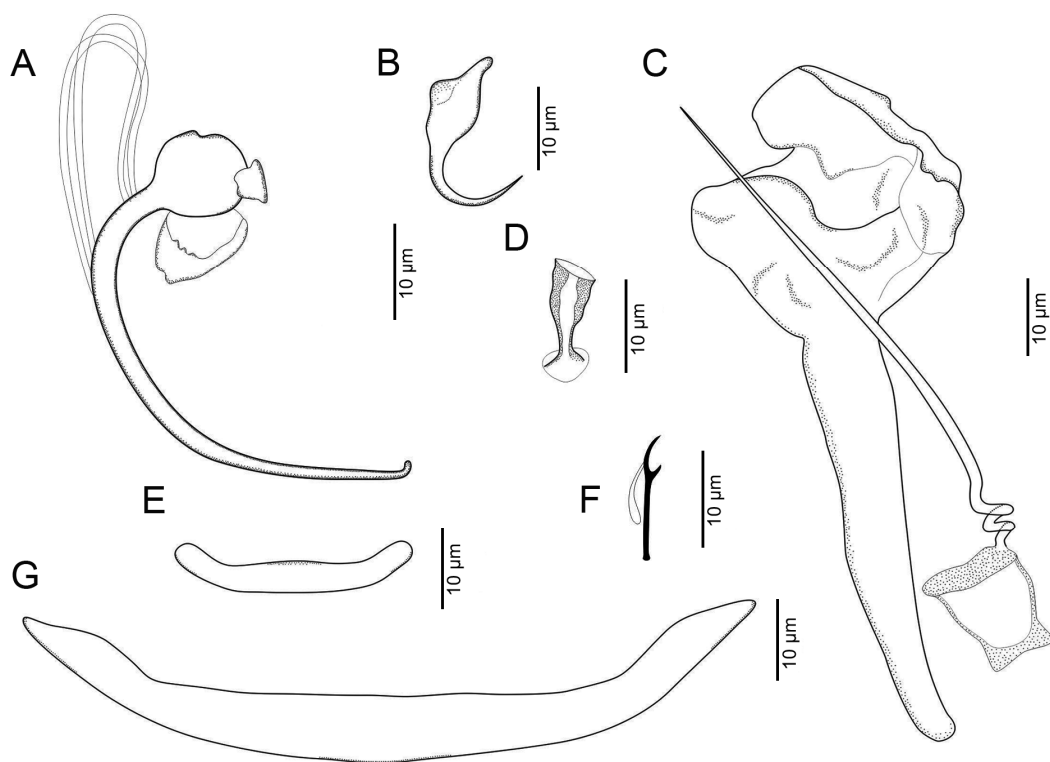


FIGURE 5. (A–F) Sclerotized structures of *Heteropriapulus bitomus* n. sp. from *Pterygoplichthys ambrosettii* (Holmberg) from the Upper Paraná River, Brazil. A – ventral anchor. B – dorsal anchor. C – male copulatory complex. D – vagina. E – dorsal bar. F – hook. G – ventral bar.

***Heteropriapulus microcleithrus* n. sp.**

(Fig. 6)

Type and only known host: *Pterygoplichthys ambrosettii* (Holmberg) (Siluriformes: Loricariidae).

Site of infection: Gills.

Type locality: Aguapeí River, Castilho (Paraná River basin), São Paulo State, Brazil (21°3'36.20"S, 51°45'38.58"W).

Type specimens: holotype – 1 whole-mounted stained specimen from *P. ambrosettii* from the Aguapeí River (host number 4 collected in January 2014) examined on 26 April 2016 (CHIOC XXX); 13 paratypes from *P. ambrosettii* from the Aguapeí River (hosts numbers 9, 17, 18, 19 and 20 collected in August 2013; 3 and 5 collected in January 2014; 8 collected in November 2014): 8 paratypes in CHIBB (XXXX) and 5 paratypes in IPCAS (XXXX).

Etymology: The name is from Greek and refers to the smallest size of the dorsal bar compared to other congeners (*micro* = small + *cleithrum* = bar).

Description [based on 10 specimens mounted in Hoyer's medium]. Body 322–590 (412) long, greatest width 57–217 (105), usually near mid-length. Accessory granules extending from level of gonads to cephalic lobes. Copulatory complex comprising non-articulated copulatory organ and accessory piece. Copulatory tube delicate, 45–54 (49) long, straight in anterior portion, forming 1½ counterclockwise spiral rings next to the rounded base. Accessory piece 32–46 (39) long, composed of 2 subunits, one long, robust, conspicuous and strongly sclerotized, another small and weakly sclerotized. Vagina short and sclerotized, 13–16 (15; n=7) long, 14–17 (16; n=7) curved length, 7–8 (8; n=7) wide, with thickened anterior and posterior margins. Eggs operculate, 45 long, 18 (n=1) wide, short polar filament opposite to opercular end. Haptor 39–67 (50) long, 65–109 (88) wide, subtrapezoidal. Ventral anchors with flattened base, 16–17 (17) long, length to notch 22–25 (24), base 10–12 (11) long, shaft moderately elongate, point 15–17 (17) long, with recurved tip extending posteroventrally from haptor, anchor filament double, well-developed, anchor patch absent. Dorsal anchors 16–18 (17) long, length to notch 15–17 (16), short deep root 1–2 (2) long, elongate superficial root 5–6 (5) long, straight point 8–9 (9) long, anchor filament double, less conspicuous. Ventral bar rounded ends, 51–62 (56) long, 3–6 (5)

wide, 6–13 (9) high. Dorsal bar small, 15–17 (16) long, 2–4 (3) wide, 3–7 (5) high, slightly thicker in middle. Seven pairs of similar hooks, 11–13 (12) long, FH loop about $\frac{3}{4}$ of shank length.

Remarks. *Heteropriapulus microcleithrus* **n. sp.** can be differentiated from its congeners by combination of the following characteristics: a sclerotized patch associated with the ventral anchor is absent, the smallest dorsal bar (other species have the bar longer than 17 μm), and an accessory piece composed of two subunits with one subunit small and weakly sclerotized, and another considerably longer, robust, strongly sclerotized. *Heteropriapulus anchoradius* **n. sp.** also possess an accessory piece composed of two subunits, but it is strongly sclerotized and differ in shape from that of the new species.

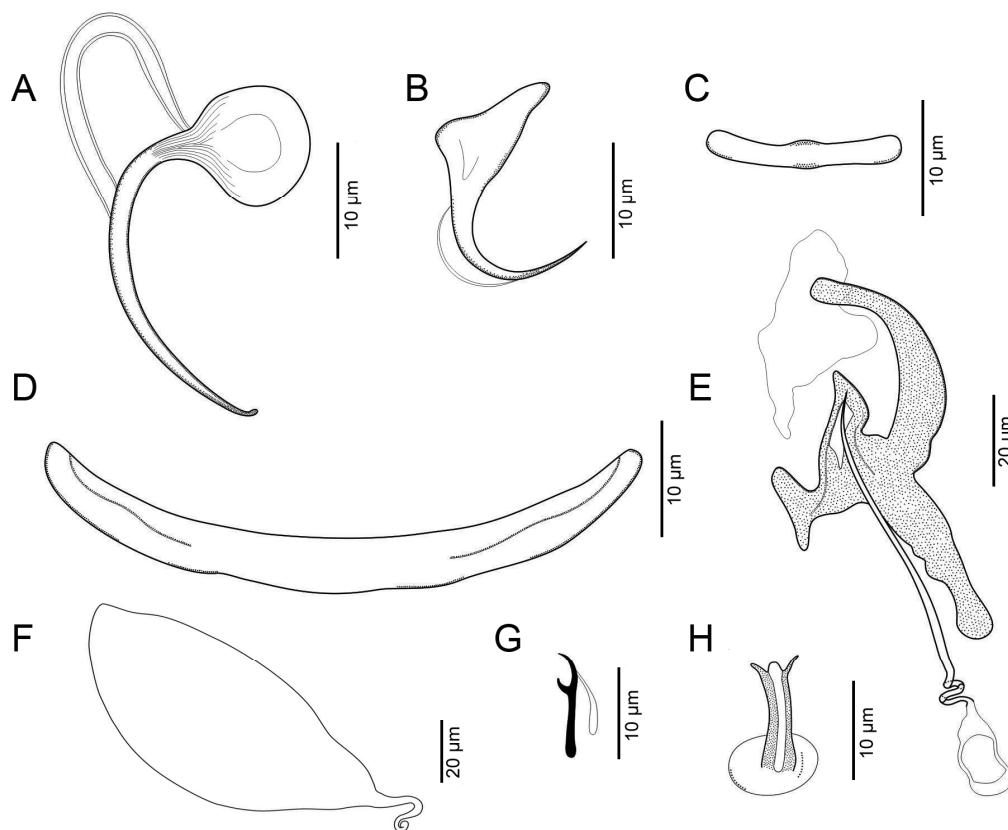


FIGURE 6. (A–H) Sclerotized structures of *Heteropriapulus microcleithrus* **n. sp.** from *Pterygoplichthys ambrosettii* (Holmberg) from the Upper Paraná River, Brazil. A – ventral anchor. B – dorsal anchor. C – dorsal bar. D – ventral bar. E – male copulatory complex. F – egg. G – hook. H – vagina.

***Heteropriapulus pterygoplichthyi* n. sp.**

(Fig. 7)

Type and only known host: *Pterygoplichthys ambrosettii* (Holmberg) (Siluriformes: Loricariidae).

Site of infection: Gills.

Type locality: Aguapeí River, Castilho (Paraná River basin), São Paulo State, Brazil (21°3'36.20"S, 51°45'38.58"W).

Type specimens: holotype – 1 whole-mounted stained specimen from *Pterygoplichthys ambrosettii* from the Aguapeí River (host number 15 collected in Jan/2014) examined on 28 April 2016 (CHIOC XXX); 11 paratypes from *Pterygoplichthys ambrosettii* from the Aguapeí River (host number 20 collected in August 2013; 4 collected in January 2014; 1, 3, 8, 9 and 11 collected in June 2014); 10 paratypes in CHIBB (CHIBB) and 1 paratype in IPCAS (M-XXX).

Etymology: The specific name refers to the generic name of its host species.

Description [based on 10 specimens mounted in Hoyer's medium]. Body 286–550 (390; n=9) long, greatest width 60–160 (83; n=9), usually near mid-length. Accessory granules extending from level of gonads to cephalic lobes. Copulatory complex comprising non-articulated copulatory organ and accessory piece. Copulatory tube delicate, 43–50 (47) long, straight in anterior portion showing 2 counterclockwise spiral rings next to rounded base. Accessory piece 42–47 (45) long, composed of 2 subunits: one long, robust, conspicuous and strongly sclerotized and another small and weakly sclerotized subunit. Vaginal tube short and sclerotized, 17–19 (18; n=5) long, 18–20 (19; n=5) curved length, 10–11 (10; n=5) wide, and thickened anterior and posterior margins. Haptor 39–62 (52; n=9) long, 77–113 (86; n=9) wide, subtrapezoidal. Ventral anchors with flattened base, total length 21–23 (22), length to notch 29–34 (31), base 14–16 (15) long, elongate shaft and point 25–31 (27) long with recurved tip extending posteroventrally, anchor filament double, well-developed, small sclerotized anchor patch present. Dorsal anchors 33–37 (35) long, length to notch 27–31 (28), superficial root 10–12 (11) long, deep root 2–3 (3) long, straight point 17–19 (18) long. Ventral bar 65–81 (73) long, 7–11 (9) wide, 11–20 (15) high, bent, rounded ends and inconspicuous median process. Dorsal bar bent, 26–32 (30) long, 5–6 (5)

wide, 8–12 (9) high, with small median process. Seven pairs of similar hooks 12–13 (13) long, FH loop about ½ of shank length.

Remarks. *Heteropriapulus pterygoplichthyi* **n. sp.** resembles *H. microcleithrus* **n. sp.** in the possession of an accessory piece composed of two subunits with one small and weakly sclerotized and another longer, more robust, conspicuous and strongly sclerotized. However, *H. pterygoplichthyi* **n. sp.** differs in the shape of the longer, subunit of the accessory piece, which is more robust than that of *P. microcleithrus*, being represented by ‘two sickles’ jointed by the base (compare Fig. 6E and Fig. 7G). *Heteropriapulus pterygoplichthyi* **n. sp.** is also typified by having an inconspicuous medial process on the ventral and dorsal bars.

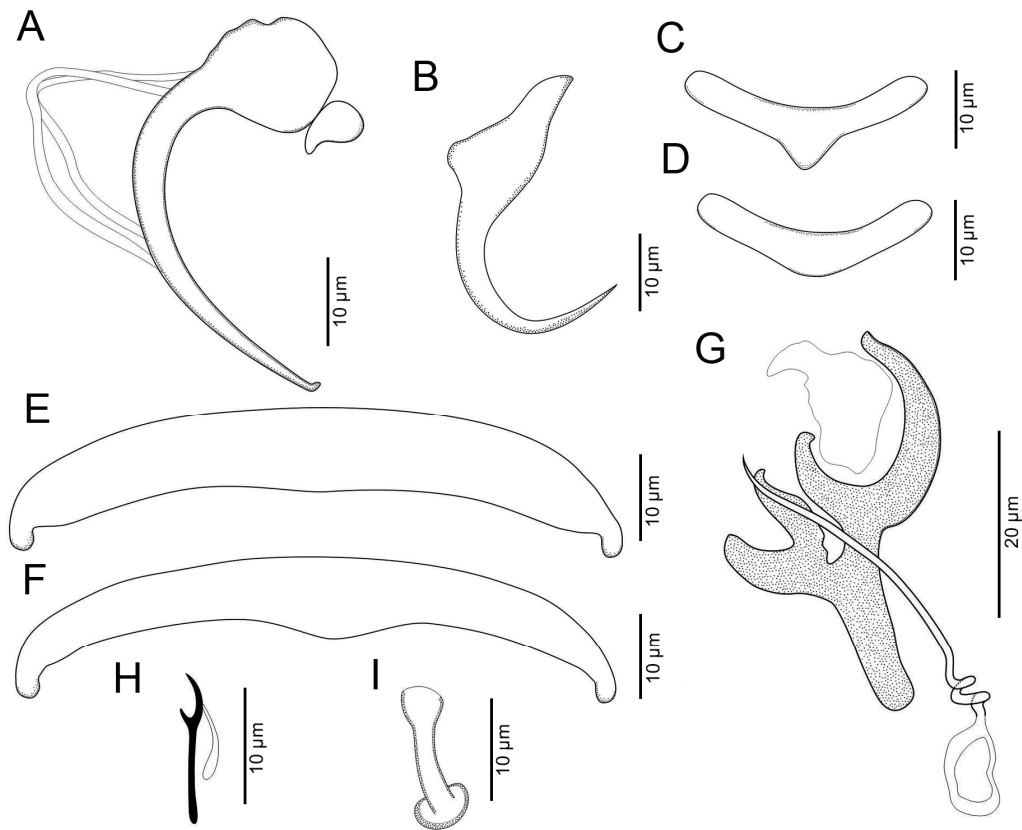


FIGURE 7. (A–I) Sclerotized structures of *Heteropriapulus pterygoplichthyi* **n. sp.** from *Pterygoplichthys ambrosettii* (Holmberg) from the Upper Paraná River, Brazil. A – ventral anchor. B – dorsal anchor. C, D – dorsal bar. E and F – ventral bar. G – male copulatory complex. H – hook. I – vagina.

***Heteropriapulius semitortus* n. sp.**

(Fig. 8)

Type and only known host: *Rhinelepis aspera* Spix & Agassiz (Siluriformes: Loricariidae).

Site of infection: Gills.

Type locality: Aguapeí River, Castilho (Paraná River basin), São Paulo State, Brazil (21°3'36.20"S, 51°45'38.58"W).

Type specimens: holotype – 1 specimen mounted in Hoyer's medium from *Rhinelepis aspera* from the Aguapeí River (host number 19 collected in November 2014) examined on 4 May 2015 (CHIOC XXX); 4 paratypes from *Rhinelepis aspera* from the Aguapeí River (host number 2 collected in January 2014; 16 and 19 collected in November 2014); 3 paratypes in CHIBB (XXXX) and 1 paratype in IPCAS (M-XXXX).

Etymology: The name is from Latin and refers to the shape of the cirrus that is half twisted (*semi* = half + *tortus* = twisted).

Description [based on 5 specimens mounted in Hoyer's medium]. Body 295–412 (336) long, greatest width 69–127 (99) usually near mid-length. Accessory granules extending from level of gonads to cephalic lobes. Copulatory complex comprising non-articulated copulatory organ and accessory piece. Copulatory tube 34–44 (40) long, straight in anterior portion, forming 2½ counterclockwise spiral rings next to rounded base. Accessory piece 23–25 (24) long, composed by single straight unit functioning as cirrus guide. Vaginal tube non-sclerotized. Haptor 40–66 (55) long, 46–111 (79) wide, subtrapezoidal. Ventral anchors with flattened base, 12–16 (14) long, length to notch 18–22 (21), base 6–8 (7), point 13–16 (14) long with recurved tip, anchor filament double, well-developed, anchor patch absent. Dorsal anchors 12–14 (13) long, length to notch 11–12 (12), superficial root 4–5 (4) long, short deep root 1–2 (2) long, straight point 5–7 (6) long, double anchor filament. Ventral bar bent, 40–45 (42; n=4) long, 3–4 (4; n=4) wide, 6–13 (11; n=4) high. Dorsal bar short, bent, 17–19 (19; n=4) long, 2–3 (2; n=4) wide, 4–7 (6; n=4) high. Seven pairs of similar hooks, 9–10 (9) long, FH loop about ¾ of shank length.

Remarks. This new species is easily differentiated from its congeners by the morphology of its copulatory complex. The accessory piece is composed of a single,

straight unit resembling that of *H. simplex* but differs by the shape of the cirrus which is sigmoid in *H. simplex* and without coils next to the base (compare Figs. 3C and 8E), whereas the accessory piece of other species is more conspicuous and robust, sickle-shaped (*H. microcleithrus* **n. sp.** and *H. pterygoplichthyi* **n. sp.**), or comprising one (*H. bitomus* **n. sp.**) or more subunits (*H. heterotylus* and *H. anchoradiatus* **n. sp.**). The cirrus of *H. semitortus* **n. sp.** forms 2½ counterclockwise spiral rings next to the rounded base, whereas that of other species contain fewer spiral rings: 1½ in *H. anchoradiatus* **n. sp.**, *H. microcleithrus* **n. sp.**, *H. heterotylus*, and *H. bitomus* **n. sp.**, and 2 in *H. pterygoplichthyi* **n. sp.**

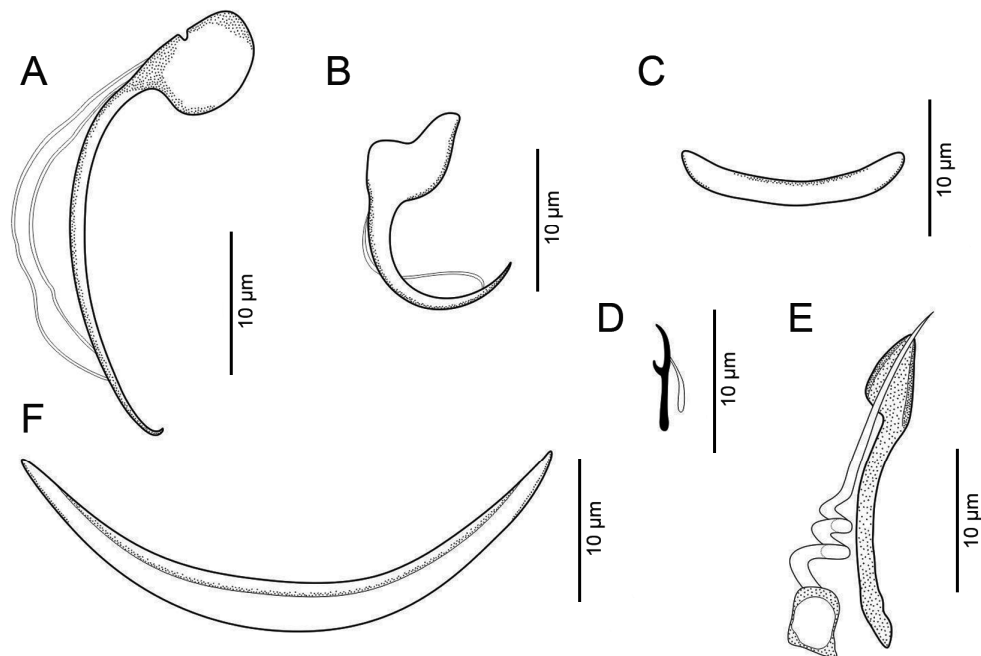


FIGURE 8. (A–F) Sclerotized structures of *Heteropriapulus semitortus* **n. sp.** from *Rhinelepis aspera* Spix & Agassiz from the Upper Paraná River, Brazil. A – ventral anchor. B – dorsal anchor. C – dorsal bar. D – hooks. E – male copulatory complex. F – ventral bar.

Based on addition of five new species to only two nominal taxa recognized before, generic diagnosis of *Heteropriapulus* is amended by adding new features for the diagnosis and placement of species in the genus. A key for identification of all the species of the genus is also provided.

Amended diagnosis. Copulatory complex comprising non-articulated copulatory organ and accessory piece; copulatory organ tubular, sclerotized; accessory piece composed of simple tubular single unit or variable number of subunits of variable size and shape. Vagina tubular, sclerotized or not. Eggs oval, operculate, short polar filament opposed to opercular end. Haptor globose to subtrapeizodal, with pair of dorsal anchors, pair of ventral anchors, dorsal bar, ventral bar and 7 pairs of similar hooks with ancyrocephaline distribution; hook with upright acute thumb and slender shank with absence or presence of round, weakly sclerotized subunit in the base; ventral anchor lacking roots or not, with elongate shaft imperceptibly joining point, well-developed anchor filament; small sclerotized basal patch present (1 or 2 pairs) or absent. Median process on dorsal bar present (slight or conspicuous) or absent.

Type species: *Heteropriapulus heterotylus* (Jogunoori, Kritsky & Venkatanarasaiah, 2004) Kritsky, 2007.

Other species: *Heteropriapulus simplex* Li & Huang, 2012, *Heteropriapulus anchoradiatus* n. sp., *Heteropriapulus bitomus* n. sp., *Heteropriapulus microcleithrus* n. sp., *Heteropriapulus pterygoplichthyi* n. sp., *Heteropriapulus semitortus* n. sp.

Key to species of *Heteropriapulus*

- 1 Vagina non-sclerotized 2
- Vagina sclerotized 3
- 2 Cirrus formed by robust, sigmoid tube *H. simplex* Li & Huang, 2012
- Cirrus formed by a narrow tube with 2½ spiral rings at the proximal end
..... *H. semitortus* n. sp.
- 3 Vaginal tube long and robust, sigmoid in shape *H. anchoradiatus* n. sp.
- Vaginal tube short, not sigmoid in shape 4
- 4 Two pairs of patches associated with the ventral anchor present
..... *H. bitomus* n. sp.
- None or single pair of patches associated with the ventral anchor present 5
- 5 No patches associated with the ventral anchor *H. microcleithrus* n. sp.
- One pair of patches associated with the ventral anchor present 6
- 6 Accessory piece divided into two subunits, one subunit strongly sclerotized composed of 'two sickles' jointed by the base, the other subunit weakly sclerotized

.....	<i>H. pterygoplichthyi</i> n. sp.
- Accessory piece with all subunits strongly sclerotized	7
7 Accessory piece divided into four subunits	
.....	<i>H. heterotylus</i> (Jogunoori, Kritsky & Venkatanarasaiah, 2004)

5.3.2. Molecular data

The ML and BI analyses yielded the same branch topology (Fig. 9). The monophyly of the genus *Heteropriapulus* is confirmed by the phylogenetic analyses with the presence of its type species. The relationship between *H. simplex* and *H. anchorradiatus* **n. sp.** is unclear, at least based on a single molecular marker. The clade with species of *Heteropriapulus* forms a monophyletic clade sister to *U. unilatus*. The species of *Heteropriapulus* and *U. unilatus* clustered with Neotropical dactylogyrids, and the Holarctic monogeneans *Ancyrocephalus percae* and *A. paradoxus* formed a sister group to the species from the Neotropics.

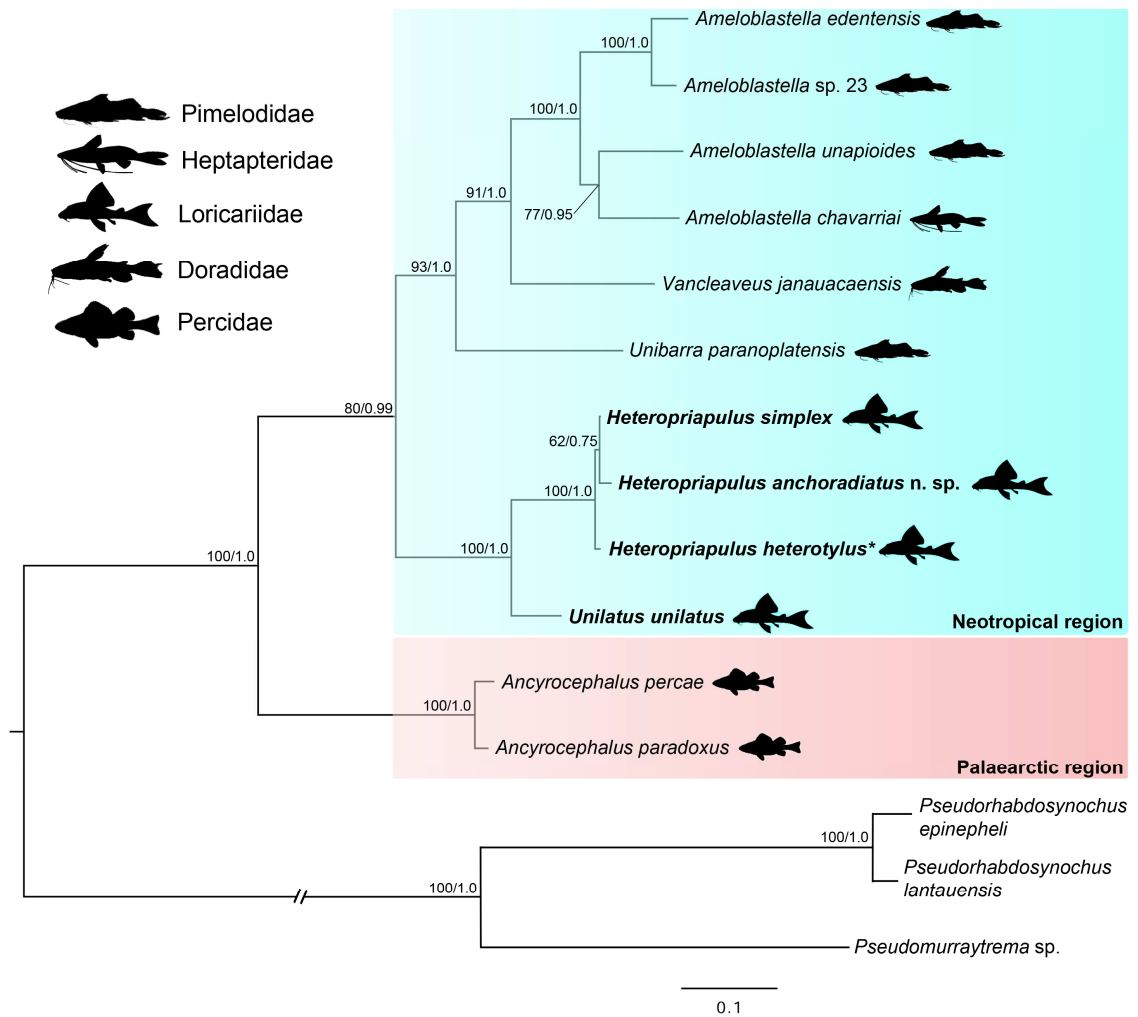


FIGURE 9. Bayesian topology based on partial 28S rDNA sequences of selected monogenean species. Newly sequenced species from this study are in bold. Asterisk indicates type-species. Maximum likelihood followed by posterior probabilities bootstrap support values are given above the branches. *Pseudorhabdosynochus epinepheli*, *P. lantauensis* and *Pseudomurraytrema* sp. were used as outgroups and had their branch shortened 4 times. Branch length scale bar indicates number of substitution per site.

TABLE 3. List of monogeneans included in the phylogenetic analyses.

Parasite species	Host	Host family	Locality	Genbank ID	Reference
Dactylogyridae					
<i>Ancyrocephalus paradoxus</i>	<i>Sander lucioperca</i>	Percidae	Morava River, Czech Republic	AJ969952	Šimková <i>et al.</i> , 2006
<i>Ancyrocephalus percae</i>	<i>Perca fluviatilis</i>	Percidae	Constance Lake, Germany	KF499080	Behrmann-Godel <i>et al.</i> , 2013
<i>Ameloblastella chavarrai</i>	<i>Rhamdia quelen</i>	Heptapteridae	Catemaco Lake, Mexico	KP056251	Mendoza-Palmero <i>et al.</i> , 2015
<i>Ameloblastella edentensis</i>	<i>Hypophthalmus edendatus</i>	Pimelodidae	Nanay River, Peru	KP056255	Mendoza-Franco <i>et al.</i> , 2016
<i>Ameloblastella unapinoides</i>	<i>Sorubim lima</i>	Pimelodidae	Iquitos, Peru	KP056254	Mendoza-Franco <i>et al.</i> , 2016
<i>Ameloblastella</i> sp. 23	<i>Hypophthalmus edendatus</i>	Pimelodidae	Nanay River, Peru	KP056233	Mendoza-Palmero <i>et al.</i> , 2015
<i>Heteropriapulus anchoradiatus</i>	<i>Pterygoplichthys ambrosettii</i>	Loricariidae	Upper Paraná River basin, Brazil		Present study
<i>Heteropriapulus heterotylus</i>	<i>Pterygoplichthys ambrosettii</i>	Loricariidae	Upper Paraná River basin, Brazil		Present study
<i>Heteropriapulus simplex</i>	<i>Pterygoplichthys ambrosettii</i>	Loricariidae	Upper Paraná River basin, Brazil		Present study
<i>Unibarra paranoplatensis</i>	<i>Aguarunichthys torosus</i>	Pimelodidae	Santa Clara, Peru	KP056219	Mendoza-Palmero <i>et al.</i> , 2015

TABLE 3. Continued.

Parasite species	Host	Host family	Locality	Genbank ID	Reference
<i>Unilatus unilatus</i>	<i>Pterygoplichthys ambrosettii</i>	Loricariidae	Upper Paraná River basin, Brazil		Present study
<i>Vancleavus januacaensis</i>	<i>Pterodoras granulosus</i>	Doradidae	Itaya River, Peru	KP056247	Mendoza-Palmero <i>et al.</i> , 2015
Diplectanidae					
<i>Pseudorhabdosynochus epinepheli</i> *	<i>Epinephelus bruneus</i>	Serranidae	Huidong, China	AY553622	Wu <i>et al.</i> , 2006
<i>Pseudorhabdosynochus lantauensis</i> *	<i>Epinephelus bruneus</i>	Serranidae	Huidong, China	AY553624	Wu <i>et al.</i> , 2006
Pseudomurraytremaidae					
<i>Pseudomurraytrema</i> sp.*	<i>Catostomus ardens</i>	Catostomidae	Snake River, Idaho, USA	AF382059	Chisholm <i>et al.</i> , 2001

Species sequenced in this study are in bold.

(*) Species used as outgroups.

5.4. Discussion

Prior to this study, the genus *Heteropriapulus* was composed of only two species, *H. heterotylus* and *H. simplex* (see Jogunoori *et al.*, 2004; Li & Huang, 2012). However, the present study provides evidence that this genus contains much more species as 5 new taxa have been added. However, it is almost certain that other species will be discovered if more loricariid catfishes are examined for monogeneans. Despite the high diversity of this catfish family in South America, only 4% of nominal species have been examined for monogeneans (Branches & Domingues, 2014). Jogunoori *et al.* (2004) assumed that the genus *Heteropriapulus* originated in the Neotropics given that both of its species known before the present study were described from hosts imported from South America as ornamental fishes. Recent studies on the monogenean fauna in the Neotropical region reveal that its current knowledge is fragmentary and many more species and genera remain to be discovered (Mendoza-Palmero *et al.*, 2012 and references therein).

The genus *Heteropriapulus* is well circumscribed and its species are well characterized by the possession of several typical features absent in other Neotropical genera of dactylogyrids. For this reason, generic assignment of as many as 5 new species to this genus was straightforward, unlike some monogeneans of other Neotropical genera from catfishes, such as *Cosmetocleithrum* Kritsky, Thatcher & Boeger, 1986, *Demidospermus* and *Urocleidoides* Mizelle & Price, 1964 (Kritsky *et al.*, 1986; Kritsky & Gutiérrez, 1998). Species composition of these and some other genera requires revision because molecular data indicate their possible polyphyly (Mendoza-Palmero *et al.*, 2015).

Phylogenetic relationships of dactylogyrids from catfishes was assessed in the Neotropical region, but their study was focused on taxa from pimelodids and did not include any species from loricariid catfishes (Mendoza-Palmero *et al.*, 2015). Therefore, this is one of the first studies that obtained molecular data for monogenean species parasitizing Neotropical loricariids. Even though sequences of only three of eight recognized species of *Heteropriapulus* were obtained, the sequence of type species, *H. heterotylus*, makes it possible to fix position of the genus within Neotropical dactylogyrids. In many other Neotropical genera of dactylogyrids including those that appeared polyphyletic in a study by Mendoza-Palmero *et al.* (2015), the absence of sequences of their type species represents a serious problem in their circumscription.

Species of *Heteropriapulus* are restricted to loricariids in the Neotropical region, but their actual host associations are not yet clear. Whereas two species, *H. heterotylus* and *H. simplex*, have been found in fishes of two different genera (*Hypostomus* and *Pterygoplichthys*), thus exhibiting stenoxenous host specificity *sensu* Euzet & Combes (1980), the remaining, newly described species are restricted to a single fish host. Interestingly, one species of fish hosts, namely *Pterygoplichthys ambrosettii*, may host as many as six congeneric species, which is not a very common phenomenon among dactylogyrids parasitizing Neotropical catfishes (Thatcher, 2006). The natural distribution area of species of *Heteropriapulus* includes the Neotropics as evidenced by the present study; records from India, Japan and Mexico are from introduced or invasive hosts of South America origin.

From the parasitological point of view, loricariids belong to the least studied Neotropical freshwater fish groups (Thatcher, 2006). However, these fishes host an interesting fauna of dactylogyrid monogeneans that are usually strictly specific to these hosts. They thus may serve as an interesting model of their coevolution, provided that they are studied using methods of phylogenetic systematics.

5.5. References

- Azevedo, R.K., Abdallah, V.D. & Luque, J.L. (2010) Acanthocephala, Annelida, Arthropoda, Myxozoa, Nematoda and Platyhelminthes parasites of fishes from the Guandu River, Rio de Janeiro, Brazil. *Check List*, 6, 659–667.
- Behrmann-Godel, J., Roch, S. & Brinker, A. (2013) Gill worm *Ancyrocephalus percae* (Ergens, 1966) outbreak negatively impacts the Eurasian perch *Perca fluviatilis* L. stock of Lake Constance, Germany. *Journal of Fish Diseases*, 37, 925–930.
<http://dx.doi.org/10.1111/jfd.12178>
- Boeger, W.A. & Belmont-Jégu, E. (1994) Neotropical Monogenoidea. 21. *Trinigyryus mourei* sp. n. (Dactylogyridae) from the gills of the Amazonian catfish *Hypostomus marginatus* (Loricariidae). *Amazoniana*, 13, 13–16.
- Branches, B. & Domingues, M.V. (2014) A new species of *Unilatus* (Platyhelminthes: Monogenoidea) from the gills of *Leporacanthicus galaxias* Isbrücker et Nijssen (Siluriformes: Loricariidae) from Brazil. *Acta Parasitologica*, 59, 91–97.
<http://dx.doi.org/10.2478/s11686-014-0213-7>

- Burguess, W.E. (1989) *An Atlas of Freshwater and Marine Catfishes – A Preliminary Survey of the Siluriformes*. T.F. H. Publications, Inc., Neptune City, NJ, USA, 784 pp.
- Chisholm, L.A., Morgan, J.A.T., Adlard, R.D. & Whittington, I.D. (2001) Phylogenetic analysis of the Monocotylidae (Monogenea) inferred from 28S rDNA sequences. *International Journal for Parasitology*, 31, 1537–1547.
- Cohen, S.C. & Kohn, A. (2008a) New data on species of *Demidospermus* (Dactylogyridae: Monogenea) parasitizing fishes from the reservoir of the Itaipu Hydroelectric Power Station, Paraná State, Brazil, with new synonymies. *Revista Brasileira de Parasitologia Veterinária*, 17, 167–170.
<http://dx.doi.org/10.1590/S1984-29612008000300011>
- Cohen, S.C. & Kohn, A. (2008b) South American Monogenea – list of species, hosts and geographical distribution from 1997 to 2008. *Zootaxa*, 1924, 1–42.
- Ergens, R. 1969. The suitability of ammonium picrate-glycerin in preparing slides of lower Monogenoidea. *Folia Parasitologica*, 16, 320.
- Euzet, L. & Combes, C. (1980) Les problèmes de l'espèce chez les animaux parasites. In: Les problèmes de l'espèce dans le règne animal. *Mémoire n° 40 de la Société Zoologique de France*, 3, 239–285.
- Ferrari-Hoeinghaus, A.P., Bellay, S., Takemoto, R.M. & Pavanelli, G.C. (2010) A new species of *Demidospermus* Suriano, 1983 (Monogenea, Dactylogyridae) parasitic on *Loricariichthys platymetopon* Isbrücker et Nijssen (Loricariidae, Siluriformes) from the Upper Paraná River floodplain, Brazil. *Acta Parasitologica*, 55, 16–19.
<http://dx.doi.org/10.2478/s11686-010-0007-5>
- Froese R. & Pauly D. 2016. FishBase. World Wide Web electronic publication. Available from: <www.fishbase.org>, version 11/2014. (accessed in: April 2016)
- Guindon, S. & Gascuel, O. (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology*, 52, 696–704.
- Gonçalves, R.A., Oliveira, M.S.B., Santos, E.F. & Tavares-Dias, M. (2014) Aspectos ecológicos da comunidade de parasitos em duas espécies de Loricariidae da bacia Igarapé Fortaleza, estado do Amapá, Brasil. *Biota Amazônia*, 4, 15–21.
<http://dx.doi.org/10.18561/2179-5746/biotaamazonia.v4n1p15-21>
- Gussev A. V. 1985. In: Bauer, O. N. (Ed). *Key to parasites of the freshwater fish fauna of the USSR. Vol 2*. Nauka, Leningrad, 424 pp. [in Russian]
- Hanek, G., Molnar, K. & Fernando, C.H. (1974) Three new genera of Dactylogyridae

- (Monogenea) from freshwater fishes of Trinidad. *Journal of Parasitology*, 60, 911–913.
- Huelsenbeck, J.P. & Ronquist, F. (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics*, 17, 754–755.
- Humason, G.L. (1979) *Animal tissue techniques*. W.H. Freeman Co., San Francisco, California, 661 pp.
- Jogunoori, W., Kritsky, D.C. & Venkatanarasaiah, J. (2004) Neotropical Monogenoidea. 46. Three new species from the gills of introduced aquarium fishes in India, the proposal of *Heterotylus* n. g. and *Diaphorocleidus* n. g., and the reassignment of some previously described species of *Urocleidoides* Mizelle & Price, 1964 (Polychoinea: Dactylogyridae). *Systematic Parasitology*, 58, 115–124.
<http://dx.doi.org/10.1023/B:SYPA.0000029422.16712.9a>
- Katoh, K. & Standley, D.M. (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution*, 30, 772–780.
<http://dx.doi.org/10.1093/molbev/mst010>.
- Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Mentjies, P. & Drummond, A. (2012) Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, 28, 1647–1649.
<http://dx.doi.org/10.1093/bioinformatics/bts199>
- Kritsky, D.C. (2007) *Heteropriapulius* nom. nov. (Monogenoidea: Dactylogyridae) for *Heterotylus* Jogunoori, Kritsky and Venkatanarasaiah, 2004, a junior homonym of *Heterotylus* Kirsch in Reitter, 1913 (Coleoptera: Curculionidae). *Systematic Parasitology*, 68, 223.
<http://dx.doi.org/10.1007/s11230-007-9111-9>
- Kritsky, D.C., Boeger, W.A. & Thatcher, V.E. (1985) Neotropical Monogenea. 7. Parasites of pirarucu, *Arapaima gigas* (Cuvier), with descriptions of two new species and redescription of *Dawestrema cycloancistrum* Price and Nowlin, 1967 (Dactylogyridae: Ancyrocephalinae). *Proceedings of the Biological Society of Washington*, 98, 321–331.

- Kritsky, D.C., Boeger, W.A. & Thatcher, V.E. (1986a) Neotropical Monogenea. 9. Status of *Trinigyrus* Hanek, Molnar & Fernando, 1974 (Dactylogyridae) with descriptions of two new species from loricariid catfishes from the Brazilian Amazon. *Proceedings of the Biological Society of Washington*, 99, 392–398.
- Kritsky, D.C., Thatcher, V.E. & Boeger, W.A. (1986b) Neotropical Monogenea. 8. Revision of *Urocleidoides* (Dactylogyridae, Ancyrocephalinae). *Proceedings of the Helminthological Society of Washington*, 53, 1–37.
- Kritsky, D.C. & Gutiérrez, P.A. (1998) Neotropical Monogenoidea. 34. Species of *Demidospermus* (Dactylogyridae, Ancyrocephalinae) from the gills of pimelodids (Teleostei, Siluriformes) in Argentina. *Journal of the Helminthological Society of Washington*, 65, 147–159.
- Kritsky, D.C. & Mizelle, J.D. (1968) Studies on monogenetic trematodes. XXXV. Some new and previously described North American species of *Gyrodactylus*. *American Midland Naturalist*, 79, 205–215.
- Li, H. & Huang, Q. (2012) One new Chinese record genera (Ancyrocephalidae) and one new species and one new recorded of Monogenea parasiting on gills of *Hypostomus plecostomus*. *Journal of Dalian Ocean University*, 27, 116–119. [In Chinese]
- Littlewood, D.T.J., Curini-Galletti, M. & Herniou, E.A. (2000) The interrelationships of Proseriata (Platyhelminthes: Seriata) tested with molecules and morphology. *Molecular Phylogenetics and Evolution*, 16, 449–466.
<http://dx.doi.org/10.1006/mpev.2000.0802>
- Lockyer, A.E., Olson, P.D. & Littlewood, D.T.J. (2003) Utility of complete large and small subunit rRNA genes in resolving the phylogeny of the Neodermata (Platyhelminthes): implications and a review of the cercomer theory. *Biological Journal of the Linnean Society*, 78, 155–171.
<http://dx.doi.org/10.1046/j.1095-8312.2003.00141.x>
- Lujan, N.K., Armbruster, J.W., Lovejoy, N.R. & López-Fernández, H. (2015) Multilocus molecular phylogeny of the suckermouth armored catfishes (Siluriformes: Loricariidae) with a focus on subfamily Hypostominae. *Molecular Phylogenetics and Evolution*, 82, 269–288.
<http://dx.doi.org/10.1016/j.ympev.2014.08.020>
- Mendoza-Franco, E.F., Caspeta-Mandujano, J.M. & Salgado-Maldonado, G. (2012) Primer reporte de *Heteropriapulius* sp. (Platelmintos, Monogenoidea) infectando

- al pez diablo *Pterygoplichthys pardalis* (Siluriformes, Loricariidae) introducido en la cuenca del Rio Lacantún de la Reserva de la Biosfera Montes Azules, Chiapas, México. *Jaina Boletín Informativo*, 23, 1–6.
- Mendoza-Franco, E.F., Mendoza-Palmero, C.A. & Scholz, T. (2016) New species of *Ameloblastella* Kritsky, Mendoza-Franco & Scholz, 2000 and *Cosmetocleithrum* Kritsky, Thatcher & Boeger, 1986 (Monogenea: Dactylogyridae) infecting the gills of catfishes (Siluriformes) from the Peruvian Amazonia. *Systematic Parasitology* 93: 847–862.
- Mendoza-Palmero, C.A., Blasco-Costa, I. & Scholz, T. (2015) Molecular phylogeny of Neotropical monogeneans (Platyhelminthes: Monogenea) from catfishes (Siluriformes). *Parasites and Vectors*, 8, 164.
<http://dx.doi.org/10.1186/s13071-015-0767-8>
- Mendoza-Palmero, C.A., Scholz, T., Mendoza-Franco, E.F., Kuchta, R. (2012) New species and geographical records of dactylogyrids (Monogenea) of catfish (Siluriformes) from the Peruvian Amazonia. *Journal of Parasitology*, 98, 484–497.
<http://dx.doi.org/10.1645/GE-2941.1>
- Mizelle, J.D. 1936. New species of trematodes from the gills of Illinois fishes. *American Midland Naturalist*, 17, 785–806.
- Mizelle, J.D. & Klucka, A.R. (1953) Studies on monogenetic trematodes. XIV. Dactylogyridae from Wisconsin fishes. *American Midland Naturalist*, 49, 720–733.
- Mizelle, J.D. & Kritsky, D.C. (1967a) Studies on monogenetic trematodes. XXX. Five new species of *Gyrodactylus* from the Pacific tomcod, *Microgadus proximus* (Girard). *Journal of Parasitology*, 53, 263–269.
- Mizelle, J.D. & Kritsky, D.C. (1967b) *Unilatus* gen. n., a unique Neotropical genus of Monogenea. *Journal of Parasitology*, 53, 1113–1114.
- Mizelle, J.D., Kritsky, D.C. & Crane, J.W. (1968) Studies on monogenetic trematodes XXXVIII. Ancyrocephalinae from South America with the proposal of *Jainus* gen. n. *American Midland Naturalist*, 80, 186–198.
- Mizelle, J.D. & Price, C.E. (1964) Studies on monogenetic trematodes. XXVII. Dactylogyrid species with the proposal of *Urocleidoides* gen. n. *Journal of Parasitology*, 50, 579–584.

- Molnar, K., Hanek, G. & Fernando, C.H. (1974) Ancyrocephalids (Monogenea) from freshwater fishes of Trinidad. *Journal of Parasitology*, 60, 914–920.
- Morand, S., Legendre, R., Gardner, S.L., & Hugot, J.-R. (1996) Body size evolution of oxyurid parasites: the role of hosts. *Oecologia*, 107, 274–282.
- Nitta, M. & Nagasawa, K. (2013) First Japanese record of *Heteropriapulius heterotylus* (Monogenea: Dactylogyridae), from the alien catfish *Pterygoplichthys disjunctivus* (Siluriformes: Loricariidae) in Okinawa. *Species Diversity*, 18, 281–284.
<http://dx.doi.org/10.12782/sd.18.2.281>
- Olson, P.D., Cribb, T.H., Tkach, V.V., Bray, R.A. & Littlewood, D.T.J. (2003) Phylogeny and classification of the Digenea (Platyhelminthes: Trematoda). *International Journal for Parasitology*, 33, 733–755.
[http://dx.doi.org/10.1016/S0020-7519\(03\)00049-3](http://dx.doi.org/10.1016/S0020-7519(03)00049-3)
- Pleijel, F., Jondelius, U., Norlinder, E., Nygren, A., Oxelman, B., Schander, C., Sundberg, P. & Thollessen, M. (2008) Phylogenies without roots? A plea for the use of vouchers in molecular phylogenetic studies. *Molecular Phylogenetics and Evolution*, 48, 369–371.
<http://dx.doi.org/10.1016/j.ympev.2008.03.024>
- Porto, D.B., Vital, J.F., Santos, A.K.S., Morais, A.M., Varella, M.B. & Malta, J.C.O. (2012) Metazoários parasitos de *Pterygoplichthys pardalis* (Castelnau, 1855) (Siluriformes: Loricariidae) da Amazônia central, Brasil. *Revista Brasileira de Zootecias*, 14, 35–40.
- Price, C.H.E. (1968) *Diaccessorius*, a new genus of Monogenea from the gills of an Amazon river teleost. *Acta Biologica Venezuelica*, 6, 84–89.
- Rambaut A. 2009. FigTree version 1.3.1 [computer program] <http://tree.bio.ed.ac.uk>
- Řehulková, E., Mendlová, M. & Šimková, A. (2013) Two new species of *Cichlidogyrus* (Monogenea: Dactylogyridae) parasitizing the gills of African cichlid fishes (Perciformes) from Senegal: morphometric and molecular characterization. *Parasitology Research*, 112: 1399–1410.
<http://dx.doi.org/10.1007/s00436-013-3291-9>
- Reis, R.E., Weber, C. & Malabarba, L.R. (1990) Review of the genus *Hypostomus* Lacépède, 1803 from southern Brazil, with descriptions of three new species (Pisces, Siluriformes, Loricariidae). *Revue Suisse de Zoologie*, 97, 729–766.

- Reitter, E. (1913). Bestimmungs-Schlüssel der mir bekannten europäischen Gattungen de Curculionidae, mit Einschluss de mir bekannten Gattungen aus dem palaearctischen Gebiete. *Verhandlungen des naturforschenden Vereines in Brünn*, 51, 1–90.
- Rodríguez-Santiago, M.A., García-Prieto, L., Mendoza-Garfias, B., González-Solís, D. & Grano-Maldonado, M.I. 2016. Parasites of two coexisting invasive sailfin catfishes (Siluriformes: Loricariidae) in a tropical region of Mexico. *Neotropical Ichthyology*, 14, 3, e160021.
<http://dx.doi.org/10.1590/1982-0224-20160021>
- Rodríguez-Santiago, M.A., Grano-Maldonado, M., Ávila, E. & Gómez, S. (2015) Occurrence of *Heteropriapulus heterotylus* (Monogenea: Dactylogyridae), ectoparasite of two invasive sailfin catfishes (Siluriformes: Loricariidae) from the southeastern Mexico. *Neotropical Helminthology*, 9, 55–64.
- Šimková, A., Matějsová, I. & Cunningham, C.O. (2006) A molecular phylogeny of the Dactylogyridae sensu Kritsky & Boeger (1989) based on the D1–D3 domains of large subunit rDNA. *Parasitology*, 133, 43–53.
<http://dx.doi.org/10.1017/S0031182006009942>
- Suriano, D.M. (1983) *Demidospermus anus* gen. nov. sp. nov. (Monogenea: Ancyrocephalinae) parasite branquial de *Loricaria* (L.) *anus* Valenciennes, 1840 (Pisces: Loricariidae) de la Laguna de Chascomus-Provincia de Buenos Aires-Republica Argentina. *Neotropica* 29, 111–119.
- Suriano, D.M. (1985) El genero *Unilatus* Mizelle y Kritsky, 1967 (Monogenea: Ancyrocephalidae) del Rio Negro, Manaus, Brasil. *Neotropica*, 31, 163–175.
- Swofford, D.L. (2002) PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4. Sinauer Associates, Sunderland, MA.
- Thatcher, V.E. (2006) *Amazon Fish Parasites* (2nd ed.) Pensoft, Sofia, Bulgaria, 508 pp.
- Weber, C. 2003. Subfamily Hypostominae (armored catfishes). In: Reis, R.E., Kullander, S.O. & Ferraris, C.J. (Eds.), *Check List of the Freshwater Fishes of South and Central America*. Edipurcs, Porto Alegre, Brazil, pp. 351–372.
- Werle, E.C., Schneider, C., Renner, M., Völker, M. & Fiehn, W. (1994) Convenient single-step, one tube purification of PCR products for direct sequencing. *Nucleic Acids Research*, 22, 4354–4355.

- Wu, X.Y., Zhu, X.Q., Xie, M.Q. & Li, A.X. (2006) The radiation of *Haliotrema* (Monogenea: Dactylogyridae: Ancyrocephalinae): molecular evidence and explanation inferred from LSU rDNA sequences. *Parasitology*, 132, 659–68.
<http://dx.doi.org/10.1017/S003118200500956X>
- Zawadzki, C.H., Weber, C., Pavanelli C.S. & Renesto, E. (2002) Morphological and biochemical comparison of two allopatric populations of *Hypostomus margaritifer* (Regan, 1907) (Osteichthyes, Loricariidae) from the upper Paraná River basin, Brazil. *Acta Scientiarum Biological Science*, 24, 499–505.
- Zica, E.O.P., Abdallah, V.D, Azevedo, R.K., Wunderlich, A.C., Carvalho, E.D. & Silva, R.J. (2012) *Unilatus unilatus* Mizelle & Kritsky, 1967 (Monogenea, Ancyrocephalinae) in *Hypostomus* spp. (Siluriformes, Loricariidae) from the Chavantes reservoir, São Paulo State, Brazil. *Helminthologia*, 49: 87–91.
<http://dx.doi.org/10.2478/s11687-012-0018-x>

***Spinitectus aguapeiensis n. sp. (Nematoda:
Cystidicolidae) from Pimelodella avanhandavae
(Siluriformes: Heptapteridae) in the Aguapeí River,
Upper Paraná River basin, Brazil***

6. *Spinitectus aguapeiensis* n. sp. (Nematoda: Cystidicolidae) from *Pimelodella avanhandavae* (Siluriformes: Heptapteridae) in the Aguapeí River, Upper Paraná River basin, Brazil¹

Abstract

Spinitectus aguapeiensis n. sp. differs from its congeners by the pattern of spinose rings arrangement; structure of mouth; vestibule long, with proximal end forming small prostom, distal end reaching between second and third rows of spines; oesophagus divided in muscular and glandular, the latter much longer and broader; nerve ring encircles muscular oesophagus between 4th and 5th rows of spines; excretory pore situated between 6th and 7th rows of spines deirids small, pit-like with central emerging structure, located between first and second rows of spines; posterior end of body remarkably spirally coiled, supported by 21 caudal pedunculate papillae: precloacal papillae - 16 subventral pairs; postcloacal papillae - 5 subventral pairs; area rugosa absent; large (left) spicule slender with slightly broadened proximal part and pointed distal end. Small (right) spicule shorter and broader than large spicule presenting unique shape that resembles a small shovel in its proximal extremity, with undulations and internal supports in mid-length, and hook-like shape of the distal end. *Spinitectus aguapeiensis* n. sp. is the first record of a nematode species in *Pimelodella avanhandavae*, the fourth record of this genus in the Paraná River basin, and the sixth species of this genus in South America.

Key-words: nematode, new species, fish parasite, Neotropical region, biodiversity.

¹ Manuscript to be submitted to the journal Systematic Parasitology.

6.1. Introduction

Spinitectus Fourment, 1883 includes parasitic nematode species that are mainly found in freshwater and marine fishes (Moravec et al., 2002). In America, 15 species of *Spinitectus* have been described from freshwater fishes, namely: *S. acipenseri* Choudhury & Dick, 1992, *S. carolini* Holl, 1928, *S. gracilis* Ward & Magath, 1917, *S. humbertoi* Caspeta-Mandujano & Moravec, 2000, *S. mariaisabelae* Caspeta-Mandujano, Cabañas-Carranza & Salgado-Maldonado, 2007, *S. mexicanus* Caspeta-Mandujano, Moravec & Salgado-Maldonado, 2000, *S. micracanthus* Christian, 1972, *S. osorioi* Choudhury & Pérez-Ponce de León, 2001, and *S. tabascoensis* Moravec, Salgado-Maldonado, Caspeta-Mandujano & González-Solís, 2009 (syn. *S. macrospinosus* Choudhury & Perryman, 2003) in North America; *S. agonostomi* Moravec & Baruš, 1971 in Central America; *S. asperus* Travassos, Artigas & Pereira, 1928 (syn. *S. jamudensis* Thatcher & Padilha, 1977), *S. multipapillatus* Petter, 1987, *S. pachyuri* Petter, 1984, *S. rodolphiheringi* Vaz & Pereira, 1934 (syn. *S. sternopygi* Petter, 1984), and *S. yorkei* Travassos, Artigas & Pereira, 1928, in South America (Moravec, 1998; Salgado-Maldonado et al., 2005; Salgado-Maldonado, 2006; Caspeta-Mandujano et al., 2007; Moravec et al., 2009, 2010). Of these, *S. multipapillatus*, *S. rodolphiheringi*, and *S. yorkei* were recorded in species of *Pimelodella* in South America (Travassos et al., 1928; Vaz & Pereira, 1934; Petter, 1987; Luque et al., 2011, and references therein).

Pimelodella Eigenmann & Eigenmann is the species-richest genus within the family Heptapteridae, with 71 valid species largely distributed in South America (Bockman & Guazzelli, 2003; Froese & Pauly, 2016). Its representatives are small to medium-sized catfishes (less than 15 to 20 mm in length), dwelling in several habitats, like streams, large rivers, surface, and even caves (Dazzani et al., 2012). *Pimelodella avanhandavae* Eigenmann is an endemic species to South America, mostly in Brazil, distributed along the Upper Paraná River, and it is popularly known as “mandizinho” and/or “mandi-chorão”. This species is non-migratory, with external fertilization, no parental care, and most likely with carnivorous feeding habit (Duke Energy, 2008). During a parasitological survey of specimens of *P. avanhandavae* sampled in a Brazilian river, a new species of *Spinitectus* was found in the intestine which is described herein.

6.2. Materials and Methods

A total of 40 specimens of *P. avanhandavae* (standard length 9–17.5 cm; weight 7.8–58.5 g) were collected by fish hooks in August 2013 and November 2014 in the Aguapeí River (21°3'36.20"S, 51°45'38.58"O), Upper Paraná River basin, located in an preserved area in the municipality of Castilho, São Paulo State, Brazil. The nematodes were recovered from intestine, fixed in 70% ethanol and cleared with glycerine for light examination. Drawings were made with the aid of a drawing tube. Measurements are given in micrometres unless otherwise stated. For scanning electron microscopy (SEM), specimens were postfixed in 1% osmium tetroxide (in phosphate buffer), dehydrated through a graded acetone series, critical point-dried and sputter-coated with gold; they were examined using a JEOL JSM-7401F scanning electron microscope at an accelerating voltage of 4 kV (GB low mode). Scientific name of the host is in accordance with FishBase (Froese & Pauly, 2016).

6.3. Results

Spinitectus aguapeiensis n. sp.

(Figs. 1 and 2)

Type-host: *Pimelodella avanhandavae* Eigenmann (Siluriformes: Heptapteridae).

Type-locality: Aguapeí River (21°3'36.20"S, 51°45'38.58"O), municipality of Castilho, São Paulo State, Brazil.

Site of infection: Intestine.

Prevalence and mean intensity: 50% (20 infected fish/40 examined fish) and 4.9 ± 1.0 (range 1–21) nematodes per infected host.

Deposition of specimens: Holotype and allotype in the Helminthological Collection of the Institute Oswaldo Cruz, Rio de Janeiro, Brazil (CHIOC XXX), paratypes in the Helminthological Collection of the Instituto de Biociências, Universidade Estadual Paulista, Botucatu, Brazil (CHIBB XXX), and the Helminthological Collection of the Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, České Budějovice (IPCAS XXX).

Etymology: The specific name relates to the river where hosts were collected.

Description (Figs. 1 and 2)

General. Whitish small-sized nematodes. Cuticle with transverse striations and longitudinal wrinkles between spinose rings. Cephalic end rounded. Mouth oval to rectangular, surrounded by 2 large lateral pseudolabia, 2 subdorsal, and 2 subventral sublabia, 4 single submedian papillae and 2 lateral amphids. Inner base of pseudolabia widening inside oral cavity and attach to inner wall of prostom (Fig. 1C; 2A b, s, p, a). Surface of body with transverse rows of spines, being interrupted by 2 lateral, 1 dorsal and 1 ventral longitudinal empty lines, thus forming 4 distinct sectors. First spiny row situated between first and second thirds of vestibule (Fig. 1B, D). Rows 1–9 with 16 well developed spines (4 per sector), rows 10–15 with 20 spines (5 per sector), row 15 and posterior most with 24–32 spines (6–8 per sector). Some rings incomplete, assymetrical and not forming a circle (Fig. 1B); some missing spines in the rings from the most posterior part of body (Fig. 2F, G). Spination weakly visible in the posterior end of body and finishing before anus/cloaca opening. Vestibule long, with proximal end forming small prostom, distal end reaching between second and third rows of spines. Oesophagus divided in muscular and glandular, the latter much longer and broader (Fig. 1A). Nerve ring encircles muscular oesophagus between 4th and 5th rows of spines (Fig. 1A, B). Excretory pore situated between 6th and 7th rows of spines (Fig. 1B; 2D, E). Deirids small, pit-like with central emerging structure (Fig. 1A, B, D; 2B, C.), located between first and second rows of spines.

Male (Based on 6 specimens, measurements of holotype in parentheses). Body length 7.12–9.21 (8.03) mm, maximum width 82–110 (98). Distance of first spinose ring 41–74 (57) from anterior end. Spines 12–17 (13–15) long and distance between rows 14–33 (15–21) both in the anterior body part; spines 7–13 (8–10) long and distance between rows 26–43 (31–35) in the mid body length. Length of vestibule, including prostom 116–135 (126); of muscular oesophagus 245–337 (287) long and 17–21 (20) wide, and glandular oesophagus 1.16–2.07 (1.72) mm long, 38–52 (52) wide. Length ratio of both oesophagus parts 1: 4–6 (6). Distance of deirids 67–110 (87), nerve ring 152–190 (173), and excretory pore 209–274 (247) from anterior body end. Posterior end of body remarkably spirally coiled, with long caudal alae 394–455 (–), supported by 21 caudal pedunculate papillae. Precloacal papillae: 16 subventral pairs; postcloacal papillae: 5 subventral pairs. A pair of lateral papillae (probably representing phasmids) at the same

level than last postcloacal pair of papillae. Area rugosa absent (Fig. 1J). Large (left) spicule slender 325–420 (347) long, 9–15 (15) wide, with slightly broadened proximal part and pointed distal end. Small (right) spicule shorter and broader than large spicule, 162–197 (162) long, 15–28 (20) wide, with hook-like distal end and two internal supports in mid-length (Fig. 1I). Length ratio of spicules 1: 1–2 (2). Tail 178–199 (181) long, with small dorsal and ventral rounded protuberances on tip (Fig. 1J).

Female (Based on 3 gravid specimens with mature eggs, measurements of allotype in parentheses). Body length 11.50–12.38 (12.38) mm, maximum width 132–169 (169). Distance of first row of spines 41–66 (52) from anterior end. Spines 8–18 (12–18) long and distance between rows 16–26 (16–21) in the anterior part; spines 8–18 (8–14) long and distance between rows 33–40 (33–37) in mid body length. Length of vestibule, including prostom 122–125 (125), of muscular oesophagus 316–489 (489) long, 19–20 (–) wide, and glandular oesophagus 1.02–2.32 (1.07) mm long, 41–45 (–) wide. Length ratio of muscular to glandular oesophagus 1: 2–7 (3). Distance of deirids 62–99 (99), nerve ring 175–181 (181), and excretory pore 231–259 (239) from anterior body end. Vulva equatorial, with non-elevated lips, 6.23–6.53 (6.53) mm from anterior end, at 53–55% of body length. Vagina muscular, shortly directed anteriorly and immediately directed posteriorly (Fig. 1F). Eggs embryonated, 29–36 (29–32) x 19–21 (20–21). Egg shell thickness 2–4 (3) (Fig. G). Tail elongate, 186–206 (206) long, with small mucron on tip (Fig. 1E).

Female (gravid specimen with immature eggs, measurements of young female in parentheses). Body length 9.065 (3.575) mm, maximum width 128 (72). Distance of first row of spines 77 (29) from anterior end. Spines 13–15 (10–12) long and distance between rows 19–25 (11–15) in the anterior part; spines 8–10 (10–13) long and distance between rows 33–35 (21–24) in mid body length. Length of vestibule, including prostom 120 (90), of muscular oesophagus 350 (173) long, 30 (18) wide, and glandular oesophagus 1.70 (1.06) mm long, 61 (36) wide. Length ratio of muscular to glandular oesophagus 1: 5 (6). Distance of deirids 102 (61), nerve ring 182 (132), and excretory pore 265 (162) from anterior body end. Vulva equatorial, with non-elevated lips, 5.255 (2.314) mm from anterior end, at 58 (65%) of body length. Vagina muscular, shortly directed anteriorly and immediately directed posteriorly. Eggs undeveloped, 17–18 x 10–13 (–). Egg shell thickness 2 (–). Tail 177 (125) long, with small mucron on tip (Fig. 1H).

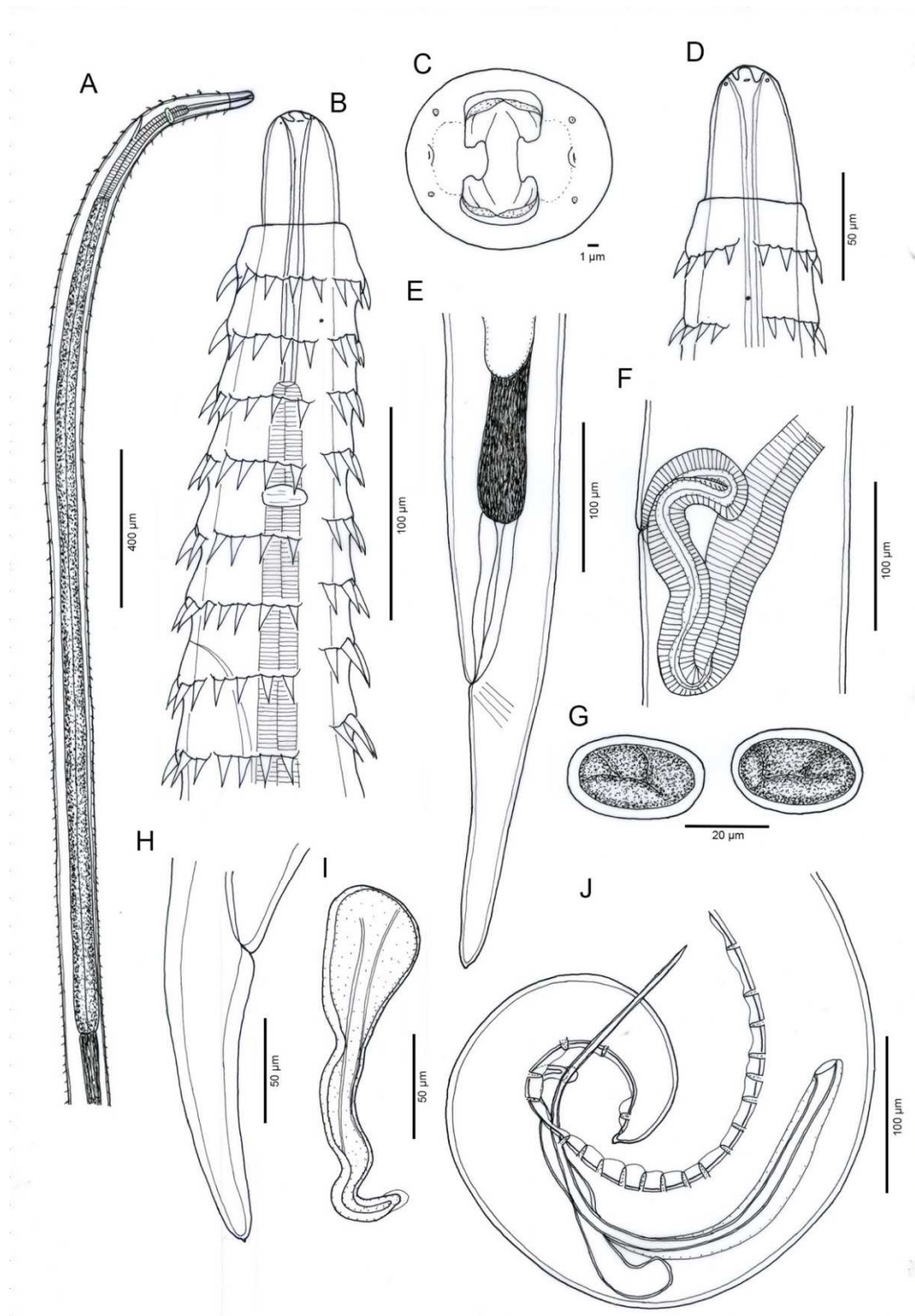


Fig. 1. *Spinitectus aguapeiensis* n. sp. from *Pimelodella avanhandavae* collected in the Aguapeí River, Upper Paraná River basin, Castilho, São Paulo, Brazil. (A) Anterior end of male, subventral view; (B) Anterior end female, sublateral view, higher magnification; (C, D) Cephalic end, apical and lateral views, respectively; (E) Posterior end of gravid female, lateral view; (F) Region of vulva, lateral view; (G) Eggs; (H) Tail of non-gravid female; (I) Small spicule, subventral view; (J) Posterior end of male.

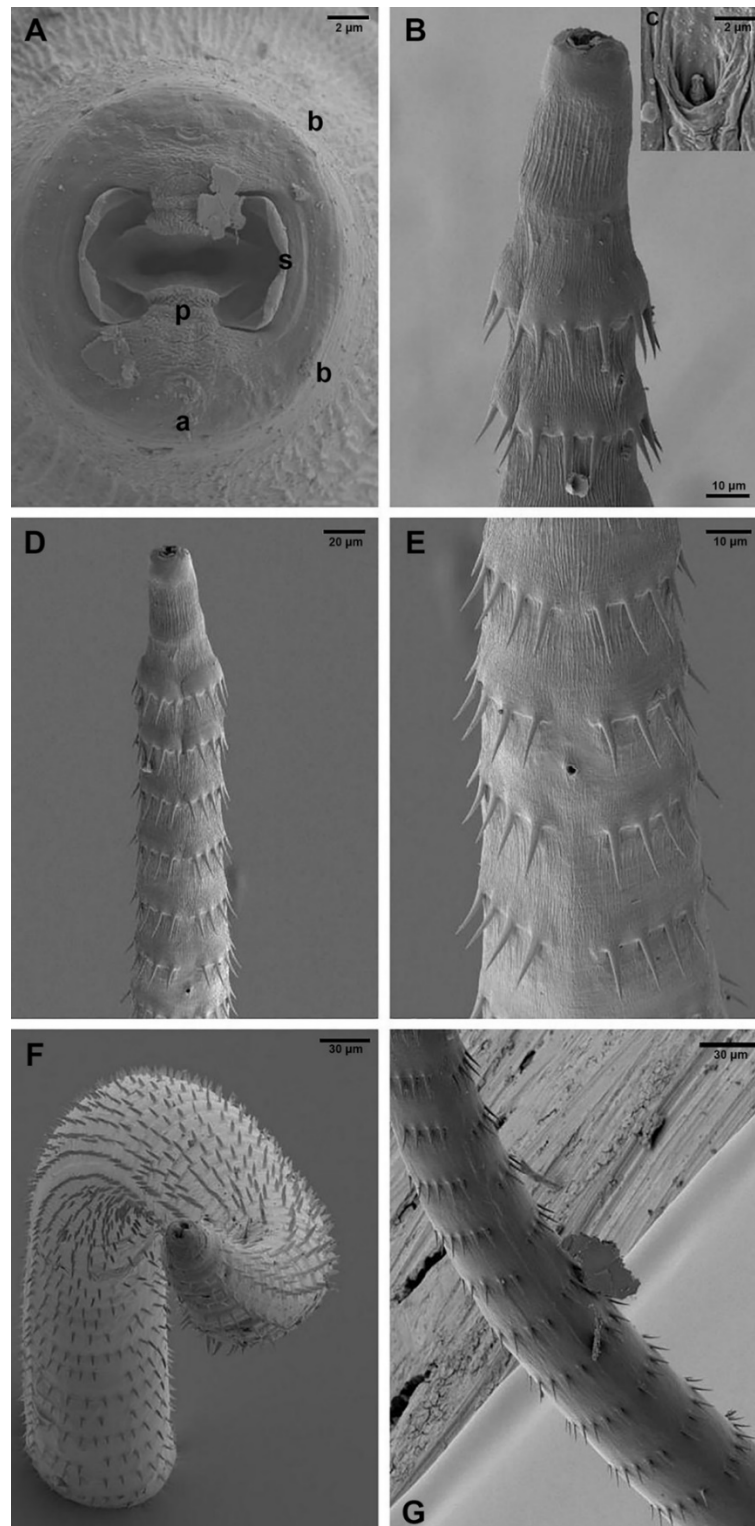


Fig. 2. Scanning electron micrographs of *Spinitectus aguapeiensis* n. sp. from *Pimelodella avanhandavae* collected in the Aguapeí River, Upper Paraná River basin, municipality of Castilho, São Paulo, Brazil. (A) Cephalic end: a – amphid, b – submedian papillae, p – pseudolabia, s – sublabia. (B) anterior end showing the deirid, (C) detail of deirid, (D, E) anterior end showing excretory pore, (F, G) spination pattern.

6.4. Remarks

The specimens described herein belong to *Spinitectus* due to the presence of a cuticle provided with spinose rings, two pseudolabia, vestibule relatively long, and caudal alae supported by pedunculate papillae (Moravec, 1998). The main morphological characteristics used to differentiate *Spinitectus* spp. are the pattern of spinose rings arrangement, structure of mouth, situation of vulva, number and distribution of male caudal papillae, length of spicules and eggs, and position of excretory pore and deirids (Moravec, 1979; Caspeta-Mandujano et al., 2000). To date, there are five valid species of *Spinitectus* recorded for South America (Moravec, 1998), namely: *S. asperus* from *Prochilodus scrofa* (Prochilodontidae), *S. multipapillatus* from *Pimelodella* sp. (Heptapteridae), *S. pachyuri* from *Pachyurus bonariensis* (Sciaenidae), *S. rodolphiheringi* and *S. yorkei* both from *Pimelodella lateristriga* (Heptapteridae) (Travassos et al., 1928; Vaz & Pereira, 1934; Petter, 1984, 1987). Of these, *S. multipapillatus* and *S. yorkei* are probably conspecific, even though the latter was inadequately described and a redescription is required (Moravec, 1998). *Spinitectus asperus*, *S. multipapillatus*, and *S. rodolphiheringi* differ from the new species by having smaller body length (males: 2.42–4.60, 6.3–7.4, and 3.7–4.9 mm, females: 6–8.3, 6.5, and 5.9–7 mm vs. males: 7.12–9.21 mm, females: 11.50–12.38 mm), higher number of spines per row (60–90, 15–25, and 30–40 vs. 16–20), different location of excretory pore, number of precloacal papillae (4, 7–8, and 4 vs. 15 pairs), and length of spicules (left: 220–230, 220–240, and 200–210, right: 90, 50–55, and 120–125 vs. left: 325–420, right: 162–197). *Spinitectus multipapillatus* also presents rows of cuticular spines divided in 4 sectors, but it differs from the new species in the above mentioned features. Even though *S. pachyuri* presents similar body length for both males and females, it differs by having 6 pairs of precloacal papillae, and smaller spicules (left: 100, right 50–55). *Spinitectus yorkei* has similar number of caudal papillae, and although it was poorly described and many important features are missing, it is clear that the species has smaller males (4.2 mm), spicules (left: 162, right: 40), and more spines per row (26–29 in males and 38–40 in females). *Spinitectus aguapeiensis* n. sp. presents a remarkably spirally coiled posterior end in males, which is not present in any of the species described for South America.

Regarding *Spinitectus* species reported in North and Central America, it is possible to find some resemblances with *Spinitectus aguapeiensis* n. sp. as for the body length, *S. micracanthus* (males: 7.6–8.2 mm), *S. gracilis* (males: 8–10, females 10–15

mm), and *S. mariaisabelae* (males: 6.56–8.28, females: 11.82–14.58 mm) are within the same range, although they have a considerably lower number of precloacal papillae pairs (2–4 pairs). *Spinitectus agostoni*, *S. humbertoi*, *S. mariaisabelae*, *S. mexicanus*, *S. osorioi*, and *S. tabascoensis* present spines disposed in four sectors as well, but they all differ by the number of precloacal papillae (4 pairs). *Spinitectus aguapeiensis* n. sp. is the second species with the highest number of precloacal papillae and that with the largest spicules amongst its congeners in South America. Moreover, it has unique shape of the small spicule that resembles a small shovel in its proximal extremity, with undulations and internal supports in mid-length, and hook-like shape of the distal end.

Interestingly, four *Spinitectus* spp. (including the new species) have been reported in *Pimelodella* spp. from South America (Travassos et al., 1928; Vaz & Pereira, 1934; Petter, 1987; Luque et al., 2011; present work). Since *Pimelodella* spp. are carnivores or omnivores fishes that consume mainly arthropods, fishes, free-living nematodes, algae, and debris, they probably get infected after feeding on arthropods or small fish. *Spinitectus* spp. normally use arthropods as intermediate host and fish might act as paratenic hosts (Anderson, 2000; Bockman & Guazzelli, 2003). Therefore, members of *Pimelodella* can be considered the definitive host for *Spinitectus* spp. and it can be suggested that certain degree of host specificity occurs between both parasite and host.

Spinitectus aguapeiensis n. sp. is the first record of a nematode species in *P. avanhandavae*, the first nematode record in the Aguapeí River, the fourth record of this genus in the Paraná River basin, and the sixth species of this genus in South America.

6.5. References

- Anderson, R. C. (2000). *Nematode Parasites of Vertebrates: Their Development and Transmission*. 2nd ed. New York: CABI Publishing, 650 pp.
- Bockmann, F. A., & Guazzelli, G. M. (2003). Family Heptapteridae (Heptapterids). In: R. E. Reis, S. O. Kullander, & C. J. Ferraris Jr. (Orgs.). *Check List of the Freshwater Fishes of South and Central America*. Porto Alegre: Edipucrs, pp. 406–431.
- Caspeta-Mandujano, J. M., Cabañas-Carranza, G., & Salgado-Maldonado, G. (2007). *Spinitectus mariaisabelae* n. sp. (Nematoda: Cystidicolidae) from the intestine of the freshwater fish *Profundulus punctatus* (Cyprinodontiformes) in Mexico. *Helminthologia*, 44, 103–106.

- Caspeta-Mandujano, J. M., Moravec, F., & Salgado-Maldonado, G. (2000). *Spinitectus mexicanus* n. sp. (Nematoda: Cystidicolidae) from the intestine of the freshwater fish *Heterandria bimaculata* in Mexico. *The Journal of Parasitology*, 86, 83–88.
- Dazzani, B., Garcia, C., Peixoto, M., Trajano, E., & Almeida-Toledo, L. F. (2012). Cytogenetic and molecular analyses in troglobitic and epigean species of *Pimelodella* (Siluriformes: Heptapteridae) from Brazil. *Neotropical Ichthyology*, 10, 623–632.
- Duke Energy. (2008). *Peixes do rio Paranapanema*. 2nd ed. São Paulo: Horizonte Geográfico, 120 pp.
- Froese, R., & Pauly, D. (2016). FishBase. World Wide Web electronic publication. <http://www.fishbase.org/summary/Pimelodella-avanhandavae.html> Accessed on 27th of June, 2016.
- Luque, J. L., Aguiar, J. C., Vieira, F. M., Gibson, D. I., & Santos, C. P. (2011). Checklist of Nematoda associated with the fishes of Brazil. *Zootaxa*, 3082, 1–88.
- Moravec, F. (1979). Redescription of the nematode *Spinitectus inermis* parasitic in eels, *Anguilla Anguilla*, of Europe. *Acta Societatis Zoologicae Bohemoslovacae*, 43, 35–42.
- Moravec, F. (1998). *Nematodes of Freshwater Fishes of the Neotropical Region*. Czech Republic: Academia Praha, 464 pp.
- Moravec, F., García-Magaña, L., & Salgado-Maldonado, G. (2002). *Spinitectus tabascoensis* sp. nov. (Nematoda, Cystidicolidae) from *Ictalurus furcatus* (Pisces) in southeastern Mexico. *Acta Parasitologica*, 47, 224–227.
- Moravec, F., Salgado-Maldonado, G., & Caspeta-Mandujano, J. M. (2010). *Spinitectus osorioi* (Nematoda: Cystidicolidae) in the Mexican endemic fish *Atherinella alvarezi* (Atherinopsidae) from the Atlantic River drainage system in Chiapas, Southern Mexico. *Memórias do Instituto Oswaldo Cruz*, 105, 52–56.
- Moravec, F., Salgado-Maldonado, G., Caspeta-Mandujano, J. M., & González-Solís, D. (2009). Redescription of *Spinitectus tabascoensis* (Nematoda: Cystidicolidae) from fishes of the Lacandon rain forest in Chiapas, southern Mexico, with remarks on *Spinitectus macrospinosus* and *S. osorioi*. *Folia Parasitologica*, 56, 305–312.
- Petter, A. -J. (1984). Nematodes de poissons du Paraguay II. Habronematoidea (Spirurida) Description de 4 espèces nouvelles de la famille des Cystidicolidae. *Revue Suisse de Zoologie*, 91, 935–952.

- Petter, A. -J. (1987). Nématodes de Poissons de l'Equateur. *Revue Suisse de Zoologie*, 94, 61–76.
- Salgado-Maldonado, G. (2006). Checklist of helminth parasites of freshwater fishes from Mexico. *Zootaxa*, 1324, 1–357.
- Salgado-Maldonado, G., Aguilar-Aguilar, R., Cabañas-Carranza, G., Soto-Galera, E., & Mendoza-Palmero, C. (2005). Helminth parasites in freshwater fish from the Papaloapan river basin, Mexico. *Parasitology Research*, 96, 69–89.
- Travassos, L., Artigas, P., & Pereira, C. (1928). Fauna helminthologica dos peixes de água doce do Brasil. *Archivos do Instituto Biológico de São Paulo*, 1, 5–68.
- Vaz, Z., & Pereira, C. (1934). Contribuição ao conhecimento dos nematoides de peixes fluviais do Brasil. *Arquivos do Instituto Biológico de São Paulo*, 5, 87–103.

Considerações finais

7. Considerações finais

Este estudo investigou os helmintos parasitos de 405 espécimes de oito espécies de peixes siluriformes do rio Aguapeí, bacia do rio Alto Paraná, município de Castilho, São Paulo, Brasil. Foram identificados 54 *taxa* de helmintos: 25 monogeneas – com 13 possíveis novas espécies, alguns novos registros de hospedeiros e novos registros de localidade; 15 digenéticos – alguns novos registros de hospedeiros e novos registros de localidade; 11 nematoides – uma espécie nova e poucos registros de novos hospedeiros; e três cestoides. Ainda, dados das relações ecológicas destes parasitos com seus hospedeiros foram apresentados. Foi realizado um estudo das relações filogenéticas de algumas espécies de dactilogirídeos com base em novos dados moleculares (13 novas sequências de 28S rDNA) e morfológicos: uma revisão da diagnose do gênero *Demidospermus* com descrição de uma nova espécie foi proposta; um novo gênero de dactilogirídeo com uma descrição de espécie também foi proposto; e foi discutido a respeito da questão do posicionamento de gênero de algumas outras espécies. Uma revisão do gênero *Heteropriapulus* foi realizado com descrição de cinco novas espécies encontrados nas brânquias de *Pterygoplychthys ambrosettii* e *Rhinelepis aspera* no qual também foi apresentado dados preliminares das relações filogenéticas de algumas espécies do gênero, incluindo a espécie tipo *Heteropriapulus heterotylus*. Ainda, uma nova espécie de *Spinitectus* é apresentada, com base em dados morfológicos. *Spinitectus aguapeiensis* n. sp. é o primeiro registro de uma nova espécie para *Pimelodella avanhandavae*, o quarto registro desta espécie na bacia do Alto Paraná e a sexta espécie do gênero descrita para América do Sul.

Com novos registros de hospedeiros e possíveis novas espécies, este estudo demonstrou que existe uma ampla diversidade de helmintos para os hospedeiros estudados do rio Aguapeí. Considerando-se que Siluriformes representa a ordem mais diversa deste rio, este estudo é apenas uma considerável pequena porção da real diversidade de parasitas que este ecossistema pode apresentar. Ainda, este estudo utilizou-se da ferramenta molecular aliada a morfologia para o grupo Monogenea, a fim de realizar trabalhos mais detalhados nas definições de gênero e espécies, e as relações filogenéticas deste grupo de helminto. Siluriformes é um grupo muito diverso e ainda existem muitas espécies desconhecidas, logo espera-se que há muito que se descobrir a respeito dos helmintos destes hospedeiros, especialmente em se tratando da evolução das suas interações hospedeiros-parasitos. Assim, estudos de biodiversidade envolvendo

análises filogenéticas e morfologia podem contribuir para o conhecimento da co-evolução destes hospedeiros e seus parasitos.