



Universidade Federal do Rio Grande
Instituto de Ciências Biológicas
Pós-graduação em Biologia de
Ambientes Aquáticos Continentais



Diversidade e padrões ecológicos de turbelários da Estação Ecológica do Taim

Jhoe Steve Reyes Pajuelo

Orientadora: Samantha Eslava Gonçalves Martins
Co-orientador: Rogério Tubino Vianna

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Dissertação apresentada ao Programa de Pós-graduação em Biologia de Ambientes Aquáticos Continentais como requisito parcial para a obtenção do título de Mestre em Biologia de Ambientes Aquáticos Continentais.

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**ATA DE DEFESA DE DISSERTAÇÃO DE MESTRADO EM BIOLOGIA DE
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Às 13:30 hs (treze horas e trinta minutos) do dia 22 (vinte e dois) do mês de setembro de 2020 (dois mil e vinte), via Webconferência (endereço eletrônico <https://conferenciaweb.rnp.br/webconf/rogerio-tubino-vianna-rogerio>) reuniram-se docentes, discentes e comunidade em geral, para a Defesa Pública de Dissertação de Mestrado do acadêmico **Jhoe Steve Reyes Pajuelo**. A dissertação intitulada “**Diversidade e padrões ecológicos de turbelários da Estação Ecológica do Taim**” foi avaliada pela Banca Examinadora composta pela Profª Dra Samantha Eslava Martins (Orientadora e Presidente da Banca); Profª. Dra. Edélti Faria Albertoni (FURG) e Profª Dra Ana Maria Leal Zanchet (UNISINOS). Após a defesa e arguição pública, a Banca Examinadora reuniu-se, para deliberação final, e considerou o acadêmico **APROVADO**. Desta forma, o acadêmico concluiu mais uma das etapas necessárias para a obtenção do grau de **MESTRE EM BIOLOGIA DE AMBIENTES AQUÁTICOS CONTINENTAIS**. Nada mais havendo a tratar, às 16:30h (dezesesseis horas e trinta minutos) foi lavrada a presente ata, que lida e aprovada, foi assinada pelos membros da Banca Examinadora, pelo Acadêmico e pelo Coordenador Adjunto do Curso.

Profª Dra Samantha Eslava Martins

Profª Dra Edélti Faria Albertoni

Profª Dra Ana Maria Leal Zanchet

Jhoe Steve Reyes Pajuelo

Profº Dr Rogério Tubino Vianna
Coordenador Adjunto do Curso

A mis padres María Pajuelo y Walter Reyes. Sin su ayuda y confianza, nada de lo recorrido habría sido posible. A mi hermana menor, Diana Reyes, por la compañía y los buenos momentos llenos de risas.

*Green indiscretion, flattery of greatness, rawness of judgement, wilfulness in folly, thoughts
vagrant as the wind, and as uncertain... a life I chose to live, a constant acceptance of
frustration.*

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RESUMO

O presente estudo determina a diversidade e os padrões ecológicos de Platyhelminthes (“turbelários”) de vida livre e comensais de *Pomacea canaliculata* em três ambientes (canal de água (C), lagoa Nicola (N) e lagoa Mangueira (M)) na Estação Ecológica de Taim (ESEC Taim). Foram realizadas amostragens padronizadas na vegetação flutuante e bentos para coletar turbelários de vida livre, assim como arrastos com redes para coletar *P. canaliculata*, hospedeiro dos turbelários comensais. Assim, foram determinadas 23 espécies de turbelários de vida livre, sendo registradas três espécies (*Myostenostomum vanderlandi*, *Macrostomum quirritium* e *Strongylostoma elongatum*) pela primeira vez no Brasil e três novas espécies para a ciência (*Macrostomum* n. sp. 1, *Gieysztorina* n. sp. 1 e *Gieysztorina* n. sp. 2). Desta forma, incrementa-se o registro para 115 espécies descritas no Brasil. Entre os três ambientes estudados, C teve a maior riqueza (observada e estimada) seguido de N e M. Contudo, os parâmetros descritivos (riqueza média, abundância e densidade) das comunidades de turbelários de vida livre nos ambientes não apresentaram diferenças significativas. Não obstante, o C e N foram relativamente semelhantes com respeito às comunidades de turbelários, se diferenciando de M. Duas espécies de turbelários comensais (*Temnocephala rochensis* e *T. iheringi*) foram encontradas infestando 81 indivíduos de *P. canaliculata*. A infestação das duas espécies comensais mostrou relação direta com o comprimento da concha do hospedeiro. Além disso, *T. rochensis* foi dominante sobre *T. iheringi* em todas as classes de comprimento da concha do hospedeiro. O presente estudo aprofunda o conhecimento da fauna de turbelários que habitam na ESEC Taim.

Palavras-chave: sitio Ramsar, região neotropical, banhados costeiros, Platyhelminthes, simbiose, turbelários.

ABSTRACT

This study determines the diversity and ecological patterns of free-living and commensal Platyhelminthes ("turbellaria") of *Pomacea canaliculata* in three environments (water channel (C), Nicola lagoon (N) and Mangueira lagoon (M)) at the nature reserve Estação Ecológica de Taim (ESEC Taim). Standardized sampling in the floating and benthos vegetation were performed to collect free-living turbellaria, as well as dragging with nets to collect *P. canaliculata*, host of the commensal turbellaria. Thus, 23 species of free-living turbellaria were determined, being recorded three species (*Myostenostomum vanderlandi*, *Macrostomum quiritium* and *Strongylostoma elongatum*) for the first time in Brazil and three new species for science (*Macrostomum* n. sp. 1, *Gieysztorina* n. sp. 1 and *Gieysztorina* n. sp. 2). In this way, it is raised to 115 the species formally described in Brazil. Among the three environments studied, C had the highest richness (observed and estimated) followed by N and M. However, the descriptive parameters (mean richness, abundance and density) of the free living turbellaria communities in the environments did not present significant differences. Nevertheless, C and N were relatively similar with respect to turbellaria communities, differing from M. Two species of commensal turbellaria (*Temnocephala rochensis* and *T. iheringi*) were found infesting 81 individuals of *P. canaliculata*. The infestation of the two species of commensals showed a direct relation with the length of the host shell. Besides, *T. rochensis* was dominant over *T. iheringi* in all length classes of the host shell. The present study deepens the knowledge of the turbellaria fauna that inhabit the ESEC Taim.

Key-words: Ramsar site, neotropical region, coastal wetlands, Platyhelminthes, symbiosis, turbellarians.

APRESENTAÇÃO

O trabalho apresentado aqui está dividido em sete seções, sendo a primeira uma introdução geral do tema estudado, seguida de objetivos e referências bibliográficas. Depois, seguem três capítulos em formato de artigo científico e escritos de acordo com as normas das revistas às quais foram/serão submetidos. Após, vem as considerações finais, e, por fim, o anexo com um artigo publicado. O primeiro capítulo intitulado **“Free-living microturbellarians (Platyhelminthes) from wetlands in southern Brazil, with the description of three new species”** foi submetido à revista “Zoological Studies”. O segundo capítulo intitulado **“Ecological patterns of free-living Platyhelminthes in coastal wetlands from southern Brazil”** será submetido à revista “Studies on Neotropical Fauna and Environment”. Finalmente, o terceiro capítulo intitulado **“Interspecific interactions in populations of *Temnocephala* spp. (Rhabdocoela: Temnocephalidae), ectosymbiont of *Pomacea canaliculata* (Caenogastropoda: Ampullariidae)”** será submetido ao periódico “Journal of Helminthology”. O anexo apresenta o artigo intitulado **“First report of *Gieysztoria falx* Brusa, Damborenea & Noreña, 2003 (Platyhelminthes: Rhabdocoela) in southern Brazil”** publicado na revista Zootaxa em 2019; e o e-mail da confirmação de submissão do manuscrito do capítulo 1.

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INTRODUÇÃO GERAL

O Brasil, maior país em território da América do Sul, apresenta grande quantidade de espécies de flora e fauna distribuídas por toda a sua extensão devido a distintos biomas de relevância (Mata Atlântica, Cerrado, Amazônia, Caatinga, Pantanal, Pampa e ambiente Marinho Costeiro) (Aguilar et al., 2016). Esta ampla biodiversidade coloca o Brasil como um dos 17 países megadiversos, que abriga quase 20% das espécies do mundo. O bioma Pampa encontra-se na região sul do Brasil. Este bioma ocupa mais da metade do território do estado do Rio Grande do Sul o qual apresenta-se principalmente como planícies ampliadas cobertas de gramíneas com alguns arbustos e raras árvores; além disso, os terrenos neste bioma são ondulados, de relevo suave, que não ultrapassam 200 metros de altitude (Aguilar et al., 2016).

Neste Bioma, a formação da Planície Costeira foi desenvolvida a partir de transgressões e regressões marinhas que resultaram em eventos de deposição ao longo do Litoral do Rio Grande do Sul (Vieira & Rangel, 1983). A hidrografia da planície costeira do Rio Grande do Sul é um sistema muito dinâmico, complexo e que possui modificações rápidas. Ao longo de toda a extensão da planície costeira encontram-se muitas lagoas interiores isoladas, e sua evolução progressiva foi formando lagoas interiores, banhados e arroios de diferentes tamanhos (Vieira & Rangel, 1988) e de reduzida profundidade (Vieira & Rangel, 1983). Além disso, os solos da planície costeira são formados por regiões permeáveis e impermeáveis, o que gera lençóis freáticos superficiais formando lagoas temporárias em ocasiões de elevados volumes pluviométricos (Krusche et al., 2002).

Segundo Meerhof & Jeppesen (2009), os corpos de água permanentes ou efêmeros que são superficiais o suficiente para permitir a penetração de luz até o fundo são definidos como lagos rasos; além disso, os sistemas rasos são definidos como polimíticos, o qual implica a circulação total da coluna de água constante ou por longos períodos de tempo sem a estratificação térmica de longa duração no verão.

De forma semelhante aos sistemas de lagos rasos, encontram-se os ecossistemas de banhados. A FEPAM (Fundação Estadual de Proteção Ambiental Henrique Luiz Roessler) (<http://www.fepam.rs.gov.br/>), órgão ambiental do Rio Grande do Sul, define os banhados como “zonas de transição terrestre-aquáticas que são periodicamente inundadas por reflexo lateral de rios e lagos e/ou pela precipitação direta ou pela água subterrânea e que resultam num ambiente físico-químico particular que leva a biota a responder com adaptações

morfológicas, anatômicas, fisiológicas, fenológicas e/ou etológicas e a produzir estruturas de comunidades características para estes sistemas". Ademais, a Convenção de Ramsar define as zonas úmidas de importância internacional como "áreas de pântano, charco, turfa ou água, natural ou artificial, permanente ou temporária, com água estagnada ou corrente, doce, salobra ou salgada, incluindo áreas de água marítima com menos de seis metros de profundidade na maré baixa." (São Paulo, 1997; Junk et al. 2013).

Nos ecossistemas dos banhados, a diversidade é determinada pelas macrófitas aquáticas que formam parte importante das comunidades contribuindo com biomassa e alta produtividade (Esteves, 2011; Wetzel, 1993). Assim, as macrófitas aquáticas afetam as características físicas e químicas da água e do sedimento, podem mudar as ciclagens de nutrientes, ser fonte de matéria orgânica (vivas ou mortas) para diversos organismos (bactérias, invertebrados, vertebrados), e alteram a estrutura espacial dos ecossistemas aquáticos devido ao incremento de complexidade espacial dos habitats e micro habitats (Thomaz & Esteves, 2011).

As estruturas dos habitats são importantes na determinação da diversidade de espécies, geralmente habitats mais complexos tem maior diversidade e maior riqueza de espécies (Bell et al. 1991; Jeffries, 1993; Taniguchi et al., 2003; Thomaz et al., 2008; Mykrä et al., 2011). Assim, a presença de vegetação num ecossistema resulta num aumento considerável da área disponível para a colonização por invertebrados (Pinder, 1995). Diversas espécies vivem associadas direta ou indiretamente às macrófitas dos banhados (Esteves 2011). Assim, os banhados da região sul do Brasil possuem grande biodiversidade. Dentre eles, encontram-se o mosaico de banhados da Estação Ecológica do Taim (ESEC Taim) (Würdig et al., 20007; Kirst et al., 2017).

Através do Poder Público (Ministério do Meio Ambiente) foram criadas as Unidades de conservação (UC's), as quais estão reguladas pelo Sistema Nacional de Unidades de Conservação (SNUC), criado pela lei N° 9.985 em junho de 2000 (Olivato & Junior, 2008). As UC's estão direcionadas para proteção da fauna, flora, microorganismos, corpos de água, solo, clima, paisagens, e todos os processos ecológicos que se desenvolvem nesses sistemas naturais, contendo grande importância na proteção da diversidade biológica e dos recursos genéticos associados (MMA, 2011). Além disso, algumas UC's podem também proteger o patrimônio histórico-cultural (Paula, 2018). Particularmente para os seres humanos, as UC's têm valiosas contribuições como: regulação da qualidade e quantidade de água para consumo;

fertilidade de solos e estabilidade das encostas; equilíbrio climático e manutenção da qualidade do ar; fornecimento de alimentos saudáveis e diversificados; base para produção de medicamentos para doenças atuais e futuras; áreas verdes para lazer, educação, cultura e religião; e fornecimento de matérias primas (Olivato & Junior, 2008).

Dentro das normas apresentadas pelo SNUC estão concebidos dois grupos de UC's: as Unidades de uso sustentável, as quais têm como objetivo a realização de pesquisa e aproveitamento dos recursos naturais; e as Unidades de proteção integral, as quais tem a função de preservar a natureza, sendo esta utilizada só para pesquisa e uso indireto de seus recursos. Dentre as UC's de proteção integral estão: Reserva Biológica (REBIO), Parque Nacional (PARNA), Monumento Natural (MN), Refúgio de Vida Silvestre (REVIS) e Estação Ecológica (ESEC), categoria a qual pertence a UC do Taim, unidade de estudo de deste trabalho.

A ESEC Taim foi criada em 21 de julho de 1986 pelo Decreto 92.963 com o objetivo de proteger os banhados da região sul e preservar a fauna ameaçada de extinção e o corredor de aves migratórias (Kurtz et al., 2003). Na ESEC Taim registra-se 49 espécies de macrófitas (Motta Marques & Villanueva 2001); 272 espécies de aves (Mälher et al., 1996; Garcia et al., 2013) das quais *Circus cinereus* encontra-se em categoria vulnerável (MMA, 2018); 63 espécies de peixes (Garcia et al., 2006; Correa et al., 2011); 21 espécies de répteis (Gomes & Krause 1982), com uma espécie (*Liolaemus occipitalis*) em categoria vulnerável (MMA, 2018); 18 espécies de anfíbios (Gayer et al., 1988); e estima-se mais de 40 espécies de mamíferos (Azambuja 2010; Sponchiado et al., 2012). Em função de sua importância internacional como zona úmida, a ESEC Taim foi designada como sítio Ramsar em 2017 (Ramsar, 2017). Neste sentido, a ESEC Taim abriga uma grande quantidade de ambientes úmidos onde os invertebrados aquáticos são importantes para a cadeia alimentar desempenhando processos funcionais na comunidade aquática (Würdig et al., 2007). Ainda assim, a maioria das pesquisas e avaliações ambientais que envolvem invertebrados aquáticos são realizadas com insetos (larvas e adultos), e muitos outros grupos, incluindo os Platyhelminthes Minot 1876 de vida livre, são usualmente deixados de fora dos estudos ambientais (Braccini et al., 2016).

Os Platyhelminthes são animais vermiformes, com simetria bilateral e hermafroditas; este Filo está composto por espécies de vida livre e parasitárias. Tradicionalmente foram classificados em quatro grandes classes: Turbellaria, Monogenea, Trematoda e Cestoda

(Brusca & Brusca, 2005). Os Platyhelminthes apresentam epiderme multiciliada. Esta característica é compartilhada entre os grupos parasitários e de vida livre. Contudo, esta característica é presente só nos Platyhelminthes parasitos em seus estados larvais, enquanto nos Platyhelminthes de vida livre (Turbellaria) estão presentes durante todo o ciclo de suas vidas (Noreña et al., 2015). Além disso, estudos recentes indicam que o grupo dos Platyhelminthes de vida livre (Turbellaria) não é monofilético e a classificação tradicional não deve ser mais usada (Egger et al., 2015). Não obstante, o presente estudo segue as recomendações de classificação baseadas no consenso geral e em evidências moleculares (Steenkiste et al., 2013; Ruggiero et al., 2015).

O grupo dos Turbellaria é formado pelos sub-filos Catenulidea e Rhabditophora (Ruggiero et al., 2015), os quais compartilham a sinapomorfia da epiderme ciliada. Os Catenulidea são compostos pela ordem Catenulida (Ruggiero et al., 2015), formada por organismos de 3-12 mm de comprimento, delicados, transparentes, com o corpo geralmente formado por zooides, podendo apresentar estatocistos e com o sistema excretor formado por um canal protonefrídio (Larsson & Jondelius, 2008; Larsson & Willems, 2010; Marcus, 1945a, b). Os Catenulida não possuem o aparelho copulador (estilete esclerotizado), o qual é usualmente utilizado para classificar o organismo em nível de espécie (Larsson & Willems, 2010).

Os Catenulida são formados por cinco famílias: Catenulidae, Stenostomidae, Chordariidae, Retronectidae e Tyrrheniellidae. As famílias Stenostomidae e Catenulidae possuem o maior número de espécies, enquanto as famílias Tyrrheniellidae e Chordariidae possuem o menor número de espécies (Tyler et al., 2016). A maior parte das espécies dos Catenulida vive em ambientes dulcícolas como poças temporárias, lagoas, córregos, rios e substratos terrestres úmidos. Os Catenulida de habitats dulcícolas são geralmente muito abundantes, enquanto as espécies marinhas são raras. A determinação taxonômica dos Catenulida é difícil, uma vez que possuem corpos muito frágeis e carecem de estruturas constantes (robustas) em seus corpos. Assim, recomenda-se que os organismos sejam estudados em vida (*in vivo*) (Larsson & Willems, 2010).

Os Catenulida representam 18% do total das espécies de Platyhelminthes de vida livre continentais registradas para o Brasil. Estas espécies foram registradas nos estados do Pará (1 sp.), São Paulo (41 spp.), Paraná (13 spp.) e Rio Grande do Sul (21 spp.); sendo os gêneros *Catenula* e *Stenostomum* os que possuem maior diversidade de espécies (Braccini et al.,

2016). A maioria das espécies registradas habita ambientes lênticos (associados às macrófitas e/ou ao sedimento), terras úmidas e campos de arroz (inclusive seus canais) (Braccini et al., 2016; Vara & Leal-Zanchet, 2013). Recentemente, nos ambientes lênticos dulcícolas permanentemente alagados na Planície Costeira do Rio Grande do Sul, quatro espécies (*Catenula evelinae*, *C. leuca*, *C. turgida* e *Stenostomum hemisphericum*) foram encontradas e registradas pela primeira vez para a região sul do Brasil (Braccini et al., 2017).

Os Rhabditophora (não Neodermata) são compostos pelas classes Macrostromorpha (Subdividida nas ordens Haplopharyngida e Macrostromida) e Neoophora (subdividida nas infraclasses Adiaphanida e Rhabdocoela) (Ruggiero et al., 2015). A classe Macrostromorpha é um grupo monofilético e apresenta características como: faringe simples coronada, sistema adesivo duoglandular, paratomía, espermatozoides em forma de filamentos e estruturas esclerotizadas no aparelho copulador masculino (Rieger, 2001). Geralmente os Macrostromorpha têm comprimentos reduzidos (1-2 mm), característica que faz difícil distinguir as estruturas internas assim sendo também difícil a determinação taxonômica dentro do grupo (Janssen et al., 2015). A identificação das espécies do Macrostromorpha é baseada nas características do sistema reprodutor masculino (Atherton & Jondelius, 2018).

Os Macrostromorpha estão compostos pelas ordens Haplopharyngida (com o menor número de espécies) e Macrostromida (com a maior diversidade). Esta última ordem compreende três famílias bem estabelecidas: Dolichomacrostromidae, Microstromidae e Macrostromidae (Atherton & Jondelius, 2018). As espécies das famílias da ordem Macrostromida se distribuem em uma ampla variedade de habitats aquáticos. Os representantes da família Microstromidae são habitantes geralmente marinhos, enquanto os representantes das famílias Dolichomacrostromidae e Macrostromidae podem habitar ambientes aquáticos dulcícolas, salobros e marinhos (Rieger, 2001).

Os Macrostromida representam 7% do total das espécies de Platyhelminthes de vida livre registradas para o Brasil. Estas espécies foram registradas nos estados de São Paulo (15 spp.) e Rio Grande do Sul (4 spp.); sendo os gêneros *Macrostromum* e *Microstromum* os que possuem mais espécies (Braccini et al., 2016). As espécies dulcícolas foram registradas em ambientes lênticos (associados às macrófitas e/ou ao sedimento) e campos de arroz (Braccini et al., 2016; Vara & Leal-Zanchet, 2013).

A classe Neoophora está subdividida nas infraclasses Adiaphanida (o qual compreende as ordens Fecampida, Prolecithophora e Tricladida) e Rhabdocoela (composta pelas ordens

Kalyptorhynchia, Endoaxonemata e Dalytyphloplanida) (Ruggiero et al., 2015). As relações filogenéticas ainda são discutíveis dentro dos Adiaphanida; as tentativas de agrupamento têm argumentos morfológicos com relação às seguintes características: intestinos diverticulados, testículos em série e repetitivos, folículos vitelogênicos aninhados e uma faringe tipo "plicatus" orientada para a parte posterior; contudo, os Adiaphanida foram chamados assim porque as ordens dentro deste grupo tem sua aparência opaca (i.e. não diáfana) (Laumer & Giribet, 2017).

Dentro dos Adiaphanida, a ordem Fecampida é uma exceção à regra dos Platyhelminthes de vida livre: eles têm o estilo de vida parasítico em crustáceos e outros invertebrados marinhos (Sudo et al., 2011). A ordem Prolecithophora habita ambientes dulcícolas e marinhos; entretanto, no Brasil não se tem espécies continentais registradas (Braccini et al., 2016).

A ordem Tricladida apresenta as características notáveis: intestino subdividido em três ramos principais, camadas musculares da faringe entrecruzadas, nefridióporos dispostos seriadamente e zona marginal adesiva (Riutort et al., 2012; Sluys et al., 2009). Além disso, as espécies deste grupo habitam ambientes terrestres (ninhada em folhas úmidas), límnicos (embaixo das pedras, macrófitas e folhas submersas), marinhos e cavernas (Carbayo et al., 2002; Leal-Zanchet et al., 2014; Noreña et al., 2015). O inventário feito em 2008 faz menção de 12 espécies límnicas registradas para o Brasil, onde as regiões Sudeste e Sul apresentam a maior quantidade de registros destas espécies, particularmente nos estados de São Paulo e Rio Grande do Sul (Carbayo & Froehlich, 2008); sendo o gênero *Girardia* o que possui maior quantidade de espécies na região Neotropical (Sluys et al., 2005).

A infraclasse Rhabdocoela, composta pelas ordens Kalyptorhynchia, Endoaxonemata e Dalytyphloplanida (Ruggiero et al., 2015), é um dos grupos com maior quantidade de espécies que apresentam características como: faringe com forma de rosa/musculosa em posição mais ou menos vertical, probóscide frontal, e comportamento simbiote comensal (Dalytyphloplanida: Temnocephalida) sobre invertebrados dulcícolas e quelônios (Van Steenkiste et al., 2013). A determinação das espécies dentro do grupo Rhabdocoela é feita analisando-se o aparelho copulador dos indivíduos, alguns grupos apresentam estruturas esclerotizadas (estiletos) os quais mostram uma forma definida para cada espécie (Young, 2001). Além disso, Van Steenkiste et al., (2013), reconhecem dois clados: Kalyptorhynchia, composto por 6530 espécies descritas, e Dalytyphloplanida (com 61.000 espécies descritas).

Os grupos Kalyptorhynchia e Dalytyphloplanida são encontrados em todo o mundo, eles são habitantes de ambientes dulcícolas, salobros e marinhos; contudo, também podem ser limnoterrestres (Houben et al., 2015; Van Steenkiste et al., 2013).

Nas regiões Sudeste e Sul do Brasil, um maior número de espécies do grupo Rhabdocoela foi registrado em ecossistemas lênticos incluindo campos e canais de arrozais, e também em ambientes lóticos, sendo que a maioria das espécies registradas habitam ambientes de água doce (Braccini et al., 2016). Recentemente, três espécies de Dalytyphloplanida (*Gieysztoria chiqchi*, *G. falx* e *Baicalellia evelinae*) foram registradas em ambientes límnicos no estado do Rio Grande do Sul (Capivarí do Sul, Osório, Tramandaí e Rio Grande), sendo *G. chiqchi* e *G. falx* encontradas pela segunda e terceira vez desde sua descrição original em Loreto, Peru (floresta amazônica) (Damborenea et al., 2005) e Berisso, Buenos Aires, Argentina (Brusa et al., 2003), respectivamente. Portanto, representam o primeiro registro destas espécies para o Brasil (Braccini et al., 2017; Reyes et al., 2019).

Dentro da ordem Dalytyphloplanida apresenta-se um grupo particular, os Temnocephalidae Blanchard, 1849 (Temnocephalida) (Tyler et al., 2016). Esse grupo apresenta adaptações morfológicas para viver sobre estruturas superficiais de invertebrados de água doce. Os temnocefalídeos apresentam uma morfologia muito distinta quando comparados aos demais platelmintos de vida livre: a epiderme não possui cílios locomotores e está organizada em placas sinciciais; além disso, a maioria das espécies apresenta tentáculos anteriores e um disco adesivo posterior para fixação no hospedeiro (Martínez-Aquino et al., 2014a).

Os Temnocephalidae representam o grupo mais diversos dentro de Temnocephalida, sendo as espécies do gênero *Temnocephala* endêmicas da região Neotropical. Cada espécie de temnocefalídeo vive sobre uma espécie de hospedeiro (invertebrado de ambiente dulcícola) ou à hospedeiros relacionados filogeneticamente (Martínez-Aquino et al., 2014a).

Na região do sul do Brasil, os temnocefalídeos têm muitos hospedeiros invertebrados como decápodes, insetos e gastrópodes, sendo uns dos mais usuais os gastrópodes do gênero *Pomacea* (Caenogastropoda: Ampullaridae) (Seixas et al., 2010a, b, c). Os caramujos do gênero *Pomacea* são organismos de água doce e habitam ambientes tropicais e subtropicais. As espécies *P. canaliculata* e *P. maculata* tem distribuição simpátrica na região do América do Sul, sendo a espécie *P. canaliculata* distribuída no sul do Brasil, Uruguai, norte de Argentina e Paraguai (Hayes et al., 2012). Estas espécies possuem organismos associados

com os quais vivem, sendo os temnocefalídeos o grupo de turbelários que desenvolveram uma relação permanente e próxima (simbiontes comensais *sensu* Damborenea et al., (2017)). Dentre todas as espécies, *T. iheringi* é a mais amplamente distribuída, se estabelecendo na cavidade paleal do manto de *P. canaliculata* (e outras espécies de *Pomacea*) e depositando seus ovos na sutura canalizada e abertura oral das conchas destes caramujos.

Os temnocefalídeos tem se relacionado com grupos de hospedeiros estritamente da água doce. Por exemplo, a espécie *T. iheringi* tem desenvolvido uma relação exclusiva com seus hospedeiros, os caramujos Ampullaridae (Martínez-Aquino et al., 2014a). Além disso, considera-se que algumas espécies de temnocefalídeos apresentam também micro endemismos, sendo que 40% das espécies reportadas para a região Neotropical são consideradas microendêmicas por terem sido encontradas em apenas uma bacia com um hospedeiro em particular (Martinez-Aquino et al., 2014b).

O Brasil é o país da América do Sul com mais espécies do gênero *Temnocephala* (18 espécies) descritas, além disso, é também o país com a maior quantidade de espécies endêmicas (nove espécies) de temnocefalídeos na região Neotropical (Martinez-Aquino et al., 2014b).

Segundo o exposto acima, os Platyhelminthes de vida livre de ambientes dulcícolas são encontrados em áreas úmidas e grandes lagoas, onde fazem parte da comunidade bentônica. A distribuição dos Platyhelminthes de vida livre está baseada em dois fatores: tipo de substrato e salinidade. Os sedimentos arenosos, arenoso-pedregoso parecem ser os substratos preferidos para a maioria das espécies (Noreña et al., 2007). Eles também podem ser encontrados em riachos, onde podem ser predadores especialmente importantes na ausência de peixes. Além disso, podem ser encontrados em diferentes tipos de substratos (por exemplo, musgo, plantas vasculares, madeira úmida, detritos, cascalho ou areia) nas áreas litorais dos rios (Noreña et al., 2015).

Espécies litorâneas de Platyhelminthes de vida livre de ambientes lóticos e lênticos, podem viver em associação com a vegetação (macrófitas) flutuante ou enraizada nas áreas litorâneas (Noreña et al., 2015) ou podem ser infaunais (se enterrando nos primeiros centímetros do sedimento). Formas maiores de Platyhelminthes de vida livre, como espécies da ordem Tricladida, podem ser encontradas sob pedras em rios ou lagoas. No entanto, algumas espécies estão sujeitas a distúrbios durante as diferentes estações do ano devido às mudanças no nível da água. Além disso, a maioria dos Platyhelminthes de vida livre de água

doce produz ovos revestidos por casulos ou cápsulas que fornecem resistência às mudanças extremas das condições ambientais, como temperatura, seca ou gelo (Young, 1972).

Os Platyhelminthes de vida livre têm alta adaptabilidade biológica ou ecológica, por isso, tem alto potencial de dispersão e colonização. Portanto, muitas espécies são cosmopolitas. Contudo, os níveis de oxigênio são um fator limitante para a sobrevivência dos organismos (Noreña et al., 2015).

Relativo aos Temnocephalidae, a espécie *T. iheringi* aproveita o movimento de água gerado pelo hospedeiro durante sua respiração, para se alimentar dos pequenos crustáceos ou algas que podem entrar na cavidade paleal do caramujo *P. canaliculata*. No entanto, ainda não se sabe quais são os fatores que determinam a presença ou prevalência destas espécies comensais em seus hospedeiros. Alguns resultados mostram que o tamanho dos hospedeiros tem uma relação direta com a quantidade de *Temnocephala* presente, isto é, hospedeiros maiores de 4 cm de comprimento tem maior prevalência (Martin et al., 2005; Damborenea et al., 2006). Além disso, Martin et al., (2005) apresentam um estudo para entender os fatores que afetam a distribuição e a abundância de *T. iheringi* entre populações de *P. canaliculata* na região dos pampas argentinos. Eles encontraram maior frequência de ocorrência de temnocefalídeos em locais lânticos com concentrações de bicarbonato menor que 6,6 mEq. l⁻¹, sugerindo que a tolerância dos epibiontes é muito menor que a do caramujo (até 9,95 mEq. l⁻¹).

A ESEC Taim é uma das áreas protegidas que abriga uma grande diversidade de fauna. Contudo, há uma carência de estudos sobre a sistemática dos Platyhelminthes de vida livre, sendo eles importantes componentes da cadeia alimentar nos ecossistemas aquáticos continentais. Neste sentido, o presente estudo procura determinar a diversidade dos Platyhelminthes de vida livre e comensais na ESEC Taim, determinando seus padrões ecológicos, incluindo inferências sobre a infestação de comensais em *P. canaliculata* em função das condições das variáveis ambientais (Martín et al., 2005). Desta forma valoriza-se a fauna de invertebrados e iniciam-se os primeiros estudos referentes ao grupo dos Platyhelminthes na ESEC Taim.

OBJETIVOS

- **Geral**

- Determinar a diversidade taxonômica da fauna de turbelários límnicos de vida livre e comensais e avaliar seus padrões ecológicos na ESEC Taim.

- **Específicos**

- Construir uma base taxonômica dos turbelários límnicos que habitam a ESEC Taim;
- Comparar a abundância e riqueza das espécies de turbelários de vida livre associados à vegetação flutuante na ESEC Taim entre diferentes ambientes aquáticos;
- Comparar a prevalência e abundância de espécies de temnocefalídeos em diferentes classes de tamanhos de *Pomacea canaliculata* em ambientes lênticos na ESEC Taim.

HIPÓTESES

- A fauna dos Platyhelminthes límnicos de vida livre é formada principalmente pelas espécies dos grupos Catenulida e Rhabdocoela seguindo a tendência observada nos corpos de água da Planície Costeira.
- A abundância e riqueza de turbelários de vida livre é alta em ambientes aquáticos pouco profundos (canais da água) e baixa em ambientes aquáticos profundos (lagoas).
- A infestação de temnocefalídeos (Platyhelminthes comensais) tem correlação positiva com o aumento do tamanho da concha em moluscos ampularídeos.

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CAPITULO 1

FREE-LIVING MICROTURBELLARIANS (PLATYHELMINTHES) FROM WETLANDS IN SOUTHERN BRAZIL, WITH THE DESCRIPTION OF THREE NEW SPECIES

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ABSTRACT

The Taim strict nature reserve (ESEC Taim) is a protected wetland (Ramsar site) in southern Brazil. Although the ESEC Taim harbours a broad conspicuous flora and fauna, this area lacks studies on free-living Platyhelminthes. Here, we describe three new species to science, one belonging to Macrostomorpha (*Macrostomum* **n. sp. 1**) and two belonging to Dalytyphloplanoida (*Gieysztoria* **n. sp. 1** and *Gieysztoria* **n. sp. 2**). *Macrostomum* **n. sp. 1** differs from their congeners for its distinct stylet in “J”-shaped. *Gieysztoria* **n. sp. 1** is distinguishable based on the crown-like penis stylet, this species possesses 12-18 spines with similar size, but two smaller spines (one a bit longer than the other). *Gieysztoria* **n. sp. 2** clearly differs from their congeners owing to its fibrous girdle which bears two groups of complexes of spines and three different-in-length ventral spines. Moreover, we identified twenty-three species, eleven belonging to Catenulida; three, to Macrostomorpha; seven, to Dalytyphloplanoida; one, to Kalyptorhynchia; and one, to Prorhynchida. We recorded three species for the first time in Brazil (*Myostenostomum vanderlandi*, *Macrostomum quiritium*

and *Strongylostoma elongatum*). The knowledge of the free-living Platyhelminthes gives to this protected area more value as natural reserve and reinforces protection actions.

Keywords

Coastal wetlands, Ramsar site, taxonomy, turbellaria, Neotropical region

BACKGROUND

Freshwater microturbellarians are free-living Platyhelminthes who live in all aquatic habitats as streams, ponds, lagoons and wetlands, where they live associated to the benthos or between roots or leaves of aquatic plants or associated to filamentous algae in permanent or temporary waters (Noreña et al. 2015; Reyes and Brusa 2017). Most freshwater microturbellarians are known from the Holarctic region owing to the intensive research carried on this region (Schockaert et al. 2008; Van Steenkiste et al. 2011; Willems et al. 2007). However, there is still a lack of knowledge when it comes to determinate biogeographical and distribution patterns of microturbellarians in other regions. In the Neotropical region most studies on microturbellarians were developed in Argentina and Brazil. In Argentina, the knowledge of microturbellarians were increased and systematized by studies of Noreña-Janssen (1995), Brusa et al. (2003; 2008), Noreña et al. (2003; 2004; 2005a; 2005b; 2005c; 2006a; 2006b), Damborenea et al. (2005; 2007; 2011), Brusa (2006), and Adami et al. (2012). In Brazil, Marcus (1943 – 1949) studied the microturbellarian fauna from a wide variety of environments, being this one of the most important works. In the Coastal Plain of Southern Brazil, freshwater microturbellarians have been studied for their diversity and composition (Braccini and Leal-Zanchet 2013; Vara and Leal-Zanchet 2013; Gamo and Leal-Zanchet 2004). Besides, Braccini et al. (2016) summarized all the records of the Brazilian microturbellarians, taking into account biogeographical and ecological aspects and suggesting actions to enhance the study of the group. More recently, six species of microturbellarians were recorded for the first time in wetlands in the Northeast region of Rio Grande do Sul. These findings increased the known diversity of freshwater microturbellarians to 109 species in Brazil (Braccini et al. 2017).

The southern region of Brazil presents a vast number of freshwater wetlands which are hot spots of biodiversity (Guadagnin 1999). In this sense, the Taim nature reserve (ESEC Taim) is a protected mosaic of wetlands in this area, also it was recently designated as a Ramsar site (Ramsar 2017). As so, the ESEC Taim harbors a broad conspicuous flora and fauna (Gomes and Krause 1982; Pires Gayer et al. 1988; Motta Marques and Villanueva 2001; Charão Kurtz et al. 2003; Garcia et al. 2006; Würdig et al. 2007; MMAB 2008; Azambuja 2010; Correa et al. 2011; Sponchiado et al. 2012).

Despite the high biodiversity found in the ESEC Taim, there are no reports on species of freshwater microturbellarians in this protected area. In this sense, the knowledge of microturbellarians allows us to add value to the state of conservation of the natural reserve and reinforces protection actions. Thus, this study displays the diversity of species of free-living microturbellarians occurring in this area, including the description of three new species of microturbellarians (Macrostomida and Dalytyphloplanida). Also, we comment on the

morphology and distribution of the species register in this protected area, some of them being recorded for the first time in Brazil.

MATERIALS AND METHODS

Research area. The area of study is located in the ESEC Taim, located in the Coastal Plain of Rio Grande do Sul, southern Brazil (32°50' S, 52° 26' W), near Brazil's border with Uruguay between the lake Mirim and the Atlantic Ocean (Bager et al. 2010) (Fig. 1). The ESEC Taim is comprised of a mosaic of ponds, channels and flooded areas (regionally called "banhados") of different lengths and depths. Also, there are the presence of lakes Nicola and Mangueira. Each sampling site is related to a water body inside the protected area. Details on the localities are given below:

Lake Nicola (Site 1) (32° 33' 18.07" S; 52° 31' 18.50" W). Located within the wetland, it is one of the main ponds of the ESEC Taim. It is a permanent pond with a notorious seasonal variation on the water regime, including the marsh vegetation on its boundaries. This lake is mainly flooded by water from lake Mangueira, which flows diffusely to lake Nicola through the swamp area. In addition, it receives water from different external channels, including agricultural water systems located in the peripheric area to the ESEC Taim.

Water channel (Site 2) (32° 35' 50.52" S; 52° 34' 5.40" W). This site is located at the western boundary within the wetland. It presents a remarkable seasonal water fluctuation throughout the year, and it is connected to lake Mangueira and lake Nicola. The water channel is quite close to the main road Brigadeiro José da Silva Paes (BR 471), which runs parallelly through almost all the western limit of the ESEC Taim. This channel presents about 16 km in length, but this measurement may vary according to seasonal rainfalls and eutrophication processes.

Lake Mangueira (Site 3) (32° 50' 11.42" S; 52° 38' 37.45" W). This lake is approximately 820 km² and its northern area is within the limits of the ESEC Taim. Lake Mangueira is by far the main hydric reservoir of the protected area, water from this lake flows through a channel parallel to the western limit of the wetland, connecting to lake Nicola (Artioli et al. 2009).

Sample collection and preparation. Sampling campaigns were carried out bimonthly from June 2018 to July 2019. Sampling was conducted randomly on two different microhabitats, i.e, benthal and phytoal environments in the ESEC Taim. Consequently, we delimited a square of 0.5 x 0.5 m to collect littoral floating vegetation on flooded areas (up to 50 cm in depth) in each sampling site. Floating vegetation was rinsed within the square area with a sieve (335 µm mesh size), washed with local water and returned to the environment. Samplings of floating vegetation were repeated three times in each site.

The benthic sampling was made also in shallow flooded areas using a core (10 cm diameter, 75 cm length). The core was introduced 10 cm in the sandy/muddy sediment, only the first 2 cm of the surface were collected. Samplings of the benthic environment were repeated five times in each site.

All material collected by the sieve and core was then deposited in plastic jars (1.25 L) with water from the sampled environment. Jars, without any fixation solution, were carried to

the laboratory and maintained with air pumps in a temperate room (~20 °C). Afterward, samples underwent Schockaert's (1996) oxygen depletion method, which consists in leaving the jar without any source of oxygen so microturbellarians tend to swim towards the superior layer of water so that they can be picked up with a pipette easily. When needed, we also examined the samples under the stereomicroscope as it is suggested by Brusa et al. (2003).

Animals extracted from jars were counted and examined *in vivo* by the squash method. We record the morphological traits of individuals through optical microscopy by making videos, photographs and sketches so we could identify the turbellarians at the species level. Whenever turbellarians present hard parts (sclerotic copulatory stylet), one or more specimens were whole-mounted using Hoyer's medium. Other specimens were fixed in AFA or alcohol 96% and were stored in alcohol 96%. Posteriorly, photographs of live and whole-mounted individuals were taken using a camera Olympus DP73/DP72 attached to a differential interference microscope Olympus CX4/BX51. In addition, drawings were made with a camera lucida based on whole-mounted specimens. Measurements (generally made in micrometers "µm") of soft and sclerotic structures were obtained using the software ImageJ (Schneider et al. 2012), figures were processed with Adobe Photoshop CS6®, dimensions were expressed as follows: average ± standard deviation (minimum value – maximum value; number of items measured "n"), unless described otherwise.

For species comparison purposes, specialized literature on turbellarians was consulted. Vouchers, holotypes and paratypes studied herein were deposited at the Helminthological Collection of the Zoology Museum "Museu de Zoologia da USP" (MZUSP), Brazil. Sampling permits were granted by the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio), permit numbers 60291-2/2017 and 64551-1/2018.

RESULTS

Order Catenulida Graff, 1905

Family Catenulidae Graff, 1905

Genus *Catenula* Dugès, 1832

Catenula lemnae Dugès, 1832

(Fig. 2A)

Synonyms: *Anortha gracilis* Leidy, 1851

Catenula gracilis Leuckart, 1854

Stenostomum gracile Graff, 1882

Stenostomum lemnae Graff, 1882

Stenostomum monocelis Schmidt, 1878

Derostoma catenula Leydig, 1854

Material. Several individuals observed alive (MZUSP PL 2257). Three squashed preparations.

Description and remarks. Whitish mature individuals 2120 ± 1158.2 µm (1301.1 – 2939 µm; n = 2) long and 105.4 ± 48.5 µm (52.2 – 147.2 µm; n = 3) wide, with three to eight zooids. When it is measured only the first zooid, they are 275.4 ± 86.7 µm (195.3 – 367.4 µm; n = 3) long. They present the typical habitus of the species, i.e., elongated body, rounded anterior region of the body forming the prostomium, pre-oral swelling with ciliated furrows,

with a statocyst located just at the ciliated furrow level (Fig. 2A). Morphology of individuals corresponds to descriptions given by Marcus (1945a; 1945b), Noreña-Janssen (1995) and Reyes et al. (2017), for the Neotropical region. Also, *C. lemnae* found here are following the descriptions, illustrations and pictures of material studied in the Palearctic and Nearctic region (Luther 1960; Gamo 1987; Noreña et al. 2007; Larsson and Willems 2010).

Distribution. Sites 1 and 3, associated with benthal and phytal microhabitats.

Previous records. The species is widespread, it is found in Sweden (Larsson and Willems 2010), USA (Ruebush 1914), Ireland (Young 2001), United Kingdom (Young 2001), Spain (Gamo 1987; Noreña et al. 2007), Perú (Reyes et al. 2017), Brazil (Marcus 1945b) and Argentina (Noreña-Janssen 1995). Also, Siberia and Japan (see Luther (1960) and references therein).

Family Stenostomidae Vejdovsky, 1880

Genus *Myostenostomum* Luther, 1960

Myostenostomum vanderlandi Rogozin, 1992

(Fig. 2B–D)

Material. Four individuals (MZUSP PL 2258), afterward whole-mounted. Individuals were photographed alive.

Description and remarks. Specimens studied alive are vermiform, the body coloration varies from light orange to whitish. The anterior region is rounded. The body, 762.7 ± 218.7 μm (550.1 – 987.1 μm ; $n = 3$) long and 114 ± 18.3 μm (92.9 – 125.2 μm ; $n = 3$) wide, consist of two to four zooids (Fig. 2B). The anterior region of the body bears two lateral ciliated pits, 48.1 ± 2.29 μm (46.4 – 50.7 μm ; $n = 3$) long and 20.4 ± 4.1 μm (17.5 – 20.3 μm ; $n = 2$) deep. The ciliated pits are connected to the anterior brain lobe which, in its anterior region, has 5 – 7 paired metameric ganglia while, in its posterior region, has two lateral trapezoidal lobes (Fig. 2C). The posterior brain lobe is somewhat triangular in shape with the apex directed to the anterior region. Besides, elongated ganglia are present at the base of both lateral angles of the posterior brain (Fig. 2D). Ventral and behind the posterior brain lobe, it is located the "V"-shaped mouth. This mouth leads to the pharynx, which is 60 ± 29.3 μm (41.7 – 93.8 μm ; $n = 3$) long. At the anterior half of the intestine, is located the muscular gut, 76.9 ± 8.4 μm (71.5 – 86.6 μm ; $n = 3$) long and 102.6 ± 28.9 μm (72.2 – 129.7 μm ; $n = 3$) wide. The intestine continues tapering to an intestine-lacking region reaching the base of the caudal appendage, which is 53.9 ± 35.9 μm (28.5 – 79.3 μm ; $n = 2$) long (Fig. 2B). Male and female reproductive systems not observed.

Myostenostomum was established by Luther (1960), this genus is differentiated by the anterior muscular gut, and consequently, it erected two species *M. bulbocaudatum* and *M. tauricum*. Later, Rogozin (1992) reviewed the genus and recognized that the anterior and posterior cerebral ganglia can be used as a diagnostic character for species discrimination so, four species were allocated in *Myostenostomum*, including *Myostenostomum* sp. (*sensu* van der Land (1970)) defined as *M. vanderlandi*. Individuals in this study are *M. vanderlandi* due to the similar morphological configuration of the cerebral ganglia, i.e., the anterior lobe has 5 – 7 paired metameric ganglia in the anterior region, while in its posterior region, has two lateral trapezoidal lobes which are in accordance with the descriptions of van der Land

(1970), Rogozin (1992) and Tokinova and Berdnik (2017). Moreover, the posterior triangular lobe with laterally elongated ganglia is also corroborated. However, on average, our specimens are smaller than those reported for the Palearctic region (Rogozin 1992; Tokinova and Berdnik 2017), except for the length of the muscular gut, which is longer. Since the type locality of *M. vanderlandi* is Suriname, we expand the range of distribution approximately 4242 km southwards in Rio Grande do Sul, Brazil, being the first record for the country.

Distribution. Sites 1, 2 and 3, associated with benthal and phytal microhabitats.

Previous records. This species was originally found in Paramaribo, Suriname (South America) (van der Land 1970). Recently, it was registered in Volga-Kama Reserve, Russia (Tokinova and Berdnik 2017).

Genus *Stenostomum* Schmidt, 1848

Stenostomum cryptops Nuttycombe and Waters, 1935

Material. A single individual, afterward whole-mounted and studied alive.

Description and remarks. The body configuration is typical of the species, small and with two pairs of plate-like light-refracting bodies, the first pair located on the anterior brain lobe, while the second pair is located at the posterior brain lobe. Club-shaped pharyngeal glands distributed along the anterior half of the pharynx. Muscular pharynx. Several excretophores laterally distributed along the intestine. Our observations are in agreement with descriptions made by Marcus (1945b), and Nuttycombe and Waters (1938). However, Marcus (1945b) stated that specimens from São Paulo bear only a pair of light-refracting bodies associated with the anterior brain lobes. Nonetheless, our specimen presents two pairs of these structures which made them more similar to individuals studied by Nuttycombe and Waters (1938) in North America. This is the second record of *S. cryptops* for the country since it was found in São Paulo (Marcus 1945b).

Distribution. Site 2, associated with phytal microhabitat.

Previous records. The species is found in Georgia, USA (Nuttycombe and Waters 1938) and São Paulo, Brazil (Marcus 1945b).

Stenostomum glandulosum Kepner and Carter, 1931

(Fig. 2E, F)

Material. Five individuals, afterward whole-mounted and studied alive.

Description and remarks. Specimens of $586.7 \pm 71.3 \mu\text{m}$ ($521.9 - 681 \mu\text{m}$; $n = 5$) long bear two zooids. The first zooid is $381.8 \pm 49.4 \mu\text{m}$ ($348.3 - 468.1 \mu\text{m}$; $n = 5$) long. With large superficial lateral ciliated pits of $39.3 \pm 6.4 \mu\text{m}$ ($32.2 - 47.8 \mu\text{m}$; $n = 4$) long. Anterior brain lobe divided into metameric masses with three pairs of plate-like light-refracting bodies (Fig. 2E, F). Rounded mouth with several club-shaped pharyngeal glands on the margin. Pharynx $66.4 \pm 13.7 \mu\text{m}$ ($54.4 - 86.8 \mu\text{m}$; $n = 5$) in length, tube-shaped, with several pharyngeal glands. Intestine with excretophores and is extended up to the caudal end. Longitudinal excretory channels extending from the anterior to the posterior region of the body (Fig. 2F).

The specimens found here agree well with the descriptions made by Kepner and Carter (1931), and Nuttycombe and Waters (1938). However, our individuals are smaller than those

described by Marcus (1945b), Kolasa and Young (1974b), but longer than those described by Kolasa and Young (1974a). The most remarkable feature of this species are the light-refracting bodies. Here, the specimens from ESEC Taim have three pairs, while Marcus (1945b), Kolasa and Young (1974b), and Kolasa and Young (1974a) mentioned 2 – 3, 1 or none, and only 2 pairs respectively, suggesting a notable variation of this feature.

Distribution. Site 2, associated with phytal microhabitat.

Previous records. The species was registered in North Europe (Lanfranchi and Papi 1978), USA (Kepner and Carter 1931; Nuttycombe and Waters 1938), Poland (Kolasa and Young 1974a), Suriname (van der Land 1970), Brazil (Marcus 1945b), and Kenya (Kolasa and Young 1974b).

Stenostomum grande Child, 1902

(Fig. 2G)

Synonyms: *Stenostomum oesophagum* Kepner & Carter, 1931

Material. Four individuals (MZUSP PL 2259) studied alive.

Description and remarks. Live specimens are whitish, with fusiform shaped. They have 1–3 zooids. The body length is $732.3 \pm 340.9 \mu\text{m}$ ($466.6 - 1226.8 \mu\text{m}$; $n = 4$). Individuals follow the descriptions given by Kepner and Carter (1931), Nuttycombe and Waters (1938), Marcus (1945a; 1945b) and Noreña et al. (2005b) for *S. grande*. Recently, molecular analyses suggest that *S. grande* and *S. leucops* could be a species complex (Rosa et al. 2015). However, there is no consensus on changing the status of *S. grande*. This species is a common inhabitant of lentic freshwater habitats (ponds and lagoons) in southern Brazil (Braccini et al. 2016; Braccini and Leal-Zanchet 2013; Vara and Leal-Zanchet 2013).

Distribution. Sites 1, 2 and 3, associated with benthal and phytal microhabitats.

Previous records. This species is widely distributed. It occurs in USA (von Graff 1911; Kepner and Carter 1931; Nuttycombe and Waters 1938), Russia (Nasonov 1923 and references therein), Finland (Nassonov 1924), Poland (Kolasa 1977), Suriname (van der Land 1970), Brazil (Marcus 1945b), Peru (Noreña et al. 2006b) and Argentina (Noreña et al. 2005b).

Stenostomum paraguayense (Martin, 1908)

(Fig. 2H)

Synonyms: *Weldonia paraguayensis* Martin, 1908

Material. Three individuals, two of them whole-mounted.

Description and remarks. Animals are elongated, live specimens are whitish. Body with 2-4 zooids. The body length is $931.5 \pm 144.1 \mu\text{m}$ ($829.6 - 1033.4 \mu\text{m}$; $n = 2$) long and $115 \pm 24.9 \mu\text{m}$ ($97.4 - 132.6 \mu\text{m}$; $n = 2$) wide. This species is easily recognizable due to the three pairs of light-refracting bodies, the transverse cells line with refringent granules located at the first third of the intestine, and the two caudal finger-like extensions (the dorsal extension longer than the ventral) (Fig. 2H). Our individuals are longer than those described by Marcus (1945b) and Noreña et al. (2005b), but shorter than those of Damborenea et al. (2011). Moreover, individuals from the ESEC Taim have the dorsal caudal extension longer than the ventral, while Noreña et al. (2005b) indicate otherwise. The dorsal caudal extension could be

absent or reduced as Marcus (1945b) noted. Also, the transverse cells line with refringent granules could be a variable feature since Damborenea et al. (2011) were not able to observe this feature in individuals from Peru.

Distribution. Site 2, associated with phytal microhabitat.

Previous records. Suriname (van der Land 1970), Brazil (Marcus 1945b), Peru (Noreña et al. 2006b; Damborenea et al. 2011), Paraguay (Martin 1908) and Argentina (Noreña et al. 2005b).

Stenostomum hemisphericum Nassonov, 1924

(Fig. 2I)

Material. Three individuals, afterward whole-mounted. Hand-drawings were made.

Description and remarks. The most remarkable feature of this species is the long pharynx which bears oblique and lateral muscle fibers associated with the pharynx. Despite few sampled individuals, morphology observed corresponds well to the descriptions made by Nasonov (1924), Marcus (1945a), Damborenea et al. (2011) and Braccini et al. (2017). This species is recorded for the third time in Brazil.

Distribution. Site 3, associated with benthal microhabitat.

Previous records. Ukraine (Nassonov 1924), Suriname (van der Land 1970), Brazil (Marcus 1945b; Braccini et al. 2017) and Peru (Damborenea et al. 2011).

Stenostomum cf. pegephilum Nuttycombe and Waters, 1938

(Fig. 3A–C)

Synonyms: *Anokkostenostomum pegephilum* (Nuttycombe and Waters, 1938)

Material. A single individual, afterward whole-mounted. Photographs and videos of a live animal.

Description and remarks. Live specimens have chains with two zooids, they are whitish. The body length is 557.6 μm long and 121.5 μm wide. Blunt anterior end with two lateral small ciliated pits (29.7 μm long). Anterior and posterior brain lobes located at the anterior region: the anterior brain lobe (ahead metameric; behind, lobes laterally paired) and the posterior brain lobe (laterally paired lobed) (Fig. 3A–B). Longitudinal mouth with associated glands (Fig. 3A–C). The muscular pharynx is 99.4 μm long, it has several small pharyngeal glands at its posterior half (Fig. 3C). Rounded posterior end with short cilia (Fig. 3B). General features are corroborated with descriptions given by Nuttycombe and Waters (1938), Marcus (1945b) and Noreña et al. (2005b). However, for two zooids, our specimen is smaller than those found by Nuttycombe and Waters (1938) (900 μm) and Marcus (1945b) (800 μm). Previous reports stated a transversal mouth (Nuttycombe and Waters 1938; Marcus 1945b), but we observed a longitudinal mouth with several surrounded glands. Nuttycombe and Waters (1938) states that the pharynx seems to form a posterior separated camera, while our specimen has two differentiated pharyngeal regions.

Distribution. Site 2, associated with phytal microhabitat.

Previous records. The species is found in Poland, Germany (Lanfranchi and Papi 1978), USA (Nuttycombe and Waters 1938), Italy (Noreña et al. 2005b and references therein), Suriname (van der Land 1970) and Brazil (Marcus 1945b).

Stenostomum saliens Kepner and Carter, 1931

(Fig. 3D)

Synonyms: *Anokkostenostomum saliens* (Kepner and Carter, 1931)

Stenostomum saltatum Kepner and Carter, 1931

Material. Two individuals, whole-mounted and photographed alive.

Description and remarks. Elongated whitish animals with 1-2 zooids, the anterior region of the body is pointed, while the posterior one is rounded (Fig. 3D1–2). The body is covered by cilia and has larger rigid cilia on the whole surface. The anterior region with brain lobes, which are connected to the lateral small ciliated pits. A V-shaped mouth is located in the first third of the body (Fig. 3D1). Intestine with excretophores located, mainly, at the anterior area of the gut (Fig. 3D2). Distal region of the intestine with lacking region. The body is $598.8 \pm 363.7 \mu\text{m}$ ($341.6 - 855.9 \mu\text{m}$; $n = 2$) long and $79.2 \pm 54.4 \mu\text{m}$ ($40.7 - 117.6 \mu\text{m}$; $n = 2$) wide. The features observed in individuals from the ESEC Taim correspond to the diagnostic features given by Kepner and Carter (1931), Nuttycombe and Waters (1938), Marcus (1945b) and Noreña et al. (2005b). In addition, our specimens are in the range of the length of individuals described by Marcus (1945b) ($400 - 600 \mu\text{m}$), but are larger than those from USA (Nuttycombe and Waters 1938) ($620 \mu\text{m}$) and Argentina (when two zooids are measured) (Noreña et al. 2005b) ($290 \mu\text{m}$).

Distribution. Site 2, associated with phytal microhabitat.

Previous records. USA (Kepner and Carter 1931; Nuttycombe and Waters 1938), Poland (Kolasa 1973a), Brazil (Marcus 1945b), Suriname (van der Land 1970), and Argentina (Noreña et al. 2005b).

Stenostomum simplex Kepner and Carter, 1931

(Fig. 3E)

Material. Two individuals, whole-mounted and photographed alive.

Description and remarks. Small whitish animals have a fusiform shape. The anterior region has two small ciliated pits connected to the brain lobes. Two lateral light-refracting bodies associated with the posterior brain lobe. The mouth is located in the first third of the body. The intestine has excretophores, which are located laterally along the entire gut. Identification is confirmed based on the habitus of live specimens and general morphology, both corresponding to the description and illustration given by Kepner and Carter (1931), Nuttycombe and Waters (1938) and Marcus (1945b).

Distribution. Site 2, associated with benthal microhabitat.

Previous records. USA (Kepner and Carter 1931; Nuttycombe and Waters 1938) and Brazil (Marcus 1945b).

Stenostomum tuberculosum Nuttycombe and Waters, 1938

(Fig. 3F–G)

Synonyms: *Anokkostenostomum tuberculosum* (Nuttycombe and Waters, 1938)

Material. Two individuals, one whole-mounted and photographed alive.

Description and remarks. Live specimens whitish with three zooids. The body length is $438 \mu\text{m}$ long. The anterior end of the body has a small projection (tubercle) (Fig. 3F–G). The

anterior brain lobe is metameric, while the posterior brain lobes are round-like. The anterior brain lobes are connected to the lateral ciliated pits. Oval mouth, rounded pharynx with two long lateral pharyngeal glands (?) (Fig. 3G). The intestine wall is dentated. This species is easily recognizable owing to the small anterior tubercle located in the frontal region of the cephalic end. Our individuals are shorter than those studied by Nuttycombe and Waters (1938), Marcus (1945b) and Noreña et al. (2005b), but it is almost in the range of individuals found by Luther (1960).

Distribution. Site 2, associated with phytal microhabitat.

Previous recods. Finland (Luther 1960), Poland (Kolasa 1973a), Germany (Lanfranchi and Papi 1978), USA (Nuttycombe and Waters 1938), Suriname (van der Land 1970), Brazil (Marcus 1945b), and Argentina (Noreña et al. 2005b).

Order Macrostomorpha Doe, 1986

Family Macrostomidae Beneden E, 1870

Genus *Macrostomum* Schmidt, 1848

Macrostomum quirritium Beklemishev, 1951

(Fig. 3H–I)

Synonyms: *Macrostomum dongyuanensis* Wang and Sun, 2015

Material. Twenty-two individuals. Six individuals observed alive on squashed preparations. The remaining, whole-mounted stylets were studied (MZUSP PL 2219–2234).

Description and remarks. Body $1671.3 \pm 712 \mu\text{m}$ ($1074.7 - 3088.3 \mu\text{m}$; $n = 6$) long and $383.5 \pm 135 \mu\text{m}$ ($194.1 - 597.7 \mu\text{m}$; $n = 6$) wide (Fig. 3H). Anterior region rounded in shape and the posterior end is spatula-shape. The female reproductive system is composed of ovaries and a female antrum. The male reproductive system presents anterior paired testes, false seminal vesicle, seminal vesicle, prostate vesicle, a penis stylet and a male gonopore. The slightly curved penis stylet, $150.8 \pm 17.7 \mu\text{m}$ ($118.9 - 172.2 \mu\text{m}$; $n = 17$) long, is tube-shaped (Fig. 3I). The shaft narrows from the base, $18.9 \pm 3.8 \mu\text{m}$ ($12.4 - 27.2 \mu\text{m}$; $n = 17$) width, to the distal opening, $4.9 \pm 0.5 \mu\text{m}$ ($4.1 - 6.4 \mu\text{m}$; $n = 17$) width. The distal tip of the stylet ends in a very slight slope (Fig. 3I). Our observations agree with descriptions for *M. quirritium* (Kolasa 1973b; Sun et al. 2015; Adami et al. 2016; Reyes and Brusa 2017).

However, individuals from the ESEC Taim have a longer penis stylet than those from the original description (Kolasa 1973b); the same trait is followed for specimens reported for Peru (Reyes and Brusa 2017) and Argentina (Adami et al. 2016). Also, variations in the size of the penis stylet are observed when comparing the proximal base and the distal opening but are within the range of the species morphology. Although measurements of the penis stylet slightly differ from descriptions given for the Neotropical region (Adami et al. 2016; Reyes and Brusa 2017), the morphology remains the same. Nevertheless, specimens from China are slightly longer (Sun et al. 2015) ($2639 \pm 274.2 \mu\text{m}$) when it comes to comparing body size. In addition, *M. quirritium* was not previously recorded for Brazil, therefore it is registered for the first time for the country and the third time for the Neotropical region. Sun et al. (2015) described the species *Macrostomum dongyuanensis* from China. However, a posterior morphological and molecular analyses demonstrated that *M. dongyuanensis* did not differ

from *M. quiritium* (Xin et al. 2019). Therefore, *M. dongyuanensis* is a junior synonym of *M. quiritium* as Xin et al. (2019) stated.

Distribution. Site 1 and 3, associated with benthal and phytal microhabitats.

Previous records. Widely distributed, the species occurs in Russia, Poland, Switzerland (Schärer et al. 2011), China (Sun et al. 2015; Xin et al. 2019), Argentina (Adami et al. 2016), and Peru (Reyes and Brusa 2017).

Macrostomum tuba Graff, 1882

(Fig. 3J)

Synonyms: *Macrostomum tuba bulbostylum* Ferguson and Jones, 1940

Macrostomum tuba gigas Okugawa, 1930

Macrostomum tuba minuta Luther, 1947

Macrostomum tuba verbekei Marcus and Marcus, 1957

Material. A single individual (MZUSP PL 2235), afterward a whole-mounted stylet was studied.

Description and remarks. *Macrostomum tuba* is a well-known species due to its simple and recognizable penis stylet. The slightly curved shaft is tube-shaped (209.2 μm long) and it narrows from the base (12.2 μm wide) to the distal tip (5.5 μm wide). Moreover, the distal tip has bulb-shaped thickened walls, which is a distinguishable feature from other species of *Macrostomum* (Fig. 3J). Also, the stylet morphology may vary according body size, being straight in smaller animals and slightly curved in larger animals (Reyes and Brusa 2017) as it has seen in other regions of the world (Hyman 1936; Ferguson 1939, 1940; Papi 1951; Du Bois-Reymond Marcus 1957; Young 1976).

Distribution. Site 1, associated with phytal microhabitat.

Previous records. Worldwide distributed (cosmopolitan) (Ferguson 1940; Marcus 1946; Papi 1951; Hyman 1955; Young 1976; Noreña et al. 2003; Gamo and Leal-Zanchet 2004; Wang et al. 2004; Adami et al. 2016; Reyes and Brusa 2017).

Macrostomum n. sp. 1 Reyes and Brusa

(Figs. 4 and 5)

Type series. Four specimens observed *in vivo*, afterward whole mounted. Holotype: one mounted specimen (MZUSP PL 2193). Paratypes: three mounted specimens (MZUSP PL 2194–2196). Also, other specimens were found at site 2 associated with phytal microhabitat.

Type locality. Site 1 (32° 33' 18.07" S; 52° 31' 18.50" W), associated with benthal microhabitat at the ESEC Taim.

Diagnosis. Prostatic vesicle globular or invert “U”-shaped, with globular prostatic glands presenting small refractive corpuscles. Penis stylet “J”-shaped, base with thick walls, gradually becomes thinner until half of the stylet length. Stylet base is 17 μm in length, the shaft gradually narrows ending in a curve in hook-shaped. Penis stylet opening is 3.6 μm long, subterminal, on the convex side of the curvature of the stylet.

Description. Body of living mature individual $1138.9 \pm 185.3 \mu\text{m}$ (957.4 – 1327.8 μm ; n=3) long and $328.9 \pm 104.3 \mu\text{m}$ (211.6 – 411.1 μm ; n=3) wide, greatest width in the mid-length. Body dorsoventrally flattened (adult) (Fig. 4A, 5A). Anterior end rounded, somewhat truncated. Posterior region of body spatula-shaped, narrow. When adhered to the substratum

the posterior end is truncated. Adhesive papillae present. The body is whitish in reflected light (Fig. 4A, D–E, 5A, D). The whole-body surface is covered by the epidermis of $10 \pm 2.8 \mu\text{m}$ ($8.30 - 13.21 \mu\text{m}$; $n = 3$) thick. Epidermis with abundant locomotory cilia. Sensory cilia sparsely distributed (Fig. 5D). Rhabdites are $10.86 \pm 1 \mu\text{m}$ ($9.9 - 11.9 \mu\text{m}$; $n = 15$) in length, rod-shaped. Three-to-ten packed rhabdites located through the body, especially in the dorsal surface. Cerebral ganglia paired at the anterior region of the body, linked by a weak commissure, sometimes is difficult to observe (Fig. 4B, 5A). Two rounded eyes, overlapping to the cerebral ganglia, separated each other by $50.2 \pm 11.8 \mu\text{m}$ ($40 - 63.12 \mu\text{m}$; $n = 3$) (Fig. 4A, D–E, 5A, D). A ciliated mouth that is $67.8 \pm 22.6 \mu\text{m}$ ($42.2 - 85 \mu\text{m}$; $n=3$) long, posterior to the eyes, opening to the pharynx simplex, which is surrounded by several thin pear-shaped pharynx glands (Fig. 4B, 5A). Blind-sac intestine, which extends towards almost the posterior end of the body.

Male reproductive system ventral. Two elongated testes, $287 \pm 50.4 \mu\text{m}$ ($204.7 - 322.4 \mu\text{m}$; $n = 5$) long, are located at the anterior region of the body, with a smooth surface, they are posterior to the pharynx and latero-ventrally to the intestine (Fig. 4A, C, 5A, D). A deferent duct was not observed. Seminal vesicle globular, $38.6 \pm 6.1 \mu\text{m}$ ($31.6 - 43.2 \mu\text{m}$; $n = 3$) in diameter, muscular thick-walled, on the central body axis, posterior to the female antrum (Fig. 4A, E, 5A, C–D). A seminal vesicle, full of spermatozoa, connects through a thick and short intervesicular duct to a smaller muscular prostate vesicle. Prostatic vesicle globular or invert “U”-shaped, $21 \pm 8.2 \mu\text{m}$ ($12 - 27.9 \mu\text{m}$; $n = 3$) in diameter (Fig. 5C). Globular prostatic glands inside the prostate vesicle, presenting small refractive corpuscles without a particular arrangement. Also, digitiform glands in the distal region of the prostate vesicle (Fig. 5C). Stylet total length is $47.3 \pm 9.6 \mu\text{m}$ ($35 - 56.4 \mu\text{m}$; $n = 4$) (“a” *sensu* Schockaert (2014)), whereas the total length from the distal tip of the stylet to the base is $46.7 \pm 9.5 \mu\text{m}$ ($34.9 - 57.5 \mu\text{m}$; $n = 4$). The ratio of the proximal diameter (base of the penis stylet) to the stylet length is 36%. Penis stylet in “J”-shaped, base with thick walls, gradually becomes thinner until almost half of the stylet length (Fig. 4F, 5B–C). Proximally, the stylet has a slightly crenelated base. Stylet base is $17 \pm 3.4 \mu\text{m}$ ($13 - 21.4 \mu\text{m}$; $n = 4$) in length, the shaft gradually narrows ending in a curve in hook-shaped, which presents an angle of $\sim 73.1 \pm 17.8^\circ$ ($50.5 - 91^\circ$; $n = 4$) (*sensu* Ferguson (1940)). Penis stylet opening is $3.6 \pm 0.7 \mu\text{m}$ ($2.8 - 4.4 \mu\text{m}$; $n = 4$) long, subterminal, on the convex side of the curvature of the stylet (Fig. 4F, 5B).

The female reproductive system. Ovaries are $236.1 \pm 17.5 \mu\text{m}$ ($214 - 253.5 \mu\text{m}$; $n = 4$) long, posterior to the testes on both sides of the body (Fig. 4A, C–E, 5A, D). Big light-brown mature oocytes can be observed containing abundant yolk. Female antrum located ventrally to the intestine and surrounded by numerous cement glands. The female antrum is connected to the ventral female gonopore (Fig. 5A).

Taxonomical remarks. As it was stated by Ferguson (1954) and Rieger (1977), the most remarkable feature for species identification within *Macrostomum* is the penis stylet. So, we discuss on the 13 species whose penis stylet resembles the morphology of the male copulatory structures of *Macrostomum* n. sp 1. (Table 1).

The above-mentioned species have a “J”-shaped stylet. However, some species within this group have a false seminal vesicle (*M. axi* Papi, 1959, *M. balticum* Luther, 1947, *M. majesticis* Schmidt and Sopott-Ehlers, 1976, *M. shenda* Wang and Xin, 2019, *M.*

thingithuense Young, 1976), while *Macrostomum* n. sp. 1 lacks this vesicle. Moreover, these species present the total stylet lengths further longer than in *Macrostomum* n. sp. 1. In addition, the lengths of the penis stylet base of *M. majestic* and *M. thingithuense* (Papi 1959; Schmidt and Sopott-Ehlers 1976; Young 1976) are shorter than in *Macrostomum* n. sp. 1.

A comparison of the length from the distal tip of the stylet to the base present notable differences between *M. distinguendum* Papi, 1951 and *M. barigoense* Young, 1976, when compared to *Macrostomum* n. sp. 1. Also, *M. distinguendum*, *M. barigoense* and *M. kepneri* Ferguson and Jones, 1940 present total lengths of the stylet longer than *Macrostomum* n. sp. 1. Also, dissimilarities are observed when comparing the distal penis stylet opening, in *M. distinguendum* (Papi 1951, 1953; Ax 2008), *M. barigoense* (Young 1976) and *M. kepneri* (Ferguson and Jones 1940) the lengths are higher than in *Macrostomum* n. sp. 1.

Macrostomum species 2 Schockaert, 2014 and *M. troubadicis*, Schmidt and Sopott-Ehlers, 1976, both inhabitants of marine environments (Schmidt and Sopott-Ehlers 1976; Schockaert 2014) are also similar to *Macrostomum* n. sp. 1. The distal tip of the stylet of *M. species 2* spirals in a counter-clockwise direction (Schockaert 2014), while the distal tip of *Macrostomum* n. sp. 1 comes from a conical straight stylet ("J"-shaped) and simply bends to a hook-shaped tip. Further, in *M. species 2* the distal stylet opening was not observed (Schockaert 2014), in *Macrostomum* n. sp. 1 it is present on the convex side. As for *M. troubadicis*, it presents a triangular seminal vesicle (Schmidt and Sopott-Ehlers 1976) which is not observed in *Macrostomum* n. sp. 1. Besides, *M. troubadicis* has a slightly "S"-shaped stylet with the base of the stylet of 4-5 µm long (Schmidt and Sopott-Ehlers 1976), much smaller than in *Macrostomum* n. sp. 1. Also, the distal end of the stylet of *M. troubadicis* is truncated, while *Macrostomum* n. sp. 1. has its distal end hook-like.

Macrostomum sileciacum Kolasa, 1973 is different from *Macrostomum* n. sp. 1 due to: the first has a slightly spirally-twisted stylet, a rounded distal tip and the distal stylet opening is on the concave side (Kolasa 1973b); while the new species, lacks these features. Compared with *Macrostomum schmitti* Hayes and Ferguson, 1940, the main difference is observed in the length of the penis stylet opening, being *M. schmitti* bigger than *Macrostomum* n. sp. 1. Moreover, the base of the stylet of *M. schmitti* is highly crenate (Hayes and Ferguson 1940), whereas *Macrostomum* n. sp. 1 is slightly crenelated.

Also, *Macrostomum platensis* Adami, Damborenea and Ronderos, 2012, is similar to the new species, but the distal end of the stylet of *Macrostomum* n. sp. 1 bends to a hook-shaped tip, while the distal end of the stylet of *M. platensis* is beveled (Adami et al. 2012). As so, the morphological differences observed, principally, in the penis stylet of the 13 above-mentioned species indicate that *Macrostomum* n. sp. 1 is a new species within the genus *Macrostomum*.

Distribution. Southern Brazil, Rio Grande do Sul state, Taim strict nature reserve (ESEC Taim).

Order Dalytyphloplanida Willems et al., 2006

Family Typhloplanidae Graff, 1905

Subfamily Mesostominae Hyman, 1955

Genus *Mesostoma* Ehrenberg, 1837

Mesostoma ehrenbergii (Focke, 1836)

(Fig. 6A)

Synonyms: *Planaria ehrenbergii* Focke, 1836

Mesostomum ehrenbergii Örsted 1844

Mesostoma wardii Woodworth, 1896

Material. Eighteen individuals (MZUSP PL 2260), 14 of which, observed in live squashed preparations.

Description and remarks. Body of live individuals are 5.4 ± 2.3 mm (1.6 – 8.7 mm; n = 14) long and 1.5 ± 0.7 mm (0.4 – 2.9 mm; n = 13) wide, flattened and leaf-shaped, yellow-whitish. Round eggs 0.24 ± 0.1 mm (n = 14) in diameter (Fig. 6A). The general appearance and anatomy of our specimens are in accordance with descriptions made for the Neotropical region (Beauchamp 1939; Marcus 1943, 1946; Ponce de León 1985; Noreña-Janssen and Faubel 1992; Gamo and Leal-Zanchet 2004; Noreña et al. 2006) and other biogeographical regions (Fuhrmann 1894; Stout 1953; Luther 1963; Young 2001). However, the body size of our specimens are smaller than those described for the Neotropical region by Beauchamp (1939) (9 mm), Marcus (1946) (15 mm), Noreña-Janssen (1995) (8 – 10 mm) and Gamo and Leal-Zanchet (2004) (3, 4 and 6 mm); but larger than those found in Peru by Noreña et al. (2006) (1-1.5 mm).

Distribution. Site 1 and 2, associated with benthal and phytal microhabitats.

Previous records. Widely distributed (see Tyler et al. 2016 for a complete overview).

Genus *Bothromesostoma* Braun, 1885

Bothromesostoma evelinae Marcus 1946

(Fig. 6B)

Material. A single individual (MZUSP PL 2261) studied alive.

Description and remarks. Body is 3.5 mm long and 0.5 mm wide, cylindrical or fusiform. Pigmentless spots, one pair dorsolateral at the anterior end of the body, connected to the peripheral unpigmented edge, while the dorsal surface has a dark blackish pigmentation (Fig. 6B). The ventral surface has clearer pigmentation than the dorsal surface. Our findings correspond to descriptions given for specimens found in the Neotropical region (Marcus 1946; Noreña-Janssen 1995; Noreña et al. 2006; Ponce de León 1986).

Distribution. Site 2, associated with phytal microhabitats.

Previous records. The species is distributed in Brazil (Marcus 1946), Uruguay (Ponce de León 1986), Argentina (Noreña-Janssen 1995) and Peru (Noreña et al. 2006).

Subfamily Typhloplaninae Luther, 1904

Genus *Strongylostoma* Örsted, 1844

Strongylostoma elongatum Hofsten, 1907

(Fig. 6C–E)

Material. Eight individuals studied alive (MZUSP PL 2262), one of them was whole mounted.

Description and remarks. Body, 1.3 mm long and 0.2 mm wide, fusiform, orange brownish. One pair of eyespots at the anterior region of the body, with rod tracks between them (Fig. 6C, E). Barrel-shaped pharynx situated in the first half of the body, anterior to the

common genital pore. The male reproductive system has a notorious feature that differentiates it from other species of the genus *Strongylostoma*. *S. elongatum* has spines that are only distributed in the distal region of the ejaculatory duct (Fig. 6D–E). Hence, species with spines along all the ejaculatory duct (*S. cirratum* Beklemischew, 1922, *S. dicorymbum* Marcus, 1946 and *S. radiatum* Müller, 1774) and lacking them (*S. simplex* Meixner, 1915 and *S. devleeschouweri* Van Steenkiste et al., 2011) are easily discriminated. Also, our specimens, when compared with *S. coecum* (Sekera 1906) Sekera 1912 (do not present eyes) and *S. gonocephalum* (see Graff 1913) (with oval spots beside and behind of the eyes) are easily differentiated. In conclusion, owing to the notable features observed, our specimens are *S. elongatum*. This is the first record for Brazil and the Neotropical region.

Distribution. Sites 1, 2 and 3, associated with benthal and phythal microhabitats.

Previous records. Widespread throughout the Palearctic and Nearctic region (see Van Steenkiste et al. 2011).

Family Dalyelliidae Graff, 1905

Genus *Gieysztoria* Ruebush and Hayes, 1939

Gieysztoria evelinae (Marcus, 1946) Luther, 1955

(Fig. 7A–C)

Synonyms: *Microdalyellia evelinae* Marcus, 1946

Material. A single individual (MZUSP PL 2236) was captured and observed alive, afterwards whole mounted.

Description and remarks. The body is 0.9 mm long and 0.2 mm wide; anterior end bluntly rounded, caudal end pointed, tail-like shape; brownish-orange pigmentation concentrated especially behind the pharynx. Two black bean-like eyes located at the anterior region of the body. The pharynx is 137.2 μm long and 111.2 μm wide. Two dorsal feathery vitellaria (357.9 μm long) with finger-like prolongations (Fig. 7A). The male reproductive system comprised by two rear lateral testes, seminal vesicle (60.7 μm long, 95.4 μm wide), prostatic vesicle (60.4 μm long, 88.3 μm wide) and a penis stylet (Fig. 7B). The penis stylet is 72.9 μm in total length, with a dorsal opened girdle and distal spines. The girdle is composed by a proximal ring (24.77 μm in height and 47.61 μm in width) united by bridges (5.4 μm in height) to a distal ring (Fig. 7C1). The distal ring bears spines that are composed of two different groups. The first group is composed of four hollow well-developed spines (41.7 μm , $n = 4$, length) with a broad triangular slightly outward-curved base (18.4 μm , $n = 1$, width) (Fig. 7C1, C4). The second group bears five to seven thin fine spines (39.7 μm , $n = 7$, length) with its distal tip slightly curved outward, located interspersed with the first group of spines and are connected by its base to the distal ring (Fig. 7C1–2). The female reproductive system is composed of a saccular ovary (113.7 μm long), a seminal receptacle, uterus and a copulatory bursa. Male and female reproductive systems discharge into the common genital pore which is located at the posterior 1/3 of the body. The above-mentioned features follow descriptions given by Marcus (1946) and Brusa et al. (2008). The body length (1700 μm) of the original description (Marcus 1946) is bigger than specimens from the ESEC Taim. Our specimens are bigger than those found in Argentina (483–583 μm) (Brusa et al. 2008). However, the total length of the stylet of our findings (72.9 μm) is smaller than those from

Argentina (90–100 μm) (Brusa et al. 2008) and São Paulo (90–100 μm) (Marcus 1946). The same trait is observed when comparing the length of distal spines, which are 50–60 μm and 60 μm for Argentinian and Brazilian specimens, respectively (Brusa et al. 2008; Marcus 1946). Although stylet dimensions are smaller than those previously described, the stylet configuration remains the same.

Distribution. Sites 3, associated with phytal microhabitat.

Previous records. The species was previously recorded from Brazil (São Paulo, Corôa lagoon) (Marcus 1946) and Argentina (Buenos Aires province, littoral of the Río de la Plata: Atalaya and La Matilde stream) (Brusa et al. 2008).

Gieysztorina falx Brusa, Damborenea and Noreña, 2003

Comments. Descriptions and remarks were made in Reyes et al. (2019).

Gieysztorina hymanae (Marcus, 1946) Luther, 1955

(Fig. 7D–F)

Synonyms: *Microdalyellia hymanae* Marcus, 1946

Material. Seven individuals (MZUSP PL 2237–2243), four of them observed alive. The remaining were whole mounted in order to study the penis stylet.

Description and remarks. Body $912.5 \pm 92.9 \mu\text{m}$ ($798.3 - 1025.6 \mu\text{m}$; $n = 4$) long. Penis stylet $49.9 \pm 8.1 \mu\text{m}$ ($43.9 - 66.8 \mu\text{m}$; $n = 7$) long. The habitus and anatomical configuration of specimens found in the ESEC Taim (Fig. 7D–E) are in accordance with original descriptions given by Marcus (1946), as well as those given by Brusa et al. (2008). Nevertheless, our individuals are larger than those found previously in Brazil (600 – 800 μm) (Marcus, 1946) and Argentina (250 – 290 μm) (Brusa et al. 2008). However, when comparing the length of the penis stylet (Fig. 7F), our specimens are smaller than specimens of the original description from Brazil and the specimens from Argentina, both with 60 μm in length (Marcus 1946; Brusa et al. 2008). The most resembling species of *G. hymanae* is *G. evelinae*, but *G. hymanae* differs from *G. evelinae* due to the latter has four robust hollow spines while *G. hymanae* has four hollow spines with no robustness. Another feature not observed in whole-mounted individuals was the proximal girdle, this structure may disappear when mounted in Hoyer's medium, but it was observed in live animals.

Distribution. Sites 3, associated with phytal microhabitat.

Previous records. The species was previously recorded only in Brazil (São Paulo, river Pirajussará) (Marcus 1946) and Argentina (Buenos Aires province, littoral of the Río de la Plata: Atalaya stream) (Brusa et al. 2008).

Gieysztorina n. sp. 1 Reyes and Brusa

(Figs. 8, 9 and 10)

Type series. Twenty specimens observed *in vivo*, afterward whole mounted. Holotype: one mounted specimen (MZUSP PL 2210). Paratypes: fifteen mounted specimens (MZUSP PL 2197–2209, 2211, 2212). Also, individuals were collected from site 2 associated with phytal microhabitats.

Type locality. Site 3, associated with phytal microhabitats at the ESEC Taim.

Diagnosis. Crown-shaped stylet $43.1\ \mu\text{m}$ long, comprises a fibrous-like girdle with a distal reinforced ring from which 12–18 spines of different lengths and widths arise. The girdle is $14.3\ \mu\text{m}$ in height and $27.7\ \mu\text{m}$ in width. Spines gradually get longer along the rim of the girdle until the opposite region of the girdle opening. In this area, spines are the longest and the widest.

Description. Body $791.2 \pm 101.8\ \mu\text{m}$ ($650.9 - 945.3\ \mu\text{m}$; $n = 11$) long and $157.4 \pm 36.75\ \mu\text{m}$ ($120 - 257.6\ \mu\text{m}$; $n = 11$) wide, rounded form (flattened whole-mounted) to elliptical; anterior region with a rounded end and caudal region tapering caudally abruptly, composed by a tail-like with 5–6 finger-like adhesive papillae (Fig. 10A–B). Body orange brown-darkish pigmentation (live adults), it can be seen spotted along the body or dispersed, but some spots are especially concentrated behind the pharynx (Fig. 8A–C). Also, brown narrow longitudinal stripes along the body are seen in the biggest animals (Fig. 8D). Ciliated epidermal cells are polygonal in shape; rigid cilia not observed. Rhabdites are arranged in groups of 3–6 over the body surface. The brain is comprised of two globular ganglia from which lateral nerves extend towards the posterior region of the body, situated ventrally to the eyes. Black bean-shaped eyes located dorsally at the anterior region of the body; they are separated from each other by $44.5 \pm 9.7\ \mu\text{m}$ ($31.2 - 58.4\ \mu\text{m}$; $n = 11$) (Fig. 8A–C, 10A). Mouth ventral, subterminal. Doliiformis pharynx (see Luther 1955), $140.7 \pm 19.3\ \mu\text{m}$ ($108.2 - 170.6\ \mu\text{m}$; $n = 11$) long and $103.7 \pm 9\ \mu\text{m}$ ($85.5 - 117.1\ \mu\text{m}$; $n = 11$) wide (Fig. 8A–C, 10A).

Male reproductive system posterior in the half body region and it is composed of testes, deferent ducts, seminal vesicle, prostatic vesicle, and penis stylet. Paired testes, $183 \pm 50.4\ \mu\text{m}$ ($127.5 - 280.5\ \mu\text{m}$; $n = 9$) long and $28.3 \pm 7.6\ \mu\text{m}$ ($21.6 - 40.4\ \mu\text{m}$; $n = 7$) wide, located at the posterior 1/3 of the body, at both sides of the body (Fig. 8A, B, 10A). Deferent ducts pair, thin and short, from the lateral-rostral region of the testes until connecting one in each side of the seminal vesicle (Fig. 8E, 10A–B). The globular seminal vesicle is $40.3 \pm 5.5\ \mu\text{m}$ ($34.7 - 47.8\ \mu\text{m}$; $n = 4$) long and $47.5 \pm 5.2\ \mu\text{m}$ ($41.3 - 53.3\ \mu\text{m}$; $n = 4$) wide. Prostatic vesicle is $31.8 \pm 1.9\ \mu\text{m}$ ($30 - 34.14\ \mu\text{m}$; $n = 4$) long and $49.6 \pm 6.4\ \mu\text{m}$ ($43.2 - 58.2\ \mu\text{m}$; $n = 4$) wide, globular with thick-walls, (Fig. 8A, E, 10A–B); saccular glands present, associated to the proximal region. Also, the prostatic vesicle is more voluminous than the seminal vesicle in mature animals (Fig. 8E, 10B). Distally, the prostatic vesicle is continued by a sclerotic penis stylet. The crown-shaped stylet ($43.1 \pm 3\ \mu\text{m}$ ($37.6 - 47.7\ \mu\text{m}$; $n = 14$) long) comprises a well-defined fibrous-like girdle that has a distal reinforced ring from which 12–18 spines of different lengths and widths arise (Fig. 9B). The dorsally-opened girdle is $14.3 \pm 2.2\ \mu\text{m}$ ($11 - 18.71\ \mu\text{m}$; $n = 14$) in height and $27.7 \pm 3\ \mu\text{m}$ ($20.8 - 331.8\ \mu\text{m}$; $n = 16$) in width. Also, the girdle is $56.8 \pm 5.3\ \mu\text{m}$ ($54 - 64.67\ \mu\text{m}$; $n = 4$) in circumference. In addition, the girdle is higher where the longest spines arise and gradually reduces in height as the spines shorten (Fig. 10C–D). Spines are fang-shaped with a long and broad funnel-shaped base, from caudal-transverse view, spines take the form from rectangular to quadrangular shape (Fig. 9A). The two smaller spines measure: $13.9 \pm 3\ \mu\text{m}$ ($8.6 - 19\ \mu\text{m}$; $n = 15$) long and $4.3 \pm 1.3\ \mu\text{m}$ ($2.3 - 6.3\ \mu\text{m}$; $n = 12$) wide; $19 \pm 3.8\ \mu\text{m}$ ($13.3 - 23.4\ \mu\text{m}$; $n = 8$) long and $5.5 \pm 2.7\ \mu\text{m}$ ($3.2 - 9.8\ \mu\text{m}$; $n = 8$) wide. Spines gradually get longer along the rim of the girdle until the opposite region of the girdle opening. In this area, spines are the longest ($27.3 \pm 4\ \mu\text{m}$ ($15.3 - 31.9\ \mu\text{m}$;

n = 15)) and the widest ($5 \pm 1.4 \mu\text{m}$ ($3.7 - 9.1 \mu\text{m}$; n = 13)) (Fig. 9A–B, 10C–D). The width of the spines was measured from its base.

Ovary single, saccular, in the left posterior 1/3 region of the body, posterior to the intestine (Fig. 8A, E, 10A–B), followed by a narrow oviduct associated to the seminal receptacle. The seminal receptacle connects to the uterus by the female duct. The uterus may contain one egg. Egg $166 \pm 5.2 \mu\text{m}$ ($162.2 - 171.9 \mu\text{m}$; n = 3) long and $112 \pm 5.5 \mu\text{m}$ ($108.3 - 118.3 \mu\text{m}$; n = 3) wide, oval, brown-yellowish (Fig. 10B). Copulatory bursa has thick walls, united to the uterus by distal region, both discharge on the common genital atrium which leads to the gonopore (Fig. 8E, 10A–B). Gonopore approximately on the 1/3 of the body length. Vitellaria $331.4 \pm 75.3 \mu\text{m}$ ($214.5 - 408 \mu\text{m}$; n = 6) long, paired (smooth), dark greenish; filling in dorsoventrally the region comprising from the posterior end of the pharynx toward the ovary, where it forms a “V”-shaped common duct (Fig. 8B, 10A–B).

Taxonomical remarks. Within *Gieysztoria*, species are grouped as Aequales and Inaequales following Luther's (1955) criteria. The first group has spines with similar shapes and sizes, while the latter, has spines with different sizes and shapes. Further, Inaequales is subdivided into Falcatae (with a large, robust falcate stylet spine, and a variable number of plate-like spines), Fenestratae (girdle with one or more openings in the girdle), Radiatae (stylet with symmetrical morphology) and Aberrantes (stylet with an aberrant configuration) (Luther 1955; Van Steenkiste et al. 2012). *Gieysztoria* n. sp. 1 belongs to the Inaequales, subgroup Aberrantes. 15 species resemble *Gieysztoria* n. sp. 1, namely: *G. faubeli* Artois et al., 2004, *G. foreli* (Hofsten, 1911), *G. macrovariata* (Weise, 1942), *G. macrovariata 9-spinosa* Luther, 1995, *G. octospinosa* Luther, 1995, *G. pseudodiadema* Noreña-Janssen, 1995, *G. pulcra* Wang and Deng, 2006, *G. qixingyanensis* Wang and Rong 2016, *G. quadrata* Noreña-Janssen, 1995, *G. reggae* Therriault and Kolasa, 1999, *G. santafeensis* Noreña-Janssen, 1995, *G. shantouensis* Zhang, Li and Wang, 2014, *G. shenzhensis* Wang and Wu, 2005, *G. tigrensis* Noreña-Janssen, 1995 and *G. uncia* (Marcus, 1946). The stylet lengths of *G. pseudodiadema*, *G. quadrata*, *G. santafeensis* and *G. tigrensis* were taken from figures in the respective descriptions, while the rest of the stylet lengths were obtained from the measurement mentioned by the respective author of each species. However, it is possible to differentiate *Gieysztoria* n. sp. 1 from other Aberrantes species due to several features. *Gieysztoria foreli* and *G. reggae* present in the girdle a distal ring joined with several bridges and spines of similar shape or size, 15–22 and 8–12, respectively (Luther 1955; Therriault and Kolasa 1999; Lu et al. 2013). *Gieysztoria tigrensis*, *G. shenzhensis* and *G. santafeensis* have bigger stylet lengths ($363.6 \mu\text{m}$, $95 \mu\text{m}$, $130.5 \mu\text{m}$ respectively); moreover, they have spines with remarkable differences in length and configuration (Noreña-Janssen 1995; Wang and Wu 2005). Contrary, *Gieysztoria* n. sp. 1 has a small stylet ($43.1 \mu\text{m}$ long) with 12–18 fang-like spines of variable lengths. Stylet lengths of *G. shantouensis* ($137 \mu\text{m}$), *G. quadrata* ($88 \mu\text{m}$), *G. macrovariata* ($102.5 - 139.5 \mu\text{m}$), *G. macrovariata 9-spinosa* ($118 - 131 \mu\text{m}$), *G. faubeli* ($73 \mu\text{m}$) and *G. pulcra* ($48 \mu\text{m}$) are therefore bigger than *Gieysztoria* n. sp. 1 ($43.1 \mu\text{m}$). In addition, these species have two long lateral spines on the edges of the girdle, while *Gieysztoria* n. sp. 1 has two smalls (but different in size) spines on the edges of the girdle. The total number of spines of *Gieysztoria* n. sp. 1 are 12–18, notably differentiated from *G. shantouensis*, *G. quadrata*, *G. macrovariata*, *G. macrovariata 9-spinosa*, *G. faubeli* and *G.*

pulcra with 7, 12, 10, 9, 16, 13, respectively (Artois et al. 2004; Luther 1955; Noreña-Janssen 1995; Wang and Deng 2006; Zhang et al. 2014). The stylet length of *Gieysztoria* n. sp. 1 (43.1 μm) is different from *G. octospinosa* (55 μm) but similar to *G. pseudodiadema* (43.8 μm) (Luther 1955; Noreña-Janssen 1995). However, *G. octospinosa* has eight spines, being the central ones larger than the rest; *G. pseudodiadema* has seven empty spines, spines at the left side are well-developed, while those at the right side are reduced (Luther 1955; Noreña-Janssen 1995). *G. octospinosa* and *G. pseudodiadema* differ from *Gieysztoria* n. sp. 1 owing to number of spines and stylet configuration. *Gieysztoria qixingyanensis* has a 54 μm -long stylet with a proximal handle-shape girdle, while *Gieysztoria* n. sp. 1 has a 43.1 μm -long stylet with a well-defined fibrous-like girdle. The girdle of *G. qixingyanensis* bears 16 interspersed spines of variable lengths and shapes (Rong et al. 2016), while the girdle of *Gieysztoria* n. sp. 1 bears 12-18 wide fang-shaped spines. The most similar species to *Gieysztoria* n. sp. 1 is *G. uncia*. The latter species has a small stylet (27 μm long). Also, from the right side of the distal rim of the girdle, four larger spines (18 μm long) are originated; from the left side, a big number of small spines (7-12 μm long) are present (they appear as an aggregated palisade) (Marcus 1946). However, the new species has a 43.1 μm -long stylet with 12-18 fang-like spines originated from the rim. Also, two small spines (one a bit longer than the other) arise from the edges of the girdle of *Gieysztoria* n. sp. 1 and, gradually, get longer until the ventral region where those reach their maximum length (27.3 μm) and width (5 μm). The above-mentioned differences, mainly on the penis stylet morphology, indicate that individuals found in the ESEC Taim represent a new species within *Gieysztoria*.

Distribution. Southern Brazil, Rio Grande do Sul state, Taim strict nature reserve (ESEC Taim).

Gieysztoria n. sp. 2 Reyes and Brusa (Figs. 11 and 12)

Type series. Eight specimens observed *in vivo*, afterward whole mounted. Holotype: one mounted specimen (MZUSP PL 2213). Paratypes: five mounted specimens (MZUSP PL 2214–2218).

Type locality. Site 1, associated with benthal microhabitats at the ESEC Taim.

Diagnosis. Stylet with a dorsally-opened fibrous girdle. The girdle has five different groups of spines: two groups of rows of spines (*a* and *b*) arising from the dorsal edges of the girdle, and three ventral spines. Spines from group *a* are a dorsal protrusion of the girdle, which bears several rows (toothbrush-like) of spines. Proximal spines are longer and have broader hollow bases, these spines gradually get shorter and thinner as spines reach its distal end. Spines from group *b* are a dorsal protrusion, distally, has several rows with a great number of very thin and short spines. Within the three ventral spines, the central one is the largest and strongest. The remaining flanking spines are shorter, but different in length.

Description. Body $798.6 \pm 164.4 \mu\text{m}$ (509.5 – 923 μm ; $n = 5$) long and $225.2 \pm 55.2 \mu\text{m}$ (164.4 – 304.6 μm ; $n = 5$) wide; habitus typical of the genus, rounded anterior end, widening towards the middle region of the body, elongated and narrowed at the end where it bears 5-6 finger-like adhesive papillae (Fig. 11C–D). Orange-brown pigment *in vivo*, randomly dispersed all over the body (Fig. 11A–C). Body surface with several rhabdites and ciliated

epidermal cells, rigid cilia not distinguished. Eyes, one pair, black kidney-like, in the anterolateral region, separated by $55.8 \pm 4.35 \mu\text{m}$ ($50.5 - 61 \mu\text{m}$; $n = 4$) (Fig. 11A, D). Brain under the eyes. Mouth subterminal, ventral at the anterior region of the body. Mouth followed by a dolliform pharynx, $162.8 \pm 18.4 \mu\text{m}$ ($137.7 - 182 \mu\text{m}$; $n = 4$) long and $125.6 \pm 12.6 \mu\text{m}$ ($112.2 - 140.2 \mu\text{m}$; $n = 4$) wide (Fig. 11A, C–D).

Ellipsoidal testes are $215.7 \pm 50.7 \mu\text{m}$ ($144.7 - 317.2 \mu\text{m}$; $n = 10$) long and $37.5 \pm 12.6 \mu\text{m}$ ($26.4 - 63.1 \mu\text{m}$; $n = 8$) wide, paired laterally. Deferent duct thin, paired, and extend from the anterior region of the testes, connecting these to the seminal vesicle (Fig. 11B, E). Seminal vesicle, $33 \pm 10.5 \mu\text{m}$ ($22 - 42.6 \mu\text{m}$; $n = 4$) long and $41.5 \pm 17.7 \mu\text{m}$ ($25.1 - 59.3 \mu\text{m}$; $n = 4$) wide, globular-shaped; circular notch in the posterior central region (Fig. 11B, E), distally connected to a prostatic vesicle. Prostatic vesicle $30.4 \pm 9.7 \mu\text{m}$ ($21.9 - 39 \mu\text{m}$; $n = 4$) long and $36.3 \pm 13.7 \mu\text{m}$ ($22.8 - 50.5 \mu\text{m}$; $n = 4$) wide, globular, with lateral external glands associated; distally is continued by a sclerotic penis stylet (Fig. 11B, E). Stylet is $34.2 \pm 3.5 \mu\text{m}$ ($29.1 - 37.6 \mu\text{m}$; $n = 6$) in axial length; is composed, proximally, by a dorsally-opened fibrous girdle, $24.8 \pm 7.3 \mu\text{m}$ ($19.4 - 39 \mu\text{m}$; $n = 6$) in width and $11.5 \pm 2.1 \mu\text{m}$ ($9.1 - 14.7 \mu\text{m}$; $n = 6$) in height, may present an oval fenestra associated to the strongest distal spine. Distally, the girdle presents five different groups of spines: two groups of rows of spines (*a* and *b*) arising from the dorsal edges of the girdle and three spines (blue, red and green colored spines) (Fig. 12C) arising from the ventral side (Fig. 12A–C). Spines from the group *a*, comprise a dorsal protrusion of the girdle, $26.7 \pm 2 \mu\text{m}$ ($23.3 - 28.3 \mu\text{m}$; $n = 6$) long, which bears several rows (toothbrush-like) of spines. Proximal spines are longer ($10.8 \pm 1.6 \mu\text{m}$ ($8.6 - 13 \mu\text{m}$; $n = 6$)) and have broader hollow bases, these spines gradually get shorter and thinner as spines reach its distal end (shortest spines are $3.7 \pm 0.8 \mu\text{m}$ ($2.4 - 4.4 \mu\text{m}$; $n = 6$)) (Fig. 12C: *a*). Spines from group *b*, comprise a dorsal protrusion which, distally, has several rows with a great number of very thin and short spines. These spines gradually align and lengthen towards its proximal area where they form a comb-like structure (Fig. 12C: *b*). A fang-shaped spine (colored in red) is $20.7 \pm 1.7 \mu\text{m}$ ($18.5 - 23.3 \mu\text{m}$; $n = 6$) long, is curved, with a rounded distal tip outward. This is the largest and strongest spine, at the ventral median side of the girdle. Proximally, this spine has a large base ($6.8 \pm 0.9 \mu\text{m}$ ($5.2 - 7.9 \mu\text{m}$; $n = 6$) wide) for muscular insertion (Fig. 12C: red). The other fang-shaped spine (colored in blue) is $16.8 \pm 1.5 \mu\text{m}$ ($15 - 19.1 \mu\text{m}$; $n = 6$) long, is slightly curved outwards, and is located between red spine and spines from the group *a*. Proximally, it presents a broad base for muscular insertion. Distally, it has a blunt tip. Also, a blue spine is associated with spines from the group *a* (Fig. 12C: blue). Spine (colored in green) is $11.9 \pm 1.2 \mu\text{m}$ ($10.4 - 13.5 \mu\text{m}$; $n = 6$) long, blade-like, between red spine and spines from group *b*. Besides, the green spine is very close to spines from group *b* (Fig. 12C: green).

The ovary is $127.3 \mu\text{m}$ long, single sac-like, at the right side of the body, posteriorly to the intestine and continued by the oviduct (Fig. 11B, E). Copulatory bursa thick-walled, releasing the contents to the common genital atrium. Seminal receptacle with sperm. Gonopore at the posterior 1/3 of the body. Vitellaria are $303.7 \pm 613 \mu\text{m}$ ($210.7 - 371.8 \mu\text{m}$; $n = 8$) long, paired, smooth dark-greenish, from the posterior side of the pharynx to the end of the intestine, where they join forming a common duct (Fig. 11C–D).

Taxonomical remarks. Because of its stylet morphology, *Gieysztoria* n. sp. 2 belongs to the Inaequales group, subgroup Aberrantes (Luther 1955). Eight species with similar stylet configuration (aberrant morphology) resemble to the new species, namely, *G. atalaya* Brusa et al., 2008, *G. complicata* (Fuhrmann, 1914), *G. falx* Brusa et al., 2003, *G. intricata* (Marcus, 1946), *G. kasasapa* Damborenea et al., 2005, *G. matilde* Brusa et al., 2008, *G. namuncurai* Damborenea et al., 2007, *G. therapaina* (Marcus, 1946). However, the features of these species are different from individuals of the new species. *Gieysztoria atalaya* has a stylet which is twice as long (62 μm) to that of *Gieysztoria* n. sp. 2 (34.2 μm). Moreover, *G. atalaya* possesses a 23 μm -long solitary spine (a) located between the two groups (b and c) of complex spines (a, b and c *sensu* Brusa et al. (2008)). While *Gieysztoria* n. sp. 2 has three ventral spines, being the longest 20.7 μm . In addition, complexes of spines protruding from the girdle in *Gieysztoria* n. sp. 2 are different from *G. atalaya* in number and spine configuration (Brusa et al. 2008). *Gieysztoria falx* is differentiated from *Gieysztoria* n. sp. 2 due to the former species has two strong pincer-like spines, while the latter has three spines, spine configuration from the protrusion of the girdle are even dissimilar. Also, the stylet of *G. falx* is larger than *Gieysztoria* n. sp. 2 (85-88, 78.9 μm , and 34.2 μm respectively) (Brusa et al. 2003; Reyes et al. 2019). The stylet of *G. complicata* and *G. intricata* are larger than *Gieysztoria* n. sp. 2 (100 μm , 120 μm and 34.2 μm respectively) (Luther 1955; Marcus 1946). Moreover, *G. complicata* has two strong parallel curved spines outwards (a), a convergent similar-in-shape crown of spines (b) and a "brush" conformed by spines (c) (proximal spines longer and broader than distal ones) (a, b and c *sensu* Marcus (1946)). Also, *G. intricata* has a single strong very-curved spine inwards with a sharp tip, this spine also bears "cuticular hairs" (a). Group complexes of spines in *G. intricata* are convergent with different lengths and orientations (b), palisade-like spines (c), and a spine associated to the palisade-like structure (d) (a, b, c and d *sensu* Marcus (1946)). Those structures are not present in *Gieysztoria* n. sp. 2 and they are, different. *Gieysztoria kasasapa* has a 75-85 μm long stylet with two groups of complexes of spines separated by a robust sclerotic arc. The first group is composed of four-convergent rows of spines, each row with 6 to 10 spines. The second group is composed of five straight and hollow spines (18-24 μm long) associated with a long (44 μm) hollow spine orientated outwards (Damborenea et al. 2005). *Gieysztoria* n. sp. 2 has no arc that separates the two-complex group of spines, three distal spines are observed instead of the longest one of *G. kasasapa*. In addition, spines originated from the protrusion of *Gieysztoria* n. sp. 2 have a different configuration than those of *G. kasasapa*. The 54-65 μm -long stylet of *G. matilde* has a unique hollow blade-like spine (25 μm long) (Brusa et al. 2008), while *Gieysztoria* n. sp. 2 shows three spines of different lengths and shapes, being the longest 20.7 μm . Moreover, *G. matilde* bears two groups of complexes of spines, each group with several rows of hollow and acicular spines (Brusa et al. 2008); instead of several rows of spines in a toothbrush-like structure or comb-like arranged spines seen in *Gieysztoria* n. sp. 2. *Gieysztoria namuncurai* has a 100 μm -long stylet with two strong curved saber-like spines (85 μm and 57 μm , respectively) oriented outwards (Damborenea et al. 2007), while *Gieysztoria* n. sp. 2 has a shorter stylet and bears three smaller spines (20.7, 16.8 and 11.9 μm , respectively). In addition, *G. namuncurai* bears three different groups of spine complexes with different

widths, lengths and orders. *Gieysztoria* n. sp. 2 has only two groups of spine complexes, being spines from the group *a* notoriously different with its toothbrush-like structure.

The most similar species to *Gieysztoria* n. sp. 2 is *G. therapaina*. This species has a 33 μm -long stylet with a discontinued girdle. Distally to the girdle, *G. therapaina*, has four groups of spines (Marcus 1946). Contrary, *Gieysztoria* n. sp. 2 has a fibrous continued girdle and bears two groups of complexes of spines and three different-in-length ventral spines. These differences are remarkable when comparing the single strong spine (20 μm long) which is curved in *G. therapaina* (Marcus 1946). The new species has, instead, three ventral spines, being the central one (red) the largest (20.7 μm) and strongest (6.8 μm width). Also, *Gieysztoria* n. sp. 2 lacks the projection originating at the distal part of the girdle that has several small spines on its apical portion as it is seen in *G. therapaina* (the “pente pedunculado” *sensu* Marcus (1946)). Instead, the new species has a long protrusion which bears several rows (toothbrush-like) of spines; their proximal hollow spines have longer and broader dimensions, these spines gradually get shorter and thinner as spines reach its distal end. Besides, *G. therapaina* shows thin spines arranged in a single row (c and d *sensu* Marcus (1946)), whereas in *Gieysztoria* n. sp. 2 the distal protrusion of the girdle (spines from group *b*) has several rows with a great number of very thin and very short spines. These spines gradually align and lengthen forming a comb-like structure. Therefore, the noteworthy features of specimens from the ESEC Taim allow us to propose that they are a new species within *Gieysztoria*.

Distribution. Southern Brazil, Rio Grande do Sul state, Taim strict nature reserve (ESEC Taim).

Order Kalyptorhynchia Graff, 1905

Family Polycystididae Graff, 1905

Subfamily Gyratricinae Graff, 1905

Genus *Gyratrix* Ehrenberg, 1831

Gyratrix hermaphroditus Ehrenberg, 1831

(Fig. 13A–C)

Synonyms: *Gyratrix hermaphroditicus* Luther, 1918

Gyrator banaticus (Graff, 1875)

Gyrator furiosum (Schmidt, 1857)

Gyrator hermaphroditus Ehrenberg, 1835

Prostomum banaticum Graff, 1875

Prostomum furiosum Schmidt, 1858

Material. Thirty-three individuals were studied alive, eleven were whole mounted (MZUSP PL 2244–2254).

Description and remarks. Body elongated and whitish, 1.7 mm long and 0.3 mm wide. The anatomical configuration of animals is following descriptions of the species made by Marcus (1945b), Karling and Schockaert (1977), Artois and Tessens (2008), Rundell and Leander (2014) and Van Steenkiste and Leander (2018) (Fig. 13A–B). Dimensions on prostate stylet III (stalk: $96.2 \pm 16 \mu\text{m}$ (81.1 – 127.7 μm ; $n = 11$)) and stylet sheath: $40.6 \pm 14 \mu\text{m}$ (27.5 – 66.4 μm ; $n = 11$)) and prostate stylet type II (penis stylet: $130.5 \pm 23.1 \mu\text{m}$ (109.7 – 178.4 μm ; $n = 11$)) are within the ranges of individuals found around the globe (Karling and

Schockaert 1977; Jouk and De Vocht 1989; Artois et al. 2000; Artois and Tessens 2008; Rundell and Leander 2014; Van Steenkiste and Leander 2018) (Fig. 13C). However, dimensions and configurations on prostate stylets and the female reproductive system vary notably within populations. This fact has been observed, suggesting complex species, a hypothesis which has not been formally tested.

Distribution. Sites 1, 2 and 3, associated with benthal and phytal microhabitats.

Previous records. Widely distributed in freshwater and marine habitats (see Tyler et al. 2016 for a complete overview).

Order Prorhynchida Laumer and Giribet, 2014

Family Prorhynchidae Hallez, 1894

Genus Prorhynchus Schultze, 1851

Prorhynchus stagnalis Schultze, 1851

Synonyms: *Opistomum serpentina* Johnston, 1865

Prorhynchus fluviatilis Leydig, 1854

Prorhynchus rivularis Fedschenko, 1872

Prorhynchus serpentinus Leuckart, 1859

Prorhynchus tenuis Girard, 1894

Planaria serpentine Dalyell, 1853

Material. Three individuals were studied alive, two of them were whole mounted (MZUSP PL 2255–2256).

Description and remarks. Body 5.1 mm long (n = 2) and 0.5 mm wide (n=2), whitish. Intestinal region dark. Stylet nail-shaped in the anterior region of the body surrounded concentrically by six bars which forms an inner structure and for ten larger bars forming an outer structure. The morphological features are according to Steinböck (1927), Fariás et al. (1995) and Marcus (1944). However, this species is considered a complex species owing to its wide distribution and the wide range of variation of stylet dimensions registered worldwide (Tyler et al. 2018 and references therein).

Distribution. Sites 2 and 3, associated with benthal and phytal microhabitats.

Previous records. Cosmopolitan (see Tyler et al. 2016).

DISCUSSION

FAUNISTIC REMARKS

The southern region of Brazil has a lot of wetlands (Maltchik et al. 2003) that harbours a great diversity of native and foreign animals (some considered at some degree of threat) (MMAB 2008; Ramsar 2017). This region has a number of records on freshwater microturbellarians (Braccini et al. 2016). In this sense, these wetlands, and, in particular the ESEC Taim protected area, are appropriate environments for microturbellarians. However, this group of soft-bodied animals presents some difficulties to be properly studied: (1) they need to be collected alive and store alive with water from the medium to carry to the laboratory; (2) it is preferable to study animals alive in order to observed and determine morphological structures as a diagnostic species features, otherwise the low taxonomic resolution will be obtained; and (3) the use of delicate laboratory equipment (microscopes)

can hardly be performed in the field (Braccini et al. 2016; Balsamo et al. 2020). These are the main reasons why they are usually left out of fauna monitoring in aquatic environments. This is especially true when it comes to evaluate invertebrate fauna at the ESEC Taim protected area. Nonetheless, we studied live animals as it is recommended (Brusa et al. 2003; Braccini et al. 2017), which allowed us to make a proper taxonomic identification of each taxa and defining a base list of microturbellarians records for the protected area.

Microturbellarian species reported here play important roles in freshwater ecosystems. In this way, species of *Mesostoma* could prey on mosquito larvae, chironomid larvae and microcrustaceans, thus altering zooplankton community structure (Blaustein and Dumont 1990; Young 2001; Tranchida et al. 2009). Also, species of *Stenostomum* could influence the structure of cladoceran communities (Nandini and Sarma 2013). Similarly, species of *Macrostomum* and *Gieysztoria* feed on rotifers, other microturbellarians, diatoms, small oligochaetes and small cladocerans (Young 2001; Reyes and Brusa 2017). Therefore, the taxonomic identification of microturbellarians in this protected area establishes the basis for understanding and deepening research on the ecological processes among microturbellarian species and their interaction with other groups of animals.

The new species reported here were described mainly on features of the penis stylet. In this regard, the stylet configuration could bring information about the type of mating behaviour of species of *Macrostomum* (Schärer et al. 2011). This is, species of *Macrostomum* that present an hypodermic mating syndrome possess the following traits: (1) a more or less "J"-shaped stylet with a sharpened bevel-like distal end and a subterminal distal opening; (2) a simple, small aflagellate sperm without bristles; and (3) a simple (without a thickened epithelium) female antrum (Schärer et al. 2011). *Macrostomum* n. sp. 1 presents a stylet with the characteristics mentioned by Schärer et al. (2011), but we could not be able to observe sperm morphology. Also, no details on the epithelium of the female antrum were observed. Thus, according to stylet configuration, it is suggested that *Macrostomum* n. sp. 1 could have a hypodermic mating syndrome. Nonetheless, more observations on the species are needed in order to confirm this hypothesis. There are few studies on the morphology of the stylet and mating behaviour of species of *Gieysztoria*. However, Damborenea et al. (2007) inferred that the robust central spines stiffen the penis papilla and help the passage of sperm during copulation. Besides, the fine spines on the crown would help to keep the gonopore opened during intercourse (Damborenea et al. 2007). These assumptions could explain the functioning of the complex stylet configuration of *Gieysztoria* n. sp. 1 and *Gieysztoria* n. sp. 2.

COMMENTS ON CONSERVATION

Intensive rice farming, livestock and silviculture around the reserve use a variety of substances such as fertilizers and pesticides (Villanueva et al. 2000) which may contain heavy metals and organic contaminants from several chemical classes in their composition. These toxic substances are spread through water flows into the wetland influencing the normal development of the resident fauna and thus the ecosystem balance in the reserve (Josende et al. 2015; Quintela et al. 2019). Therefore, baseline studies on the diversity of microturbellarians are highly recommended not only for understanding the ecological role

that these organisms play in the protected area, but for the increment of formally known species present there. Therefore, taxonomical and ecological knowledge brings a valuable contribution to determine potential deleterious consequences caused by anthropogenic activities that take place in the limits of the ESEC Taim.

CONCLUSIONS

Our study is the first report on microturbellarians from southern Brazilian protected wetlands (ESEC Taim). These areas harbour three new species to science and 23 species reported at the ESEC Taim. As so, the new species *Macrostomum* n. sp. 1 differs from their congeners for its distinct stylet in “J”-shaped, with a presumably hypodermic mating syndrome. In addition, two new species (*Gieysztoria* n. sp. 1 and *Gieysztoria* n. sp. 2) present a remarkable complex penis stylet configuration that resembles a crown in an irregular fashion. Furthermore, we recorded three species for the first time in Brazil (*Myostenostomum vanderlandi*, *Macrostomum quirritium* and *Strongylostoma elongatum*). Consequently, we rise the number of registered species of microturbellarians to 115 in Brazil. The documentation of freshwater microturbellarians for this protected area increase the value as a natural reserve by adding information on soft-bodied animals generally disregarded on environmental monitoring. As such, the knowledge of free-living Platyhelminthes reinforces protection actions on the ESEC Taim.

LIST OF ABBREVIATIONS

Anterior brain lobe: **ab**; Brain: **b**; Bursa: **bu**; Caudal appendage: **ca**; Caudal glands: **cg**; Cement glands: **cg**; Cilia: **c**; Ciliated furrow: **cf**; Ciliated pits: **cp**; Common genital atrium: **cga**; Common genital pore: **gp**; Copulatory bursa: **cb**; Copulatory organ: **co**; Deferent duct: **dd**; Egg: **eg**; Ejaculatory duct: **ed**; Eyes: **e**; Female antrum: **fa**; Female duct: **fd**; Female gonopore: **fg**; Girdle: **gd**; Intestine: **i**; Intracapsular seminal vesicle: **isv**; Light-refracting bodies: **lb**; Male gonopore: **mg**; Metameric ganglia: **meg**; Mouth: **m**; Muscular gut: **mgu**; Ovary: **o**; Oviduct: **ov**; Posterior brain lobe: **pb**; Proboscis: **p**; Prostate glands: **pg**; Prostatic vesicle: **pv**; Protonephridial duct: **pd**; Protonephridium: **pr**; Penis stylet: **ps**; Penis stylet opening: **psv**; Pharynx: **ph**; Pharynx glands: **phg**; Rod tracks: **rt**; Ring: **r**; Rhabdite: **rh**; Seminal receptacle: **rs**; Seminal vesicle: **sv**; Sperm: **sp**; Spine: **s**; Statocyst: **st**; Testes: **t**; Transverse cells line: **tc**; Uterus: **u**; Vitellaria: **vi**; Zooid: **z**.

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Availability of data and materials: Type and non-type specimens are deposited in the Museu de Zoologia, Universidade de São Paulo (MZUSP).

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Figure captions

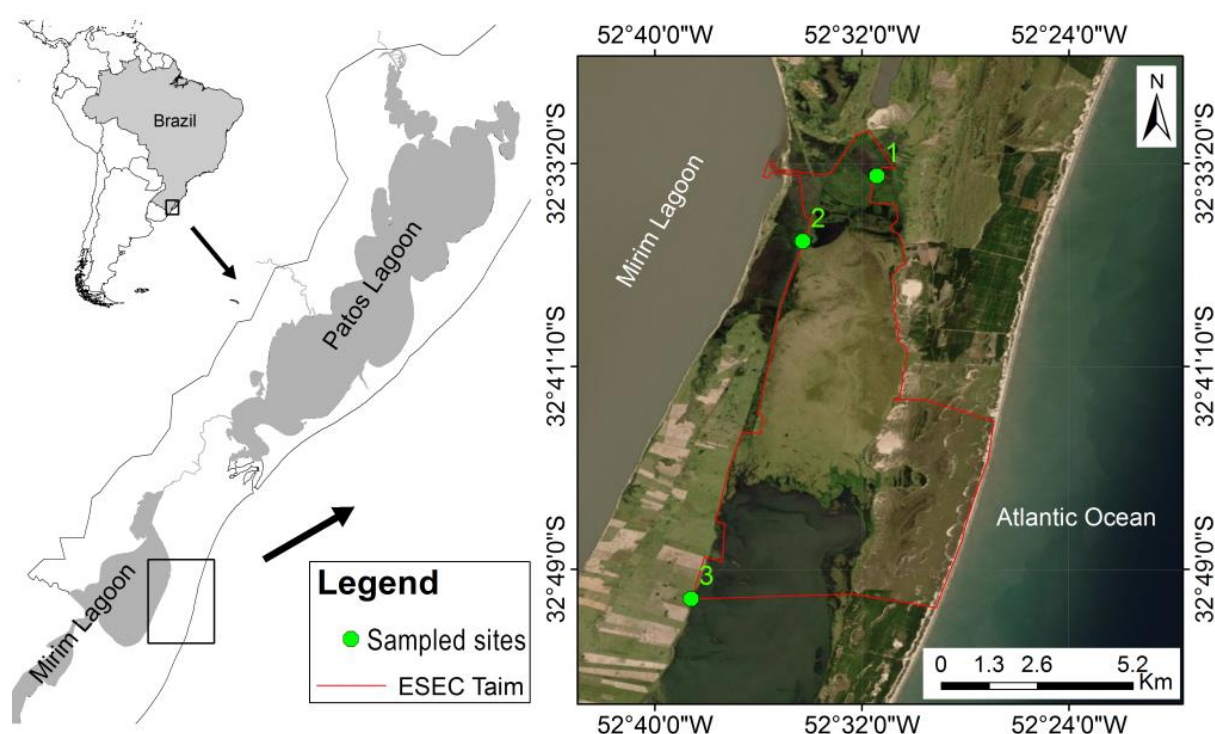


Figure 1 . Sampling sites map showing the collecting sites at the Taim nature reserve (ESEC Taim), Rio Grande do Sul, Brazil.

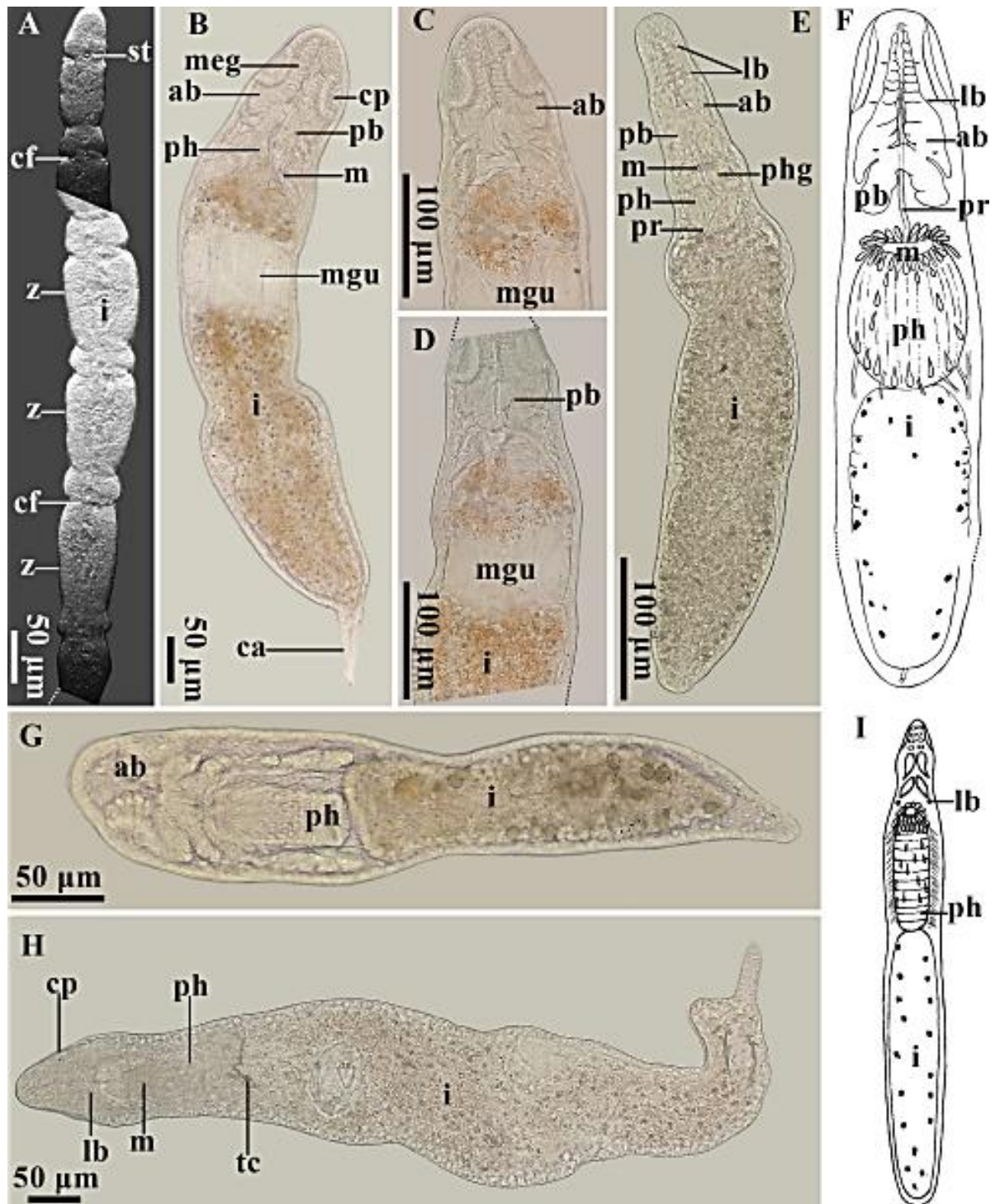


Figure 2. Live specimens of Catenula. (A) *Catenula lemna*, chain two zooids. (B–D) *Myostenostomum vanderlandi*: (B) habitus of whole-mounted individual, (C) detail of the anterior brain lobe and (D) detail of the posterior brain lobe. (E–F) *Stenostomum glandulosum*: (E) live individual, (F) free-hand schematic representation. (G) *Stenostomum grande*, a single zooid. (H) *Stenostomum paraguayense*, intestine with some food items. (I) *Stenostomum hemisphericum*, free-hand schematic representation.

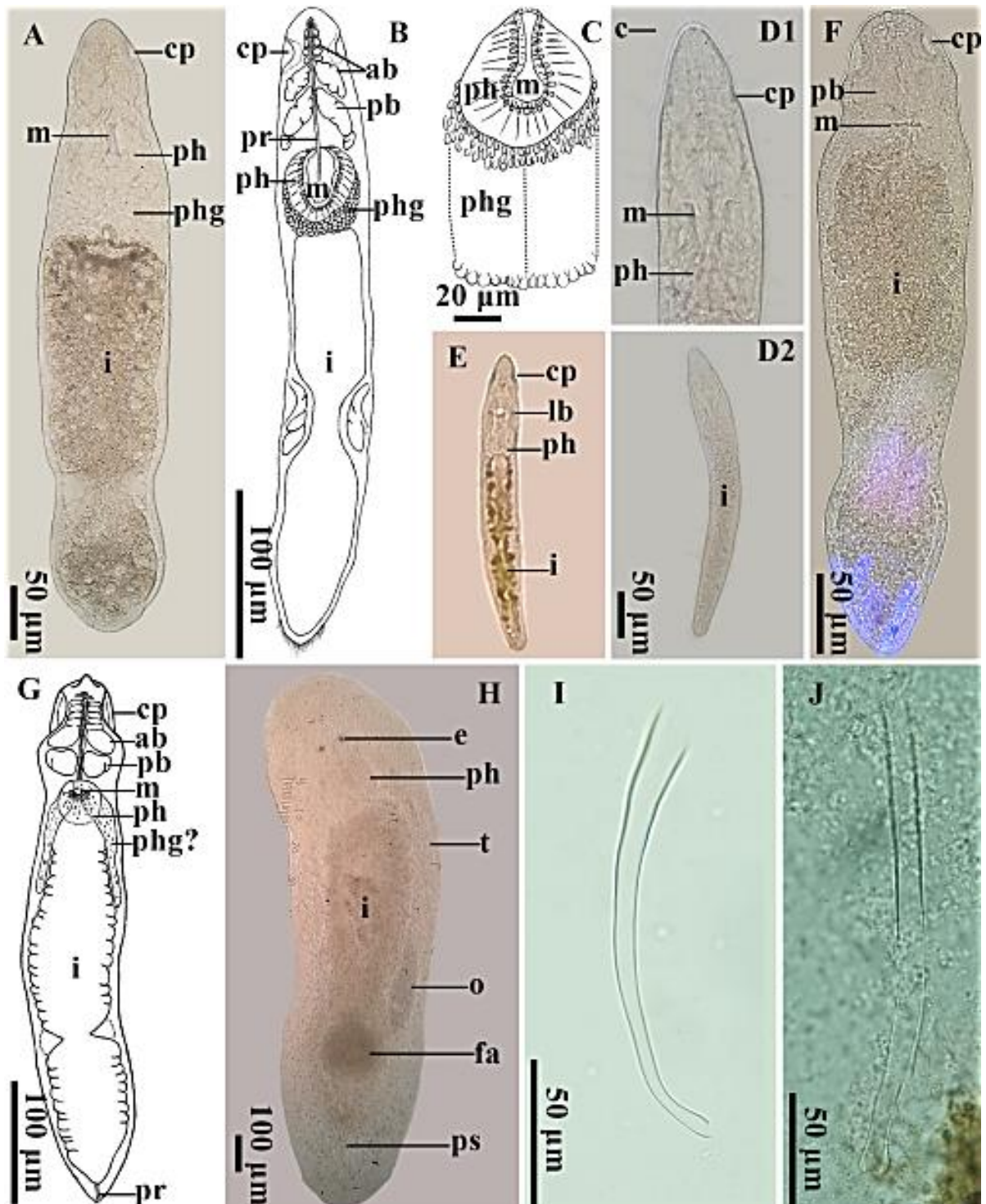


Figure 3. Live specimens of Stenostomidae and Macrostomidae. (A–C) *Stenostomum* cf. *pegephilum*: (A) chain with two zooids, (B) schematic representation, (C) detail of the muscular pharynx. (D1–2) *Stenostomum saliens*: (D1) detail of the anterior region of the body and (D2) dorsal view of the habitus of a specimen. (E) *Stenostomum simplex* (scale bar not available). (F–G) *Stenostomum tuberculosum*: (F) individual with three zooids, (G) schematic representation. (H–I) *Macrostomum quiritium*: (H) dorsal view of a mature specimen, (I) detail of the penis stylet. (J) *Macrostomum tuba*, detail of the penis stylet.

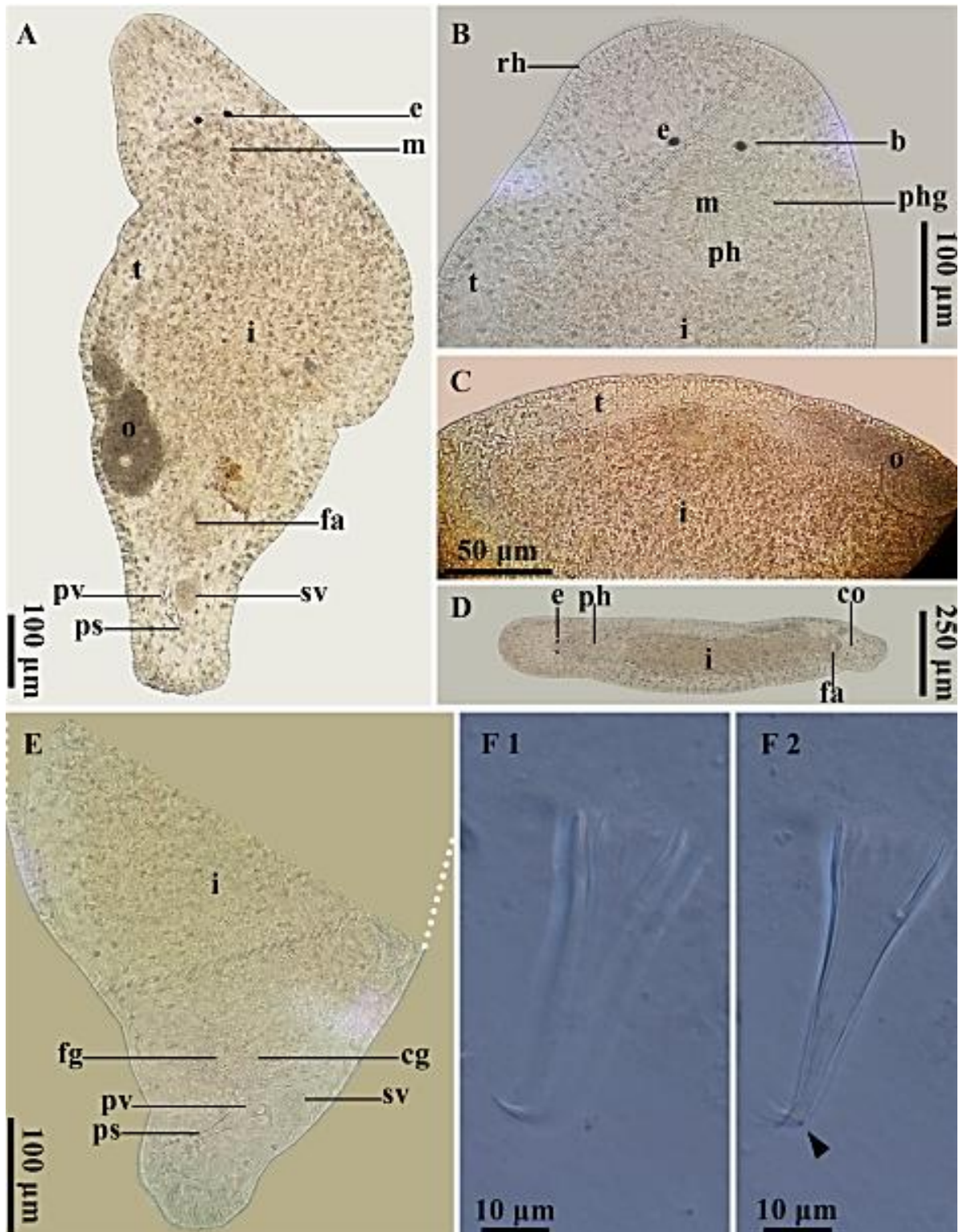


Figure 4. Live specimen of *Macrostomum* n. sp. 1 Reyes and Brusa (A) Dorsal view of a mature individual. (B) Detail of the anterior body region. (C) Detail of the testes and ovary. (D) A free-swimming specimen. (E) Posterior body region with details of the copulatory organs. (F1–2) Detail of the penis stylet in two different focal planes (black arrowhead indicating the penis stylet opening).

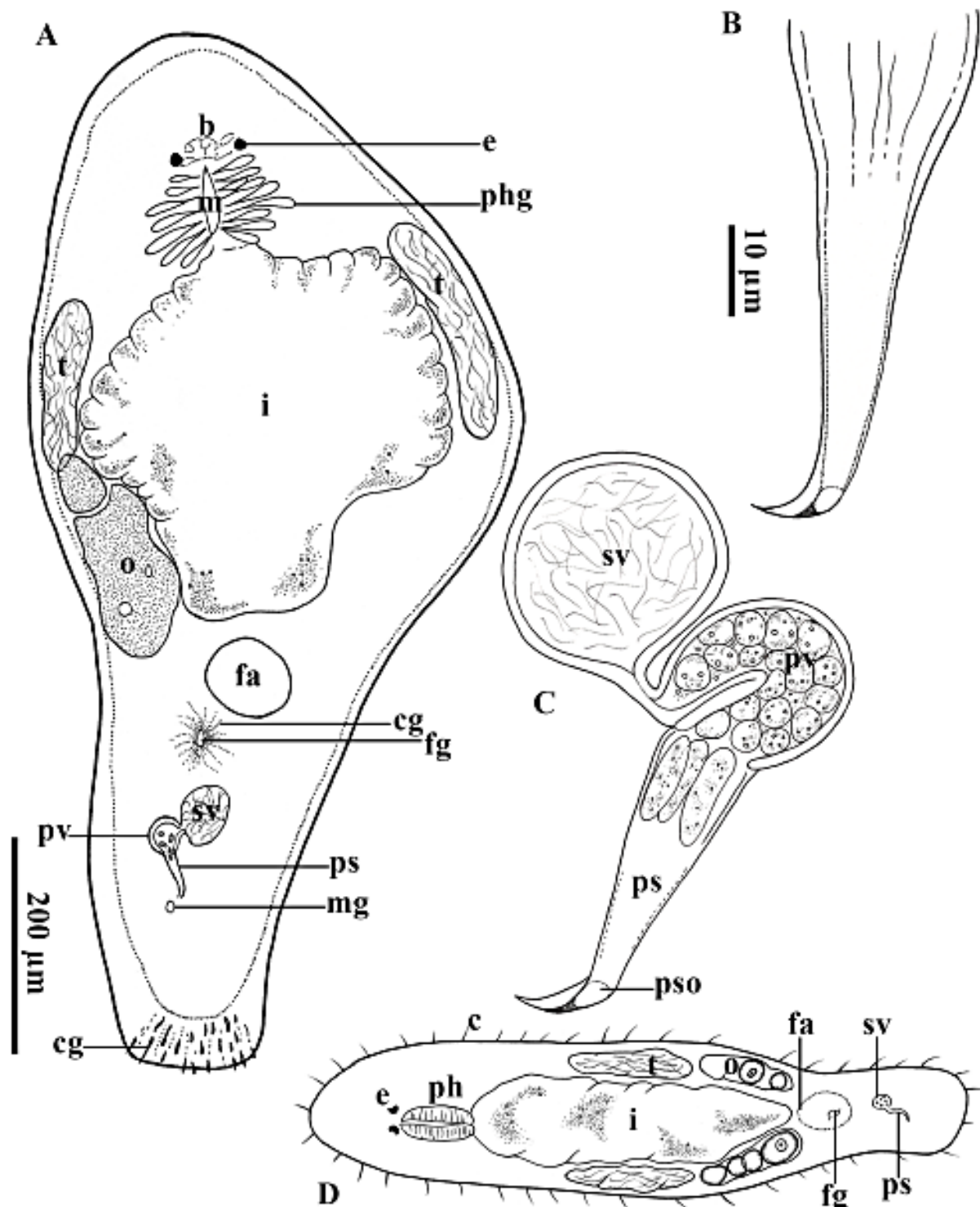


Figure 5. *Macrostomum* n. sp. 1 Reyes and Brusa (A) Schematic reconstruction of the body. (B) Schematic representation of the penis stylet (from the holotype). (C) Detail of the male copulatory system. (D) Free-hand drawing of a swimming individual.

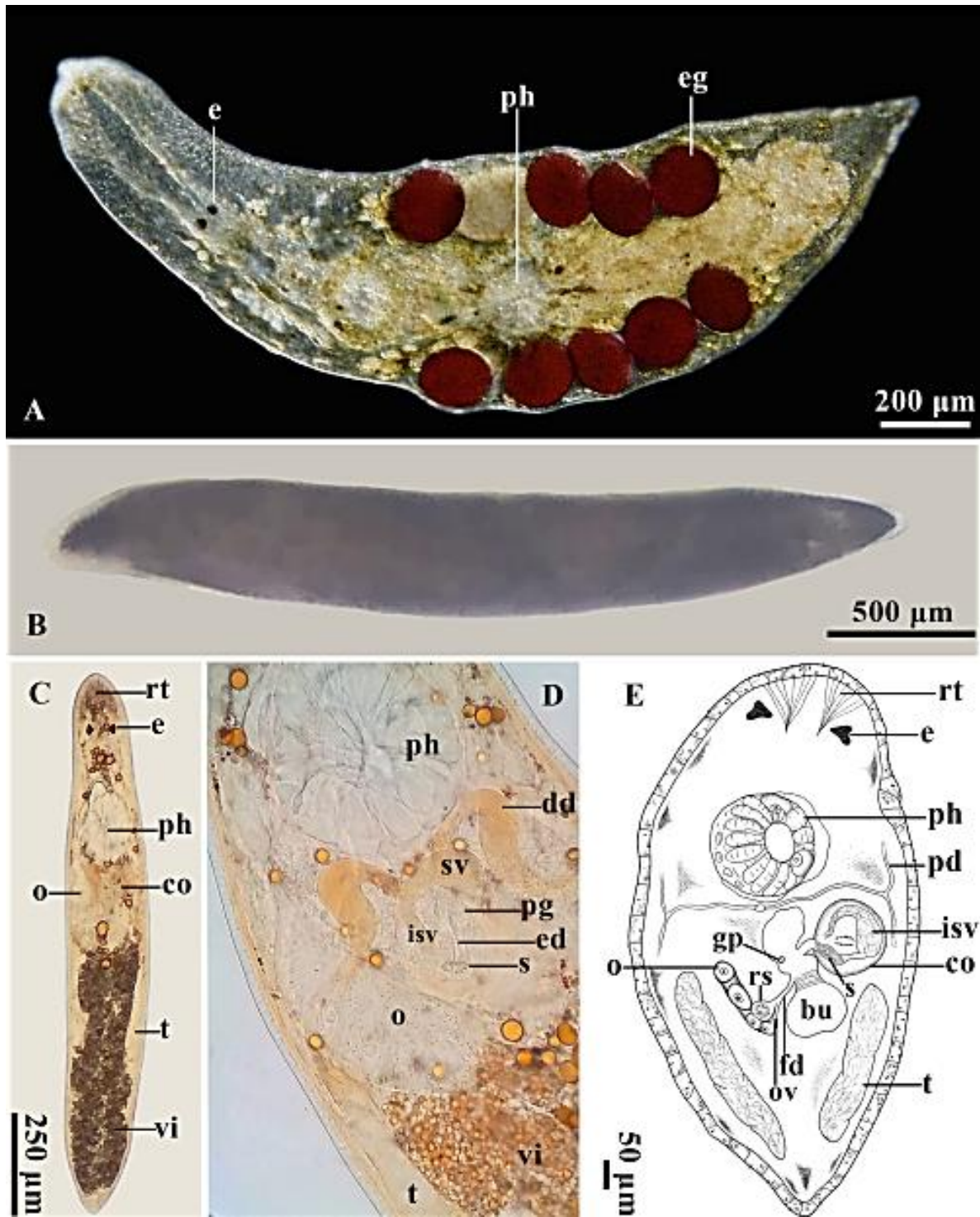


Figure 6. Live specimens of Typhloplanidae. (A) *Mesostoma ehrenbergi*, dorsal view of a mature individual observed in dark field. (B) *Brothromesostoma evelinae*, dorsal view. (C–E) *Strongylostoma elongatum*: (C) dorsal view of a mature specimen, (D) detail of the male copulatory organ (scale bar not available), (E) schematic representation of the habitus.

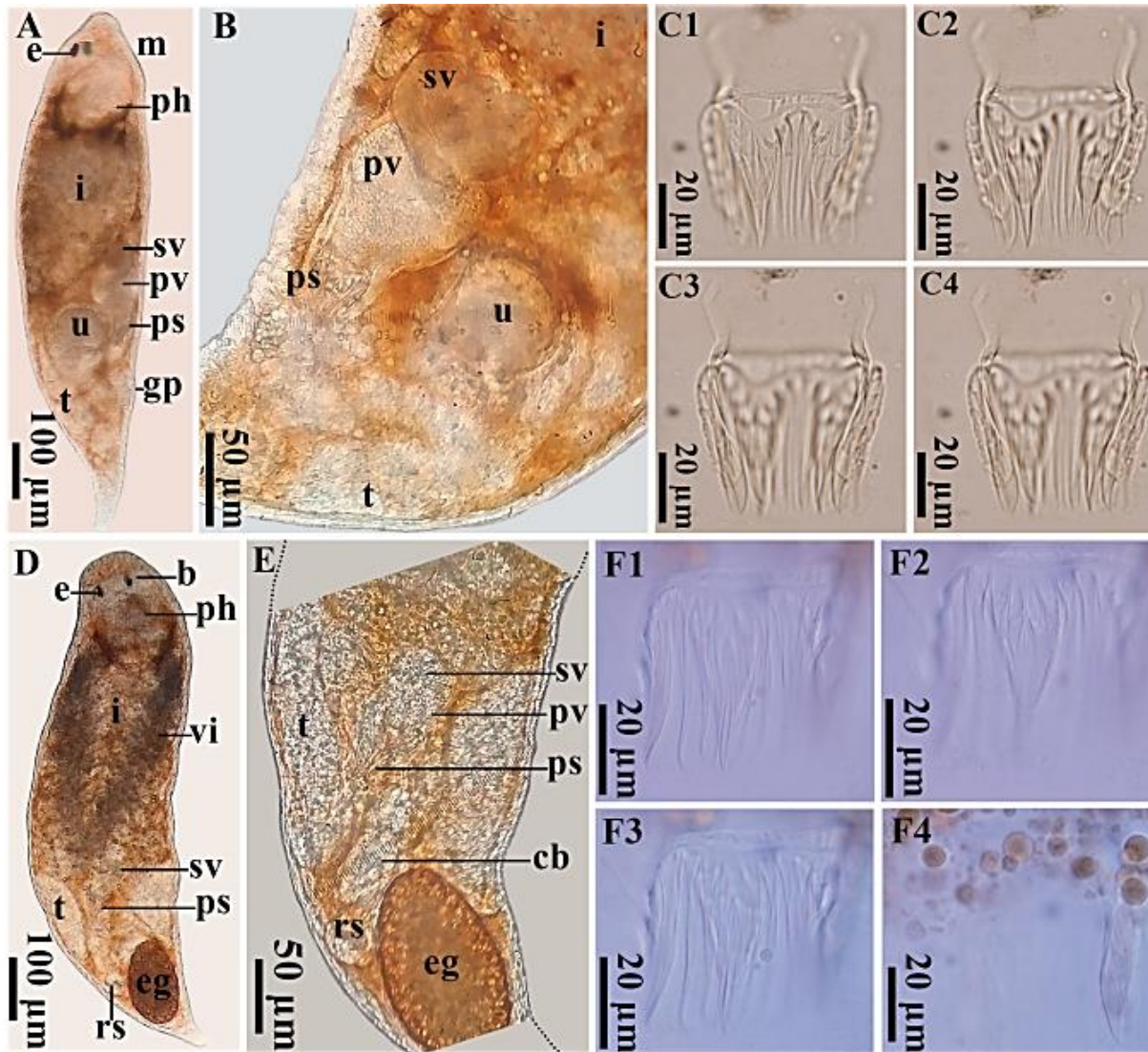


Figure 7. Live specimens of *Gieysztorina*. (A–C) *Gieysztorina evelinae*: (A) lateral view of a free-swimming individual, (B) detail of the posterior body region with details of the copulatory organs and (C1–4) detail of the penis stylet in different focal planes. (D–F) *Gieysztorina hymanae*: (D) dorsal view of a mature individual, (E) posterior body region with details of the copulatory organs and (F1–4) detail of the penis stylet in different focal planes.

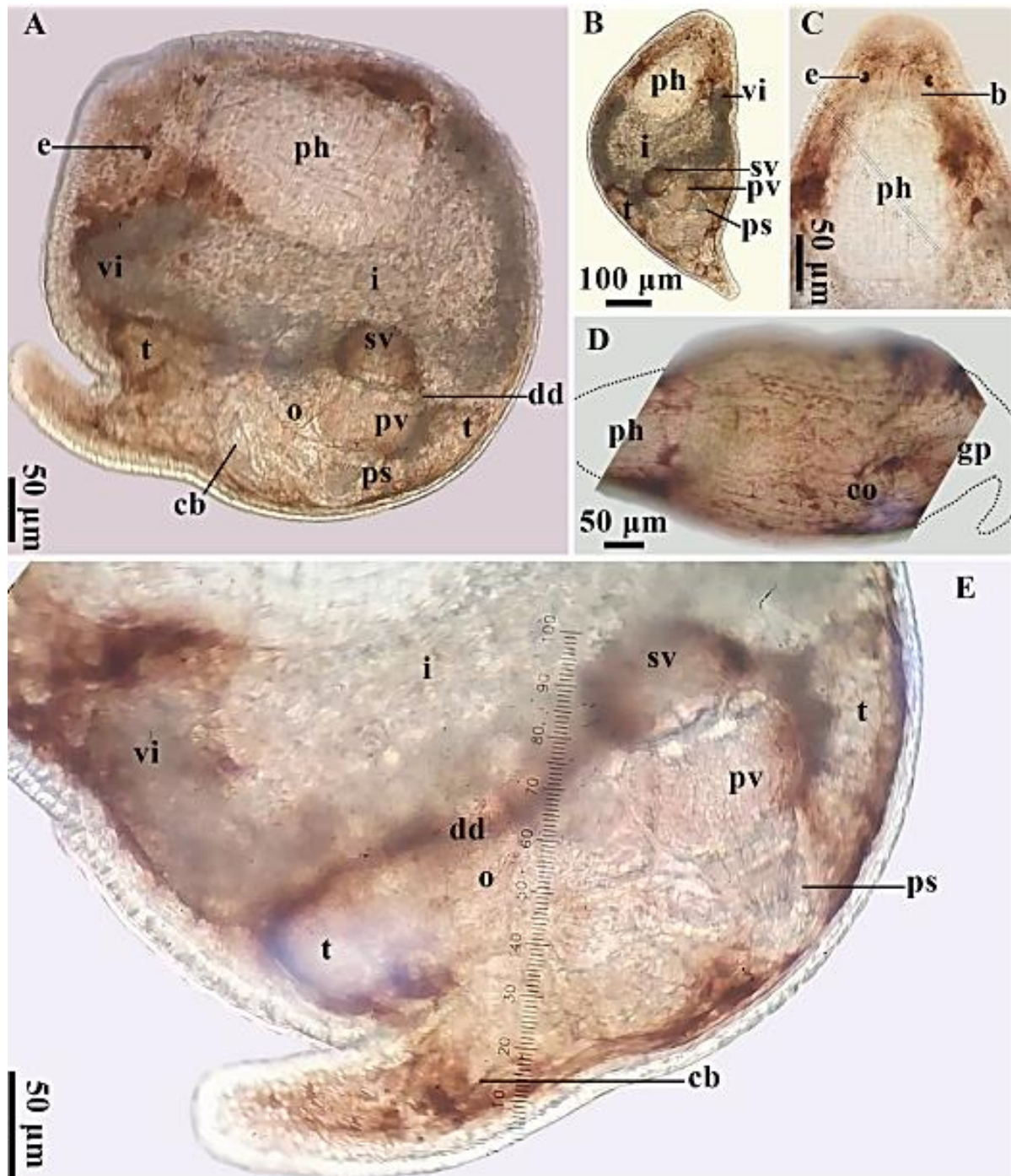


Figure 8. Live specimens of *Gieysztoria* n. sp. 1 Reyes and Brusa (A) Internal organisation from a live animal based on a whole-mounted preparation, dorsal view. (B) Habitus from live animal, dorsal view. (C) Detail of the anterior region of the body. (D) Detail of the epidermis surface, ventral view (anterior region of the body oriented at the left side). (E) Posterior body region with details of the copulatory organs.

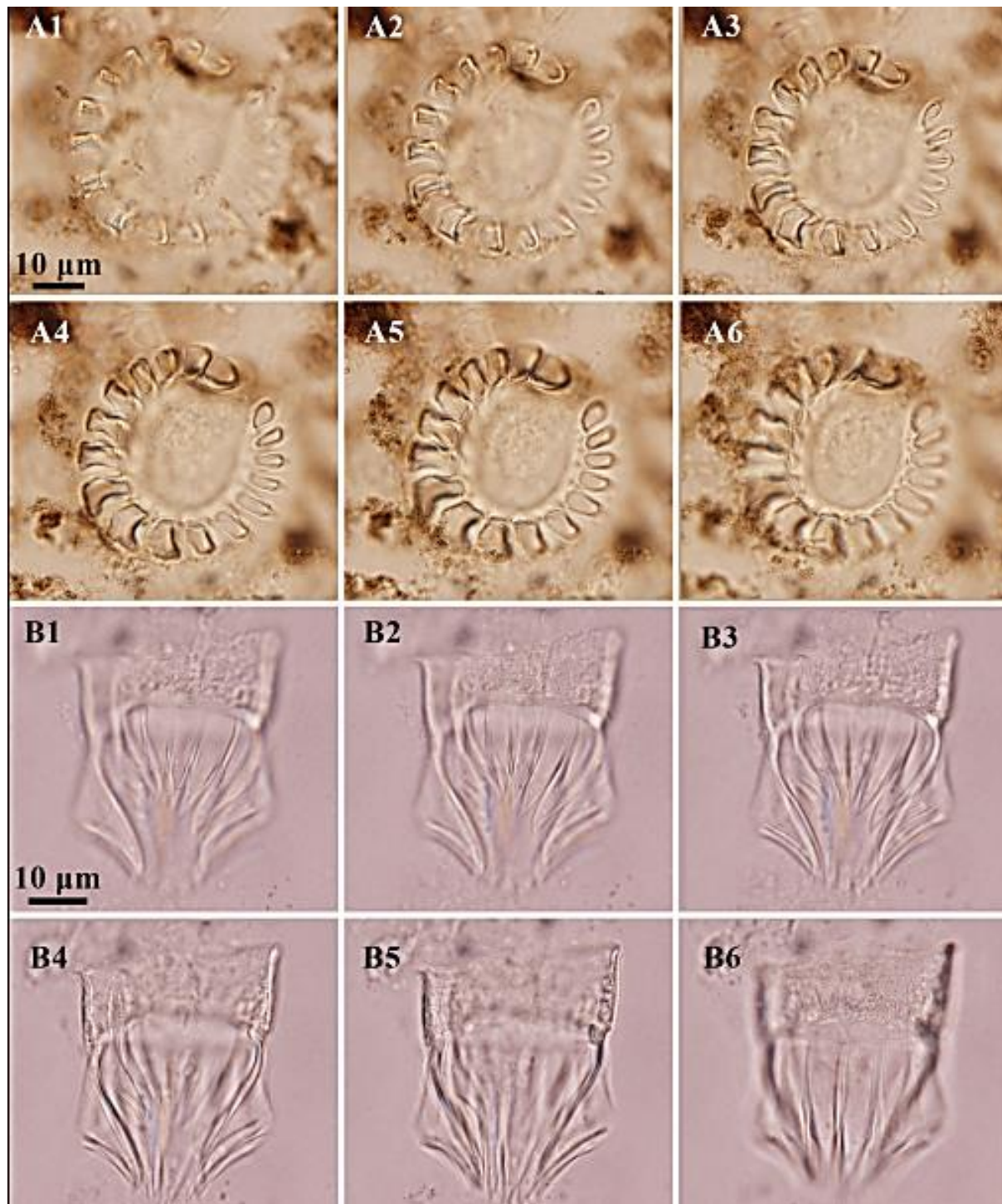


Figure 9. *Gieysztoria* n. sp. 1 Reyes and Brusa. Penis stylet in different focal planes. (A1–6) caudal view and (B1–6) lateral view (same scale bar for all photographs in A and B, respectively).

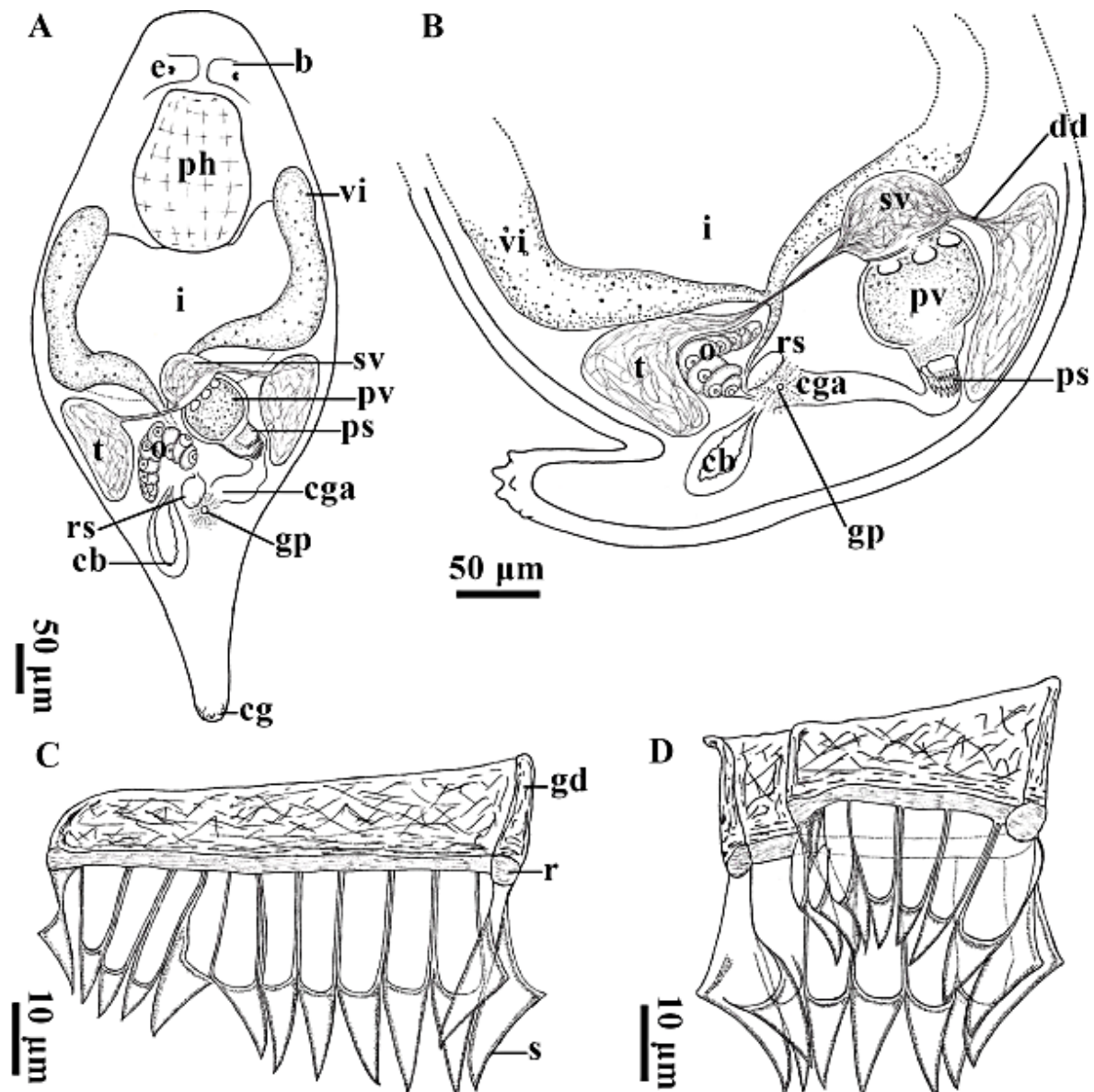


Figure 10. *Gieysztoria* n. sp. 1 Reyes and Brusa. (A) Schematic representation of the habitus from a live animal based on a whole-mounted preparation, dorsal view. (B) Detail of the male and female copulatory systems, dorsal view. (C) Schematic representation of a squashed stylet. (D) Penis stylet (from the holotype).

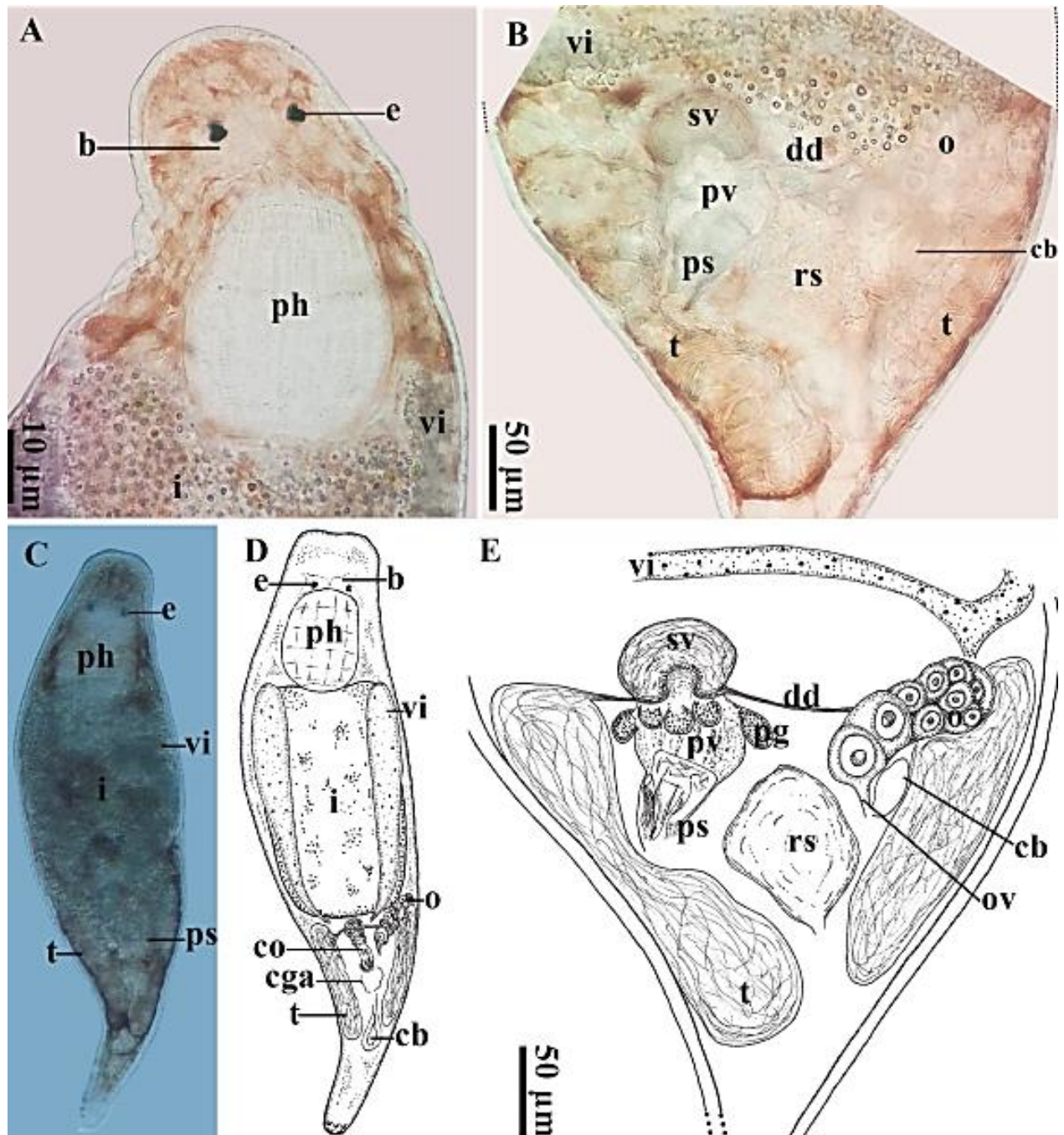


Figure 11. *Gieysztoria* n. sp. 2 Reyes and Brusa. (A) Detail of the anterior region of the body. (B) Posterior body region with details of the copulatory organs. (C) Habitus from live animal, dorsal view. (D) Schematic representation of the habitus from a live animal. (E) Schematic representation of the male and female copulatory systems.

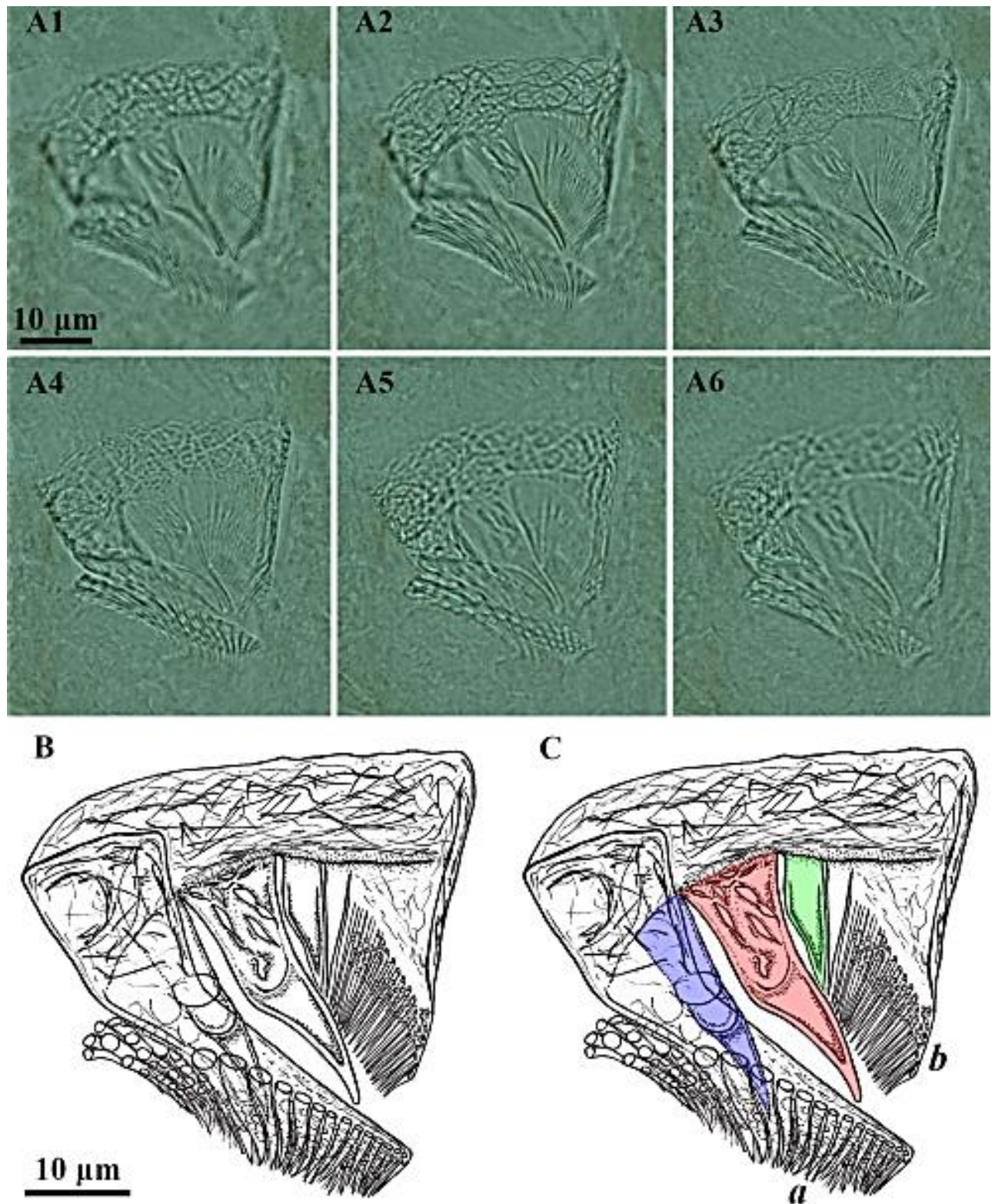


Figure 12. *Gieysztoria* n. sp. Reyes and Brusa. (A 1–6) Penis stylet in different focal planes (same scale bar for all photographs). (B) Schematic representation of the penis stylet. (C) Graphic explanation of dorsal spines: group *a* and *b*, and ventral spines: blue, red and green spines.

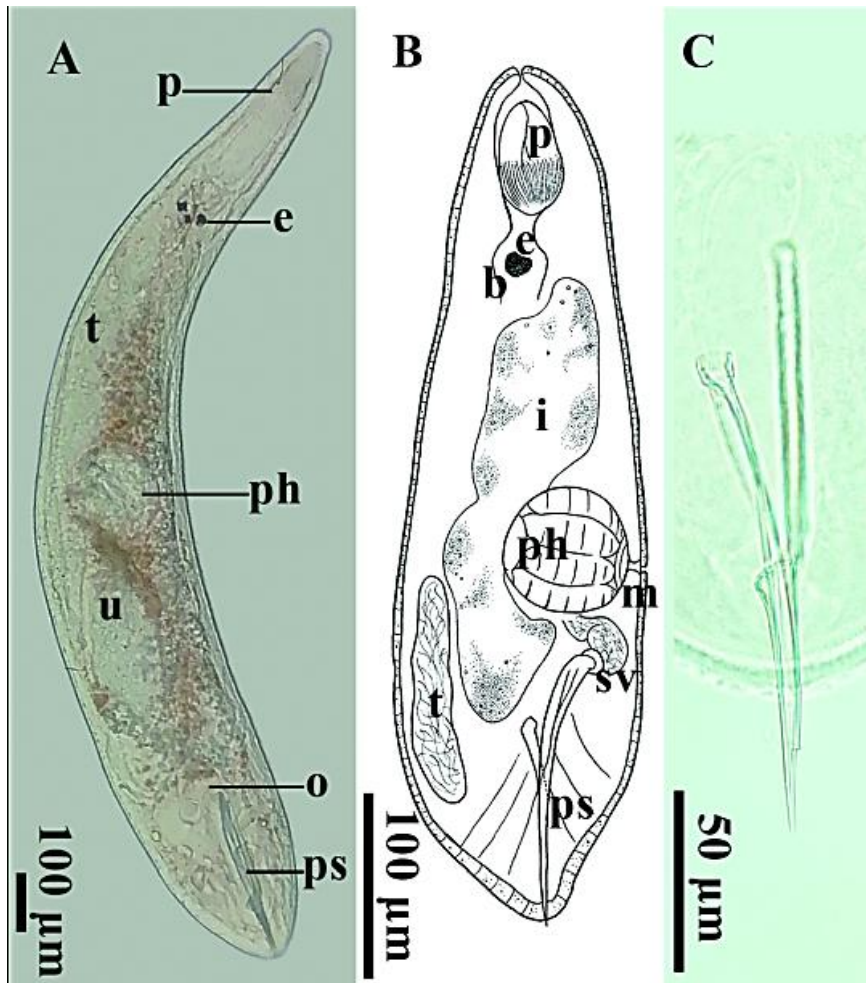


Figure 13. Specimens of Polycystididae. (A–C) *Gyratrix hermaphroditus*: (A) habitus of a live mature animal, (B) schematic representation of the habitus and (C) penis stylet.

Table 1. Comparison between *Macrostomum* n. sp. 1 and other similar species within the genus *Macrostomum*.

Species	Total length of the stylet (µm)	Length of the base of the stylet (µm)	Length of the stylet opening (µm)	Ratio of stylet base to stylet length (%)	Comments	Habitat	Distribution	Reference
<i>M. axi</i>	88	12	N.A	13.6		Freshwater	Italy	Papi 1959
<i>M. balticum</i>	73 – 76	11.5*	4.6*	15		Marine	Widely distributed	Luther 1960
<i>M. majesticis</i>	37 – 45	9 – 12	2 – 2.5	24 – 26.7	False seminal vesicle	Marine	Ecuador	Schmidt and Sopott-Ehlers 1976
<i>M. shenda</i>	56 ± 3.1	14 ± 3.8	8 ± 1.0	25		Brackish-water	China	Xin et al. 2019
<i>M. thingithuense</i>	58 (55 – 59)	8	8	13.8		Freshwater	Kenya	Young 1976
<i>M. barigoense</i>	85; 73 – 78	11; 17	5; 9	15 – 21.8		Freshwater	Kenya	Young 1976
<i>M. distinguendum</i>	100 – 102	30	8	29.4 – 30	Higher lengths of the distal penis stylet opening	Marine, Freshwater	Widely distributed	Ax 2008
<i>M. kepneri</i>	67.5	15	15	22.2		Marine	USA	Ferguson and Jones 1940
<i>M. platensis</i>	47 (37 – 52)	21.00	10	44.7	Distal end of the stylet is bevelled	Freshwater	Argentina	Adami et al. 2012
<i>M. schmitti</i>	58.48*	18.5	14.10	31.6	Bigger length of the penis stylet opening	Freshwater	USA	Hayes and Ferguson 1940
<i>M. silesiacum</i>	42 – 56	24 – 32	N.A	57.10	Slightly spirally-twisted stylet	Freshwater	Poland	Young 1973
<i>M. species 2</i>	36	13	N.A	36.10	Stylet spirals in a counter-clockwise direction	Marine	Portugal	Schockaert 2014
<i>M. troubadicis</i>	43	4 – 5	N.A	10.5	"S"-shaped stylet	Marine	Ecuador	Schmidt and Sopott-Ehlers 1976
<i>Macrostomum</i> n. sp.	47.3	17	3.6	36		Freshwater	Brazil	This study

1

(*): Measurement based on images and scales from the references. NA: not available

CAPÍTULO 2

ECOLOGICAL PATTERNS OF FREE-LIVING PLATYHELMINTHES IN COASTAL WETLANDS FROM SOUTHERN BRAZIL

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Keywords: Ramsar site, Turbellaria, neotropical region, Aquatic invertebrate, Diversity

ABSTRACT

Free-living Platyhelminthes (also known as turbellarians) live in marine and freshwater environments. Freshwater Platyhelminthes are abundant and diverse invertebrates that inhabit lakes, ponds, streams and wetlands. Although these animals occur in most freshwater ecosystems, they are little considered in ecological studies. The Coastal Plain in Southern Brazil harbours a great diversity of fauna and flora. As so, we analysed the structure of turbellarian communities in floating vegetation in three different-in-size limnic environments (shallow lakes and water channels) located inside a strict nature reserve, aiming to determine differences in abundance, density and richness among these environments. We found 1,225 individuals of turbellarians, belonging to 34 species and 12 genera, distributed among 6 orders. The most abundant order was Tricladida with 49.4 % of relative abundance, the remaining (Catenulida, Dalytyphloplanida, Macrostomida, Kalyptorhynchia and Prorhynchida) were present in lesser percentages. We found no significant differences among limnic environments with respect to abundance, density nor richness. We determined that small shallow lakes are a potential rich environment, but we did not record the estimated richness as observed species, while water channels are the richest environment with observed and estimated richness. As for bigger shallow lakes, they present a naturally not very diverse environment of turbellarian species. Similarities on turbellarian community among close limnic systems could be explained by inhabiting same aquatic macrophytes and hydrological subsystem, while bigger shallow lakes have a different community as it has its own contributing basin and could be influenced by other factors.

INTRODUCTION

Humid areas such as wetlands cover an approximate area of 30,300 km² in southern Brazil (Maltchik et al., 2003). In this sense, the Coastal plain of Southern Brazil is characterized by presenting several limnic ecosystems (shallow lakes, ponds and wetlands) (Marques et al., 2013), which determine diversity, with the aquatic macrophytes as an important part of communities, contributing to high biomass and high productivity (Esteves, 2011; Wetzel, 1993). Aquatic macrophytes affect the physical and chemical characteristics of water, are a source of organic matter (living or dead) for various organisms (bacteria, invertebrates and vertebrates), and alter the spatial structure of aquatic ecosystems due to the increased of spatial complexity of habitats (Thomaz & Esteves, 2011). In addition, shallow lakes are characterized by a coastal zone that maintains a strong interaction with the pelagic and pleuston zones, thus favouring a high richness of species (Meerhoff & Jeppesen, 2009).

Habitat structures are important in determining species diversity, usually more complex habitats have greater diversity (Bell et al. 1991; Jeffries, 1993; Tanaguchi et al. 2003; Thomaz et al. 2008; Mykrä et al. 2011). Thus, the presence of vegetation in an aquatic ecosystem results in a considerable increase in the area available for invertebrate colonisation (Pinder, 1995). Therefore, shallow lakes and wetlands in southern Brazil are likely to present a great biodiversity of aquatic invertebrates (Braccini & Leal-Zanchet 2013).

Aquatic invertebrates are important components of food chain and perform other functional processes in limnic aquatic community (Würdig et al., 2007). However, most environmental research and assessments involving aquatic invertebrates are conducted using insects (larvae and adults), and many other groups, including the free-living Platyhelminthes, are usually left out of environmental studies (Braccini et al., 2016).

Free-living Platyhelminthes (also known as turbellarians) are acoelomate bilaterians that present a ciliated epidermis and a blind-sac intestine. They are hermaphroditic and some have

the capacity of asexual reproduction (Noreña et al., 2016; Tyler & Hooge, 2004). According to body size, and for practical reasons, turbellarians are grouped into microturbellarians (~1 mm) and macroturbellarians (0.2 – 10 cm). Microturbellarians are represented by Catenulida and most of Rhabditophora, while larger animals belonging to Tricladida and Polycladida are considered macroturbellarians (Noreña et al., 2015; Schockaert et al., 2008). A conservative approach reveals that approximately 1,300 species of turbellarians inhabit freshwater environments (Schockaert et al., 2008). As so, turbellarians are inhabitants of several inland ecosystems such as wetlands, lakes and rivers (Braccini & Leal-Zanchet, 2013; Gamo & Leal-zanchet, 2004; Noreña-Janssen, 1995; Noreña et al., 2005; Reyes & Brusa, 2017), but they are also found in wet agricultural environments (Vara & Leal-Zanchet, 2013). Turbellarians associated to pleuston community live among floating aquatic plants (Damborenea et al., 2005; Noreña et al., 2006).

Turbellarians are abundant and diverse in aquatic environments; however, most investigations were made on taxonomic issues disregarding ecological aspects (Kolasa, 2000; Schockaert et al., 2008). The main reasons comprise the need of living turbellarians (specially microturbellarians) for the study, the paucity of researches in the area, and the lack of interest on the group when it comes to perform biodiversity assessments (Eitam et al., 2004; Schockaert et al., 2008). Nonetheless, some efforts were made in order to understand turbellarian communities in different-in-sizes pools. For instance, in Israel, Eitam et al. (2004) determined that species richness and community dissimilarity were positively related to surface area, but community dissimilarity was positively related to permanence. Young (2001) indicated that abiotic variables such as temperature, food availability and water regime in the ecosystem influence the seasonal occurrence of microturbellarians.

With respect to the Neotropical region, sporadic studies were made assessing the turbellaria fauna on lentic waters (Heckman, 1998). Noreña-Janssen (1995) indicated that abundance

dynamics of the microturbellarian species depend, mainly, on the type of life cycle adopted by the species, on water flow regime, and coverage of aquatic plants. Also, the highest population densities were found within macrophytes (Noreña-Janssen, 1995). Similarly, Braccini and Leal-Zanchet (2013) studied different-in-size permanent coastal wetlands in southern Brazil, and they found that the highest estimated species richness was present rather in smaller than in more intermediate and larger wetlands. Also, summer influences the variation in abundance among wetlands. The fact that freshwater turbellarians are found abundant and diverse makes them an important group in community structuring of other freshwater invertebrates (e.g., mosquito larvae, chironomid larvae and crustaceans) causing oscillations in the abundance dynamics (Blaustein & Dumont, 1990; Dumont et al., 2014; Maly et al., 1980; Schwartz & Hebert, 1986). Also, triclads can be important predators in small streams when fish are not present (Noreña et al., 2015).

The Southern Brazil is a hotspot of biodiversity (Guadagnin, 1999), presenting a great mosaic of flooded areas (about 37,300 km²) (Schwarzbolt & Schäfer, 1984). This coastal ecosystem harbors high abundance and diversity of fauna (Artioli et al., 2009; Garcias & Bager, 2009; Kirst et al., 2017; Moreira et al., 2014; Stenert et al., 2012) and aquatic vegetation (Maltchik et al., 2004; Rolon et al., 2010) as a function of the heterogenic geography (Vieira & Rangel, 1983) and the seasonal hydrographic modifications (Vieira & Rangel, 1988). As so, the complexity of environmental factors (especially aquatic rooted and floating vegetation) in these temporal and permanent flooded areas of Southern Brazil could represent an adequate habitat for turbellarians. However, so far, there are only a few studies on the ecology of turbellarians (Braccini & Leal-Zanchet, 2013). In this context, we indagated the species richness and abundance of turbellarians in different shallow limnic systems at the Coastal plain of Southern Brazil. In addition, we test whether the abiotic factors such as temperate and pH could explain turbellarian abundance and richness in distinct environments. To do so, we

used as a model the nature reserve of Taim (ESEC Taim) that is one of the most outstanding flooded areas in southern Brazil. This area is composed by a mosaic of temporal and permanent ponds, including part of a large lake within its area (Charão Kurtz et al., 2003). The ESEC Taim shelters a great diversity of vertebrates (including endangered species) (Azambuja, 2010; Correa et al., 2011; Gomes & Krause, 1982; Garcia et al., 2006; MMA, 2018; Pires Gayer et al., 1988; Sponchiado et al., 2012) and aquatic plants (Motta Marques & Villanueva, 2001). For its importance as a humid ecosystem, the ESEC Taim was recently designated as a Ramsar site (Ramsar, 2017).

MATERIALS AND METHODS

Research area

The study area is situated at the Taim nature reserve (ESEC Taim), located in the Coastal Plain of Rio Grande do Sul, southern Brazil. The ESEC Taim is a strict protected area situated between lake Mirim and the Atlantic Ocean. The ESEC Taim has a plain relief and it is composed by several shallow freshwater ecosystems (channels, lakes and wetlands). Also, this area present sand dunes and grass fields (Waechter & Jarenkow, 1998). We performed sampling collections in three different environments: in a small shallow lake (site 1), in a water channel (site 2) and in a large shallow lake (site 3) (Fig. 1). Water temperature and pH were registered for each sampling site using a multiparameter HANNA© HI 9126. Details on collecting sites are given as follows.

Lake Nicola (Site 1) (S1) (32° 33' 18.07" S; 52° 31' 18.50" W): It is a 2.58 km² shallow lake, located in the northernmost area of the ESEC Taim. This lake has a remarkable seasonal water volume variation. Seasonally, lake Nicola is covered with different kind of marsh

vegetation generally arranged in banks. Diffusely, lake Nicola receives water from external agricultural channels and from lake Mangueira (Würdig et al., 2007).

Water channel (Site 2) (S2) (32° 35' 50.52" S; 52° 34' 5.40" W): Located at the western limit of the protected area, this channel was formed as a result of the construction of the main road Brigadeiro José da Silva Paes (BR 471), which runs parallelly throughout almost all the western limit of the ESEC Taim. Depending on the rainfall regime and water availability, this channel diffusely connects Lake Nicola to Lake Mangueira. This channel is long and presents floating and rooted plants according to seasonal rainfalls and eutrophication processes.

Lake Mangueira (Site 3) (S3) (32° 50' 11.42" S; 52° 38' 37.45" W): This is a vast freshwater body (820 km²), which diffusely supply water to lake Nicola. The north area of the lake is within the boundaries of the ESEC Taim. Littoral aquatic plants are composed by rooted and floating vegetation (Würdig et al., 2007).

Sample collection and preparation

Sampling campaigns were carried out between September 2018 and July 2019. We performed five samplings in sites S1 (Sept., Nov. 2018; Feb., May, Jul. 2019) and S2 (Oct., Nov. 2018; Mar., Apr., May 2019), while S3 (Oct. 2018; Feb., Apr., Jun. 2019) was sampled four times. All samples were made approximately every two months in order to remove bias that the frequency of sampling campaigns could influence the determination of turbellarian community at these sampled sites. We used a 0.25 m² square to set a known area in order to collect turbellarians, from which free-living Platyhelminthes on floating vegetation microhabitats (0.5 m maximum depth) were randomly collected.

Floating vegetation was gathered using a plankton sieve (335 µm mesh size), then washed up with water from the environment and transferred to 1.25 L plastic jars. The sampling procedure was randomly repeated three times in each site. Samples were carried live to the laboratory.

Once in the laboratory, samples were stored in a temperate room ($\sim 20^{\circ}\text{C}$) with air pumps for posterior analyses. Posteriorly, samples underwent oxygen depletion method (Schockaert, 1996). In brief, samples were left without any source of oxygen causing turbellarians to swim towards the surface of the jar in order to reach dissolved oxygen at the upper layers of water, where turbellarians can be picked up easily. Besides, we looked for turbellarians among leaves, roots and detritus as suggested by Brusa et al. (2003).

Live animals picked up from jars were observed *in vivo* using an optical microscopy in order to identify turbellarians (Young, 2001), whenever possible, to the species level. When needed, after live observations, some specimens were whole mounted using Hoyer's medium (Humason, 1979). We compared all species found in the ESEC Taim against specialized literature concerning turbellarians fauna mainly from the Neotropics, but also, from other biogeographic regions. All specimens were counted and registered according to the site they were gathered. Sampling permits were granted by the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio), permits number 60291-2/2017 and 64551-1/2018.

Data analyses

We estimated species richness, abundance, and density of individuals ($/\text{m}^2$) for each sampling site. We defined species abundance according to Chodorowski (1959), with modifications: a) rare (only 1 individual); b) lesser abundant (2 – 19 individuals); and c) abundant (more than 20 individuals). The composition of the turbellarian assemblages was compared among the sites by cluster analysis, using species abundance data in order to explore differences in turbellarian assemblage composition between sampling sites. Posteriorly, we used a Similarity percentage analysis (SIMPER test) to identify the most important turbellarian species to generate the patterns of dissimilarity observed between sites. Both analyses were based on the Bray-Curtis similarity coefficient (Gotelli & Ellison, 2004).

The simplest method to assess diversity in a locality is by counting species. However, the use of non-parametric richness estimation such as Chao, Jackknife and Bootstrap has been suggested as a solution to determine richness in many cases involving freshwater organisms (Foggo et al., 2003; Melo & Froehlich, 2001). Besides, rarefaction curves are also used to estimate if samplings were performed properly in order to determine richness (Gotelli & Colwell, 2001). Both richness estimators and rarefaction curves were previously used in southern coastal wetlands in Brazil to define an estimation of freshwater turbellarian diversity in these environments, suggesting that they are efficient tools to portrait turbellarian richness (Braccini & Leal-Zanchet, 2013).

Species rarefaction curves for each site were built based on accumulation of individuals (abundance) (Colwell & Elsensohn 2014), so that biases on comparison due to differences in abundance in some turbellarians species was corrected (Gotelli & Colwell 2001). In addition, because the ESEC Taim has only one previous published record of turbellarian species (Reyes et al., 2019), we tested the performance of five non-parametric richness estimators, based on abundance (Chao 1) and incidence (Chao 2, jackknife 1, jackknife 2 and Bootstrap) in order to determine the completeness of species inventory. As so, richness estimators were used in order to understand the alfa diversity in each sampling site and considering the ESEC Taim as a whole (14 samples). Species accumulation curves and richness estimators were computed using Past 3.x (Hammer et al., 2001) performing 999 randomizations in each analysis. Furthermore, Analysis of variance (ANOVA) were performed to test whether richness or density of turbellarian differ among sampling sites. When necessary, the data were log-transformed to fulfil ANOVA assumptions. Additionally, a multiple regression analysis (GLMs) (McCullagh & Nelder 1989) was conducted to examine the effects of temperature and pH (independent variables) on density and richness (dependent variables) of turbellarian.

RESULTS

In the floating vegetation microhabitat, 1,225 turbellarians were found. They were identified as follows: 34 species belonging to 12 genera, distributed among 6 orders. The order Tricladida had the highest relative abundance (49.4 %), while the orders Catenulida, Dalytyphloplanida, Macrostomida, Kalyptorhynchia and Prorhynchida were less abundant (Table 1).

Triclads were represented by the most abundant species, *Girardia* sp. 1, this species was abundant at all sampling sites (Table 1). We found 12 species of the order Catenulida, this order was the second most abundant with 27.7 % of relative abundance. Within this order, the most abundant species was *Stenostomum* sp. 1, it had 14.4 % of relative abundance and was present in all sampling sites. The remaining species within Catenulida appeared with lesser relative abundance ($\sim 0.1 - 7\%$), being *S. cryptops* and *S. cf. pegephilum* rare species (Table 1).

Thirteen species of the order Dalytyphloplanida were found and was the third most abundant order with 16.2 % of relative abundance. Within Dalytyphloplanida, the three most abundant species found were *Gieysztorina* sp. 4, *G. falx* and *Gieysztorina* sp. 2 with 6.2, 4.4 and 1.6 % of relative abundance, respectively. While the rarest species were *Gieysztorina evelinae*, *Gieysztorina* sp. 3 and *Bothromesostoma evelinae* ($\sim 0.1\%$ each one) (Table 1).

The orders with the lowest relative abundances were Macrostomorpha (3.8 %), Kalyptorhynchia (2.7 %) and Prorhynchida (0.2 %). Within Macrostomorpha, *Macrostomum tuba* and *Microstomum* sp. 1, were rare species. Within Kalyptorhynchia, *Gyratrix hermaphroditus* was abundant. As for Prorhynchida, only two individuals were found (Table 1).

Turbellarian mean density at each sampling site was 34 ± 18.4 (S1), 156.6 ± 165.9 (S2) and 160.3 ± 74.7 (S3) individuals / m² (Table 1). Turbellarian density had no significant differences among the three sampling sites (ANOVA, $F=1.48$; $df=2$; $p>0.05$).

Turbellarian species richness in each sampling site varied from 13 to 25 (Table 1) without significant differences among these habitats (ANOVA, $F=0.61$; $df=2$; $p>0.05$). Rarefaction curves based on individual abundance showed that S2 and S3 reached their asymptote of alfa diversity, while S1 did not reach its asymptote. It was observed that S3 presented less richness than S1 and S2 (Fig. 2). These results are similar to those from observed richness. The microhabitat at S1 showed 18 observed species against 22 predicted species by the Bootstrap estimator, representing 83 % of completeness; while Chao 1 and Chao 2 predicted 31 each one, this is, same percentage of completeness (59 %). On average, 66 % of turbellarian fauna was observed at S1 (Fig 3A). The microhabitat at S2 presented 25 observed species, while Bootstrap estimated 30 species, computing 83 % of completeness in respect to the observed species. Chao 2 and Jackknife 2 calculated 41 and 42 species, respectively, estimating 60% of completeness. On average, 71 % of completeness was registered at S2 (Fig. 3B). In the microhabitat at S3, 13 species were found, the same richness was predicted by estimator Chao 1, amounting 100 % of completeness in relation to the observed species. Jackknife 2 calculated 18 species, estimating 74 % of completeness. On average, 85 % of completeness at S3 was calculated (Fig. 3C) (Table 2). When the analyses were performed with all samples, the estimator Chao 1 and Chao 2 calculated 76 % and 61 % of completeness, respectively. Jackknife 1 and Jackknife 2 calculated 49 and 58 species, respectively, representing 70 % and 59 % of completeness. Bootstrap, represented 84 % of completeness, calculating 40 species, estimated the highest percentage of species at the ESEC Taim. Considering the five estimators, the species richness found in the present study represented an average of 70% of completeness of the inventory of species for this protected area (Fig. 4 and Table 2).

During the study, water temperature varied from 10.6 to 26.1 °C (mean 20.09 ± 5.12), and pH ranged from 6.5 to 8.2 (mean 7.37 ± 0.69). These abiotic factors did not affect the turbellarian richness and density at the ESEC Taim (Table 3).

The cluster analysis grouped seven (A–G) categories indicating that there was not a chief factor in faunal similarity, especially in S1 and S2. However, samples from S3 fell into the same category in the analysis, suggesting similar turbellarian composition (Fig. 5). Groups A, B and C were different from the remaining, as they were conformed by one sample each and had distinct assemblages composed of a very few individuals from a few species. At the 39.7 % level of similarity, group D was composed by three samples dominated by *Stenostomum* sp. 1 (42.3 %). Group E is gathering, at 45 % level of similarity, S1, being the dominant species *Gieysztorina falx* (41.6 %). Similarly, group F (61.9 % similarity) is comprised by samples of S2, in which *Girardia* sp. 1 contributed with 32.6 %. At the 52.7 % level of similarity, group G clustered all samples from S3, the species *Girardia* sp. 1 was dominant in all samples, contributing in 70% to the formation of this group (Fig. 5, Table 4). The species that contributed the most to the dissimilarities (on average 67.9 %) among the groups were *Girardia* sp. 1, *Gieysztorina falx* and *Stenostomum* sp. 1 (Table 5).

DISCUSSION

The southern part of the Neotropical region has few studies dealing with ecological aspects of turbellarian assemblages because the individuals must be observed alive, in order to identify diagnostic characteristics to determine them to the species level (Braccini & Leal-Zanchet, 2013; Noreña et al., 2015). The most abundant order was Tricladida (*Girardia* sp. 1). *Girardia* sp. 1 occurred in all three sampling sites, more abundant at lake Mangueira (S3) (802 km²). Contrary, Braccini and Leal-Zanchet (2013) found lower number of individuals of the genus *Girardia* in large southern Brazilian coastal wetlands (2 – 6 km), in both in benthic

substrate and vegetation. Lake Mangueira (S3) is a large coastal lake (820 km²) where the littoral zone is covered by aquatic vegetation (Marques et al., 2013) and, since aquatic vegetation influence the macroinvertebrate colonization (De Szalay & Resh, 2000; Pinder, 1995), it could be responsible for the abundance of *Girardia* sp. 1 at S3. It is noteworthy that in the northern hemisphere the temperature of water may limit the abundance of triclads species (Harrath et al., 2004; Reynoldson, 1977; Reynoldson & Young, 2000), while southern relatives stand greater thermal windows (9 °C to 31 °C), maintaining their abundance (Lenguas-Francavilla et al., 2018). In this sense, a combination of factors seems more likely to influence the abundance of triclads in subtropical coastal wetlands. As for the lesser dominant species, such as microturbellarians, most of them are worldwide distributed and well-known. We found a few are representatives of the Neotropical region (e.g. *Gieysztoria falx*, *G. evelinae*, *G. hymanae*, *Stenostomum paraguayense* and *Bothromesostoma evelinae*) as other authors earlier reported (Brusa et al, 2003; Martín 1908; Marcus 1946; Reyes et al., 2019). Microturbellarian abundance in the neotropics depends mainly on lifecycle and overflows (Noreña-Janssen, 1995). Moreover, these animals take advantage of the expansion of water vegetation growing on littoral areas of the water body, where they find protection and a suitable substratum for community support (Noreña-Janssen, 1995). In addition, turbellarians are much diverse in vegetated littoral zones than deeper habitats (Young, 2001). Wetlands like the ones in the ESEC Taim are highly variable systems due to the presence of macrophytes, detritus abundance and water hydrodynamics (Marques et al., 2013; Würdig et al., 2007). In the present study, no significant differences between sampling sites were observed with respect to mean richness, abundance and density. The rarefaction curves showed that lake Nicola (S1) did not reach its asymptote and could have an incomplete species inventory. On the other hand, the richness estimators (66% of completeness) predicted 27 species in S1, bringing it to the second highest diverse site at the ESEC Taim. In fact, the

S1 environment is highly diverse in microhabitats and present notably water fluctuations (Würdig et al., 2007). Braccini and Leal-Zanchet (2013) also reported that large (2 – 24 km perimeter) wetlands have lower estimated richness of turbellarian species. If we follow the considerations of Braccini and Leal-Zanchet (2013), lake Nicola (S1) could be considered as a large body of water. Therefore, a low turbellarian diversity within lake Nicola (S1; 2.58 km²) would be expected, contrary to the estimated results by the rarefaction curves. In this sense, our results could represent the real alpha diversity at S1 without a bias in sampling effort.

As for sites S2 and S3, they reached their asymptotes in the rarefaction curves. For the water channel (S2), estimators amounted an average of 71 % of completeness, showing that S2 is expected to have 35 species and comprises the highest diverse site at the ESEC Taim. In addition, depending on the season, the water channel (S2) could be interrupted along its surface by marsh or rooted vegetation generating small "ponds". The water channel (S2) presents diverse floating and rooted plants all over the littoral zone. This feature is considered as an important factor for diversification and generation of diversity and density (Panatta et al., 2006; Pinder, 1995; Thomaz et al., 2008) since small ponds may harbor high species diversity than larger and less isolated bodies of water (Braccini & Leal-Zanchet, 2013; Scheffer et al., 2006).

As mentioned above, the rarefaction curve of lake Mangueira (S3) reached its asymptote with less species and individuals examined indicating that diversity at S3 is naturally low. Similarly, on average, estimators computed 85 % of completeness over four (4) campaigns. It is also noticeable that Chao 1, an estimator that consider abundance, computed 100% of completeness for S3. Therefore, it would be expected that no more species would be recorded in new surveys. These results are also in accordance with those of Braccini and Leal-Zanchet (2013), who indicated that larger wetlands present lower richness estimated of turbellarian species. So, this principle could be observed in larger coastal lakes such as S3. When all sites

were gathered for analysis, the estimators predicted 70 % (on average) of completeness of the inventory of species. This result indicates that the collected samples represent most of the turbellarian fauna at the ESEC Taim, considering that S1, S2 and S3 are different habitats with different factors affecting the development of species communities (Würdig et al., 2007). Water temperature varied from 10.6 to 24.1 °C, while pH varied from 6.5 to 8.2 between the three sampling sites. These factors did not affect richness and density of turbellarians. The main factors that influence turbellarian distribution are oxygen content and availability of food (Noreña et al., 2015), other factors such as temperature and water regimen could be related to reproduction of turbellarians (laying eggs) (Noreña et al., 2015; Young, 2001). In addition, several microturbellarians can stand ecological changes and variation on temperature (Gamo García, 1987; Noreña et al., 2006; Young, 1976). As for pH, turbellarians can stand wide bands of pH (Noreña et al., 2004; Young, 2001). Thus, new samples measuring oxygen or food items would be necessary to understand if turbellarian communities could be influenced by these factors at the ESEC Taim.

The cluster analysis revealed that the turbellarian community at lake Nicola (S1) and water channel (S2) was relatively similar, while the community at lake Mangueira (S3) was constant and notably differentiated. This could be explained due to S1 and S2 are relatively close to each other sharing similar aquatic macrophytes. In addition, it has been suggested that habitat complexity due to aquatic macrophytes affects species richness and abundance (Bell et al. 1991; Jeffries, 1993; Thomaz et al., 2008). Thus, it would be expected a similar turbellarian community. Besides, water diffusion between these two bodies of water (S1 and S2), could contribute to the sharing of species and connectivity (Würdig et al., 2007).

Interestingly, group G clustered all samples from lake Mangueira (S3) suggesting that turbellarian community at this Lake is mostly the same along the sampling campaigns. This result could be explained owing to lake Mangueira belongs to its own contributing basin

(hydrological South subsystem) (Würdig et al., 2007), having its own community composition. In addition, turbellarian assemblages in larger bodies of water are known to have low turbellarian species diversity (Braccini & Leal-Zanchet, 2013). Since S3 present a well-established vertebrate fauna such as fish communities (Correa et al., 2011; Garcia et al., 2006), it is likely that these vertebrates may shape the lake Mangueira invertebrate communities, influencing the abundance and species richness as it was stated for other shallow lakes (Meerhoff & Jeppesen, 2009; Scheffer et al., 2006).

In regard of dissimilarities, *Girardia* sp. 1, *Gieysztoria falx* and *Stenostomum* sp. 1 were the species that contributed the most to the dissimilarity between sites S1, S2 and S3. These differences, based on these three species, should be taken with caution because two species were recognized according to the appreciable morphological structures. As so, the genus *Stenostomum* is very diverse, but difficult to diagnostic taxonomically (Larsson & Willems, 2010), while *Girardia* is less diverse (Carbayo & Froehlich, 2008), but could be found inhabiting with sibling species. This uncertainty could generate misinterpretations in determining turbellarian diversity and their abundance at different sites. The present analysis of the turbellarian community on floating vegetation microhabitats provides an overview that is useful in understanding the dynamics of ecological systems in wetlands in Southern Brazil, particularly inside the nature reserve ESEC-Taim. As so, this study represents the first contribution to the knowledge of the diversity and assemblage structure of this scarce-studied group of invertebrates in a protected area in southern Brazil. Therefore, a continuous monitoring of the fauna of turbellarians along the following years will be valuable in order to have a better understanding of how this ecosystem behave and develop with respect to these free-living flatworms.

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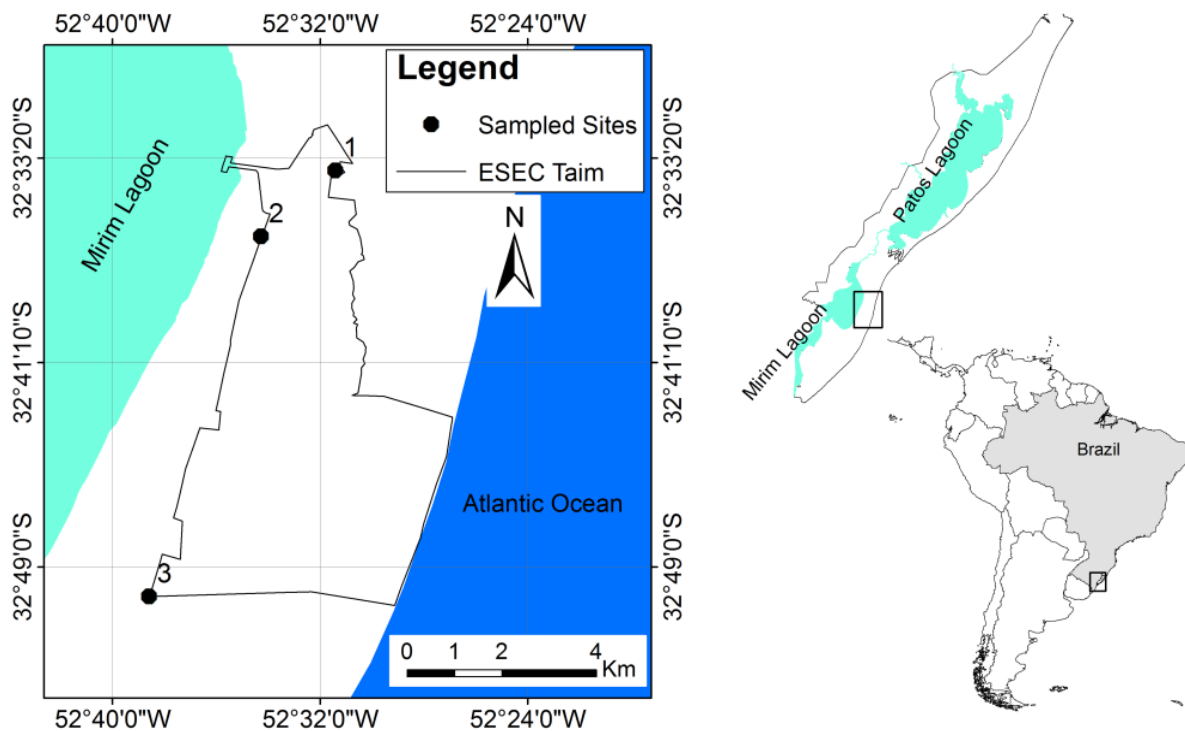


Figure 1. Sampling sites map showing the collecting sites at the Taim nature reserve (ESEC Taim) in the Coastal Plain of southern Brazil.

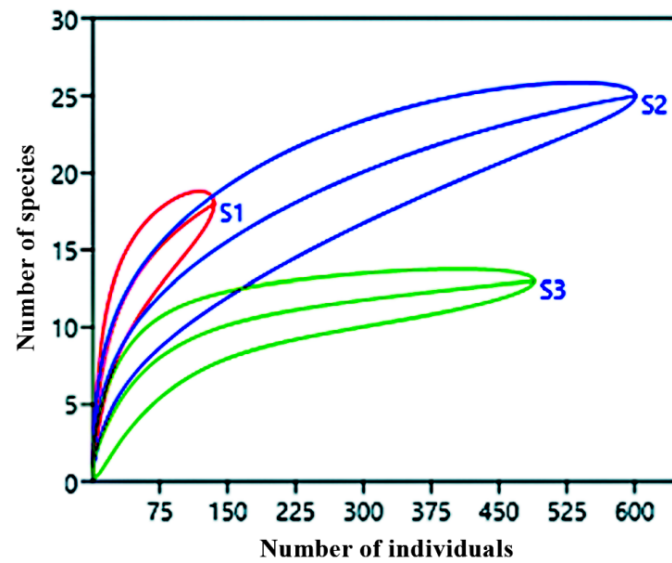


Figure 2. Species accumulation curves with their respective confidence intervals for lake Nicola (S1), the water channel (S2), and lake Mangueira (S3).

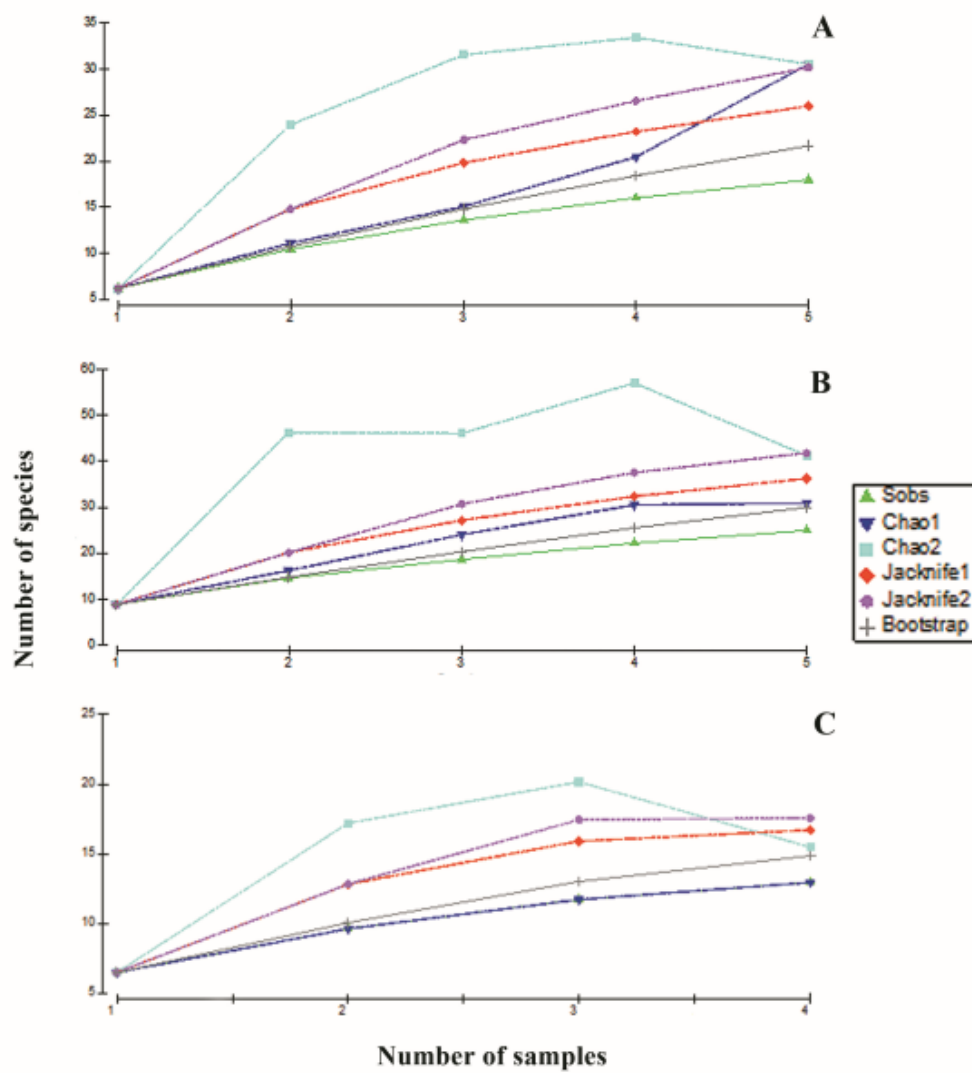


Figure 3. Completeness of species inventory determined by different richness estimators at the Taim nature reserve (ESEC Taim). A, Estimations for lake Nicola (S1); B, Estimations for the water channel (S2); C, Estimations for lake Mangueira (S3).

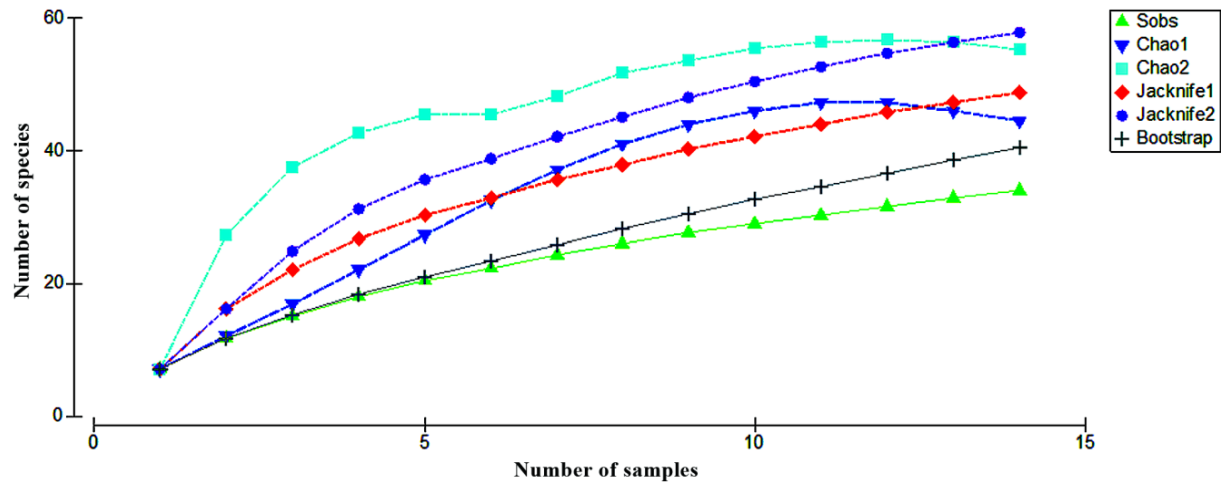


Figure 4. Completeness of species inventory determine by different richness estimators (using lumped samples) at the Taim nature reserve (ESEC Taim).

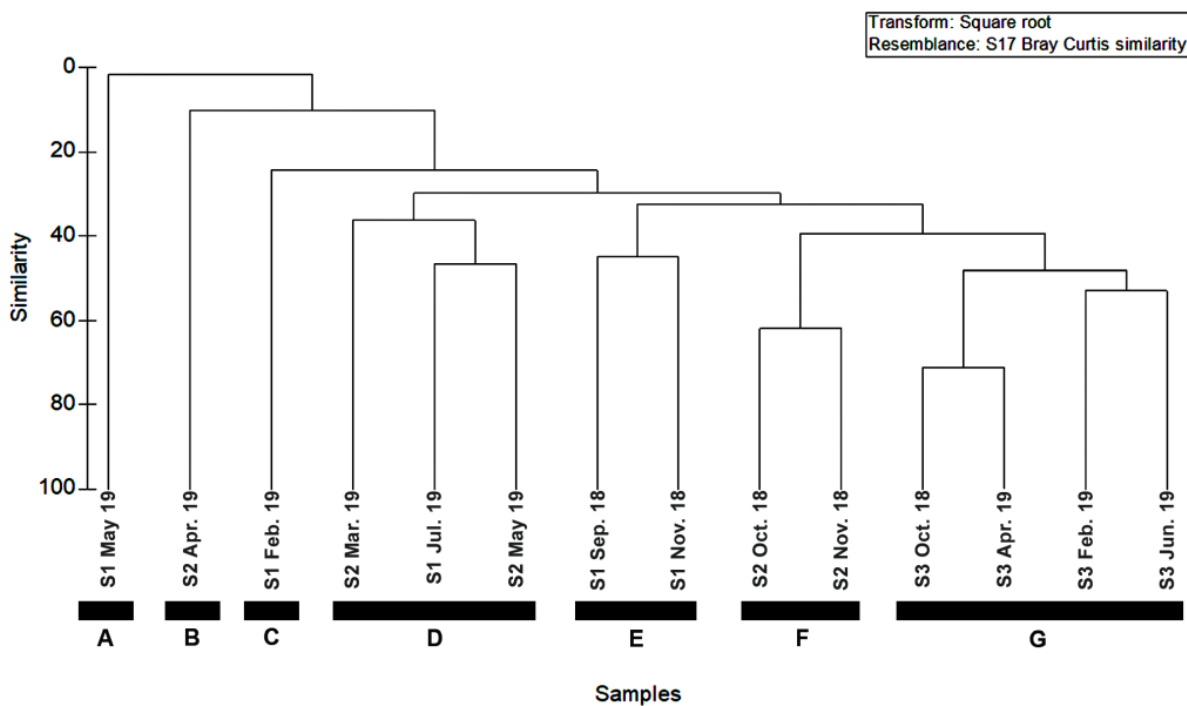


Figure 5. Cluster diagram by group-average clustering based on the Bray-Curtis similarity index using the abundance of species.

Tables captions

Table 1. Turbellaria total abundance and density in three floating vegetation microhabitats (S1, S2 and S3) of the ESEC Taim.

Species	Abundance			Density (indiv./m2)		
	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3
Catenulida						
<i>Catenula lemnae</i>	29	0	8	7.7 ± 14.4	0	2.7 ± 3.3
<i>Stenostomum grande</i>	11	80	3	2.9 ± 3.3	21.3 ± 36.4	1 ± 1.3
<i>Stenostomum cryptops</i>	0	1	0	0	0.3 ± 0.6	0
<i>Stenostomum cf. pegephilum</i>	0	1	0	0	0.3 ± 0.6	0
<i>Stenostomum saliens</i>	0	5	0	0	1.3 ± 3	0
<i>Stenostomum paraguayense</i>	0	3	0	0	0.8 ± 1.2	0
<i>Stenostomum tuberculosum</i>	0	2	0	0	0.5 ± 0.7	0
<i>Stenostomum glandulosum</i>	0	6	0	0	1.6 ± 3.6	0
<i>Stenostomum sp. 1</i>	17	136	30	4.5 ± 5.6	36.3 ± 39.5	10 ± 13
<i>Stenostomum sp. 2</i>	0	2	0	0	0.5 ± 0.7	0
<i>Stenostomum sp. 3</i>	0	2	0	0	0.5 ± 1.2	0
<i>Myostenostomum vanderlandi</i>	2	1	0	0.5 ± 0.7	0.3 ± 0.6	0
Macrostomorpha						
<i>Macrostomum quiritium</i>	9	0	8	2.4 ± 5.37	0	2.7 ± 2.9
<i>Macrostomum sp. 1</i>	1	1	0	0.3 ± 0.6	0.3 ± 0.6	0
<i>Macrostomum sp. 2</i>	9	2	15	2.4 ± 5.4	0.5 ± 0.7	5 ± 6.9
<i>Macrostomum tuba</i>	1	0	0	0.3 ± 0.6	0	0
<i>Microstomum sp. 1</i>	1	0	0	0.3 ± 0.6	0	0
Tricladida						
<i>Girardia sp. 1</i>	17	196	392	4.5 ± 7.3	52.3 ± 69	130.7 ± 90.9
Dalytyphloplanida						
<i>Gieysztoria falx</i>	18	36	0	4.8 ± 6.6	9.6 ± 15.2	0
<i>Gieysztoria evelinae</i>	0	0	1	0	0	0.3 ± 0.7
<i>Gieysztoria hymanae</i>	0	0	7	0	0	2.3 ± 4.7
<i>Gieysztoria sp. 1</i>	3	0	0	0.8 ± 1.8	0	0
<i>Gieysztoria sp. 2</i>	0	15	5	0	4 ± 8.9	1.7 ± 2
<i>Gieysztoria sp. 3</i>	1	0	0	0.3 ± 0.6	0	0
<i>Gieysztoria sp. 4</i>	5	60	11	1.3 ± 2.3	16 ± 20.2	3.7 ± 7.3
<i>Mesostoma ehrenbergii</i>	2	2	0	0.5 ± 1.2	0.5 ± 1.2	0
<i>Mesostoma sp. 1</i>	0	7	0	0	1.9 ± 4.2	0

<i>Mesostoma</i> sp. 2	0	2	0	0	0.5 ± 1.2	0
<i>Strongylostoma elongatum</i>	5	0	1	1.3 ± 3	0	0.3 ± 0.7
<i>Strongylostoma</i> sp. 1	1	15	1	0.3 ± 0.6	4 ± 4.6	0.3 ± 0.7
<i>Bothromesostoma evelinae</i>	0	1	0	0	0.3 ± 0.6	0
Kalyptorhynchia						
<i>Gyratrix hermaphroditus</i>	3	22	7	0.8 ± 1.2	5.9 ± 11	2.3 ± 3.2
<i>Gyratrix</i> sp. 1	0	1	0	0	0.3 ± 0.6	0
Prorhynchida						
<i>Prorhynchus stagnalis</i>	0	2	0	0	0.5 ± 1.2	0

Table 2. Number of species expected and percentages of inventory completeness according to different richness estimators at the three sampling sites (S1, S2 and S3) and lumped samples (ESEC Taim).

	S1	S1 (%)	S2	S2 (%)	S3	S3 (%)	ESEC Taim	ESEC Taim (%)
Cumulative alfa diversity	18		25		13		34	
Chao 1	31	59	31	81	13	100	45	76
Chao 2	31	59	41	60	16	84	55	61
Jackknife 1	26	69	36	69	17	78	49	70
Jackknife 2	30	60	42	60	18	74	58	59
Bootstrap	22	83	30	83	15	87	40	84
Average of inventory completeness		66		71		85		70

Table 3. Summary results of a GLM - multiple regression analysis, testing the relationship between richness and density of freshwater turbellarians as a function of temperature and pH (physical variables).

Physical variables	Richness					Density				
	r2	Df	MS	F	p	r2	Df	MS	F	p
Ph	5.057	1	5.057	0.241	0.634	140.900	1	140.900	0.010	0.922
Temperature	16.805	1	16.805	0.801	0.392	29489.639	1	29489.639	2.116	0.176
Error	209.846	10	20.985			139376.223	10	13937.622		

Table 4. Percentage contribution of typifying taxa to within-group similarity for the identified turbellarian assemblages by cluster analysis (cut off for low contributions: 90%). The first two species with the greatest contribution are shown.

Group D		
Average similarity: 39.74	Average Abundance	Contribution (%)
Species		
<i>Stenostomum</i> sp. 1	3.35	42.29
<i>Girardia</i> sp. 1	1.76	24.12
Group E		
Average similarity: 45.02		
Species		
<i>Gieysztorina falx</i>	3.46	41.56
<i>Catenula lemnae</i>	1.57	14.61
Group F		
Average similarity: 61.86		
Species		
<i>Girardia</i> sp. 1	11.27	32.6
<i>Stenostomum</i> sp. 1	8.7	23.18
Group G		
Average similarity: 52.70		
Species		
<i>Girardia</i> sp. 1	10.85	70.32
<i>Stenostomum</i> sp. 1	2.72	13.43

Table 5. Dissimilarity values among clusters of sampling campaigns (Bray-Curtis coefficient was used as a measure of similarity). The first three species with the greatest contribution are shown.

Pairwise comparison	SIMPER		
	Average dissimilarity (%)	Species	Contribution (%)
D-F	72.3	<i>Girardia</i> sp. 1	18.81
		<i>Stenostomum</i> sp. 1	10.69
		<i>Gieysztoria falx</i>	9.26
D-G	68.1	<i>Girardia</i> sp. 1	35.28
		<i>Gieysztoria</i> sp. 4	8.55
		<i>Macrostomum</i> sp. 2	6.45
E-F	65.5	<i>Girardia</i> sp. 1	19.85
		<i>Stenostomum</i> sp. 1	13.8
		<i>Stenostomum grande</i>	12.42
E-G	68.6	<i>Girardia</i> sp. 1	32.82
		<i>Gieysztoria falx</i>	12.86
		<i>Gieysztoria</i> sp. 4	6.63
E-D	72.1	<i>Gieysztoria falx</i>	14.16
		<i>Gieysztoria</i> sp. 4	10.9
		<i>Girardia</i> sp. 1	8.56
F-G	60.6	<i>Stenostomum</i> sp. 1	13.46
		<i>Stenostomum grande</i>	12.77
		<i>Gieysztoria falx</i>	10.29

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CAPÍTULO 3

INTERSPECIFIC INTERACTIONS IN POPULATIONS OF *TEMNOCEPHALA* SPP. (RHABDOCOELA: TEMNOCEPHALIDAE), ECTOSYMBIONT OF *POMACEA CANALICULATA* (CAENOCASTROPODA: AMPULLARIIDAE)

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ABSTRACT

Platyhelminthes are known parasiting invertebrates among other animals. However, a group of Platyhelminthes, such as *Temnocephala*, have developed an intimate ectosymbiotic relationship with the freshwater snails *Pomacea canaliculata* to such an extent that they need this snail to be able to develop their complete lifecycle; Nonetheless, the ecological parameters of these ectosymbionts are not well understood. Thus, we describe the ecological relationships between snails and *Temnocephala* these in freshwater wetlands. We analysed the size (shell) and sex of the host against various population parameters of two co-occurrent *Temnocephala* species (*T. rochensis* and *T. iheringi*). We found high prevalence of both species of ectosymbionts, *T. rochensis* is predominant over *T. iheringi* in most of the population parameters (abundance, intensity of infection and mean intensity). We also observed that population parameters were positively related to host size, but not to the sex of the host. These findings allow us to raise new hypotheses about the behaviour of co-occurrent species of *Temnocephala* in mollusc hosts.

Keywords:

Platyhelminthes, ectosymbiosis, apple snail, population dynamics, wetlands

INTRODUCTION

Flatworms (Platyhelminthes) are usually known for parasiting a variety of vertebrate hosts including human beings. However, there are some free-living groups of Platyhelminthes, including a clade which presents a symbiotic habit with other animals. Particularly, species from the genus *Temnocephala* Blanchard, 1849 are commensal symbionts Platyhelminthes, this is, these animals are strictly associated to its host, they do not benefit from its host, but from the host's attributes. *Temnocephala* have been found living in association with both invertebrates (Decapoda, Insecta, Gastropoda) and vertebrates (Chelonia) (Martínez-Aquino et al., 2014) hosts in freshwater ecosystems. These worms inhabit the internal and external cavities of the host's body, sheltered by rigid structures where they lay their eggs.

The taxonomy, systematics and phylogeography of Temnocephalida have been well studied in the neotropics (Damborenea & Cannon, 2001; Martinez-Aquino et al., 2014; Braccini et al., 2016; Martínez-Aquino et al., 2017). On the other hand, the ecological studies on the association of these ectosymbiotic flatworms with their hosts are scarce (Zivano et al., (2020) and references therein). Studies have evidenced that in the neotropics, *Temnocephala* spp.

dwelling on the paleal mantle cavity of the apple snail *Pomacea canaliculata* (Lamarck, 1822) (Seixas et al., 2010a, 2010b, 2010c), which is an aquatic gastropod widely distributed in tropical and subtropical areas (Martín et al., 2005; Hayes et al., 2012). Nevertheless, the ecological relationships between *P. canaliculata* and its ectosymbionts (e.g., *Temnocephala iheringi* Haswell, 1893; *T. rochensis* Ponce de Leon, 1979 and *T. haswelli* Ponce de Leon, 1989) are still poorly understood. Damborenea (1996, 1998) analysed the population prevalence, abundance and distribution of *T. iheringi* and found that the snail's size was positively related to *T. iheringi* abundance, generally in warmer months. Damborenea et al. (2006) have found that the abundance of *T. iheringi* was different according to the sampling site despite the high prevalence of the temnocephalids in almost all sampling sites. They also observed no differences in host sexes on *T. iheringi* distribution, as well as a positive correlation between abundance and host size. Martín et al. (2005) found that the prevalence of *T. iheringi* was high. In regard of fecundity, the number of eggs laid by *T. iheringi* on its host was different between distinct snail populations, but not between sexes. Besides, higher number of eggs were found in snails inhabiting lentic environments (Martín et al., 2005).

There are even less ecological studies on other co-occurring temnocephalids species that live on the same host. Seixas et al., (2010b, 2010c) have found *T. rochensis* and *T. haswelli* dwelling on *P. canaliculata*, but studies on the ecological relationships of the infrapopulations co-occurring in the same host populations have not been performed. In this sense, the present study focuses on filling knowledge gaps on the behavior and use of resources of co-occurring commensal symbiont species and their interactions with their host in freshwater environments ecosystems inside a nature reserve (ESEC Taim). Thus, the objective of this study was to determine the relationships between the population parameters of the co-occurring commensal symbionts (*Temnocephala* spp.) and the characteristics of their host (*P. canaliculata*).

MATERIALS AND METHODS

Research area

The study was developed in the Taim nature reserve (ESEC Taim). This area is placed in the Coastal Plain of Rio Grande do Sul state, at the southernmost extreme of Brazil (32°50' S, 52° 26' W). The ESEC Taim is a set of channels, shallow ponds and lakes (Bager et al., 2010). Three sampling sites were taken in representative ecosystems along the reserve in order to

record the largest number of snails. The sampling sites were Nicola lake (Site 1), an artificial water channel (Site 2), and Mangueira lake (Site 3) (Fig. 1).

Sample collection and preparation

In the present study, the apple snail *Pomacea canaliculata* was used as the host model, and the commensal symbiont species *T. rochensis* and *T. iheringi* were used for studying the ecological patterns involving infrapopulations of co-occurring species of symbionts in a single host specimen. Sampling permits were granted by the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio), permits number 60291-2/2017 and 64551-1/2018. Seven sampling campaigns were performed along one year, visiting each sampling site at least twice. Specimens of *P. canaliculata* were collected from floating and submersed vegetation (1.5 m depth) by dragging a net (mesh size 500 μ m) through the aquatic vegetation during 3 min, in triplicate. Specimens were transferred to labelled buckets with water from the sampled environment and transported alive to the laboratory.

In the laboratory, the total length of the shell (from the apex to the basal extreme of the aperture) of the snail was measured (Damborenea, 1996) with a digital caliper. Afterwards, each snail was cryoanesthetized, dissected and sexed as follows: with penis (males), with coloured albumen gland (females); snails lacking these structures were considered juveniles with undetermined sex. The mantle cavity was exhaustively examined under the stereoscope in order to obtain temnocephalids. Similarly, the sutural line on the shell was searched and the laid eggs over the structure were collected. Eggs were not differentiated from the co-occurring species because of the similarity between them. After collecting the temnocephalids, they were observed alive under the optical microscope and identified as *T. rochensis* and *T. iheringi* (Fig. 2) (Damborenea, 1992; Damborenea et al., 1997; Seixas et al., 2010a, 2010b, 2010c; Martinez-Aquino et al., 2014). Both live animals and eggs were counted to determine their abundance.

Data analyses

Mean abundance, prevalence and intensity were calculated according to Bush et al., (1997). Linear regressions were performed to determine the relationship between number of eggs and the length of the shell. Analysis of covariance (ANCOVA) was used to check the intensity of the co-occurring species in relation to the length of the shell (covariate).

Besides, according to Damborenea (1996), the shell length was categorized within nine size classes with 5 mm interval each. An analysis of variance (two-way ANOVA) was used to compare the mean abundance of the co-occurring species throughout the snails' size classes.

"Co-occurring" (with two levels: *T. iheringi* and *T. rochensis*) and "size classes" (with nine levels: size class from 5-10 mm to >45 mm) were used as fixed factors. Significant differences were analysed by Tukey's test ($p < 0.05$).

The difference in the length of the shell between male and female snails were checked by a *t*-Student test. Posteriorly, the data were log-transformed ($\log_{10}(x+1)$) to attend the statistical criteria for parametric analysis, and whether temnocephalids prefer male or female snails, was determined using a two-way ANOVA with fixed factors, "sex" (with two levels: male and female) and "co-occurring species" (with two levels: *T. iheringi* and *T. rochensis*). All data were expressed as: minimum value – maximum value (mean ($\bar{x}$) $\pm$ standard deviation (sd)); "n"), unless described otherwise.

RESULTS

A total of 81 specimens of *P. canaliculata* with shell lengths from 9 to 51.9 mm (mean size = 27.3 ± 11 mm) were collected, of which 35 were females, 30 males and 16 undifferentiated juveniles (Table 1). There was no significant difference between male and female sizes (*t*-Student test: $t = -0.04$, $df = 63$, $p = 0.97$). The number of eggs varied from 0 to 458 (mean = 100.4 ± 112.3 , $n = 81$), distributed over 71 shells. The number of eggs increased according to the length of the shell ($R^2 = 0.51$) (Fig. 3). Sites 1, 2 and 3 showed a mean abundance of eggs of 175, 60 and 15.5 eggs per snail in shell sizes of 9 – 51.9 mm (mean = 28.9 ± 12.6 , $n = 40$), 14.8 – 44 mm (mean = 32.9 ± 6.4 , $n = 21$) and 12.5 – 26.5 mm (mean = 18.1 ± 3.7 , $n = 20$), respectively.

A total of 2557 temnocephalids were recorded in 79 hosts; 1775 were identified as *T. rochensis*, and 782 were *T. iheringi* (Fig. 2). All examined hosts showed that the prevalence of *T. rochensis* was 98 %, while the prevalence of *T. iheringi* was 80 %. Thus, the prevalence of *T. rochensis* was a 22.5 % higher than of *T. iheringi*. The intensity of both *Temnocephala* species showed a positive relation with the length of the shell of the host (Fig. 4). The intensity of *T. rochensis* was greater than that of *T. iheringi* (ANCOVA: $F = 18.81$, $p < 0.05$). Also, when analysed the mean abundance, the two-way ANOVA supported these results (Fig. 5 and Table 2). Hosts with the length of the shell smaller than 20 mm showed 237 individuals of *Temnocephala* being 80.6 % *T. rochensis* and 19.4 % *T. iheringi*; hosts presented a mean abundance of 7.1 individuals of *T. rochensis* and 1.7 of *T. iheringi* per snail. Hosts from 20 to 30 mm in shell length showed 539 individuals of *Temnocephala* being 70.7 % *T. rochensis* and 29.3 % *T. iheringi*; host presented a mean abundance of 20.1 individuals of *T. rochensis*

and 8.3 of *T. iheringi* per snail. Finally, hosts larger than 30 mm in the length of the shell showed 1781 individuals of *Temnocephala* being 67.5 % *T. rochensis* and 32.5 % of *T. iheringi*; hosts presented a mean abundance of 34.4 individuals of *T. rochensis* and 16.5 of *T. iheringi* per snail.

Considering all 81 specimens of *P. canaliculata* captured, 79 (97.5%) were infested by specimens belonging to *Temnocephala*. The prevalence of *T. rochensis* was 100 % in males and females, and 87.5 % in juveniles of *P. canaliculata*, corresponding to 655, 1048 and 72 individuals of *T. rochensis* infesting males, females and juveniles of *P. canaliculata*, respectively. For *T. iheringi*, the prevalence was 90 %, 88.6 % and 43.8 % in males, females and juveniles of *P. canaliculata*, respectively. In this sense, 342, 430 and 10 individuals of *T. iheringi* infested males, females and juveniles of *P. canaliculata*, respectively. No significant differences were found in mean abundance of both species of *Temnocephala* between male and female snails, but this parameter was higher in *T. rochensis* compared to *T. iheringi* in all hosts, regardless of the sex of the snail (Fig. 6 and Table 2).

DISCUSSION

The genus *Temnocephala* is endemic from the Neotropics (Martinez-Aquino et al., 2014). As commensal symbiotic organisms, they developed a close and intimate relationship with their host; species diversify according to host evolution, anatomy and life habits (Damborenea et al., 2017; Martínez-Aquino et al., 2017; Zivano et al., 2020). Among the hosts, species of *Temnocephala* (*T. rochensis*, *T. iheringi* and *T. haswelli*) have evolved in a close relationship with the gastropod mollusc *P. canaliculata* (Seixas et al., 2010a, 2010b, 2010c), inhabiting their mantle cavity where they find food items carried by the currents generated by the snail breathing (Damborenea et al., 2017). Co-occurring species of *Temnocephala* infesting the same specimen of *P. canaliculata* have been reported (Seixas et al., 2010a), but to the extent of our knowledge, the present study is the first to report relationships between commensal symbiotic infrapopulations of *Temnocephala* spp.

In regard to the abundance of eggs over the host shell suture lines, a positive relationship between the host size and number of eggs was observed. Similar findings were made by Damborenea (1996) (in warmer months). The species identification through the analysis of eggs is challenging as eggs from *T. rochensis* and *T. iheringi* are very similar (Seixas et al., 2010a), so they were not differentiated in the present study. Martín et al. (2005) found more

eggs laid on the shell of snail populations living in lentic habitats than those from lotic environments, but no relationship between host size and eggs was explored. Interestingly, high mean abundance of eggs and higher mean shell size were observed in hosts of sampling sites 1 (shallow lake) and 2 (artificial water channel), while site 3 (a relatively deep lake) had the lowest mean abundance of eggs and mean shell size. Our findings might indicate contradictions to the evidence found by Martín et al. (2005) regarding egg infestation in lentic and lotic environments. However, in the absence of more lotic or lentic environments analysed in the present study, it can be said that host size (maturity) influence egg infestation (Damborenea, 1996) along with other environmental factors beyond the objectives of this study.

High abundance and prevalence of *T. iheringi* was positively related to host size, in which snails larger than 30 mm had the highest level of infestation. Similar results were previously observed in other limnic environments in Southern Brazil (Damborenea, 1996, 1998; Damborenea et al., 1997; Martín et al., 2005; Damborenea et al., 2006). According to Damborenea (1996), bigger hosts (shell length > 40 mm) present higher mean abundance of temnocephalids due to the activity and characteristic of adult snails (copulation and available space in mantle cavity).

For *T. rochensis*, the prevalence was also size-dependent and higher than that for *T. iheringi*. As this is a pioneer study concerning ecological patterns of *T. rochensis* inhabiting *P. canaliculata*, there are no previous studies for comparison of the results. However, it seems that the prevalence and mean abundance of *T. rochensis* follow the patterns shown by other species of temnocephalid (i.e. *T. iheringi*) (Damborenea, 1996, 1998; Damborenea et al., 1997; Damborenea et al., 2006; Martín et al., 2005).

The predominance of *T. rochensis* over *T. iheringi* becomes clear when infrapopulation parameters such as intensity and mean abundance are examined. These parameters tend to be positively related to host size, with a predominance of *T. rochensis* over *T. iheringi* observed in all size classes. Although there are several species of metazoarians symbionts associated to *P. canaliculata* living in its mantle cavity (Damborenea et al., 2017), species with analogous behaviour to *Temnocephala* spp. (in the same host species) such as *Helobdella* spp., have been reported with high richness but low abundance; interactions among six species of leeches in the mantle cavity revealed a marked dominance pattern of a single species (*H. ampullariae*) over others from the same genus (Damborenea & Gullo, 1996). Therefore, it is

likely that co-occurring species of *Temnocephala* follow similar population patterns in the same environment (mantle cavity).

In this sense, it is important to note that factors other than the host size may influence the predominance of *T. rochensis* over *T. iheringi*, such as availability of food items, availability of space on the mantle cavity and host aggregation (possibility to exchange ectosymbionts) (Damborenea, 1996; Damborenea et al., 2017). Thus, species of *Temnocephala* might compete for resources on the mantle cavity, inhibiting competing sibling species or interacting with other symbionts in the host. Damborenea et al., (2006) examined a set of commensals and parasitic species of Platyhelminthes inhabiting *P. canaliculata* and found that the composition of symbionts can characterize hosts in different environments, being the abundance of temnocephalids correlated with sex and size of the host. The present study corroborates the importance of the host size, but the results showed that no preference of any species of *Temnocephala* for male or female snails was found and therefore the host sex was not a determinant factor when it refers to establish the predominance of one species of *Temnocephala* over another.

Finally, freshwater environments within the ESEC Taim undergo a marked hydroperiod (Vieira, 1984; Marques et al., 2013) and variations in the water level generate stress in the snails leading to unfavorable conditions for the settlement of their symbionts (burial, aestivation, etc.) (Damborenea, 1996; Damborenea & Gullo, 1996; Hayes et al., 2015). In this sense, the processes by which species of *Temnocephala* maintain their populations and share/compete for resources throughout the life cycle of their mollusc hosts might be driven by the favorable conditions of the environment (warmer months) as it has been reported for other invertebrate symbionts (Damborenea, 1996; Damborenea & Gullo, 1996). However, the discovery of two species of *Temnocephala* in specimens of *P. canaliculata*, gives rise to the hypothesis whether there is a preferential use of resources (e.g. food items) in the snail or if there are areas of preference in the mantle cavity.

In summary, determining the ecological relationships between commensal symbionts (*Temnocephala* spp.) and their hosts (*P. canaliculata*) allows us to elucidate the intricate trophic links that occur in aquatic environments of the ESEC Taim. In this sense, interesting associative processes between these organisms are shown, they can be used as models for studies in other groups of invertebrate commensal symbionts in mollusc hosts in coastal aquatic environments. Thus, the importance of the conservation of wetlands within and around the ESEC Taim is reiterated.

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Figure captions

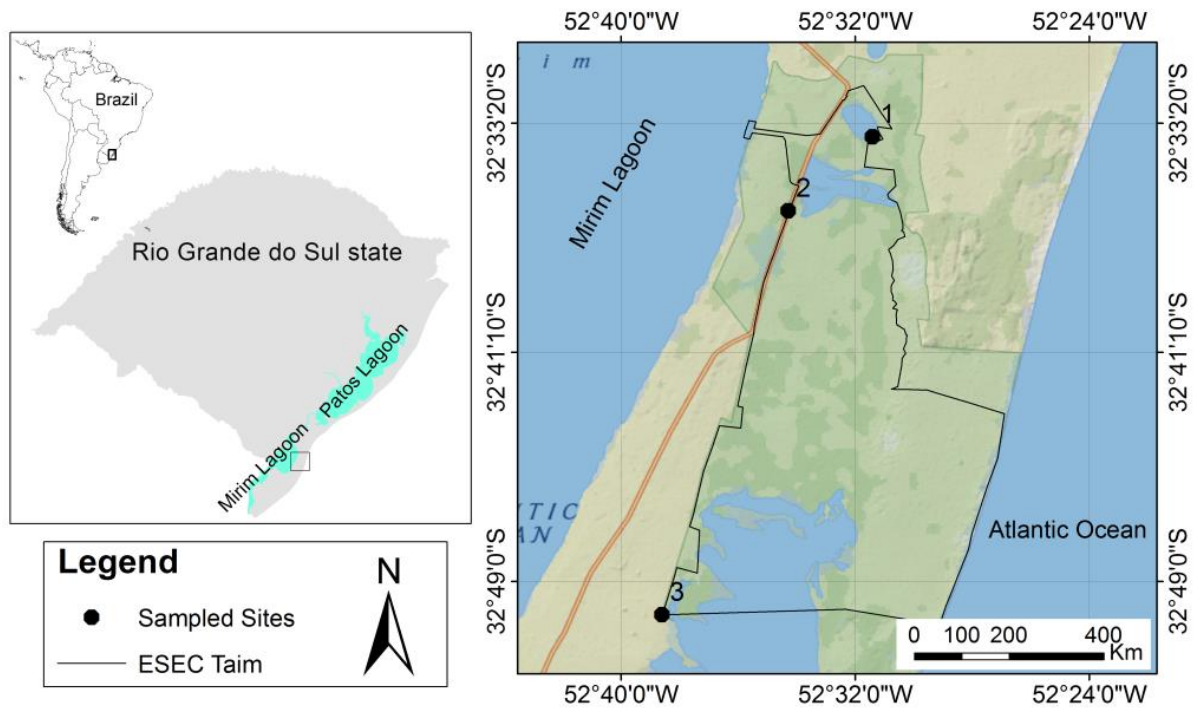


Figure 1. Sampled sites at the Taim nature reserve (ESEC Taim): Nicola lake (Site 1), an artificial water channel (Site 2) and Mangueira lake (Site 3).

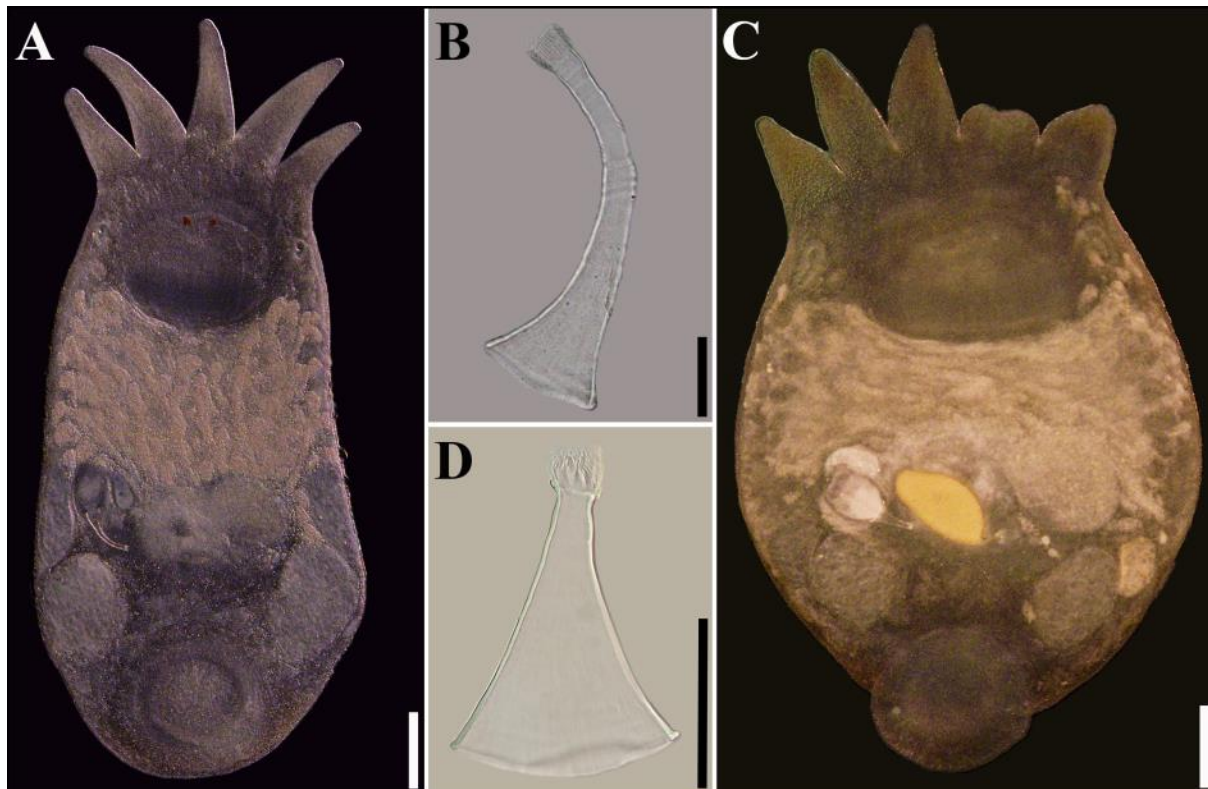


Figure 2. Photographs of live specimens of the co-occurring species of *Temnocephala* infesting *P. canaliculata*. *Temnocephala rochensis* (A) Whole-mounted individual observed on dark field, (B) male copulatory apparatus (cirrus) observed on Hoyer's medium. *Temnocephala iheringi* (C) Whole-mounted individual observed on dark field, (D) cirrus observed on Hoyer's medium. Scales bars: A and C = 200 μ m, B and D = 50 μ m.

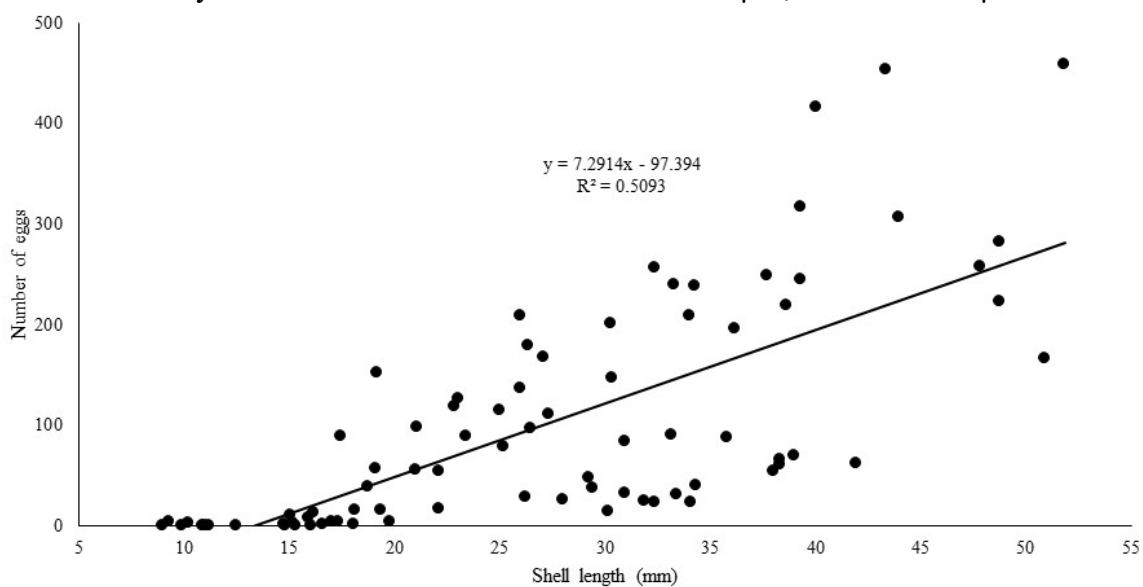


Figure 3. Laid eggs of *Temnocephala* spp. as a function of the size of the host snail *Pomacea canaliculata*.

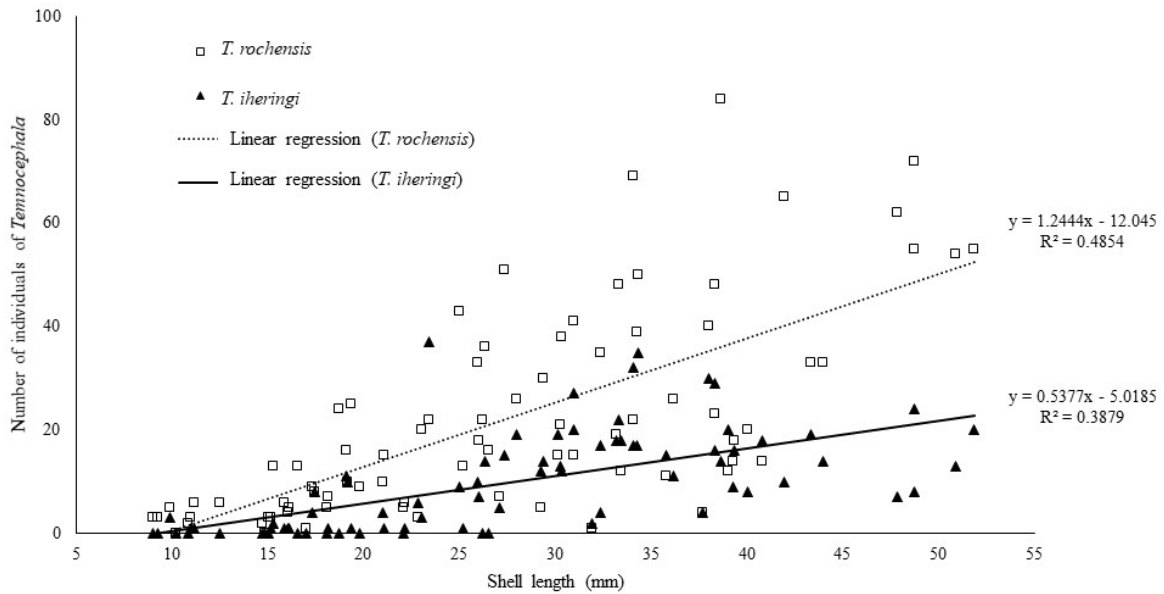


Figure 4. Intensity of both *T. rochensis* and *T. iheringi* with respect to the shell length of *Pomacea canaliculata*.

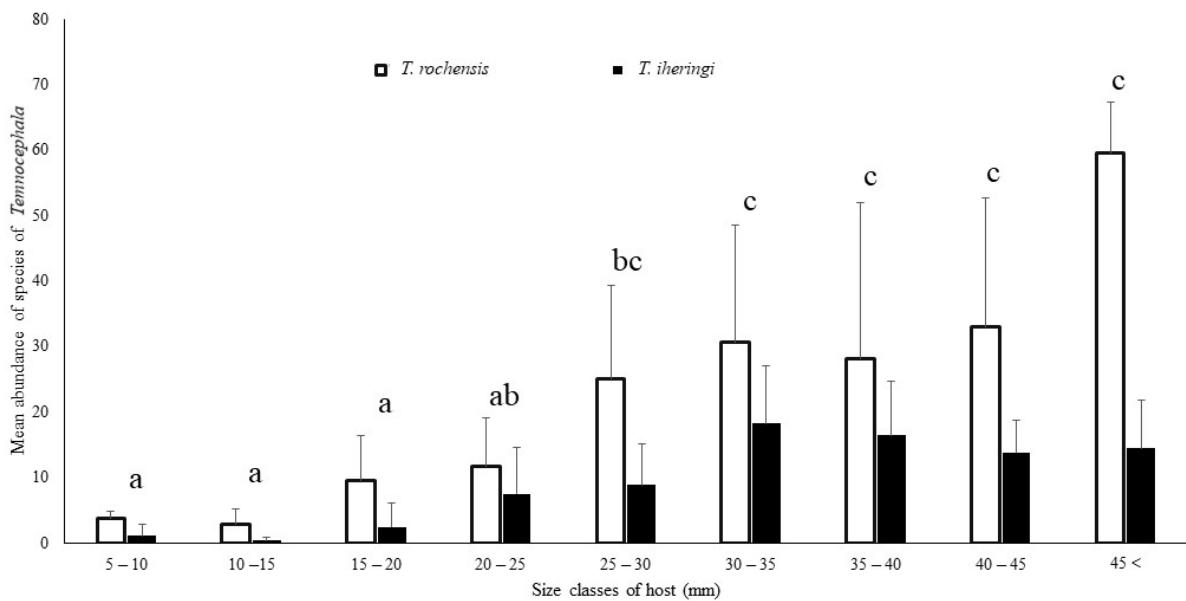


Figure 5. Mean abundance of *Temnocephala rochensis* and *T. iheringi* across the nine size classes of the host *Pomacea canaliculata*. Bars with the same letters are not significantly different (two-way ANOVA, Tukey's Honestly Significant Difference post hoc test, $n=9$, $p < 0.05$).

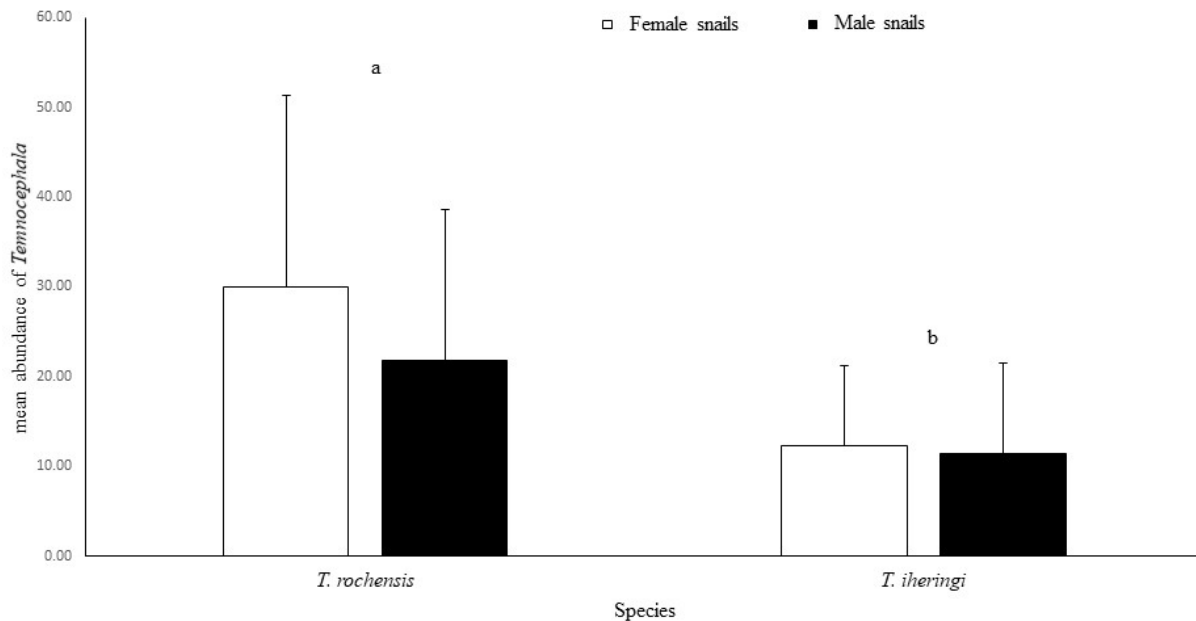


Figure 6. Mean abundance *Temnocephala rochensis* and *T. iheringi* in males and females individuals of *Pomacea canaliculata* at the ESEC Taim (white, female; black, male snails). Bars with different letters are significantly different.

Table captions

Table 1. Number ("n"), total length with minimum and maximum value (mean $\pm$ sd) of males, females and undifferentiated juveniles of the snail *Pomacea canaliculata* from the ESEC Taim.

Sex of the snail	"n"	Total length
Male	30	16.1 - 43.4 (30.9 $\pm$ 8.3)
Female	35	17.3 - 51.9 (30.8 $\pm$ 10.2)
Juvenil	16	9 - 16.6 (13 $\pm$ 2.7)

Table 2. Summary of the two-way ANOVA comparing A: the host size classes and the co-occurring species, and B: sex of the host between the co-occurring species. The host size classes is a factor with nine levels: from 5-10 mm to >45 mm. The co-occurring species is a factor with two levels: *T. rochensis* and *T. iheringi*. The sex of the host is a factor with two levels: male and female.

		df	MS	F	p-value
A	Size classes	8	2.6928	25.207	0.0000
	Co-occurring species	1	4.9224	46.077	0.0000
	Host size vs Co-occurring species	8	0.1450	1.357	0.2203
	Error	144	0.1068		
B	Sex of the host	1	0.5176	2.950	0.0883

Co-occurring species	1	4.3654	24.879	0.0000
Sex of the host vs Co-occurring species	1	0.0336	0.191	0.6625
Error	126	0.1755		

GUIDE FOR AUTHORS (*JOURNAL OF HELMINTHOLOGY*)

Scope

Journal of Helminthology publishes original papers, review articles and short communications on all aspects of pure and applied helminthology, particularly those helminth parasites of environmental health, medical or veterinary importance. Research papers on helminths in wildlife hosts, including plant and insect parasites, are also published along with taxonomic papers contributing to the systematics of a group. The journal will be of interest to academics and researchers involved in the fields of human and veterinary parasitology, public health, microbiology, ecology, epidemiology and biochemistry.

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The Editor welcomes original, creative, high-quality contributions suitable for the journal's international readership. The novelty and importance of submitted papers should be made clear in the Introduction.

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Subheadings: typed in italic (except for those words and symbols which would be italicized in the text), e.g. 'Sample collection'.

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Book

Bailey, N.T.J. (1995) *Statistical methods in biology*. 3rd edn. 255 pp. Cambridge, Cambridge University Press.

Chapter in a book

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Use an initial capital in column headings and row headings. Type the table number and a short title at the top of the table.

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CONSIDERAÇÕES FINAIS E PERSPECTIVAS

A grande diversidade de ambientes aquáticos, que são encontrados dentro dos limites da ESEC Taim, fornece habitats apropriados para o desenvolvimento de diversas espécies de Platyhelminthes de vida livre e simbioses (“turbelários”). Neste sentido, a fauna dos turbelários de vida livre da ESEC Taim foi registrada revelando a presença de 23 taxa identificados ao nível específico, sendo seis espécies encontradas pela primeira vez no Brasil: três espécies (*Myostenostomum vanderlandi*, *Macrostomum quirritium* e *Strongylostoma elongatum*) registradas pela primeira vez no país; e três espécies novas para a ciência (*Macrostomum* **n. sp. 1**, *Gieysztoria* **n. sp. 1** e *Gieysztoria* **n. sp. 2**). Desta forma, o conhecimento das espécies de turbelários é aumentado, sendo atualmente 115 espécies formalmente descritas para o Brasil, considerando a presente contribuição.

Em relação às comunidades de turbelários na ESEC Taim, a riqueza média, abundância e densidade, não apresentaram diferenças significativas entre os ambientes estudados. Nenhuma variável ambiental teve influência na riqueza ou abundância. O ambiente canal de água foi o que apresentou maior riqueza (observada e estimada), seguido da lagoa Nicola e da lagoa Mangueira. Além disso, e de acordo com a comunidade de turbelários, a lagoa Nicola e o canal de água são relativamente semelhantes, se diferenciando da lagoa Mangueira.

Duas espécies de turbelários simbioses (*Temnocephala rochensis* e *T. iheringi*) foram reportadas pela primeira vez infestando a mesma população de caramujos *Pomacea canaliculata*. Se encontrou que a infestação dos simbioses aumenta à medida que o comprimento da concha do caramujo aumenta. A espécie *T. rochensis* foi dominante sobre *T. iheringi*.

O estudo da diversidade dos turbelários gera maior conhecimento sobre os invertebrados de corpo macio que usualmente não são estudados nos monitoramentos da fauna aquática. Assim, valoriza-se o estado da área protegida. Neste sentido, um contínuo monitoramento desta fauna é necessário para entender como as comunidades de turbelários se relacionam com os ecossistemas dentro da reserva e, inclusive, determinar a presença de potenciais novas espécies. Além disso, o resultado inédito da presença de duas espécies de *Temnocephala* sobre a cavidade paleal do mesmo hospedeiro gera intrigantes perguntas: como é que as duas espécies compartilham (ou competem) o espaço da cavidade paleal nos caramujos? Existem sítios específicos de infestação quando duas espécies infestam uma mesma população de hospedeiros? Quais são as condições necessárias para que duas espécies infestem uma mesma população de caramujos? E desta

maneira, estimula a continuidade de estudos sobre as relações entre espécies destes grupos entre si e com seu hospedeiro.

Uma vantagem para considerar é que os ambientes alagados dentro da ESEC Taim estão localizados relativamente perto da FURG o que faz que a coleta das amostras e o posterior processamento no laboratório dos espécimes seja realizado apropriadamente, podendo observar os espécimes vivos por mais tempo. Neste sentido, o aproveitamento deste atributo torna a ESEC Taim em uma reserva protegida ideal para o desenvolvimento de estudos de longo prazo sobre Platyhelminthes tanto de vida livre quanto simbioses comensais.

ANEXO

Artigo publicado em qualidade de “**Correspondence**” na revista **Zootaxa**, como parte do Capítulo 1.

First report of *Gieysztoria falx* Brusa, Damborenea & Noreña, 2003 (Platyhelminthes: Rhabdocoela) in southern Brazil

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Within Dalyelliidae Graff, 1905, *Gieysztoria* Ruebush & Hayes, 1939 is the most widely distributed and speciose genus, members of which live in marine, brackish, or freshwater habitats (Van Steenkiste *et al.*, 2012). *Gieysztoria* is composed by ~97 free-living species (Tyler *et al.* 2016), and species identification is mainly made on the male copulatory system, which has an armed penis (stylet) with different configurations of spines (Noreña *et al.*, 2016). The stylet configuration of *Gieysztoria* species is traditionally split into two groups: *Aequales* (spines of similar shape and size) and *Inaequales* (spines of different shape and size) (Luther, 1955). In the neotropics, specimens of *Gieysztoria* belong to both the *Aequales* and *Inaequales* group and are well represented (Damborenea *et al.*, 2005; Noreña *et al.*, 2003). According to Braccini *et al.* (2016, 2017), there are 16 species of *Gieysztoria* in Brazil, mainly distributed in the southern region. South Brazil harbours a mosaic of wetlands that are considered hot spots of biodiversity, including the Taim Ecological Station (ESEC Taim) protected area that was designated as a Ramsar site (Ramsar, 2017). In the present study, *Gieysztoria falx* Brusa, Damborenea & Noreña, 2003 is registered in the ESEC Taim, located in Rio Grande do Sul. It represents the first record of *G. falx* in Brazil. Illustrations and comments on stylet configuration are given.

Samples were taken monthly from 2018.09.19 to 2018.11.30, in three sampling sites (32° 33' 18.07" S, 52° 31' 18.50" W; 32° 35' 50.52" S, 52° 34' 5.40" W; 32° 50' 11.42" S, 52° 38' 37.45" W) that comprises of freshwater ponds and channels in the ESEC Taim. Samples of floating vegetation were collected using a small net (mesh size 335 µm). After collection, samples were deposited in plastic jars with water from the sampled environment and then examined on a Petri dish using a stereomicroscope. All specimens found were picked up with a glass pipette, placed on a slide and fixed using Hoyer's medium in order to do observations and measurements of the stylet under the light microscope. Identification of the specimens were based on stylet morphology only. The specimens studied were deposited at the Helminthological Collection of Museu de Zoologia da USP (MZUSP PL 2165-2183), Brazil. Sampling permits were granted by the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio), permits number 60291-2/2017 and 64551-1/2018.

A brief description of the stylet morphology is provided. The axial length of the stylet is 67.11 – 83.6 µm ($n = 18$; $\bar{x} = 78.9$ µm). The stylet is a laterally opened girdle, its proximal part is reticulated and fenestrated (Fig. 1, B). The distal region of the stylet has (1) a pair of large grooved spines, notably curved resembling a pincer-like structure and (2) a group of very fine straight spines (Fig. 1, A and B). In the first group of spines, the outermost has a bulb-like structure on its proximal side, while the second has forked tips. The second group of spines protrude from both distal edges of the girdle forming a “brush”-like structure. The “brush” has several fine straight spines, each spine has a longitudinal groove and their tip is slightly orientated to the peripheral (Fig. 1, A).

The stylet keeps the configuration described by Brusa *et al.* (2003), in spite of being slightly shorter (78.9 µm) compared to those registered by the latter authors (85 – 88 µm). In addition, the length of the girdle is bigger than the total length of the region of the spines, in accordance with the original description. *Gieysztoria falx* is placed into the *Inaequales* group, *i.e.* spines of the stylet with different shape and size (Luther 1955, Brusa *et al.*, 2003). *Gieysztoria* has several species in South America with complex stylets (Brusa *et al.*, 2003) and it is thought that species distribution

Confirmação da submissão do manuscrito do Capítulo 1 para a revista **Zoological Studies**.

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Date: seg., 29 de jun. de 2020 as 14:57

Subject: [ZS] 200096 Acknowledgment

To: <rtvanna@gmail.com>

Dear Dr. Rogerio Tubino Vanna,

This is to acknowledge the receipt of the above referenced manuscript, submitted by you for a possible publication in the Zoological Studies. The manuscript has now been assigned to our editors to handle the review process.

On behalf of the Editorial Board of the Journal, I would like to thank you for submitting your work to this Journal.

Paper No. : 200096 (1st)

Paper Title: Free-living microtubellarians (Platyhelminthes) from wetlands in southern Brazil, with the description of three new species

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