



Gyrodactylidae (Monogenea: Platyhelminthes) gill parasites of Tetragonopterinae (Characiformes: Characidae) from the upper Paraná River floodplain, Brazil

Gyrodactylidae (Monogenea: Platyhelminthes) parasitos branquiais de Tetragonopterinae (Characiformes: Characidae) na planície de inundação do alto rio Paraná

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Abstract: Aim: This study records *Gyrodactylus neotropicalis* and *Anacanthocotyle anacanthocotyle* in new hosts: *Astyanax lacustris* and *Moenkhausia forestii*, on the upper Paraná river floodplain. Besides, ecological aspects of the parasites distribution are explored. **Methods:** Both host species were collected between March and September 2014; monogeneans were removed from the gills using a stereo-microscope and mounted in Hoyer or stained with Gomori's trichrome and mounted in Balsam of Canada. Illustrations were prepared on a phase contrast microscope. Monogeneans were identified using the appropriate literature. Prevalance, abundance, mean intensity and intensity range were calculated to parasite species in each host. **Results:** *Gyrodactylus neotropicalis* and *Anacanthocotyle anacanthocotyle* are recorded in new geographic area, upper Paraná River floodplain, in new host species, *A. lacustris* and *M. forestii* and in new infection site, gills. *A. anacanthocotyle* occur in higher prevalence, abundance and intensity parasitizing *A. lacustris*, while *G. neotropicalis* presented higher prevalence, abundance and intensity in *M. forestii*. **Discussion:** *Gyrodactylus neotropicalis* described by Kritsky & Fritts, 1970, parasite of *Astyanax fasciatus* (Cuvier), presented a different shield position between anchors than the specimens studied here; *A. anacanthocotyle* also described by Kritsky & Fritts, 1970 presented spicules in the cephalic region, what was not observed in the specimens studied here. Plasticity in parasitism may explain the difference between monogenean in different hosts. **Conclusion:** Until now, there was no record of this Gyrodactylidae species parasitizing Characidae fish in South America. Those parasites are the first Gyrodactylidae monogeneans described from *A. lacustris* and *M. forestii*.

Keywords: taxonomy; parasitology; freshwater; *Moenkhausia forestii*; *Astyanax lacustris*.

Resumo: Objetivo: Este estudo apresenta novos registros de ocorrência de *Gyrodactylus neotropicalis* e *Anacanthocotyle anacanthocotyle* parasitando *Astyanax lacustris* e *Moenkhausia forestii*, na planície de inundação do alto rio Paraná. Além disso, são discutidos aspectos ecológicos referentes à distribuição dos parasitos em seus hospedeiros. **Métodos:** Ambos os hospedeiros foram coletados entre março e setembro de 2014; os monogênicos foram removidos das brânquias sob estero-microscópio e preservadas em Hoyer ou corados com Tricrômio de Gomori e preservados em Bálsamo do Canadá. As ilustrações foram preparadas em microscópio de contraste de fases. Os monogênicos foram identificados e descritos segundo a literatura pertinente. Foram calculadas a prevalência, a abundância,



a intensidade média e a amplitude da intensidade para cada espécie de parasito em cada hospedeiro. **Resultados:** *Gyrodactylus neotropicalis* e *A. anacanthocotyle* são registradas em nova região geográfica, planície de inundação do alto rio Paraná; em novos hospedeiros, *A. lacustris* e *M. forestii* e em novo sítio de infecção, brânquias. *A. anacanthocotyle* ocorreu em maior prevalência, abundância e intensidade parasitando *A. lacustris*, enquanto *G. neotropicalis* apresentou maior prevalência, abundância e intensidade em *M. forestii*. **Discussão:** *Gyrodactylus neotropicalis* descrito por Kritsky & Fritts, 1970, parasito de *Astyanax fasciatus* (Cuvier), apresenta o escudo do haptor em posição diferente do que os espécimes encontrados aqui; *A. anacanthocotyle* também descrito por Kritsky & Fritts, 1970 apresenta espículas na região cefálica, o que não foi encontrado nos espécimes estudados aqui. A plasticidade no parasitismo pode explicar a diferença na distribuição das espécies de monogenético entre os diferentes hospedeiros. **Conclusões:** Até agora não havia registros de Gyrodactylidae parasitando Characidae na América do Sul. Essas espécies de parasitos são os primeiros monogenéticos girodactilídeos descritos para *A. lacustris* e *M. forestii*.

Palavras-chave: taxonomia; parasito; água doce; *Moenkhausia forestii*; *Astyanax lacustris*.

1. Introduction

The fish fauna of the Neotropical region is the most diversified of the world, and represent about 24% of the global marine and freshwater fish species (Vari & Malabarba, 1998). The upper Paraná River floodplain has 182 fish species; of these, around 40 species belongs to the family Characidae (Graça & Pavanelli, 2007). Some Characidae species are widely distributed in Brazil, as *Astyanax lacustris*¹ (Lütken, 1875) and *Moenkhausia forestii* Benine, Mariguela & Oliveira, 2009, as known as “lambaris” or “piabas” (Benine et al., 2009). Although these fish species are not large commercially exploited, they present great ecological importance, as main items in the feeding of commercially valuable fish (Silva et al., 2012). They are also used as baits in sport fishing, ornamental fish, in fighting larvae of mosquitoes (Sato et al., 2006) and contribute to seeds dispersion (Andrian et al., 2001).

Kritsky & Fritts (1970) described two Gyrodactylidae species on the external surface of *Astyanax fasciatus* (Cuvier) in Costa Rica: *Anacanthocotyle anacanthocotyle* and *Gyrodactylus neotropicalis*. Mendoza-Franco et al. (1999) registered those species in México, also parasitizing *Astyanax fasciatus* external surface. Salgado-Maldonado et al. (2005) registered *A. anacanthocotyle* parasitizing fins of *Astyanax aenus* in Mexico and Rodríguez-Ortiz et al. (2014) found *G. neotropicalis* in the external surface of *Poecilia sphenops*, in Costa Rica (Kohn et al., 2006).

In South America, Jara (1986) described a new occurrence of the *Anacanthocotyle* genus in Peru, parasite of *Bryconamericus peruanus* (Müller & Troschel, 1845). However, there is no identification at the specific level and the species is registered

as *Anacanthocotyle* sp. Therefore, until now, *Anacanthocotyle* genus is composed exclusively by *A. anacanthocotyle*, the type species.

With more than 450 species, *Gyrodactylus* von Nordmann, 1832 is one of the most diverse genus within the Monogenea (Harris et al., 2004). The high diversity of Characiformes contrasts with the scarcity of described species of the genus *Gyrodactylus* infecting these fishes. Currently, only six nominal species of *Gyrodactylus* have been recorded in Characiformes from the Americas (Razo-Mendivil et al., 2016). Besides, there is no registration for Gyrodactylidae parasitizing Tetraodonopterinae in South America (Thatcher, 2006; Cohen et al., 2013).

During a helminthological study on the freshwater fishes *Astyanax lacustris* and *Moenkhausia forestii*, from the upper Paraná River floodplain, *Anacanthocotyle anacanthocotyle* and *Gyrodactylus neotropicalis* were found and recorded from the gills.

2. Material and Methods

Specimens of *Astyanax lacustris* (n=28) and *Moenkhausia forestii* (n=20) were collected in the upper Paraná River floodplain (22°50'-22°70'S and 53°15'-53°40' W), southern Brazil (Figure 1), in partnership with the ILTER (International Long Term Ecological Research) /CNPq Project (site 6), between March and September 2014.

Monogeneans were removed from the gills using a stereomicroscope, killed in a 1: 4000 formalin solution and preserved in 5% formalin. Some specimens were mounted unstained in Hoyer's medium to study sclerotized structures. Other specimens, stained with Gomori's trichrome, were used to observe internal organs (Eiras et al., 2006).

Measurements are in micrometers (µm), with means followed by the range and number (n) of

¹ Previously described as *Astyanax altiparananae* (Garutti & Bristki, 2000), redescribed by Lucena & Soares (2016).

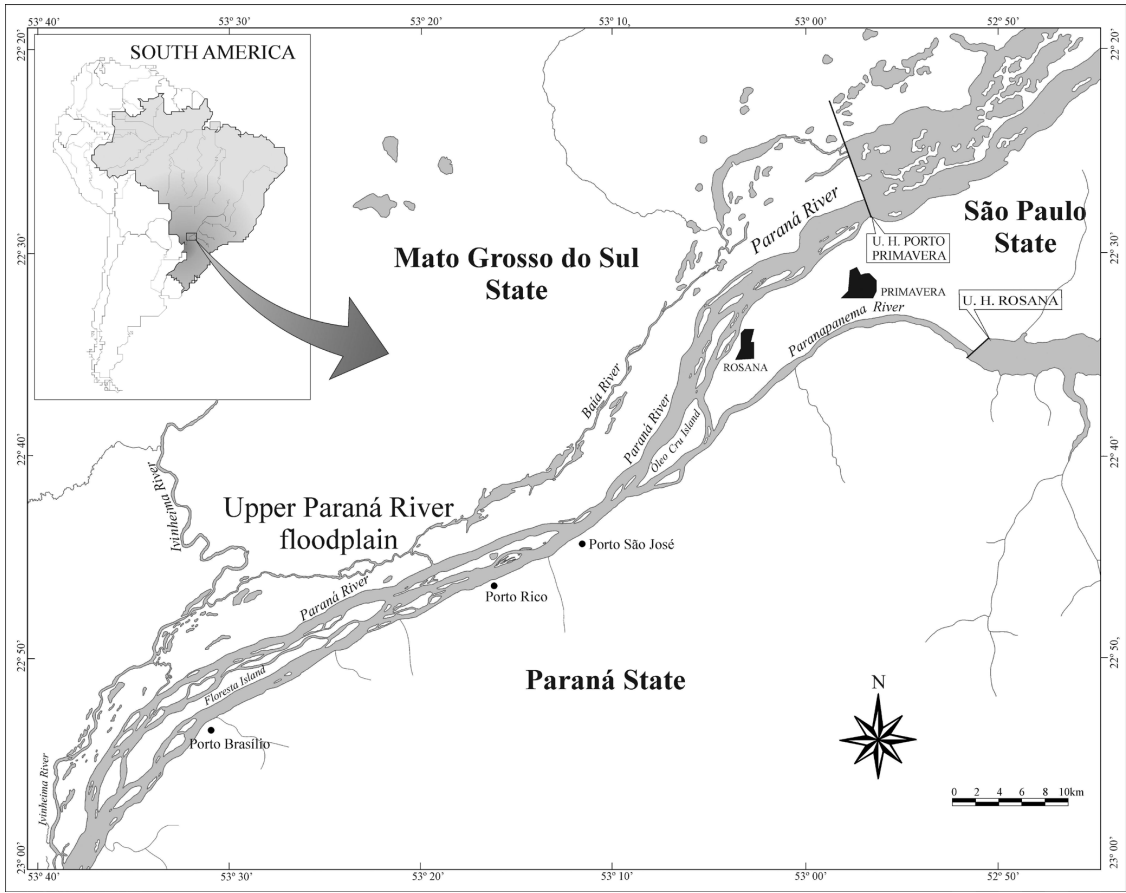


Figure 1. Map of the localization of the upper Paraná River floodplain.

specimens or structures measured in parentheses. Illustrations were prepared on a Nikon (Eclipse E200) phase contrast microscope. After that, drawings were scanned and assembled using InkScape 0.91. Voucher specimens were deposited in the Instituto Oswaldo Cruz Helminthological Collection (CHIOC), Rio de Janeiro State, Brazil.

Monogenean population of each species were described as proposed by Bush et al. (1997), based on parasite prevalence (P%), mean intensity (MI), mean abundance (MA) and intensity range (IR).

3. Results and Discussion

Anacanthocotyle anacanthocotyle (n=56) was found parasitizing three specimens of *Astyanax lacustris* (SL: 2.2-9.0 cm) and two specimens of *Moenkhausia forestii* (SL: 2.6-3.3 cm). Considering the same hosts, two specimens of *A. lacustris* and two specimens of *M. forestii* were parasitized by *Gyrodactylus neotropicalis* (n = 5). Comparing the distribution of *A. anacanthocotyle* and *G. neotropicalis* in each host, it is possible to note that *A. anacanthocotyle* occur in higher prevalence, abundance and intensity parasitizing *A. lacustris*,

while *G. neotropicalis* presented higher prevalence, abundance and intensity in *M. forestii* (Table 1).

The distribution of *Anacanthocotyle anacanthocotyle* and *Gyrodactylus neotropicalis* in each host species was similar (Table 1) and only mean intensity (MI) was higher for *A. anacanthocotyle* parasitizing *Astyanax lacustris*, what can be explained by the aggregation of monogenean distribution. The variability in abundance among populations of the same host species used to be as large as that among different host species. In this case, parasites distribution could be a reflection of the importance of local abiotic and biotic effects, independent of the identity of the host species (Poulin, 1998).

Taxonomic description

Monogenea (Van Beneden, 1858)

Gyrodactylidae (Van Beneden & Hesse, 1863)

Isancistrinae (Fuhrmann, 1928)

Anacanthocotyle anacanthocotyle Kritsky & Fritts, 1970 (Figure 2)

Hosts: *Astyanax lacustris* (Lütken, 1875) and *Moenkhausia forestii* Benine, Mariguela & Oliveira, 2009

Table 1. Gyrodactylidae parasites of *Astyanax lacustris* and *Moenkhausia forestii*, from the upper Paraná River floodplain.

| Host | Monogenea | P (%) | MA (\pm SD) | MI (\pm SD) | IR |
|-----------------------------|--|-------|--------------------|----------------------|------|
| <i>Astyanax lacustris</i> | <i>Anacanthocotyle anacanthocotyle</i> | 10.71 | 1.85 (\pm 9.43) | 17.33 (\pm 28.29) | 1-50 |
| | <i>Gyrodactylus neotropicalis</i> | 7.14 | 0.1 (\pm 0.41) | 1.5 (\pm 0.70) | 1-2 |
| <i>Moenkhausia forestii</i> | <i>Anacanthocotyle anacanthocotyle</i> | 10 | 0.2 (\pm 0.69) | 2 (\pm 1.41) | 1-3 |
| | <i>Gyrodactylus neotropicalis</i> | 10 | 0.15 (\pm 0.48) | 1.5 (\pm 0.70) | 1-2 |

P = prevalence; MA = mean abundance; MI = mean intensity; SD = standard deviation; IR = intensity range.

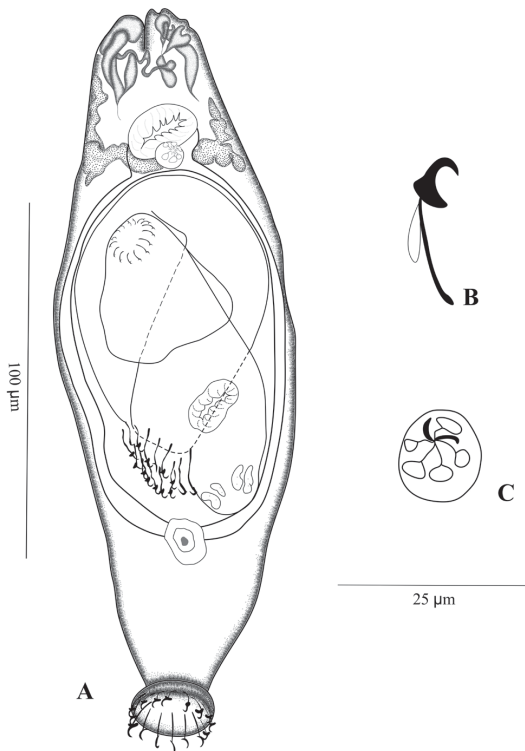


Figure 2. *Anacanthocotyle anacanthocotyle* Kritsky & Fritts, 1970. (A) Composite drawing of whole-mount (ventral view); (B) Hook; (C) Male Copulatory organ (MCO).

Locality: upper Paraná River floodplain; Brazil (22°50' – 22°70'S and 53°15' – 53°40'W)

Site: gill filaments

Specimens deposited: Voucher specimens CHIOC 338870 and CHIOC 38873

Description. (Based on 28 specimens). Body elongated, 269 (202-422; n=28) long; 90 (60-115; n=28) greater width, near midlength. Tegument thick. Cephalic lobes moderate to incipient, spicules not observed. Head organs well developed, longitudinally striated; cephalic glands moderate to well developed. Eyes absent. Pharynx muscular, ventral and anterior opening, 28 (23-34; n=26) length; 31 (24-44; n=26) width. Male copulatory organ (MCO) muscular and spherical, near pharynx region, spinelets radially arranged, MCO 12 (8-19; n=25) diameter.

Confluent intestinal cecum, in half of trunk length. Peduncle moderate to broad. Haptor opening posteriorly or posteroventrally, 26 (19-35; n=27) length; 43 (35-59; n=27) width. Anchors and bars absent. Hooks 16, radially arranged and with similar size and shape. Hook shank with slight proximal enlargement; hooklet point open or slightly recurved, thumb with shelf and pointed tip; hook length 17 (15-23; n=27). FH loop extends more than half shank length, with one looping; 5 (3-6; n=21) length. Hooks SFH indistinct. Viviparous, with two embryo generation observed.

Remarks. *Anacanthocotyle anacanthocotyle* specimens were identified according to the morphologic characters presented by Kritsky & Fritts (1970); the species can be defined by the combination of the following characters: viviparous; two cephalic lobes; anchors and bars absent; 16 hooks, radially arranged. *Anacanthocotyle anacanthocotyle* is the only species described for the genus (Thatcher, 2006) and this is the first report from Brazil.

Kritsky & Fritts (1970) described spicules in the cephalic region, above each cephalic lobe. However, spicules were not observed in the specimens analyzed here. Mendoza-Franco et al. (1999) described *A. anacanthocotyle* in a new geographic locality, but they did not mention the presence of spicules and the illustrations did not show spicules. Therefore, we did not consider such single character relevant enough to justify a new *Anacanthocotyle* species.

Gyrodactylinae (Fuhrmann, 1928)

Gyrodactylus neotropicalis Kritsky & Fritts, 1970 (Figure 3)

Hosts: *Astyanax lacustris* (Lütken, 1875) and *Moenkhausia forestii* Benine, Mariguela & Oliveira, 2009

Locality: upper Paraná River floodplain; Brazil (22°50' – 22°70'S and 53°15' – 53°40'W)

Site: gill filaments

Specimens deposited: Voucher specimens CHIOC 38872 and CHIOC 38873

Description. (Based on 6 specimens). Body elongated; 368 (324-427; n=6) long; 128 (89-142; n=6) greater width, near midlength.

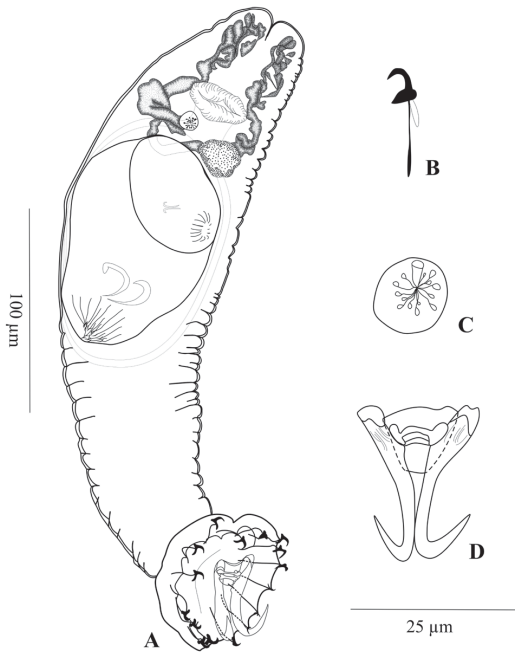


Figure 3. *Gyrodactylus neotropicalis* Kritsky & Fritts, 1970. (A) Composite drawing of whole-mount (ventral view); (B): Hook; (C) Copulatory organ (MCO); (D) Anchors, bars and shield on haptor; ventral view.

Tegument thick. Two cephalic lobes moderate to well developed, spicules absent. Head organs and cephalic glands conspicuous. Eyes absent. Pharynx muscular, ventral and anterior opening, 30 (23-34; n=5) long; 43 (38-54; n=5) width. MCO muscular and spherical, near pharynx region, with 17 spinelets radially arranged; 16 (15-17; n=2) diameter. Intestinal cecum confluent, extending to approximately two-thirds of total body length. Peduncle conspicuous. Haptor opening posteriorly or posteroventrally; 73 (64-83; n=6) long; 72 (68-89; n=6); width. Single pair of anchors; 40 (26-44; n=6) length; 30 (24-34; n=6) width. Anchors connected from the root by a shield, 15 (13-17; n=5) width; 12 (11-16; n=4) length; and two bars: superficial bar, 12 (11-13; n=5) length; deep bar, 10 (9-13; n=5) length. Hooks 16, radially arranged and with similar size and shape. Proximal hook shanks uniform; hooklet shaft and point evenly recurved; base with rounded proximal margin, shelf prominent, 26 (24-29; n=6) length. FH loop extends about half shank length, with one looping; 6 (5-8; n=6) length. Hooks SFH not observed. Viviparous, with two embryo generation observed.

Remarks. Species that belongs to the genus *Gyrodactylus* (Nordmann, 1832) are viviparous and presents: peduncle conspicuous; copulatory organ muscular, bulbous, with spines, represented only

by the MCO; ventral anchor present; superficial bar present, rod-shaped, with or without a shield; parasites of gills and external surfaces of species of Clupeiformes, Cyprinodontiformes, Perciformes, Siluriformes and Characiformes (Thatcher, 2006).

Gyrodactylus neotropicalis Kritsky & Fritts, 1970 can be defined by the combination of the following characters: spherical and ventral MCO, near the pharynx, with spinelets radially arranged; single pair of anchors, without filament, connected at their roots to each other by a shield and two bars; 16 hooks with similar size and shape, radially arranged (Kritsky & Fritts, 1970).

However, there is a difference between the *Gyrodactylus neotropicalis* from Kritsky & Fritts, (1970), parasite of *Astyanax fasciatus* (Cuvier), and the specimens observed here: shield position between anchors in haptor. Kritsky & Fritts (1970) described the shield connecting anchor at the middle, with shield situated below the deep root; but we observed the shield above the deep root (Figure 3). Secondary filament hook (SFH) was not observed, like expected according to the species description. Mendoza-Franco et al. (1999) illustrated shield above the deep root, and hooks without SFH, as we describe here.

Even though there are morphological differences between *Gyrodactylus neotropicalis* specimens, we did not consider these characters relevant enough to justify a new *Gyrodactylus* species. Since *Astyanax lacustris* and *Moenkhausia forestii* were not exclusive hosts for *A. anacanthocotyle* and *G. neotropicalis*, plasticity in parasitism may explain the difference between monogenean in different hosts (Poulin, 1998).

Anacanthocotyle anacanthocotyle and *Gyrodactylus neotropicalis* was first described parasitizing the external surface of *Astyanax fasciatus* (Cuvier) by Kritsky & Fritts (1970), in Costa Rica. Mendoza-Franco et al. (1999) described these parasite species in a new geographical locality, Yucatan Peninsula, Mexico, for the same host, but parasitizing fins.

Jara (1986), made the only register until now for *Anacanthocotyle* genus in South America, parasitizing *Bryconamericus peruanus* on Peru. There are registers of *Gyrodactylus* from South America, including Tetragonopterinae or Characidae fishes (Thatcher, 2006) but they are not specified, they are identified as *Gyrodactylus* sp. Rodríguez-Ortiz et al. (2014) described the occurrence of *G. neotropicalis* parasitizing the external surface of *Poecilia sphenops* (Valenciennes, 1846) in Costa Rica.

Anacanthocotyle anacanthocotyle and *G. neotropicalis* are the first Gyrodactylidae monogeneans parasites described from *Astyanax lacustris* and *Moenkhausia forestii*. This occurrence in the upper Paraná river floodplain, southern Brazil, expands the parasites species geographical distribution and infection sites.

Although the present study provided new data on host spectrum and distribution of Gyrodactylidae species, the information about the fauna of monogeneans in south Brazil, specially related with small fishes, like Tetragonopterinae, is still limited. This information, together with data on the occurrence of monogeneans in South America, is necessary for better understanding the species composition, distribution, history and phylogeny of these parasites of freshwater fishes.

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