International Journal for Parasitology: Parasites and Wildlife 24 (2024) 100968

Contents lists available at ScienceDirect

International Journal for Parasitology: Parasites and Wildlife

journal homepage: www.elsevier.com/locate/ijppaw

Diversity and ecological relationships of Cestoda and **Monogenoidea** parasites of freshwater stingrays (Myliobatiformes, Potamotrygonidae), in the upper Paraná River, Brazil

Jumma Miranda Araújo Chagas[a,*], Douglas de Castro Ribeiro[a],
Thalita Fischer Santini Mendes[b], Felipe Chinaglia Montefeltro[b], Luciano Alves dos Anjos[b]

[a] *Instituto de Biociências, Letras e Ciências Exatas, Campus São José do Rio Preto, Ibilce-UNESP, Rua Cristóvão Colombo, 2265, São José do Rio Preto, São Paulo, 15054-000, Brazil*
[b] *Faculdade de Engenharia de Ilha Solteira, Departamento de Biologia e Zootecnia, FEIS-UNESP, Passeio Monção, 830, Ilha Solteira, São Paulo, 15385-000, Brazil*

ARTICLE INFO

Keywords:
Taxonomy
Parasite ecology
Helminths
Allochthonous species

ABSTRACT

The Neotropical freshwater stingrays of *Potamotrygon* genus present a unique and complex natural history and biogeographical pattern that can be traced to a marine origin and the colonization of the continental environment during the Miocene. During the evolution of potamotrygonids, several species of the parasitic fauna coevolved and co-opted concomitantly to their hosts during the colonization of the new environments. One striking example can be observed during the colonization of the upper Paraná River region. However, few studies explored the ecological and taxonomic aspects of potamotrygonid parasites. In this work, we investigate aspects of the ecology and taxonomy of the species of Monogenea and Cestoda that are parasites the species of freshwater stingrays of the genus *Potamotrygon* in the upper Paraná River. Our results indicate that at least six species of parasites are present in potamotrygonids in the region. Two of the observed parasites are putative new species and three of the parasitic species were identified for the first time in the region, hence expanding their geographic distributions. We quantified ecological aspects at different levels of communities for the collected parasite species. We compared the diversity in different locations and hosts and performed an exploratory analysis to investigate the differences in parasite abundance. Additionally, an identification key for the Monogenea and Cestoda species of the sampled region is provided.

1. Introduction

It is estimated that almost 90% of helminth diversity are unknown to science (Carlson et al., 2020). The global description of the parasite fauna is complex, involving a series of stages such as survey, collection and description of diversity, geographic distribution data and ecological associations with the host species. It is a time-consuming process in which distorted analyses generate quantitative differences between the diversity of recognized species and the expected diversity (Carlson et al., 2020). One of the groups with the most information are cestodes parasites of Chondrichthyes. However, the available information totals only 28% of the number of species expected in this group (Carlson et al., 2020).

Potamotrygon Garman 1877 is the most specious genus of freshwater stingrays in the Potamotrygonidae family in South America, with 30 valid species (Fricke et al., 2024), with most species restricted to the Amazon and Prata basin drainages (Carvalho et al., 2013, 2016). The colonization of potamotrygonids in the upper Paraná River region in the 1970s was possible after the flooding of the Sete Quedas falls, currently submerged by the Itaipu hydroelectric reservoir. The Sete Quedas falls represented a natural barrier separating the fauna of the lower portion of the Paraná River from the higher (Loboda and Carvalho 2013). Seven species of the genus *Potamotrygon* are recorded in the Prata basin (i.e. Paraná, Paraguay, and Uruguay Rivers drainages), they are: *Potamotrygon amandae* Loboda and Carvalho (2013), *Potamotrygon falkneri* Caswell, 1978, *Potamotrygon motoro* (Müller and Henle, 1841), *Potamotrygon histrix* (Müller and Henle, 1839), *Potamotrygon schroederi* Fernández-Yépez 1958, *Potamotrygon brachyura* (Günther, 1880) and *Potamotrygon pantanensis* Loboda and Carvalho (2013). The species most commonly found in the upper Paraná River are *P. amandae* and

---

* Corresponding author.
E-mail address: jumma.chagas@unesp.br (J.M.A. Chagas).

https://doi.org/10.1016/j.ijppaw.2024.100968
Received 2 June 2024; Received in revised form 22 July 2024; Accepted 23 July 2024

P. falkneri and more rarely *P. schroederi* (Garrone Neto, 2010). *P. amandae* was described in 2013, having previously been identified as *P. motoro* in the region, and is the species with the greatest geographic distribution among the other species recorded in the Prata basin (Loboda and Carvalho, 2013).

The parasite fauna of the Potamotrygonidae is mostly represented by helminths Monogenea van Beneden 1858; Cestoda van Beneden 1849. Two genera of Monogenea are recognized in Potamotrygonidae: *Potamotrygonocotyle* Mayes, Brooks & Thorson 1981 and *Paraheteronchocotyle* Mayes, Brooks & Thorson 1981. These genera are in families with representatives that are, for the most part, found in marine elasmobranchs (i.e. Monocotylidae Taschenberg, 1879 and Hexabothriidae Price, 1942). However, the lineages of Monogenea and Cestoda that colonized the freshwater environment are restricted to potamotrygonids and are not found in other host lineages. This fact suggests a simultaneous colonization event of South American rivers by the ancestors of parasites and their hosts (Domingues and Marques, 2007).

Cestodes adults are obligatory parasites of the digestive tract of vertebrates, and in larval stages they can infest other groups of vertebrates and invertebrates (Stunkard, 1953). In elasmobranchs, tapeworms are the most diverse group; they are generally the final hosts of the cycle, being commonly located in the spiral valve of these animals (Caira and Healy, 2004). Cestodes are frequently found parasitizing potamotrygonids, with groups of typically marine parasites (e.g. *Acanthobothrium* van Beneden, 1849; *Anindobothrium* Marques, Brooks & Lasso 2001; *Rhinebothrium* Linton, 1889 and *Paraoncomegas* Campbell, Marques & Ivanov 1999) and groups restricted to potamotrygonids in freshwater (e.g. *Potamotrygonocestus* Brooks and Thorson, 1976; *Nandocestus* Reyda and Marques, 2011 and *Rhinebothroides* Mayes, Brooks & Thorson, 1981).

In this work, we investigate the taxonomic diversity of Cestoda and Monogenea parasites in freshwater stingrays of the genus *Potamotrygon* in the upper Paraná River and describe some ecological relationships between these helminths and their hosts.

2. Material and methods

2.1. Sampling of hosts and parasites infracommunities

Two host species of Potamotrygonidae (i.e. *P. amandae* and *P. falkneri*) were analyzed. The potamotrygonids were collected between May 2015 and 02/2016, using a casting net, harpoon, and artisanal fishing in two locations on the upper Paraná River, between the states of São Paulo and Mato Grosso do Sul (Fig. 1). Collection site 1 (S1, three points, −20.52; −51.48, −20.46; −51.43 and −20.40; −51.37) corresponds to the upper stretch of the Paraná river where there is a well-established population of freshwater stingrays (Dagosta et al., 2024; Langeani et al., 2007). This location has only two significant tributaries, the Tietê and Sucuriú Rivers. In this location, the main channel of the river is approximately 53 km long, with a water regime strongly controlled by two large hydroelectric dams, the Ilha Solteira dam upstream, and the Engenheiro Souza Dias dam downstream. Site 2 (S2, -20.96; −51.70) is also delimited by two dams, the Engenheiro Souza Dias dam (upstream) and the Porto Primavera dam (downstream), approximately 240 km long and with a greater number of tributaries, also with aquatic biodiversity and more diverse allochthonous when compared to S1 (Dagosta et al., 2024).

In the field, the stingrays were temporarily placed in 25-L plastic boxes. Subsequently, the animals were euthanized with 800 mg/l^{-1} of benzocaine in ethanol (1:10, v:v) intracardiacally (Leary, 2013; West et al., 2007) and the parasites were collected.

To collect monogeneans, the gill chamber of the hosts was exposed by removing the integument, followed by separation of the gill arches. Subsequently, the gill arches were fixed in a 4% formaldehyde solution in sodium phosphate buffer at 60 °C for 48h. After fixation, the gill arches were washed in distilled water (on a 20 μm steel sieve) and preserved in 70% ethanol until the screening and removal of the parasites under a stereoscope. The collected monogeneans were mounted on slides and coverslips with Gray & Wess or Hoyer's and stained with Gomory's trichrome (Domingues and Marques, 2007).

To collect the cestodes, the host's spiral valves were removed, opened longitudinally, and fixed in a 4% formaldehyde solution in

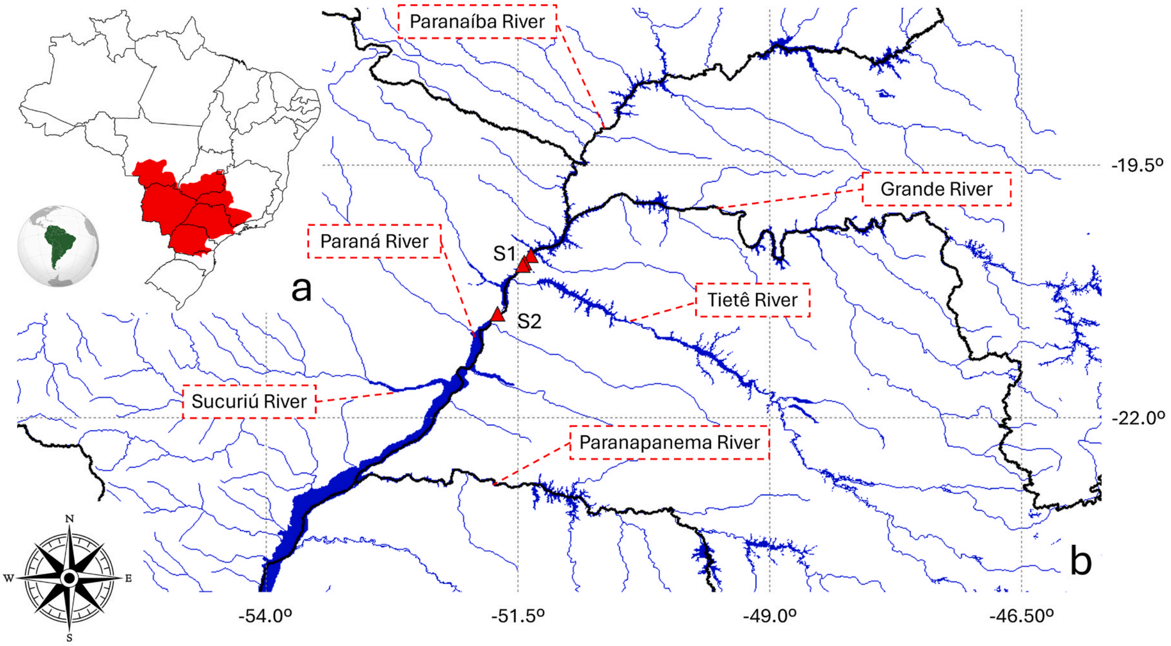

Fig. 1. Collection area for potamotrygonids and their parasites. (a) Highlight (red) of the upper Paraná River system (Brazilian portion). (b) Collection sites (red triangles), S1 with three points and S2 with one point, in the upper Paraná River, between the states of São Paulo and Mato Grosso do Sul, Brazil. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

sodium phosphate buffer at 60 °C for 48 h. Subsequently, the material was carefully washed in distilled water and preserved in 70% ethanol and screened using a stereoscope to separate the parasites from the intestinal contents. The cestodes were rehydrated in a regressive alcohol series followed by two baths with distilled water (15 min each step), and stained with Delafield's hematoxylin, dehydrated in a progressive alcohol series, diaphanized in Methyl Salicylate and mounted on a slide and coverslip with Canada Balm (Marques, 2000). The slides with parasites were analyzed and photographed using a Leica DM 2500 microscope equipped with a Leica DMC 2900 camera.

Some of the specimens were analyzed using Scanning Electron Microscopy (SEM). These specimens were fixed in 2.5% Glutaraldehyde solution and stored in a refrigerator until dehydration. Dehydration was carried out in an increasing alcohol series (7.5, 15, 30, 50, 70, 80, 90 and 100%) with two baths of 15 min each. Finally, the material was submitted to the Critical Point using liquid CO2 (Balzers Union), mounted on metal bases (stubs) and metallized with gold-palladium ions (Metalizer MED 010, Balzers Union). The material was examined and electron micrographed using an SEM QUANTA 200 Scanning Electron Microscope.

All procedures followed the guidelines and policies for ethical conduct in the use of animals in Brazil, approved by the Universidade Estadual Paulista - UNESP and Conselho Nacional de Controle de Experimentação Animal – CONCEA (authorization number CEUA/FEIS 15/2017), and registered in Sistema Nacional de Gestão do Patrimônio Genético e Conhecimento Tradicional Associado – SisGen (registration number A001CBE). Permission for collection was provided by Instituto Brasileiro de Meio Ambiente e Recursos Naturais Renováveis – IBAMA and Instituto Chico Mendes de Conservação da Biodiversidade – ICMBio (authorization number SISBIO 50019-4).

Vouchers of the hosts were deposited in DZSJRP fish collection (Departamento de Ciências biológicas of the Instituto de Biociências, Letras e Ciências Exatas) of the São Paulo State University 'Júlio de Mesquita Filho', São José do Rio Preto, São Paulo, Brazil. Vouchers of the parasites were deposited in LAPEISA collection (Departamento de Biologia e Zootecnia of the Faculdade de Engenharia de Ilha Solteira) of the São Paulo State University 'Júlio de Mesquita Filho', Ilha Solteira, São Paulo, Brazil.

Parasites species names follow the World Register of Marine Species (WoRMS Editorial Board 2024).

2.2. *Parasites biodiversity quantifies and data analysis*

Parasite biodiversity was characterized by parameters of infection, richness, and diversity. The definitions of prevalence, abundance and degree of intensity of infection follow Bush et al. (1997). The terms infracommunity, component community, supracommunity with respect to scale follow Poulin and Morand (2000). The community status (or degree of importance) of the taxa within the parasite communities was classified based on Caswell (1978) and Hanski (1982) adapted by Bush and Holmes (1986), considering species with prevalences greater than 66% as core species, prevalences between 33% and 66% as secondary species, and prevalences lower than 33% as satellite species. Additionally, we evaluated the frequency of parasite dominance.

We used Quantitative Parasitology v. 3.0 (Reiczigel et al., 2019) to quantification of ecological parameters. The dominance (Simpson index - Ranges from 0 (all taxa are equally present) to 1 (one taxon dominates the community completely) and evenness index were calculated in the software Past v. 4.09 (Hammer et al., 2001). We compared parasite richness between hosts and locations using the diversity *t*-test (Magurran, 1988) with 1000 bootstrap replicas in software Past v. 4.09 (Hammer et al., 2001; Faul et al., 2009). Additionally, we calculated the test power and effect size using the software GPower v. 3.1 (Faul et al., 2009).

Component community richness and meta-community richness was estimate using the sample-size-based diversity accumulation curves using hosts as unit of sampling and Hill numbers. Diversity metrics were species richness, Shannon Index and Simpson Index values (Chao et al., 2014). Values were calculated and visualized for hosts (to visualize component community richness) and sites (to visualize meta-community richness). The iNEXT function of package iNEXT (Hsieh et al., 2016) was used to extrapolate species richness these curves (asymptotic estimate). We used 100 bootstrap iterations, and 95% confidence intervals generate by function iNEXT.

We used generalized linear mixed modeling (GLMM) with a negative binomial distribution and log link (estimated using Maximum Likelihood and Nelder-Mead optimizer) to determine if infracommunity abundance varied between sampling (function glmer.nb of package lmer4, Bates et al., 2014). The fixed part of the model has as its response variable the abundance of all parasite species together. We used as predictors the disc length (DL), sex (males or females), gonadal development stages (immature, initial development, advanced development, mature (including pregnant females) and resting) and Condition Factor (used as syntax for the length-weight relationship, calculated according to Peig and Green (2009)). We assume that the host species (i.e. *P. amandae* or *P. falkneri*) and collection site (S1 or S2) did not set up independent samples and were included in the model as random variables. Graphs of GLMM results were made with the package effects (Fox et al., 2016).

Version 4.2.2 of R software (R Core Team, 2020) was used to perform statistical analyzes of the mentioned packages.

3. Results

3.1. *Parasite/host ecological relationships*

A total of 84 host specimens were analyzed, of which 39 specimens of *P. falkneri* and 45 specimens of *P. amandae*. The disc length of the animals ranged from 20.5 to 52.0 cm (35.99 ± 5.65) in *P. amandae* and 18.5–71.0 cm (34.97 ± 11.65) in *P. falkneri.* In *P. falkneri*, 87% of the specimens were parasitized. In *P. amandae* 42% of individuals were parasitized in S1 and 75% in S2. The supracommunity in the hosts analyzed was 6.643 parasite specimens.

The richness found in the host community was six species of helminths, consisting of one species of Monogenea (*Potamotrygonocotyle tsalickisi* Mayes, Brooks & Thorson, 1981) and five species of Cestoda (*Acanthobothrium quinonesi* Mayes, Brooks & Thorson, 1978, *Rhinebothrium paratrygoni* Rego and Dias, 1976, *Rhinebothroides glandularis* Brooks, Mayes & Thorson 1981, *Potamotrygonocestus* sp. 1 and *Potamotrygonocestus* sp. 2). Infracommunities richness ranged from one to five parasite species and no co-parasitism was observed between Cestoda and Monogenea. Information by parasite species is available in Table 1. The component community in *P. falkneri* is made up of five species of cestodes, with *R. paratrygoni* being the species with the highest prevalence and abundance. In *P. amandae* component community was composed of four taxa from both groups, Cestoda and Monogenea, with *P. tsalickisi* being the species with the highest prevalence and abundance (Table 1).

The results of the diversity *t*-test suggest a statistically significant difference in parasite diversity between locations (S1 $<$ S2, $t = -40.00$; $p = < 0.001$; $d = 3.76$; $\beta = 0.99$) and between host species (*P. amandae* $<$ *P. falkneri,* $t = -29.68$; $p = < 0.001$; $d = 0.52$; $\beta = 0.21$) (Fig. 2). Rarefaction and extrapolation values of the diversity estimates generated by iNEXT for different sites and host are presented in Fig. 3.

In S1, *P. tsalickisi* was considered a core species and *A. quinonesi* and *R. paratrygoni* were considered satellite species. In S2, in *P. falkneri, R. paratrygoni* was recognized as the core species, *A. quinonesi* and *Potamotrygonocestus* sp. 1 were considered secondary species and *R. glandularis* and *Potamotrygonocestus* sp. 2 as satellite species. In *P. amandae, P. tsalickisi* was considered secondary species and *A. quinonesi*, *R. paratrygoni* and *Potamotrygonocestus* sp. 2 as satellite species.

Table 1

Ecological aspects of parasites found in species of the genus *Potamotrygon* in the upper Paraná River, Brazil. Abbreviations: Ab = Abundance, AF = Analyzed Fishes, Do = Dominance, Ev = Evenness, In = Intensity, PF = Parazited Fishes, Pr (%) = Prevalence (percent).

Taxa	Infection Sites	*Potamotrygon amandae*							*Potamotrygon falkneri*							*Potamotrygon amandae*						
		Site 1							Site 2							Site 2						
		AF	PF	Pr (%)	Ab	In	Do	Ev	AF	PF	Pr (%)	Ab	In	Do	Ev	AF	PF	Pr (%)	Ab	In	Do	Ev
Monogenea																						
Potamotrygonocotyle tsalickisi	Gills	11	11	100	4810	153	0.25	0.69	11	0	–	–	–	–	–	9	6	66.7	1026	126	0.33	0.75
Cestoda																						
Acanthobothrium quinonesi	Spiral valve	36	5	13.9	61	4	0.54	0.53	39	22	56.4	77	2.5	0.07	0.93	9	2	22.2	3	2	1	–
Rhinebothrium paratrygoni	Spiral valve	36	4	11.1	70	7	0.59	0.50	39	31	79.5	392	6	0.07	0.85	9	2	22.2	1	1	–	–
Rhinebothroides glandularis	Spiral valve	36	0	–	–	–	–	–	39	12	30.8	24	1	0.12	0.96	9	0	–	–	–	–	–
Potamotrygonocestus sp. 1	Spiral valve	36	0	–	–	–	–	–	39	18	46.2	167	1	0.48	0.47	9	1	11.1	1	1	–	–
Potamotrygonocestus sp. 2	Spiral valve	36	0	–	–	–	–	–	39	5	12.8	11	2	0.18	1.02	9	0	–	–	–	–	–

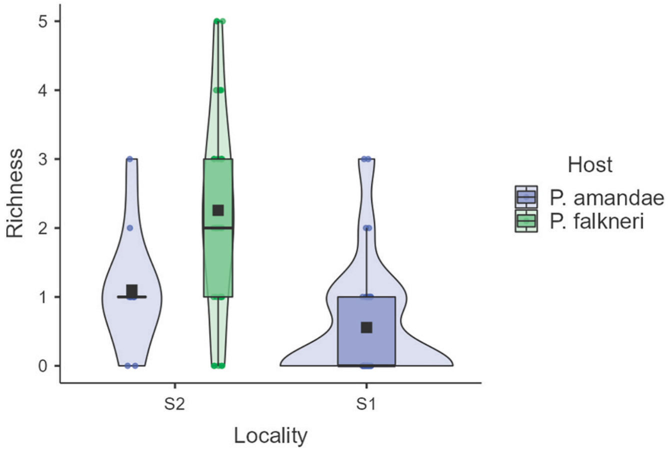

Fig. 2. Component community richness and meta-community richness of helminths species in potamotrygonids from the upper Paraná River. Results of diversity *t*-test suggest a statistically significant difference between sites (S1 < S2, t = −40.00; p = < 0.001; d = 3.76; β = 0.99) and between host (*Potamotrygon amandae* < *Potamotrygon falkneri*, t = −29.68; p = < 0.001; d = 0.52; β = 0.21). Mean and Median values are indicated by black square and horizontal black line respectively.

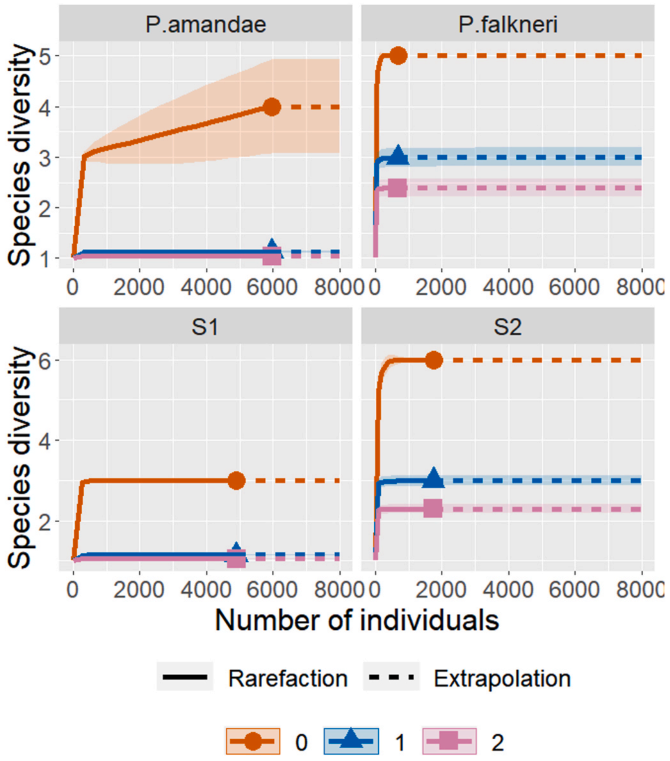

Fig. 3. Rarefaction and extrapolation of component community richness and meta-community richness of helminths species in potamotrygonids from the upper Paraná River. Sample-size-based diversity accumulation curves (with 95% confidence intervals of lower and upper limits) using hosts as unit of sampling and Hill numbers. Diversity metrics were species richness (0), Shannon Index (1) and Simpson Index (2) values.

The results of GLMM (Table 2 and Fig. 4 show total explanatory power is substantial (conditional $R^2 = 0.62$) and the part related to the fixed effects alone (marginal R^2) is of 0.61. The effect of sex and gonadal development were statistically non-significant and positive. On other hand, the effect of DL and Condition Factor were statistically significant (positive and negative respectively) (see Fig. 5).

Table 2

Results of Generalized Linear Mixed Model (GLMM) with negative binomial distribution and log link explaining the abundance of helminth species. Disc length (DL), sex (males or females), gonadal development stages (immature, initial development, advanced development and resting) and Condition Factor are predictors. Host species and collection site are random variables. Marginal significance statistical (*) and significant statistically (**) of *p-values* are indicates.

Fixed effects	Category	Estimate (SE)	z value	p value
Intercept		3.86 (2.03)	1.89	0.0585*
Disc length		0.10 (0.05)	2.18	0.0287**
Sex				
Male *vs.*	Female	0.35 (0.69)	0.49	0.6208
Gonadal development		0.59 (0.33)	1.74	0.0819
Condition Factor (K)		−5.24 (1.61)	−3.23	0.0012**
Random effects		**Variance (SE)**	**Correlation**	
Locality	(intercept)	0.01 (0.11)		
Host				
Potamotrygon amandae vs.	*Potamotrygon falkneri*	0.39 (0.62)	−1.00	

3.2. Identification key for Monogenea parasites of Potamotrygon spp. of the upper course of the Paraná River

(One species) Haptor with one central loculus and eight peripheral loculi; haptor septa with slightly sclerotized margins; two pairs of dorsal accessory structures, associated with four posteriors peripheral loculi; each dorsal haptoral accessory structure with sclerotized margins; anterior pair of dorsal haptoral accessory structures bilobate; male copulatory organ with subterminal aperture, without an accessory piece.	*Potamotrygonocotyle tsalickisi*

3.3. Identification key for species of Cestoda parasites of Potamotrygon spp. of the upper course of the Paraná River

1.1. Hooks of the scolex absent.	2
1.2 Sclerotized hooks of the scolex present.	3
2. Fusiform bothridia, without central constriction; medial longitudinal septum of the scolex originating from the posterior margin of the anterior loculus to the posterior margin of the bothridium; strobila craspedote; ovarian lobes symmetrical; paravaginal glandular cells absent	*Rhinebothrium paratrygoni*
2.1 Pedunculated bothridia; marginal and transverse septa of the scolex forming loculi; strobila acraspedote; ovarian lobes asymmetrical; paravaginal glandular cells present	*Rhinebothroides glandularis*
3. Septate bothridia, triloculated, with a pair of bifid hooks, medial and lateral branches of the hook subequal, with the distal cusp of the lateral branch surpassing the median region of the medial branch; loculi asymmetrical, anterior loculus conspicuously larger, reaching or exceeding the medial region of the scolex, posterior loculus well reduced, with the anterior edge entering the medial loculus; testes surpassing the cirrus sac, reaching the anterior margin of the ovary; vitellaria extending beyond the anterior margin of the ovary	*Acanthobothrium quinonesi*
3.1 Non-septate bothridia, with a pair of simple, non-bifurcated hooks; testes not exceeding the cirrus sac; vitellaria not exceeding the anterior margin of the ovary	4
4. Proximal portion of hook at an angle greater than 130° in relation to the anterior process of the base; furcation laterally arched, with cusp directed posteromedially; posterolateral genital pore, not exceeding the posterior portion of the ovary lobes	*Potamotrygonocestus* sp. 1
4.1 Proximal portion of hook at an angle of approximately 100° in relation to the anterior process of the base; furcation arched anteriorly, with cusp directed medially; terminal genital pore, surpassing the posterior portion of the ovary lobes	*Potamotrygonocestus* sp. 2

(continued on next column)

(continued)

3.4. Systematic parasitology

Monocotylidea (*sensu* Boeger and Kritsky 1993)
Monocotylidae Taschenberg 1879
Potamotrygonocotyle Mayes, Brooks & Thorson 1981
Potamotrygonocotyle tsalickisi Mayes, Brooks & Thorson 1981 Fig. 5

Remarks: Four species of the genus *Potamotrygonocotyle* are described for the Prata Basin (i.e. *Potamotrygonocotyle chisholmae* Domingues and Marques (2007), *Potamotrygonocotyle dromedarius* Domingues and Marques (2007), *Potamotrygonocotyle rionegrensis* Domingues and Marques, 2007 and *Potamotrygonocotyle tsalickisi* Mayes, Brooks & Thorson 1981. However, only *P. tsalickisi* was recorded in the present study. Domingues and Marques (2007) describe *Potamotrygonocotyle eurypotamoxenus* and *Potamotrygonocotyle uruguayense* in the Prata Basin. Subsequently, Domingues and Marques (2011) proposed these species as junior synonyms of *P. tsalickisi* and *P. chisholmae*, respectively. *P. tsalickisi* readily differentiates itself from its congeners from the Prata Basin due to the morphology of the copulatory organ, with a subterminal opening (*vs.* terminal opening in *P. dromedarius*, *P. rionegrensis* and *P. chisholmae*). *P. tsalickisi* has a wide distribution in the Amazon and Prata Basins and relatively low parasite specificity (Domingues and Marques 2007).

Rhinebothriidea Healy, Caira, Jensen, Webster & Littlewood 2009
Rhinebothriidae Euzet 1953
Rhinebothrium Linton 1890
Rhinebothrium paratrygoni Rego and Dias 1976 Fig. 6

Remarks: *Rhinebothrium paranaensis* Menoret & Ivanov 2009 was considered a junior synonym of *Rhinebothrium paratrygoni* by Reyda and Marques (2011), but this has not been accepted by Ruhnke et al. (2017) and is still considered valid in the Global Cestode database (GCD) and in the WoRMS (WoRMS Editorial Board, 2024). *Rhinebothrium paranaensis* together with *Rhinebothrium paratrygoni* corresponds to the two valid species of the genus *Rhinebothrium* described for the Prata Basin. *Rhinebothrium paratrygoni* can be differentiated from other congeners by the following characteristics: strobilous conspicuously craspedote (*vs.* acraspedote in *Rhinebothrium brooksi* Reyda and Marques 2011 and *Rhinebothrium copianullum* Reyda and Marques, 2011) and the smaller size of microtrichia (<5 μm *vs.* >7 μm in *R. brooksi* and *R. copianullum*). Additionally, *R. paratrygoni* can be readily differentiated from *Rhinebothrium fulbrighti* Reyda and Marques (2011) and other congeners by the greater number of testes per proglottid (four or more *vs.* two to three in *R. fulbrighti, R. brooksi* and *R. copianullum*). *Rhinebothrium paratrygoni* is widely distributed in the Prata Basin, being recorded mainly in the upper and middle Paraguay River, upper Paraná River and upper Uruguay River. Additionally, there are reports of the species in the upper Amazon River, in the Boca do Acre and Juruá Rivers, in the state of Amazonas (Reyda and Marques, 2011).

Rhinebothriidea Healy, Caira, Jensen, Webster & Littlewood 2009
Rhinebothriidae Euzet 1953
Rhinebothroides Mayes, Brooks & Thorson 1981
Rhinebothroides glandularis Brooks, Mayes & Thorson 1981 Fig. 7

Remarks: Two valid species of the genus *Rhinebothroides* are recorded in the Prata Basin, *Rhinebothroides glandularis* Brooks, Mayes & Thorson

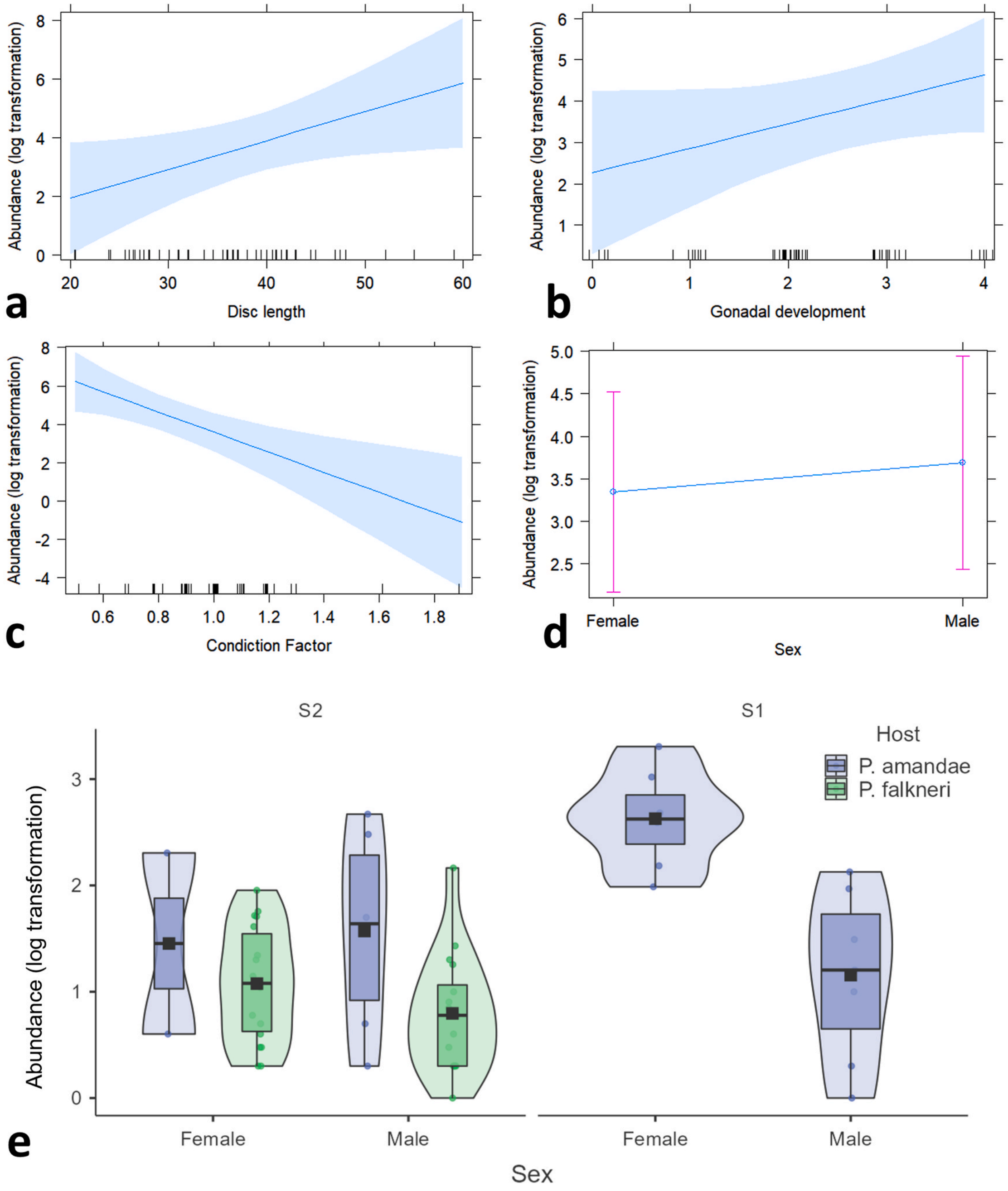

Fig. 4. Relationship of infracommunity abundance of helminths inferred by mixed generalized linear modeling (GLMM) in potamotrygonids of the upper Paraná River. Abundance *vs.* (a) disc length (DL), (b) gonadal developmental stages (immature = 0, early development = 1, advanced development = 2, mature = 3 and rest = 4), (c) condition factor and (d) sex (males or females) (e) Host species (i.e. *Potamotrygon amandae* or *Potamotrygon falkneri*). and collection sites (S1 or S2) are random variables.

1981, *Rhinebothroides freitasi* (Rego, 1979). *Rhinebothroides glandularis* was originally described as endemic to the Orinoco River in Venezuela, parasitizing *Potamotrygon histrix* (see Ruhnke, 2017) (originally identified as *Potamotrygon orbignyi* by Marques and Brooks, 2003). However, it is currently known that *R. glandularis* is widely distributed in the Amazon, Orinoco and Paraguay Rivers (Marques and Brooks 2003). Among the species recorded in the Prata Basin, *R. glandularis* can be readily differentiated from its congeners, except *Rhinebothroides mclennanae* Brooks and Amato (1992), by the presence of glandular cells close to the vagina region. *R. mclennanae* is described parasitizing *Potamotrygon motoro* in the Paraguay River (Brooks and Amato, 1992), and is the third species present in the Prata Basin. Some authors (e.g. Bueno, 2010; Marques and Brooks, 2003), recognize *R. mclennanae* as a synonym of *R. glandularis*, but this has not been accepted by Ruhnke (2017). Therefore, *R. mclennanae* is still considered valid in the GCD and in the WoRMS (WoRMS Editorial Board, 2024).

Onchoproteocephalidea Caira and Jensen, 2017
Onchobothriidae Braun 1900
Acanthobothrium Blanchard, 1848
Acanthobothrium quinonesi Mayes, Brooks & Thorson 1978 Fig. 8

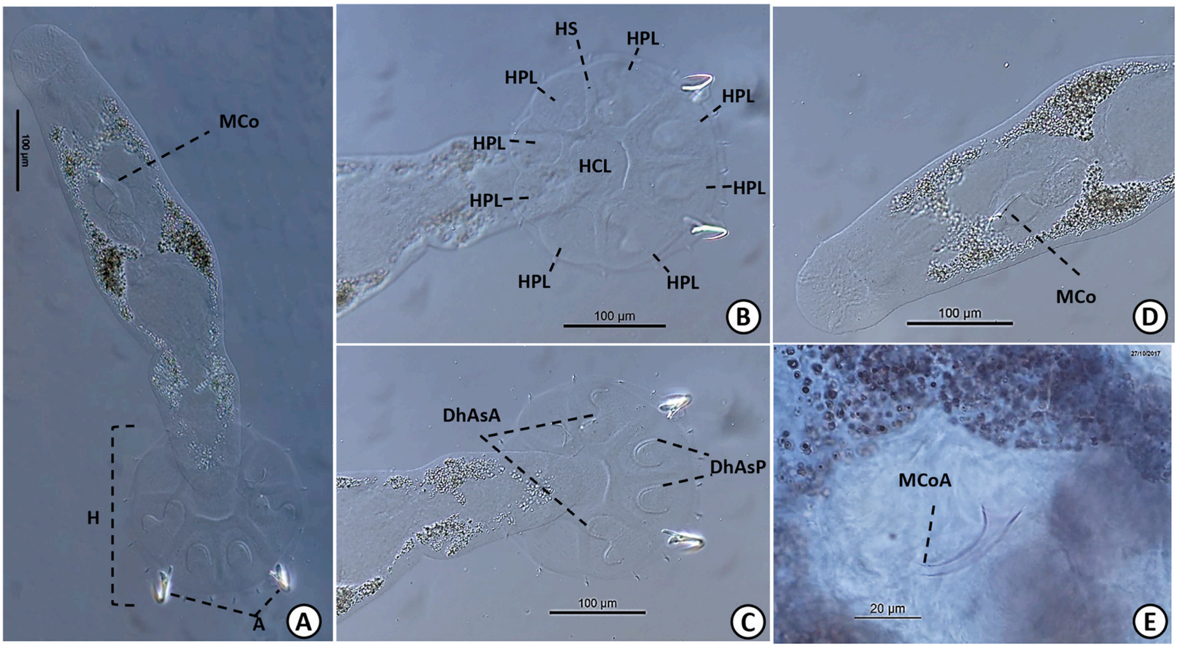

Fig. 5. Morphology of *Potamotrygonocotyle tsalickisi.* Whole specimen (a); haptor (b and c), and male copulatory organ (d and e). Abbreviations: A = anchor; DhAsA = anterior dorsal haptoral accessory structure; DhAsP = posterior dorsal haptoral accessory structure; H = haptor; HCL = haptor central loculi; HPL = haptor peripheral loculi; HS = haptoral septa; MCo = male copulatory organ, and MCoA = male copulatory organ aperture.

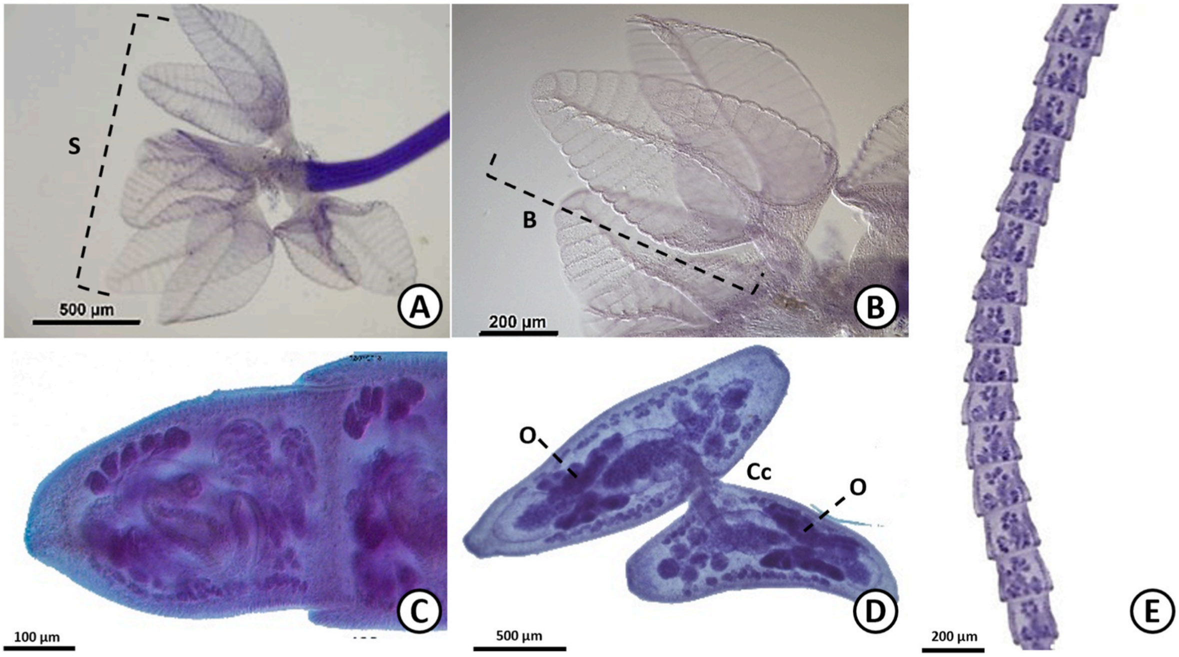

Fig. 6. Morphology of *Rhinebothrium paratrygoni.* Morphology of scolex (A); Details of bothridium (B); Terminal mature proglottid (C); Cross-copulation between mature proglottids (D), and partial strobila (E). Abbreviations: B = bothridia; Cc = Cross-copulation; O = ovary, and S = scolex.).

Remarks: *Acanthobothrium quinonesi* was originally described as parasitizing the spiral valve of *Potamotrygon magdalenae* (Duméril, 1965), in the Magdalena river in Colombia (Mayes et al., 1978). Cardoso Jr. (2010) in his unpublished thesis suggests *Acanthobothrium regoi* Brooks and Amato, 1992 and *Acanthobothrium peruviense* Reyda and Marques, 2011 as junior synonyms of *Acanthobothrium quinonesi.* This has not been accepted by Caira and Jensen (2017) and *A. peruviense* is still considered valid in the GCD and in the WoRMS (WoRMS Editorial Board 2024). In the Prata Basin, *A. quinonesi* differs from *Acanthobothrium terezae* Rego and Dias 1976 the only other species recorded in the region, due to its relatively small scolex with heterometric loculi; anterior loculus conspicuously larger than the others, reaching or exceeding the medial region of the scolex; posterior loculus reduced, with the anterior edge entering the medial loculus. Additionally, *A. quinonesi* differs from *A. terezae* by the reduced number of testes (22–100 *vs.* 43–114), with 6–39 pre-vaginal testes, 2–16 post-vaginal testes and 11–50 aporal testes (*vs.* 18–49 pre-vaginal testes, 1–15 post-vaginal testes and 30–65 aporal testes). *A. quinonesi* has the largest biogeographical distribution among the species of the genus in freshwater habitats, with records in the drainages of the Amazon,

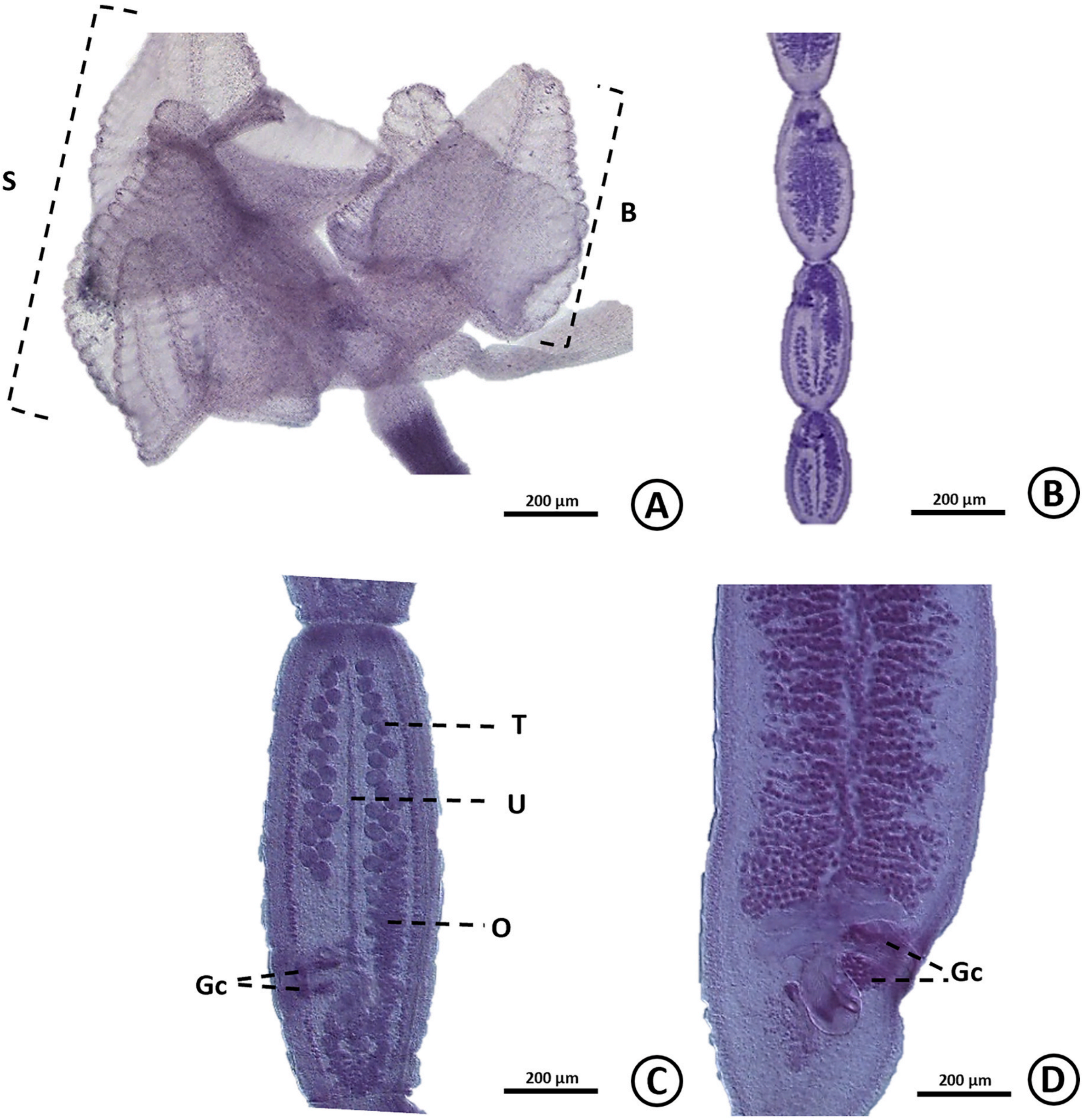

Fig. 7. Morphology of *Rhinebothroides glandularis.* Morphology of scolex (A); partial strobila (B); Mature proglottid (C); Gravid proglottid (D). Abbreviations: B = bothridia; Gc = gland cells; O = ovary; S = scolex; T = testes, and U = uterus.

Tocantins-Araguaia, and Prata drainage Basin. This taxon is considered a species with low specificity (Cardoso Jr., 2010).

Onchoproteocephalidea Caira and Jensen, 2017
Onchobothriidae Braun 1900
Potamotrygonocestus Brooks and Thorson 1976
Potamotrygonocestus sp. 1 Fig. 9

Remarks: Luchetti (2011) identified inconsistencies related to circumscription of some taxa identified for the genus *Potamotrygonocestus* in the work of Marques et al. (2003). Reevaluation of specimens from the Paraguay River suggest *Potamotrygonocestus* sp. 1 as a putative undescribed species (or species complex) based on the presence of rounded hooks with reduced furcation width immediately after insertion into the hook base in the midposterior portion of the base, at an obtuse angle between the furcation base and the length; genital pores at the height of the ovary, never exceeding the posterior limit of the ovarian lobes (Luchetti, 2011, unpublished data). To date, *Potamotrygonocestus* sp. 1 is widely distributed in the Amazon and Prata Basins and may also be present in the Orinoco River drainage (Luchetti, 2011). This new lineage has already been confused with another species of the genus in the Prata Basin. Lacerda et al. (2009) suggested the presence of *Potamotrygonocestus travassosi* Rego 1979 in *P. falkneri* in the floodplain of the upper Paraná River. However, the specimens collected by Lacerda et al. (2009) are specimens of *Potamotrygonocestus* sp. 1 by the presence of furca of the hook inserted in the center-lateral region of the base, decreasing in width immediately after its insertion in the base (Luchetti, 2011). *Potamotrygonocestus* sp. 1 differs from *Potamotrygonocestus* sp. 2 (putative new species) due to the combination of characteristics described in the identification key (see 4. and 4.1).

Onchoproteocephalidea Caira and Jensen, 2017
Onchobothriidae Braun 1900
Potamotrygonocestus Brooks and Thorson 1976
Potamotrygonocestus sp. 2 Fig. 10

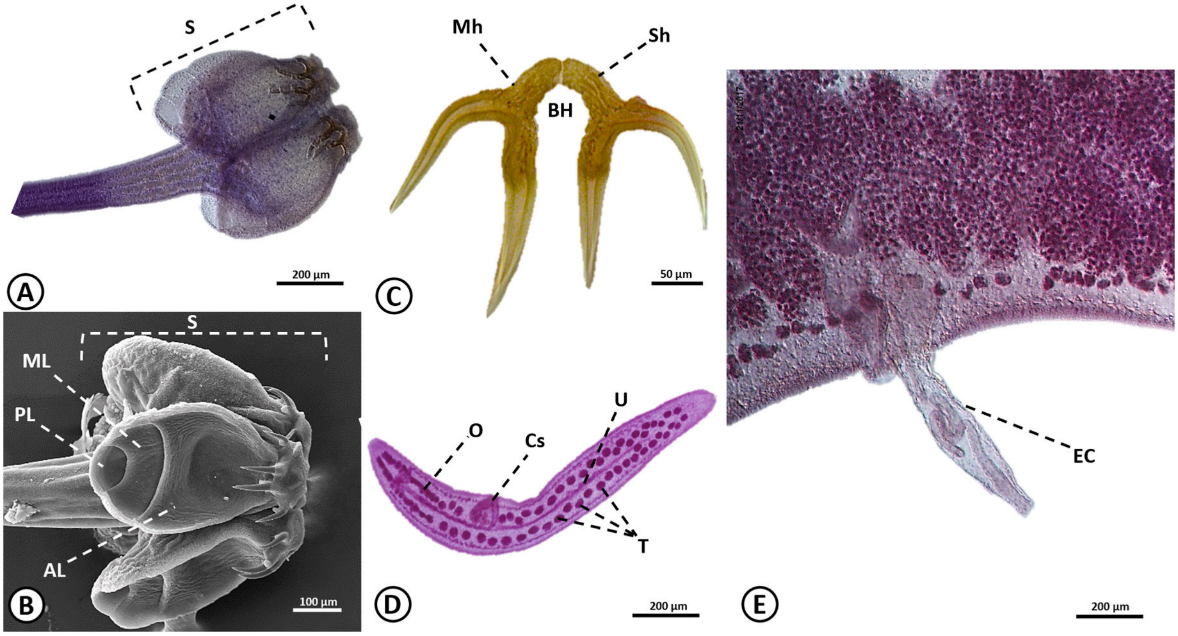

Fig. 8. Morphology of *Acanthobothrium quinonesi.* Morphology of scolex by light microscopy (A) and SEM (B); Isolated bothridia hooks (C); Mature proglottid (D); Cirrus sac (E). Abbreviations: AL = anterior loculus; BH = bothridia hooks; Cs = cirrus sac; EC = everted cirrus; Lh = lateral hook; Mh = medial hook; ML = middle loculus; O = ovary; PL = posterior loculus; S = scolex; T = testes, and U = uterus.

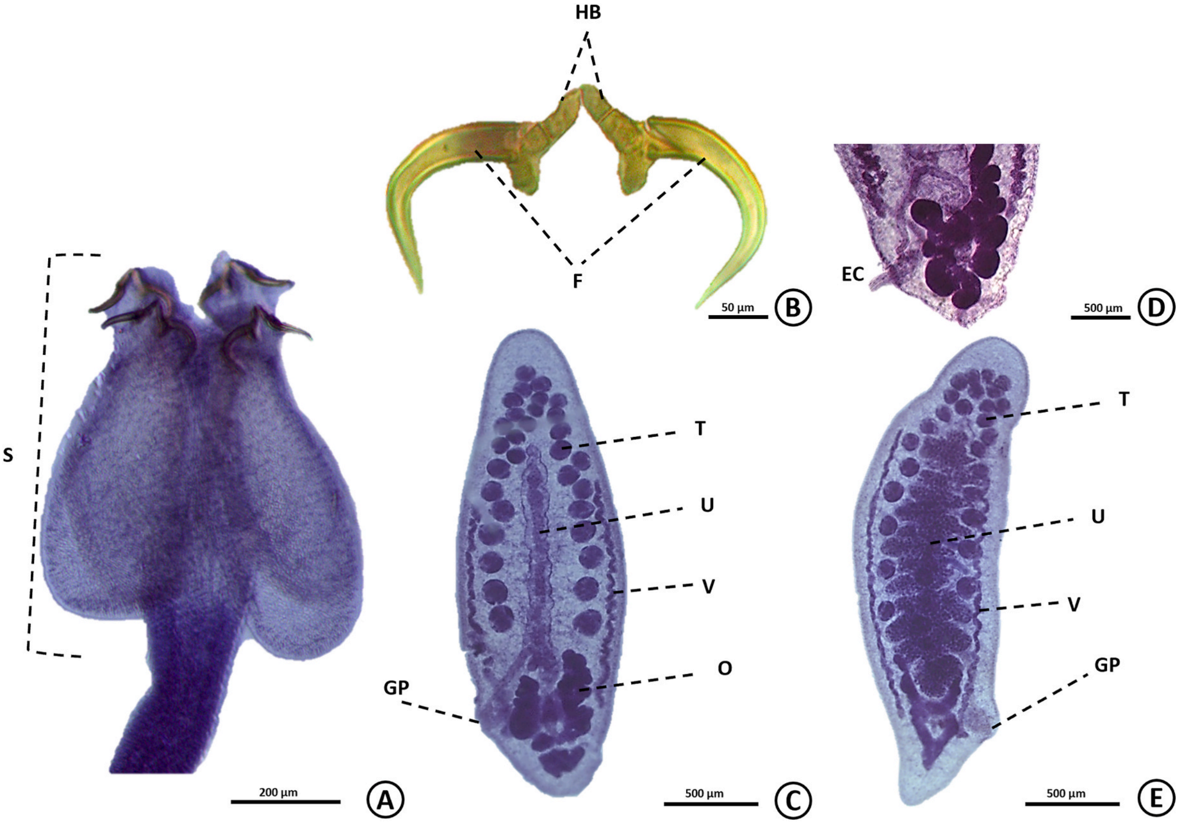

Fig. 9. Morphology of *Potamotrygonocestus* sp.1. Morphology of scolex (A); Isolated bothridia hooks (B); Mature proglottid (C); Cirrus sac (D); Gravid proglottid (E). Abbreviations: EC = everted cirrus; F = furca; GP = genital pore; HB = hook base; O = ovary; S = scolex; T = testes; U = uterus, and V = vitellaria.

Remarks: *Potamotrygonocestus* sp. 2 was initially identified by Luchetti (2011) in the spiral valve of *P. motoro* in the Paraguay River, Mato Grosso do Sul, Brazil. Additionally, additional hosts like *P. falkneri*, *P. histrix* and *P.* cf. *motoro* (=*P. amandae*) were recognized. *Potamotrygonocestus* sp. 2 can be differentiated from *Potamotrygonocestus* sp. 1 (putative new species), sympatric species in upper Paraná River, due to the morphology of the hooks (see identification key 4. and 4.1). *Potamotrygonocestus* sp. 2 is restricted to the drainages of the Paraguay

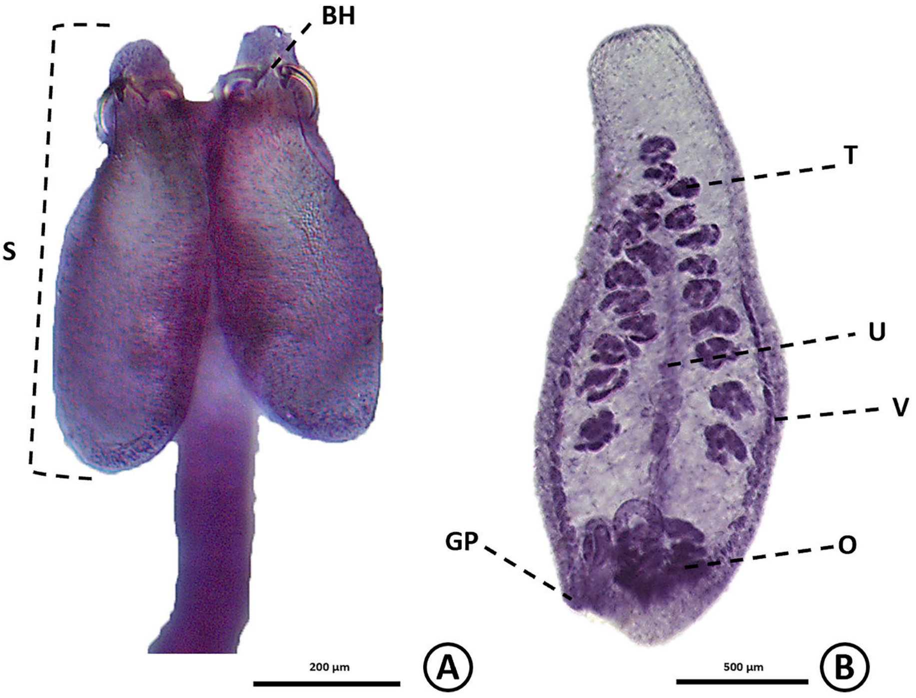

Fig. 10. Morphology of *Potamotrygonocestus* sp.2. Morphology of scolex (A); Mature proglottid (B). Abbreviations: BH = bothridia hooks; GP = genital pore; O = ovary; S = scolex; T = testes; U = uterus, and V = vitellaria.

and Paraná Rivers (Luchetti, 2011).

4. Discussion

Considering all parasite species, more than half of the analyzed hosts were parasitized (67%), with significant differences between parasite species in the different regions sampled. The result of the effect size suggests a difference with high magnitude between the diversity observed in the two collection sites (Fig. 3). *Potamotrygonocotyle tsalickisi* was the only monogenean species found in the analyzed hosts. Lehun et al. (2020), published a Checklist of parasites in fish from the floodplain of the upper Paraná River, not reporting records of monogeneans in potamotrygonids. However, in S1 *P. tsalickisi* is the most abundant species in our study. In general, monogeneans tend to exhibit a relatively high degree of host specificity when compared to other parasite groups. *P. tsalickisi* is restricted to Neotropical freshwater stingrays, not occurring in other freshwater hosts. The presence of this parasite in the sampled region may indicate that the allochthonous host is well adapted to the new environment, suggesting the hypothesis that these ectoparasites remained present during the colonization process of the region.

Despite the high abundance of *P. tsalickisi* in *P. amandae,* this monogenean was not identified parasitizing *P. falkneri*, a fact already observed by other authors (e.g. Alvarenga et al., 2009; Lacerda et al., 2009). This condition is curious, taking into account that *P. tsalickisi,* has already been described parasitizing *P. falkneri* in the Salobra River, Mato Grosso do Sul state (Domingues and Marques, 2007). There are proposed hypotheses in an attempt to explain the absence of monogeneans in *P. falkneri* in the upper Paraná River (e.g. Alvarenga et al., 2009). The absence by translocation (i.e., the loss of parasites during habitat changes from the lower/middle Paraná River to the upper portion of this river) here is refuted, since at least one species (i.e. *P. tsalickisi*) is present in the region of the upper Paraná River and probably colonized the region together with the potamotrygonids.

On the other hand, the fact that *P. tsalickisi* is restricted to *P. amandae* in the sampled region, even in places where there is abundance of *P. falkneri,* suggests two possible hypotheses, the first regarding the specificity of *P. tsalickisi*, which is unlikely when we analyze the work of Domingues and Marques (2007), that defines the species with has a wide distribution in the Amazon and Prata Basins and relatively low parasite specificity. As an alternative hypothesis a possible interspecific competition between Monogenea and Cestoda. In general, parasites present the specificity of microhabitats, living and feeding in specific regions of the host. This is most evident in groups such as Monogenea and Cestoda that feature a restricted variety of microhabitats. However, nothing prevents competition between these two groups, can influence their specificity (Adamson and Caira, 1994). The absence of monogeneans in *P. falkneri* may be more related to the abundance of cestodes than the specificity of these parasites in relation to the host. The results indicate an evident pattern of interspecific competition of parasitism, in which a host parasitized by cestodes, does not have monogeneans parasites, and if parasitized by monogeneans, are not parasitized by cestodes. The effects of this competition can be presented in two ways: parasites can modify their microhabitat in the presence of the other (Interactive site Selection) (Adamson et al., 1992; Bush and Holmes, 1986; Stock and Holmes, 1988), or they tend not to occur in certain hosts (Competitive Exclusion) (Bates and Kennedy, 1990; Poulin, 2001). Both hypotheses are speculative, requiring additional studies.

Cestoda is a very abundant group of *P. falkneri* in the sampled region, but few specimens were collected in *P. amandae.* In cestodes, this divergence may be related to the diet of the host species. *P. amandae* and *P. falkneri* present high trophic niche breadth and significant differences in diet composition, possibly associated with body and oral size this species (Pagliarini et al., 2020). The diet is an expected predictor of parasite diversity in vertebrates (Poulin and Morand, 2000) and tapeworms are generally acquired via ingestion of an infected prey, one would assume that tapeworm diversity is influenced by host diet. Furthermore, parasite diversity is not randomly distributed and is in part affected by host diet (Poulin, 1995). Hosts with broader diets generally harbour a greater parasite diversity (Poulin, 1995).

The species of cestodes recorded in the sampled region has phylogenetic affinities more related to cestodes parasitic on marine elasmobranchs than to cestodes parasitic on freshwater teleosts. In our work, the most abundant taxon of cestodes is the genus *Rhinebothrium*. About

41 species are described for the genus *Rhinebothrium,* however, only eight parasites potamotrygonids (Ruhnke et al., 2017). In the present study, *R. paratrygoni* was the only species found, parasitizing mainly *P. falkneri* and, unlike its congeners, is widely distributed in the Prata Basin (Lacerda et al., 2009; Lehun et al., 2020; Takemoto et al., 2009).

Rhinebothroides glandularis has long been considered restricted to the Orinoco Delta and upper Paraguay River, exclusively parasitizing *P. orbignyi* and *P. motoro* (Brooks and Amato, 1992; Brooks and Amato, 1992). However, more recently the distribution of *R. glandularis* was revised and expanded to the lower Amazon River, Negro River, Tocantins River, Branco River, Parnaíba River and Solimões River, parasitizing at least six different species of potamotrygonids (Marques and Brooks, 2003). Our results suggest *R. glandularis* was found to parasitize only *P. falkneri,* even where the populations of *P. amandae* and *P. falkneri* are syntopic, in this work we reported the first record of *R. glandularis* in the region of the upper Paraná River.

Our results suggest only the presence of *Acanthobothrium quinonesi* in the upper Paraná River region. However, for the Prata Basin is recorded a second species, *A. terezae*, widely distributed in the lower Paraná River, Paraguay and Araguaia Rivers drainages, and several locations in the Amazon Basin (Cardoso Jr., 2010). The diversity of the genus *Acanthobothrium* in freshwater is relatively low when compared to marine diversity. Only seven species of the genus are recorded in freshwater environment, all known species being unique parasites of potamotrygonids (Caira and Jensen, 2017). In freshwater environments the diversity of the genus *Acanthobothrium* may be underestimated (Caira and Jensen, 2017; Cardoso Jr., 2010; Fyler et al., 2009). On the other hand, the evidence available so far does not allow to rule out or confirm the hypothesis of reduced phylogenetic diversity during the colonization process of its hosts to the continental environment.

The genus *Potamotrygonocestus* was the only one represented by more than one species in the sampled region. Two putative new species were identified, *Potamotrygonocestus* sp. 1 and *Potamotrygonocestus* sp. 2, both parasitizing only *P. falkneri.* The occurrence of *Potamotrygonocestus* sp. 1 in *Potamotrygon falkneri* in the upper Paraná River was previously reported by other authors (Lacerda et al., 2009; Luchetti, 2011). *Potamotrygonocestus* sp. 1 is widely distributed in the Paraguay, Paraná and Amazonas Rives drainages (Luchetti, 2011). *Potamotrygonocestus* sp. 2, on the other hand, is geographically restricted to the Prata Basin, occurring in the Paraguay River and lower Paraná River (Luchetti, 2011), being the first record of *Potamotrygonocestus* sp. 2 in the upper Paraná system. The nomenclature used follows the proposed by Luchetti (2011), but additional information on molecular aspects the species of the genus *Potamotrygonocestus* is necessary for a more robust taxonomic identification.

Our results detected high prevalence rates for *A. quinonesi* and *R. paratrygoni* in *P. falkneri.* The high prevalence of these species was also observed in other locations in the upper Paraná River (see Lacerda et al., 2009). The prevalence of *A. quinonesi* and *R. paratrygoni* in *P. amandae* was substantially lower, even in locations where these hosts are syntopic and with high prevalence rates in *P. falkneri* (Table 1). *Potamotrygonocotyle tsalickisi,* the only species of Monogenea collected, also has high prevalence rates, reaching 100% in some locations (Table 1). Our work brings the first data on ecological aspects of monogenean parasites of potamotrygonids for the region of the upper Paraná River.

According to Caswell (1978) and Bush and Holmes (1986), only core species (in equilibrium) have predictable ecological patterns, while satellite species are unstable. The presence of core species in both collection sites suggests a competitive dynamic between the different groups (or species) of parasites. In this context, any changes in these subunits may modify the hierarchical community status in the community. Regarding the community status within the sampled communities, most species of parasites were considered satellites, however, two species stand out as central, *R. paratrygoni* in *P. falkneri* and *P. tsalickisi* in *P. amandae* (in S1).

The exploratory analysis with GLMM suggests some preliminary data about the parasitic abundances observed in potamotrygonids. In general, larger animals have higher abundance rates when compared to smaller animals (Table 2 and Fig. 4). However, these conjectures require more specific investigations for a better understanding of these ecological patterns. In part, the observed pattern can be explained by the fact that larger fish are able to carry more parasites, since growth is associated with a direct increase in the surface area to which parasites can attach. Therefore, the greater abundance in larger host may be the result of an accumulation of parasitic infection over time (i.e. with age) in conjunction with the increased surface area of the microhabitat available to parasites (Hagmayer et al., 2020). Another relevant abundance correlation is with the Condition Factor, where in healthier animals (i.e. with higher K values) the abundance tends to decrease substantially (Table 2 and Fig. 4). Considering that parasites are harmful to their hosts, this negative correlation between parasite abundance and K value is expected and was also observed in other Chondrichthyes (Tavares-Dias et al., 2008).

All potamotrygonids present histotrophic viviparity as reproductive mode (Garrone Neto, 2010; Hamlett, 2005; Lodé, 2012; Rangel et al., 2020). This reproductive mode requires a high expenditure of maternal energy reserves to supply vitellogenesis and gestational demand (Hamlett, 2005; Rangel et al., 2020). In parasitic environments, hosts can allocate a greater amount of energy resources to the immune system and defense measures against parasites and other pathogens (Sheldon and Verhulst, 1996). Given this, it is expected that females presented greater abundance when compared to males. This pattern was observed only in *P. amandae* in S1 and can be attributed only to monogenean infection. May suggest that the parasitic fauna presents a certain opportunism during the reproductive period of its hosts, at a time when a large part of the energy resources and photodynamic would be directed towards pregnancy, damaging the hosts natural countermeasures and favoring the recruitment of parasites (Barber et al., 2000).

Finally, it is worth mentioning that studies related to parasitic fauna in potamotrygonids have been based on reduced numbers of host specimens and localities (Brooks and Amato, 1992; Ivanov, 2004). In recent decades, several research groups have been dedicated to clarifying aspects of taxonomy and systematics of potamotrygonids parasites (e.g. Bueno 2010; Cardoso Jr., 2010; Domingues and Marques, 2007; Fehlauer-Ale, 2009; Luchetti, 2011; Marques, 2000), resulting in a few studies that address information related to the freshwater ecology of the parasite/host relationship of the group, even in well-sampled regions such as the upper Paraná River (e.g. Alvarenga et al., 2009; Lacerda et al., 2009). Additionally, in this work, we present the first information on parasitic ecological aspects for *Potamotrygon amandae* in the upper Paraná System.

Investigating ecological aspects in freshwater stingrays requires a lot of time and sampling effort, requiring a very specific logistics of host capture, processing, and analysis of their parasites, mainly because the host species are in general, medium to large size, along with the fact that some species of parasites have high abundance rates (>2000 specimens per host). These difficulties reflect an underestimated taxonomic and ecological diversity for the species that parasitize potamotrygonids. As evidence, we present the first records of *R. glandularis* and *P. tsalickisi* for the upper Paraná River system. Additionally, a putative new species is also recorded, *Potamotrygonocestus* sp. 2, being also its first record in the upper Paraná system.

The composition of the parasitic fauna is a product resulting from the interactions of biotic and abiotic factors, and possibly other intrinsic attributes inherent in this dynamic. We hope that our work encourages more researchers to investigate the parasitic fauna in Potamotrygonidae, increasing the knowledge about the taxonomy and ecology of the parasites of this group, attempt to reconstruct in part the natural history of the complex historical relationships between parasite/host of freshwater stingrays.

CRediT authorship contribution statement

Jumma Miranda Araújo Chagas: Writing – review & editing, Writing – original draft, Visualization, Validation, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Douglas de Castro Ribeiro:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation. **Thalita Fischer Santini Mendes:** Methodology, Investigation. **Felipe Chinaglia Montefeltro:** Writing – review & editing, Supervision, Project administration, Methodology. **Luciano Alves dos Anjos:** Writing – review & editing, Supervision, Project administration, Methodology, Investigation, Funding acquisition.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Jumma Miranda Araujo Chagas reports financial support was provided by CNPq - Conselho Nacional de Desenvolvimento Científico e Tecnológico - Brazil (Process nº 163765/2015-4). If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors would like to thank Dr. Cristiéle S. Ribeiro, Dr. João F.P. Bastos, and Dr. Marcos Chiquitelli for their help during the fieldwork. We thank Dr. Fernando P.L. Marques and Dr. Marcus V. Domingues for help in identifying and processing parasites.

References

Adamson, M.L., Buck, A., Noble, S., 1992. Transmission pattern and intraspecific competition as determinants of population structure in Ppinworms (oxyurida: nematoda). J. Parasitol. 78 (3), 420. https://doi.org/10.2307/3283638.

Adamson, M.L., Caira, J.N., 1994. Evolutionary factors influencing the nature of parasite specificity. Parasitology 109 (S1), S85–S95. https://doi.org/10.1017/S0031182000085103.

Alvarenga, F.M.S., Lacerda, A.C.F., Takemoto, R.M., Pavanelli, G.C., 2009. Ausência de parasitas metazoários branquiais em Potamotrygon falkneri (Potamotrygonidae) e suas implicações ecológicas na planície de inundação do alto rio Paraná, Brasil, pp. 589–592.

Barber, I., Hoare, D., Krause, J., 2000. Effects of parasites on fish behaviour: a review and evolutionary perspective. Rev. Fish Biol. Fish. 10 (2), 131–165. https://doi.org/10.1023/A:1016658224470.

Bates, D., Mächler, M., Bolker, B., Walker, S., 2014. Fitting linear mixed-effects models using lme4. arXiv. http://arxiv.org/abs/1406.5823. (Accessed 2 March 2024).

Bates, R.M., Kennedy, C.R., 1990. Interactions between the acanthocephalans *Pomphorhynchus laevis* and *Acanthocephalus anguillae* in rainbow trout: testing an exclusion hypothesis. Parasitology 100 (3), 435–444. https://doi.org/10.1017/S0031182000078732.

Boeger, W., Kritsky, D., 1993. Phylogeny and a revised classification of the monogenoidea bychowsky, 1937 (Platyhelminthes). Syst. Parasitol. 26, 1–32. https://doi.org/10.1007/BF00009644.

Brooks, D.R., Amato, J.F.R., 1992. Cestode parasites in potamotrygon motoro (natterer) (Chondrichthyes: Potamotrygonidae) from Southwestern Brazil, including Rhinebothroides mclennanae n. sp. (Tetraphyllidea: Phyllobothriidae), and a revised host-parasite checklist for helminths inhabiting neotropical freshwater stingrays. J. Parasitol. 78 (3), 393. https://doi.org/10.2307/3283633.

Bueno, V.M., 2010. Delimitação de espécies em *Rhinebothroides* Mayes, Brooks & Thorson, 1981(Cestoda: Tetraphyllidea) com ênfase no complexo *Rhinebothroides freitasi* (Rego, 1979). Dissertação (mestrado). Instituto de Biociências da Universidade de São Paulo, Departamento de Zoologia.

Bush, A.O., Holmes, J.C., 1986. Intestinal helminths of lesser scaup ducks: patterns of association. Can. J. Zool. 64 (1), 132–141. https://doi.org/10.1139/z86-022.

Bush, A.O., Lafferty, K.D., Lotz, J.M., Shostak, A.W., 1997. Parasitology Meets Ecology on Its Own Terms: Margolis et al. Revisited. J. Parasitol. 83 (4), 575. https://doi.org/10.2307/3284227.

Caira, J., Healy, C., 2004. Elasmobranchs as hosts of metazoan parasites. In: Musick, J., Carrier, J., Heithaus, M. (Eds.), Biology of Sharks and Their Relatives, 20043354. CRC Press, pp. 523–551. https://doi.org/10.1201/9780203491317.ch18.

Caira, J.N., Jensen, K. (Eds.), 2017. Planetary Biodiversity Inventory (2008-2017): Tapeworms from Vertebrate Bowels of the Earth, Special Publication No. 25. Natural History Museum, the University of Kansas, Lawrence, Kansas.

Cardoso, Jr.M., 2010. Taxonomia das linhagens de *Acanthobothrium* Van Beneden, 1850 (Eucestoda: Tetraphyllidea) parasitas de Potamotrigonidae (Chondrichthyes: Myliobatiformes). In: Dissertação (Mestrado). Instituto de Biociências da Universidade de São Paulo, Departamento de Zoologia.

Carlson, C.J., Dallas, T.A., Alexander, L.W., Phelan, A.L., Phillips, A.J., 2020. What would it take to describe the global diversity of parasites? Proc. R. Soc. A B. 287 (1939), 20201841 https://doi.org/10.1098/rspb.2020.1841.

Carvalho, M., Paulo, J., Da Silva, J.P., Loboda, T., Fontenelle, J.P., Silva, F., Ragno, M., Soares, M., Laurini, C., Shibuya, A., Lúcia, M., Araújo, G., Marques, F., 2013. Systematics and Evolution of the Highly Diverse and Morphologically Complex Neotropical Freshwater Stingrays (Chondrichthyes: Potamotrygonidae).

Carvalho, M.R.D., Rosa, R.S., Araújo, M.L.G.D., 2016. A new species of neotropical freshwater stingray (Chondrichthyes: Potamotrygonidae) from the rio Negro, Amazonas, Brazil: the smallest species of potamotrygon. Zootaxa 4107 (4), 566. https://doi.org/10.11646/zootaxa.4107.4.5.

Caswell, H., 1978. A general formula for the sensitivity of population growth rate to changes in life history parameters. Theor. Popul. Biol. 14 (2), 215–230. https://doi.org/10.1016/0040-5809(78)90025-4.

Chao, A., Gotelli, N.J., Hsieh, T.C., Sander, E.L., Ma, K.H., Colwell, R.K., Ellison, A.M., 2014. Rarefaction and extrapolation with Hill numbers: a framework for sampling and estimation in species diversity studies. Ecol. Monogr. 84 (1), 45–67. https://doi.org/10.1890/13-0133.1.

Dagosta, F.C.P., Monção, M.S., Nagamatsu, B.A., Pavanelli, C.S., Carvalho, F.R., Lima, F.C.T., Langeani, F., Dutra, G.M., Ota, R.R., Seren, T.J., Tagliacollo, V., Menezes, N.A., Britski, H.A., Pinna, M.D., 2024. Fishes of the upper rio Paraná basin: diversity, biogeography and conservation. Neotrop. Ichthyol. 22 (1), e230066 https://doi.org/10.1590/1982-0224-2023-0066.

Domingues, M.V., Marques, F.P.L., 2007. Revision of *Potamotrygonocotyle* Mayes, Brooks & Thorson, 1981 (Platyhelminthes: monogenoidea: Monocotylidae), with descriptions of four new species from the gills of the freshwater stingrays *potamotrygon* spp. (Rajiformes: Potamotrygonidae) from the La plata river basin. Syst. Parasitol. 67 (3), 157–174. https://doi.org/10.1007/s11230-006-9086-y.

Domingues, M.V., Marques, F.P.L., 2011. Phylogeny and taxonomy of *Potamotrygonocotyle* Mayes, Brooks & Thorson, 1981 (Monogenoidea: Monocotylidae) with a description of four new species. J. Helminthol. 85 (4), 353–380. https://doi.org/10.1017/S0022149X10000684.

Faul, F., Erdfelder, E., Buchner, A., Lang, A.-G., 2009. Statistical power analyses using G*Power 3.1: tests for correlation and regression analyses. Behav. Res. Methods 41 (4), 1149–1160. https://doi.org/10.3758/BRM.41.4.1149.

Fehlauer-Ale, K.H., 2009. Sistemática Molecular e Biogeografia dos Monogenoidea (Platyhelmintos: Cercomeromorpha), parasitas das brânquias de Potamotrygonidae (Condrichthyes: Rajiformes: Myliobatoidei. Tese (Doutorado). Instituto de Biociências da Universidade de São Paulo, Departamento de Zoologia.

Fricke, R., Eschmeyer, W.N., van der Laan, R., 2024. Eschmeyer's Catalog of Fishes: Genera, Species, References. http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp?_gl=1*jdbeq4*_ga*MTI3OTQyMzE2MC4xNzA0NDY2NDE1*_ga_6Y72VP61VZ*MTcwNDQ3MTA2NS4yLjAuMTcwNDQ3MTA3MC41NS4wLjA. (accessed 5 January 2024).

Fyler, C.A., Caira, J.N., Jensen, K., 2009. Five new species of Acanthobothrium (Cestoda: Tetraphyllidea) from an unusual species of Himantura (Rajiformes: Dasyatidae) from northern Australia. Folia Parasitol. 56 (2), 107–128. https://doi.org/10.14411/fp.2009.016.

Garrone Neto, D., 2010. Considerações sobre a reprodução de duas espécies de raias (Myliobatiformes, Potamotrygonidae) na região do Alto Rio Paraná, Sudeste do Brasil. Pan-Am. J. Aquat. 5, 101–111.

Hagmayer, A., Furness, A.I., Pollux, B.J.A., 2020. Parasite infestation influences life history but not boldness behavior in placental live-bearing fish. Oecologia 194 (4), 635–648. https://doi.org/10.1007/s00442-020-04795-6.

Hamlett, W.C. (Ed.), 2005. Reproductive Biology and Phylogeny of Chondrichthyes: Sharks, Batoids, and Chimaeras. Science Publishers, Enfield, NH.

Hammer, Ø., Harper, D.A.T., Ryan, P.D., 2001. Past: Paleontological Statistics Software Package for Education and Data Analysis.

Hanski, I., 1982. Dynamics of regional distribution: the core and satellite species hypothesis. Oikos 38 (2), 210. https://doi.org/10.2307/3544021.

Hsieh, T.C., Ma, K.H., Chao, A., 2016. iNEXT: an R package for rarefaction and extrapolation of species diversity (H ill numbers). MEE 7 (12), 1451–1456. https://doi.org/10.1111/2041-210X.12613.

Ivanov, V.A., 2004. A new species of *Rhinebothroides* Mayes, Brooks & Thorson, 1981 (Cestoda: tetraphyllidea) from the ocellate river stingray in Argentina, with amended descriptions of two other species of the genus. Syst. Parasitol. 58 (3), 159–174. https://doi.org/10.1023/B:SYPA.0000032933.71645.1e.

Lacerda, A.C.F., Takemoto, R., Pavanelli, G., 2009. Ecology of endoparasites of the fluvial stingray Potamotrygon falkneri (Chondrichthyes: Potamotrygonidae) from the upper Paraná River floodplain, Brazil. Braz. J. Biol. 69 (2), 297–303. https://doi.org/10.1590/S1519-69842009000200009.

Langeani, F., Castro, R.M.C.E., Oyakawa, O.T., Shibatta, O.A., Pavanelli, C.S., Casatti, L., 2007. Diversidade da ictiofauna do Alto Rio Paraná: composição atual e perspectivas futuras. Biota Neotropica 7 (3), 181–197. https://doi.org/10.1590/S1676-06032007000300020.

Leary, S.L. (Ed.), 2013. AVMA Guidelines for the Euthanasia of Animals, 2013 edition, 2013 edition. American Veterinary Medical Association, Schaumburg, IL.

Lehun, A.L., Hasuike, W.T., Silva, J.O.S., Ciccheto, J.R.M., Michelan, G., Rodrigues, A.D.F.C., Nicola, D.N., Lima, L.D.D., Correia, A.N., Takemoto, R.M., 2020. Checklist of parasites in fish from the upper Paraná River floodplain: an update. Rev. Bras. Parasitol. Vet. 29 (3), e008720 https://doi.org/10.1590/s1984-29612020066.

Loboda, T.S., Carvalho, M.R.D., 2013. Systematic revision of the Potamotrygon motoro (Müller & Henle, 1841) species complex in the Paraná-Paraguay basin, with description of two new ocellated species (Chondrichthyes: Myliobatiformes: Potamotrygonidae). Neotrop. Ichthyol. 11 (4), 693–737. https://doi.org/10.1590/S1679-62252013000400001.

Lodé, T., 2012. Oviparity or viviparity? That is the question…. Reprod. Biol. 12 (3), 259–264. https://doi.org/10.1016/j.repbio.2012.09.001.

Luchetti, N.M., 2011. Revisão Taxonômica de *Potamotrygonocestus* Brooks & Thorson, 1976 (Eucestoda, Tetraphyllidea). Dissertação (mestrado). Instituto de Biociências da Universidade de São Paulo, Departamento de Zoologia.

Magurran, A.E., 1988. Ecological Diversity and its Measurement. Croom Helm, London Sydney.

Marques, F.P.L., 2000. Evolution of Neotropical Freshwater Stingrays and Their Parasites: Taking into Account Space and Time. (Thesis). University of Toronto.

Marques, F.P.L., Brooks, D.R., 2003. Taxonomic revision of Rhinebothroides (Eucestoda: Tetraphyllidea: Phyllobothriidae), parasites of neotropical freshwater stingrays (Rajiformes: Myliobatoidei: Potamotrygonidae). J. Parasitol. 89 (5), 994–1017. https://doi.org/10.1645/GE-3059.

Marques, F.P.L., Brooks, D.R., Araújo, M.L.G., 2003. Systematics and phylogeny of Potamotrygonocestus (Platyhelminthes, Tetraphyllidea, Onchobothriidae) with descriptions of three new species from freshwater potamotrygonids (Myliobatoidei, Potamotrygonidae). Zool. Scripta 32 (4), 367–396. https://doi.org/10.1046/j.1463-6409.2003.00111.x.

Pagliarini, C.D., Da Silva Ribeiro, C., Spada, L., Delariva, R.L., Chagas, J.M.A., Dos Anjos, L.A., Ramos, I.P., 2020. Trophic ecology and metabolism of two species of nonnative freshwater stingray (Chondrichthyes: Potamotrygonidae). Hydrobiologia 847 (13), 2895–2908. https://doi.org/10.1007/s10750-020-04283-1.

Poulin, R., 1995. "Adaptive" changes in the behaviour of parasitized animals: a critical review. Int. J. Parasitol. 25 (12), 1371–1383. https://doi.org/10.1016/0020-7519(95)00100-X.

Poulin, R., 2001. Interactions between species and the structure of helminth communities. Parasitology 122 (S1), S3–S11. https://doi.org/10.1017/S0031182000016991.

Poulin, R., Morand, S., 2000. The diversity of parasites. Q. Rev. Biol. 75 (3), 277–293. https://doi.org/10.1086/393500.

Rangel, B., Hussey, N., Niella, Y., Martinelli, L., Gomes, A., Moreira, R., 2020. Neonatal nutritional strategy of a viviparous elasmobranch with extremely low reproductive output. Mar. Ecol. Prog. Ser. 638, 107–121. https://doi.org/10.3354/meps13261.

Reiczigel, J., Marozzi, M., Fábián, I., Rózsa, L., 2019. Biostatistics for parasitologists – a primer to quantitative Parasitology. Trends Parasitol. 35 (4), 277–281. https://doi.org/10.1016/j.pt.2019.01.003.

Reyda, F.B., Marques, F.P.L., 2011. Diversification and species boundaries of Rhinebothrium (Cestoda; Rhinebothriidea) in South American freshwater stingrays (Batoidea; Potamotrygonidae). PLoS One 6 (8), e22604. https://doi.org/10.1371/journal.pone.0022604.

Ruhnke, T.R., Reyda, F.B., Marques, F.P.L., 2017. Rhinebothriidea healy, Caira, jensen, webster & Littlewood, 2009. In: Planetary Biodiversity Inventory (2008-2017): Tapeworms from Vertebrate Bowels of the Earth. https://www.marinespecies.org/aphia.php?p=sourcedetails&id=286017. (Accessed 26 May 2024).

Sheldon, B.C., Verhulst, S., 1996. Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology. TREE 11 (8), 317–321. https://doi.org/10.1016/0169-5347(96)10039-2.

Stock, T.M., Holmes, J.C., 1988. Functional relationships and microhabitat distributions of enteric helminths of grebes (Podicipedidae): the evidence for interactive communities. J. Parasitol. 74 (2), 214. https://doi.org/10.2307/3282447.

Stunkard, H.W., 1953. Life histories and systematics of parasitic worms. Syst. Zool. 2 (1), 7. https://doi.org/10.2307/2411565.

Takemoto, R., Pavanelli, G., Lizama, M., Lacerda, A., Yamada, F., Moreira, L., Ceschini, T., Bellay, S., 2009. Diversity of parasites of fish from the upper Paraná River floodplain, Brazil. Braz. J. Biol. 69 (2 Suppl. l), 691–705. https://doi.org/10.1590/S1519-69842009000300023.

Tavares-Dias, M., Martins, M., Moraes, F., Kronka, S., 2008. Fator de condição e relação hepato e esplenossomática em teleósteos de água doce naturalmente parasitados. Acta Sci. Biol Sci. 22 https://doi.org/10.4025/actascibiolsci.v22i0.2944.

West, G., Heard, D.J., Caulkett, N.A., 2007. Zoo Animal and Wildlife Immobilization and Anesthesia. Ames (Iowa). Blackwell publ.

WoRMS Editorial Board, 2024. World register of marine species. Available from: https://www.marinespecies.orgatVLIZ https://doi.org/10.14284/170.