

First record of helminth parasites of *Piabina thomasi* (Characiformes: Characidae) from Salta, Argentina: population and community data

Primer registro de helmintos parásitos de *Piabina thomasi* (Characiformes: Characidae), Salta, Argentina: datos poblacionales y comunitarios

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ABSTRACT: The aim of the present study was to analyze the composition and the population and component community structure of helminth parasites of *Piabina thomasi* (Characiformes, Characidae) from Puerta de Díaz Reservoir, Northwestern Argentina. Eight taxa were found in 105 specimens collected between November 2008 and April 2009: *Gyrodactylus* sp., Dactylogyridae gen. sp., *Magnivitellinum simplex*, *Petasiger* sp., Echinostomatidae gen. sp., Gryporhynchidae gen. sp., *Procamallanus (Spirocamallanus) hilarii*, and *Contracaecum* sp. Four taxa were autogenic, three were allogenic and one could be autogenic or allogenic species. Dactylogyridae gen. sp. (among adults) and *Petasiger* sp. (among larvae) were the taxa with higher prevalence and mean intensity. Significant variations were found between seasons in infrapopulations of *Petasiger* sp., *Magnivitellinum simplex*, Dactylogyridae gen. sp. and *Gyrodactylus* sp. Only the abundance of Dactylogyridae gen. sp. and Echinostomatidae gen. sp. showed significant correlation with length of host. For the first time, *Piabina thomasi* is reported as a new host for *Gyrodactylus* sp., Dactylogyridae gen. sp., *M. simplex*, *Petasiger* sp., Echinostomatidae gen. sp., Gryporhynchidae gen. sp., *P. (S.) hilarii*, and *Contracaecum* sp. and Salta, as a new locality for the former six.

KEYWORDS: Helminth, *Piabina thomasi*, Characidae, Northwestern Argentina.

RESUMEN: El objetivo de este estudio fue establecer la composición y estructura de la población y la comunidad componente de los helmintos parásitos de *Piabina thomasi* (Characiformes, Characidae) en el embalse Puerta de Díaz, Salta, al Noroeste de Argentina. Se encontraron ocho taxa en 105 especímenes colectados entre noviembre de 2008 y abril de 2009: *Gyrodactylus* sp., Dactylogyridae gen. sp., *Magnivitellinum simplex*, *Petasiger* sp., Echinostomatidae gen. sp., Gryporhynchidae gen. sp., *Procamallanus (Spirocamallanus) hilarii* y *Contracaecum* sp. Cuatro taxones fueron autógenos, tres fueron alogénicos y uno no se pudo confirmar. Entre los adultos, Dactylogyridae gen. sp. fue el taxón con los mayores valores de prevalencia e intensidad media y las metacercarias de *Petasiger* sp. lo fueron entre las larvas. Se hallaron variaciones estacionales significativas en las infrapoblaciones de *Petasiger* sp., *Magnivitellinum simplex*, Dactylogyridae gen. sp. and *Gyrodactylus* sp. Sólo dos especies parásitas (Dactylogyridae gen. sp. and Echinostomatidae gen. sp.) mostraron correlación significativa entre su abundancia y el tamaño del hospedador. *Piabina thomasi* constituye un nuevo hospedador para todas estas especies parásitas y, con excepción de *P. (S.) hilarii* y *Contracaecum* sp., todas se registran por primera vez para la provincia de Salta.

PALABRAS CLAVES: Helmintos, *Piabina thomasi*, Characidae, Noroeste argentino

INTRODUCTION

Piabina thomasi (Fowler) (Characiformes, Characidae) (Thomaz *et al.*, 2015) is a small freshwater fish that lives in Pasaje-Juramento-Salado River and upper Bermejo River basins ranging from south of Bolivia to northwest of Argentina (Miquelarena and Aquino, 1995), in the Neotropical region. They feed on algae, vegetable debris, and principally on aquatic insect (larvae of chironomids, trichopterids and beetles)

(Monasterio de Gonzo, 2003).

Previous reports of fish helminth parasites in northwestern Argentina, were for adults of *Procamallanus (Spirocamallanus) hilarii* Vaz & Pereira, 1934, larvae of *Contracaecum* sp. and metacestodes parasitizing *Jenynsia alternimaculata* (Fowler) (Cyprinodontiformes, Anablepidae) from the Isasmendi stream (Ailán Choke *et al.*, 2014). Also, intestinal

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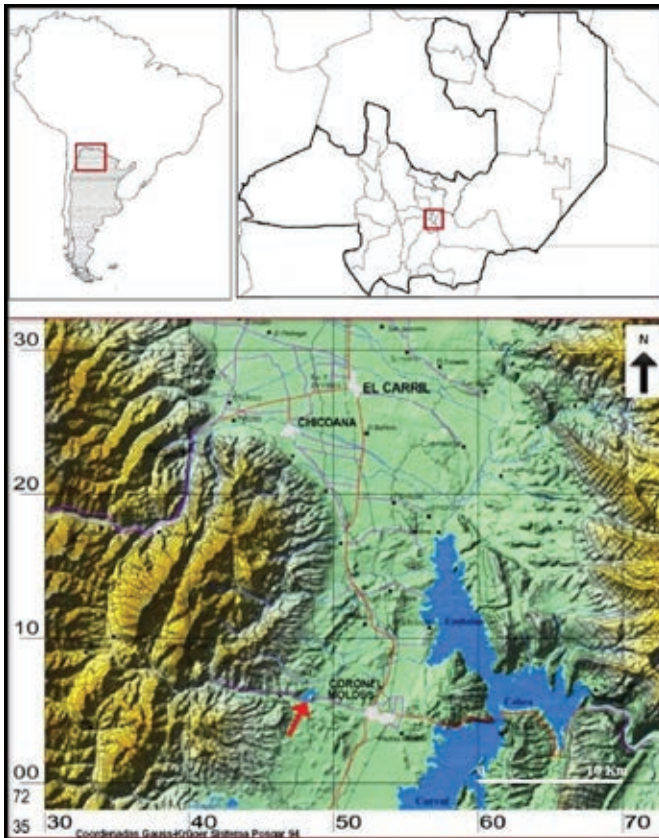


Figure 1. Location of sampling site Puerta de Díaz Reservoir (red arrow), province of Salta, Argentina.

unidentified adult nematodes and metacercariae of *Diplostomulum* were found in the brain of *Odontesthes bonariensis* (Cuvier and Valenciennes) (Atheriniformes, Atherinopsidae) captured in Puerta de Díaz reservoir (Sueldo *et al.*, 1980), while *Rhabdochona fabiana* Ramallo, 2005 (Nematoda, Spirurida) has been registered in the intestine of *Bryconamericus iheringii* (Boulenger) (Characiformes, Characidae) from Medina River (Ramallo, 2005a).

In Peru, metacercariae of *Pygidiopsis* sp. from *Piabina peruanus* (Müller and Troschel) were also registered (Jara and Escalante, 1986).

Considering the helminth fauna of *P. thomasi* is unknown, the objective of this paper is to provide information on the helminth fauna of this fish species in Northwestern Argentina, its relationships with length and sex of the host, and seasons at population and community level.

MATERIALS AND METHODS

Fish specimens were caught at Puerta de Díaz reservoir (25°16'S, 65°31'W) (Figure 1), built on Chuñapampa River that belongs to Juramento River basin (province of Salta, Northwestern Argentina) for parasitological analysis. Samples were taken with baited traps submerged near the reservoir coast during daylight from November 2008 to April 2009 (November, N=5; December, N=10; January, N=27; February, N=20; March, N=22 and April, N=21). Samples from

November to December were assigned to late spring, from January to February to summer, and from March to April to early autumn. Fish were individually placed in plastic bags with water of the reservoir and transported to the laboratory. All specimens were maintained alive in aerated containers until examination. Weight and standard length were recorded for each fish. All internal organs were examined for helminth parasites. Each organ was placed in a Petri dish and examined under a stereomicroscope. Sex was recorded by gonad inspection and the fish specimens classified as male, female or indeterminate sex, this last category was assigned to specimens with insufficient gonad development or to those that had just spawn. Helminths were removed, identified, counted, and fixed in 70% ethanol (nematodes) or 10% hot formalin (monogeneans, trematodes, cestodes). For identification were used Yamaguti (1971, 1975), Khalil *et al.* (1994), Moravec (1998), Anderson (2000), Gibson *et al.* (2002), Jones *et al.* (2005), Bray *et al.* (2008) and, Cohen *et al.* (2013). Monogeneans, trematodes and cestodes were stained with hydrochloric-carmin, cleared in creosote, and mounted in Canada balsam as permanent slides. Nematodes were cleared in lactophenol, and mounted as temporary mounts. Echinostomatidae metacercariae were extracted from cyst mechanically. Voucher helminth specimens were deposited in the Collection of Instituto para el Estudio de la Biodiversidad de Invertebrados (IEBI), Facultad de Ciencias Naturales, Universidad Nacional de Salta, (Argentina) (UNSa IEBI).

Prevalence, mean abundance, mean intensity, relative dominance, parasite species richness (S), Shannon's Diversity Index (H) and Berger-Parker Dominance Index (d) were calculated for each infracommunity of parasites (Magurran, 1988). Also, parasites were classified as autogenic or allogenic species.

The following statistical test were performed: Spearman correlation coefficient (rs) was used to verify possible correlations between host standard length with parasites abundance, Shannon's (H), Berger-Parker (d) and richness (S); Kruskal-Wallis test (KW) was used to detect possible effects of seasons on abundance and parasite species richness; Mann-Whitney test (U) to determine possible effects of host sex on abundance. Also, a Chi-square test with Yates' adjustment for independence was calculated to compare differences in prevalence of each taxon among seasons.

RESULTS

A total of 105 specimens of *Piabina thomasi* (standard length 25-51; 38.64±5.40 mm); weight (0.3-3.6; 1.53±0.73 g) were caught, from those 50

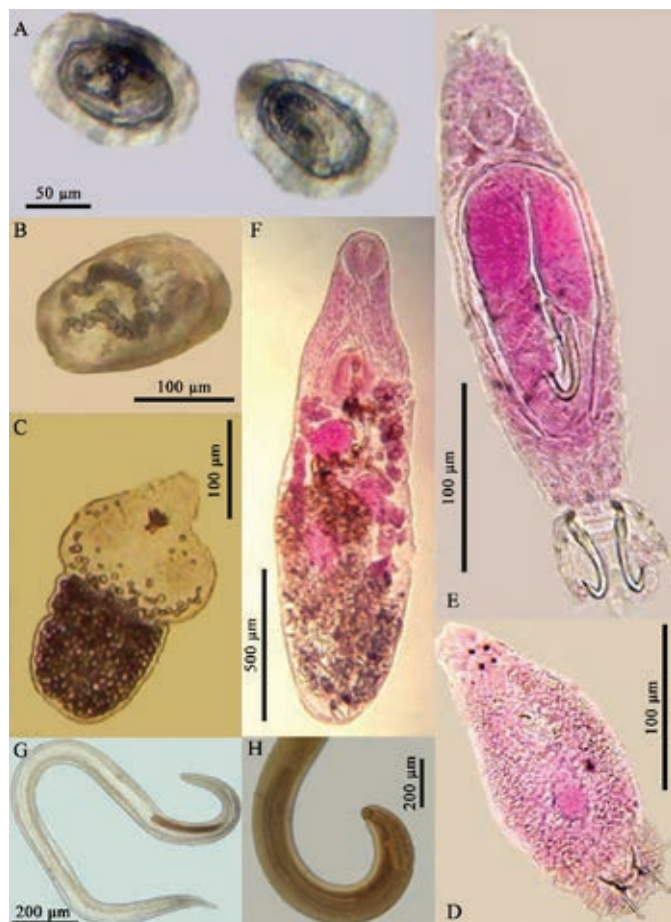


Figure 2. Helminths of *Piabina thomasi*: **A:** *Petasiger* sp., **B:** Echinostomatidae gen. sp., **C:** Gryporhynchidae gen. sp., **D:** Dactylogyridae gen. sp., **E:** *Gyrodactylus* sp., **F:** *Magnivitellinum simplex*, **G:** *Contracaecum* sp., **H:** *Procamallanus (Spirocamallanus) hilarii* (anterior end).

were males, 43 females and 12 indeterminate.

The helminthological records comprises Dactylogyridae gen. sp. (Monogenea, Dactylogyridae) (UNSA IEBI-P 142/1-2), *Gyrodactylus* sp. (Monogenea, Gyrodactylidae) (UNSA IEBI-P 141/1-2), *Magnivitellinum simplex* Kloss, 1966 (Digenea, Alloglossidiidae) (UNSA IEBI-P 140/1-3), *Petasiger* sp. (Digenea,

Echinostomatidae), Echinostomatidae gen. sp. (Digenea, Echinostomatidae), Gryporhynchidae gen. sp. (Cestodea, Cyclophyllidea, Gryporhynchidae) (UNSA IEBI-P 143/1), *Procamallanus (Spirocamallanus) hilarii* (Nematoda, Chromadorea, Camallanidae) (UNSA IEBI-P 144/1), and *Contracaecum* sp. (Nematoda, Chromadorea, Anisakidae) (UNSA IEBI-P 145/1) (Figure 2). A total of 10,090 parasites were collected, accumulating *Petasiger* sp. the huge number of specimens (N= 5964) (Table 1).

Four taxa were autogenic (AU), three were allogenic (AL) and one could be autogenic or allogenic species (Table 1). From the total, 63.5 % were larvae and 36.5% were adults. The 100 % of fish (N=105) had at least two parasite species, and the 41.9% (N=44) harbored four parasite species.

Dactylogyridae gen. sp. had the highest prevalence (99 %), *Petasiger* sp. had the highest mean abundance, mean intensity and relative dominance, and *Procamallanus (S.) hilarii* had the lowest prevalence, mean abundance, mean intensity and dominance (Table 1). For the infracommunities, the Shannon Diversity Index (H) value was 0.34 ± 0.11 (0.03-0.56) and the Berger-Parker Dominance Index value was 0.66 ± 0.14 (0.38 - 0.99).

Richness of the component community was eight species, but the infracommunities had a minimum of two species (N=13), and a maximum of seven species of helminths (N=1), with a mean number of species per host of 3.88 ± 1.10 .

Standard length of the hosts had a weak relationship with abundance in Dactylogyridae gen. sp. ($r_s=0.414$, $p<0.0001$) and Echinostomatidae gen. sp. ($r_s=0.357$, $p<0.001$) while no correlation was observed between length and abundance in *Gyrodactylus* sp. ($r_s=-0.055$, $p=0.576$), *Magnivitellinum simplex* ($r_s=-0.125$, $p=0.205$), *Petasiger* sp. ($r_s=0.242$, $p=0.013$), *Contracaecum* sp. ($r_s=-0.085$, $p=0.387$), and Gryporhynchidae gen. sp. ($r_s=0.001$, $p=0.999$).

Table 1: Number of parasites (NP), Relative Dominance (RD), Prevalence (P), Mean Abundance (MA), and Mean Intensity (MI) from *Piabina thomasi* (Puerta de Díaz Reservoir, Salta, Argentina).

Taxon and infection site*	Stages and colonization strategy**	NP	RD (%)	P (%)***	MA***	MI***
MONOGENEA						
Dactylogyridae gen. sp. (F,G)	Adults, AU	3545	35.133	99 (95.5-99.1)	33.76 (30.38-37.73)	34.09 (30.85-38.13)
<i>Gyrodactylus</i> sp. (F)	Adults, AU	29	0.287	14.3 (9.3-21.4)	0.28 (0.17-0.43)	1.93 (1.53-2.60)
DIGENEA						
<i>Magnivitellinum simplex</i> (I)	Adults, AU	110	1.090	42.9 (34.8-51.2)	1.05 (0.78-1.36)	2.44 (2.02-3.04)
<i>Petasiger</i> sp. (G,P)	Metacercariae, AL	5964	59.108	92.4 (86.6-96.1)	56.80 (48.02-68.32)	61.48 (52.80-74.48)
Echinostomatidae gen. sp. (G, S)	Metacercariae, AL	127	1.258	47.6 (39.2-56.1)	1.20 (0.91-1.57)	2.52 (2.08-3.16)
CESTODEA						
Gryporhynchidae gen. sp. (GB, IW, M, PC)	Metacestode, AL	289	2.864	73.3 (66.3-81.1)	2.74 (2.23-3.34)	3.74 (3.13-4.45)
NEMATODA						
<i>Procamallanus (S) hilarii</i> (I)	Adult, AU	1	0.009	1.0 (0.0-4.2)	-	1-

* Infection site: F=Fins; G=Gills, GB=Gall bladder; I=Intestine; IW= Intestine wall; M=Mesenteries; P=Pharynx; PC= Pyloric caeca wall; S=Skin. ** AU=Autogenic; AL=Allogenic. ***Prevalence, Mean Abundance, and Mean Intensity with Confidence Interval (90%).

dae gen. sp (rs=0.008, p=0.937).

No correlation was found between standard length and species richness (S), (rs=0.047, p=0.635), Dominance Berger-Parker Index (d), (rs=0.049, p=0.619), and Diversity Index (H), (rs=-0.083, p=0.397).

Significant differences between seasonal prevalence were found in three species: *Gyrodactylus* sp. ($X^2=9.421$, p=0.009), Dactylogyridae gen. sp. ($X^2=6.073$, p=0.048) and *Petasiger* sp. ($X^2=9.657$, p=0.008). Results of Kruskal-Wallis test indicate significant differences between seasonal abundance of six helminth parasite species (Table 2). No significant differences were found between seasons and richness (H=2, p= 0.3679).

No relationship was found between host sex and abundance of helminth species (Dactylogyridae gen. sp. U=1046, p=0.8261; *Gyrodactylus* sp. U=1068, p=0.9337; *M. simplex* U=1025, p=0.666; *Petasiger* sp. U=886.5, p=0.147; Echinostomatidae gen. sp. U=873.5, p=0.094; Gryporhynchidae gen. sp. U=947.5, p=0.318; *Contracaecum* sp. U=947.5, p=0.318).

DISCUSSION

This study represents the first report of helminths in the characid *P. thomasi*, including also population and community comparisons of the metazoan parasites with standard length and sex of the host and seasons.

The richness of parasite species found in *P. thomasi* (S=8) was higher than the reported in the characids *Astyanax bimaculatus*, *Astyanax paraguayae* and *Oligosarcus hepsetus* (S=2) from Guandu River (Abdallah et al. 2004), but lower than in *Astyanax altiparanae* (S=23) from Paraná River (Lizama et al. 2008), both records in Brasil.

From all the species found, there are no previous records of Dactylogyridae and *Gyrodactylus* sp. in Northwestern Argentina, but they were cited in Central region (Suriano 1981, 1997; Rossin and Timi, 2014) and Patagonia (Viozzi and Gutiérrez 2001;

Viozzi and Brugni 2003, 2004; Cohen and Kohn, 2008; Vega et al., 2011; Waicheim et al. 2014). In relation to digeneans, *Petasiger* sp. was reported emerging from *Biomphalaria orbigny* in Puerta de Díaz reservoir (Davies, 2014). *Magnivitellinum simplex* was previously reported in *A. bimaculatus*, *Astyanax fasciatus*, *Astyanax eigenmanniorum* and *Oligosarcus jenynsii* from Buenos Aires in Argentina (Lunaschi, 1989). Data from molecular biology analysis has changed the taxonomy of *M. simplex* from Family Macroderoididae (Yamaguti, 1975; Font and Lotz, 2008) to a new Family Alloglossidiidae. The only Planorbidae that lives in Puerta de Díaz Reservoir is *B. orbigny*, and is probably the first intermediary host for this species (Davies, 2014), considering members of the Family Macroderoididae infect snails, then form cysts in other invertebrates and posteriorly, can be eaten by fish completing the life cycle. In relationship to nematodes, larvae of *Contracaecum* are common in both freshwater and marine fishes, found throughout the world. In Argentina, they were recorded in the north of the country parasitizing freshwater fishes (Ramallo and Torres, 1995; Hamann, 1999; Ailán Choke et al., 2014) that acquired the infection by eating copepods. Adults of *Contracaecum* are in other fishes, piscivorous birds (e.g. cormorants) and mammals (Anderson, 2000). *Procamallanus* (S.) *hilarii* was reported in characid fishes from Northwestern Argentina, in provinces of Tucumán, Santiago del Estero (Ramallo, 1997; 2005b), and Salta (Ailán Choke et al., 2014). This nematode has its larval phase in copepods (Anderson, 2000), that constitutes the diet of *P. thomasi*.

Component community of *P. thomasi* in Puerta de Díaz Reservoir includes autogenic (*M. simplex*, *P. (S.) hilarii*, Dactylogyridae gen. sp., *Gyrodactylus* sp., *P. (S.) hilarii*), allogenic (Gryporhynchidae gen. sp., *Petasiger* sp. and Echinostomatidae gen. sp.) and one that can be either (*Contracaecum* sp.). The dominance of allogenic species indicates an intermediate position of *P. thomasi* in the trophic web

Table 2. Results of the Kruskal-Wallis test (H) for abundance of taxa between seasons in *Piabina thomasi* (Puerta de Díaz Reservoir, Salta, Argentina) with a posteriori Mann-Whitney test (P<0.05).

	H, p	Spring/Summer U, p	Summer/Autumm U, p	Spring/Autumm U, p
Dactylogyridae sp.	23.11 <0.0001	181.5, 0.005	438, 0.007	262, 0.286
<i>Gyrodactylus</i> sp.	3.137, 0.014	292, 0.164	797.5, 0.006	315, 0.006
<i>Magnivitellinum simplex</i>	5.361, 0.036	300.5, 0.363	789, 0.04	211, 0.02
<i>Petasiger</i> sp.	21.34, 0.02	109, 0.06	845, 0.182	79.5, 0.02
Echinostomatidae gen. sp.	12.15, 0.0008	150, 0.000	931, 0.500	150, 0.000
Gryporhynchidae gen. sp.	9.764, 0.006	167.5, 0.001	967, 0.724	168.5, 0.005
<i>Contracaecum</i> sp.	0.727, 0.412	301.5, 0.194	977, 0.662	285, 0.335

in Puerta de Díaz Reservoir. All the endohelminths detected were heteroxenous, mainly acquired by ingestion of intermediate hosts such as arthropods (e. g. *Contracaecum* sp., *P. (S.) hilarii*, Gryporhynchidae gen. sp., *M. simplex*) which are items of the diet of *P. thomasi*.

Significant differences between seasonal abundances according Kruskal-Wallis test were found. The taxa that contributed to these differences were: *Gyrodactylus* sp., Dactylogyridae gen. sp., *M. simplex* and Echinostomatidae gen. sp. (showed a maximum in summer), *Petasiger* sp. (showed a progressive increase with the maximum in autumn) and Gryporhynchidae gen. sp. (whose abundance increased in summer and stabilized in autumn).

The abundance of larvae of *Petasiger* sp. and its increment on each seasons suggests a cumulative effect, and monogeneans high values in summer could be explained by changes in water quality. Also, changes in prevalence of monogeneans and metacercariae in relation to seasons of the year might be a consequence of changes in the environment and, in habits of fish species, or a summary of all these factors. Values of community parameters (dominance, richness and diversity) cannot explain variations in length of hosts and in seasons as they were no correlated.

Changes in the structure of parasite populations and communities can occur with age, sex, behavior of host, and according to environmental factors such as season of the year or habitat (Poulin, 1998). In this study only changes between length and abundance in two species: Echinostomatidae gen. sp. and Dactylogyridae gen. sp. were recorded and no differences were registered between sex of the hosts with abundance of helminth parasites or with richness, dominance or diversity.

Studies, in other freshwater environments, of the helminths of *P. thomasi* all year round including a big number of specimens perhaps could provide a better explanation of the structure and dynamics of the populations and communities of metazoan parasites of this fish species.

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