



First report of *Diplostomum lunaschiae* (Digenea, Diplostomidae) parasitizing five fish species from a stream in the Caatinga domain, Brazil

WALLAS BENEVIDES BARBOSA DE SOUSA, MARIA FERNANDA BARROS GOUVEIA DINIZ, MARIA NAIANE MARTINS DE CARVALHO, BRUNO ANDERSON FERNANDES DA SILVA & FÁBIO HIDEKI YAMADA*

Laboratório de Ecologia Parasitária (LABEP), Departamento de Ciências Biológicas, Universidade Regional do Cariri (URCA), Crato, Ceará, 63105-000, Brazil.

* Corresponding author: fhyamada@hotmail.com

Abstract. The aim of this study was to report new records and provide a comparative morphometry of the metacercariae *Diplostomum lunaschiae* parasitizing eyes of five fish species in a stream from the Caatinga domain, in Brazil.

Keywords: Freshwater fish, helminths, Neotropical region, trematodes.

Primeiro registro de *Diplostomum lunaschiae* (Digenea, Diplostomidae) parasitando peixes de riachos do domínio Caatinga, Brasil. Resumo: O objetivo deste estudo foi relatar novos registros e fornecer uma morfometria comparativa das metacercárias *Diplostomum lunaschiae* parasitando olhos de cinco espécies de peixes em um riacho do domínio Caatinga, no Brasil.

Palavras-chave: Peixes de água doce, helmintos, região Neotropical, trematódeos.

Introduction

The watersheds under the Caatinga domain have specific characteristics, as intermittent and seasonal regime of its rivers, in response to scarce and irregular rainfall. These factors play an important role in the organization and functionality of aquatic ecosystems (Ab'saber 1995, Leal *et al.* 2003). In this domain, there are few inventories of ichthyofauna, the latter carried out by Rosa *et al.* (2003) accounted for 240 species.

In aquatic environments, fish and invertebrates, share habitat and resources in space and time (Marcogliese 2002). Due to a long evolutionary history on earth and for inhabiting aquatic ecosystems, fish are the vertebrates with the longest exposure time and adaptation to microorganisms that favored parasitism (Thatcher 2006). Thus, constituting the group of vertebrates with the largest number of parasite species still unknown to science (Malta 1984, Luque & Poulin

2007). Parasitism is an ecological relationship in which there is an interaction between two individuals of different species, where the parasite depends on the host to survive having an intimate and lasting association (Von Zuben 1997, Rey 2011).

Among taxonomic groups of fish parasites, trematodes are endoparasites with heteroxenic life cycle, containing more than one intermediate host (Luque 2004, Neves *et al.* 2016). Commonly, in life cycle of these parasites, mollusks act as intermediate hosts, and piscivorous fish or birds as definitive hosts: however, fish can be parasitized by both adult and larval stages also acting as intermediate hosts (Pavanelli *et al.* 2008, Takemoto *et al.* 2009). The metacercariae (larval stage) is usually more pathogenic than adults, causing serious tissue damage during migration until they reach the site of infection or when they become encysted in the muscle, nervous system, gonads, eyes and among

other organs (Luque 2004, Takemoto *et al.* 2004, Pavanelli *et al.* 2008).

Among the main groups of trematodes, the Superfamily Diplostomoidea Poirier, 1886 is composed of more than 250 described species, 88 genera, 16 subfamilies and 6 families, encompassing among them the family Diplostomidae (Niewiadomska 2002). The metacercariae of this family are found parasitizing the fish eyes, being able to penetrate the retina, aqueous humor, vitreous humor or crystalline lens (Chappell 1995, Niewiadomska 1996). *Diplostomum* Lutz, 1928 is the genus frequently found parasitizing freshwater fish in Brazil (Pavanelli *et al.* 2013). Species of this genus also tend to be very abundant, it may contain dozens or hundreds of specimens infecting a single host (Chappell 1969, Valtonen *et al.* 1997, Valtonen & Gibson 1997). *Diplostomum lunaschiae* Locke, Drago, Núñez, Rangel e Souza & Takemoto, 2020 was observed parasitizing the intestine of *Tigrisoma lineatum* (Boddaert, 1783) in Argentina (Locke *et al.* 2020), and metacercariae was found in the eyes of several species of Siluriformes fish in Brazil

(Pelegrini *et al.* 2019, Locke *et al.* 2020). Herein, we report new records and provide a comparative morphometry of the metacercariae *D. lunaschiae* parasitizing eyes of five fish species in a stream from the Caatinga domain, in Brazil.

A total of 162 hosts were collected (50 *Astyanax bimaculatus* (Linnaeus, 1758); six of *Psalidodon fasciatus* (Cuvier, 1819); 43 *Hoplosternum littorale* (Hancock, 1828); one *Leporinus piau* Fowler, 1941 and 20 *Prochilodus brevis* Steindachner, 1875) between December 2018 to August 2019, in the Carás stream (7°06'37.23"S; 39°24'21.33"W), municipality of Crato, State of Ceará, Brazil (Fig. 1). The fish collection was authorized under a Permanent License for the Collection of Zoological Material (SISBIO #61328-1). All animal procedures were performed in full compliance with the Ethics Committee for Animal Experimentation (CEUA/protocol #00165/2018.1) of the Universidade Regional do Cariri (URCA). Classification and nomenclature of hosts followed Britski *et al.* (1988), Graça & Pavanelli (2007) and Leal *et al.* (2003).

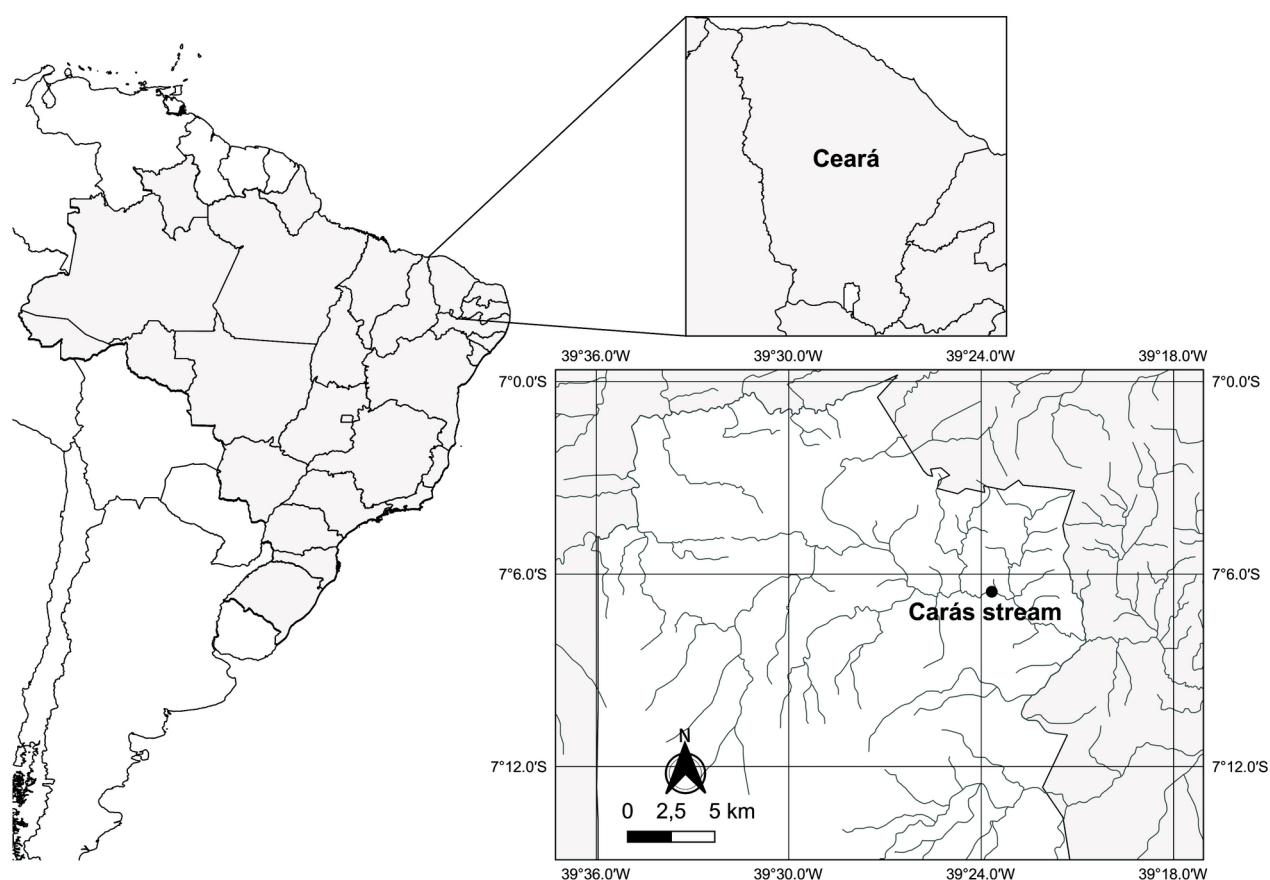


Figure 1. Sampling area in Carás stream, municipality of Crato, State of Ceará, Brazil.

The collected fish specimens were individually stored in plastic bags and placed in a Styrofoam box with ice for immediate transportation to the laboratory, where they were necropsied. The eyes were removed and analyzed fresh when possible or placed in vials containing 70% ethanol. Specimens of metacercariae were stained with hydrochloric carmine and diaphanized in eugenol medium according to Eiras *et al.* (2006). All measurements are presented in micrometers (μm) and are expressed as mean followed by standard error (SE) and the range in parentheses. Morphological and morphometrical analyses of parasites were carried out using the Motic Images Plus™ software, version 2.0. The drawings were made with the aid of a camera lucida mounted on a Leica DMLS microscope with phase contrast optics. The prevalence, mean abundance and mean intensity of infection were calculated in accordance with Bush *et al.* (1997).

A total of 1,064 metacercariae of *D. lunaschiae* (Fig. 2) were recovered from the five hosts species analyzed (651 in *A. bimaculatus*, 43 in *P. fasciatus*, 151 in *H. littorale*, 152 in *L. piau* and 67 in *P. brevis*) (Table I). Morphological and morphometrical analyses of parasites were based on 20 specimens from each analyzed host species (Table II).

The metacercariae of *D. lunaschiae* recovered from the five host species examined in the present study, are similar in morphology and measures, and also were in same site infection described by Pelegrini *et al.* (2019) (see in Table II). Pelegrini *et al.* (2019) described this metacercariae as Diplostomidae gen. sp., subsequently, Locke *et al.* (2020) from a phylogenetic analysis described the metacercariae of *D. lunaschiae*. In the present study, the metacercariae of *D. lunaschiae* has shown lower prevalence level than the hosts evaluated by Pelegrini *et al.* (2019). Even when comparing the host species *H. littorale*, fish species present in both studies, the prevalence was lower in specimens of Carás stream. According to Kennedy (2009), the local conditions may have considerable influence on the dynamics of the population of its parasites.

To date, *D. lunaschiae* have been found only parasitizing fish species belonging to Siluriformes *H. littorale*, *Hypostomus albopunctatus* (Regan, 1908), *Hypostomus ancistroides* (Ihering, 1911), *Hypostomus hermanni* (Ihering, 1905), *Hypostomus iheringii* (Regan, 1908), *Hypostomus paulinus* (Regan, 1905), *Hypostomus regani* (Ihering, 1905), *Hypostomus strigaticeps* (Regan, 1908), *Loricaria*

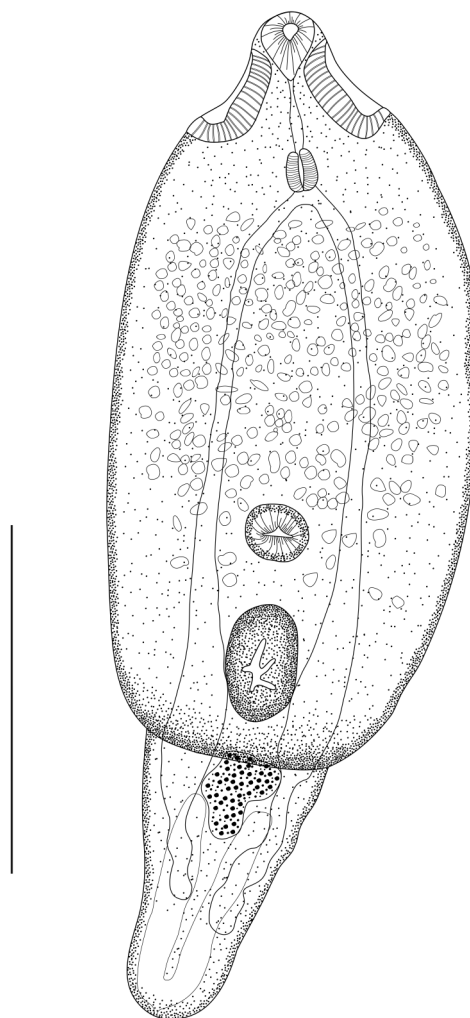


Figure 2. Metacercariae of *Diplostomum lunaschiae* Locke, Drago, Núñez, Rangel e Souza & Takemoto, 2020 from fishes collected in the Carás stream, municipality of Crato, Ceará state, Brazil. Scale-bar: 200 μm

piracicabae (Ihering, 1907), *Loricaria prolixa* (Isbrücker & Nijssen, 1978) and *Rhamdia quelen* (Quoy & Gaimard, 1824) (Pelegrini *et al.* 2019), and *Trachelyopterus galeatus* (Linnaeus, 1766) (Locke *et al.* 2020).

Parasite of the family Diplostomidae exhibit low host-specificity since many intermediate fish hosts have already been found to be infected (Zica *et al.* 2011, Ramos *et al.* 2013, Kudlai *et al.* 2017). In fact, several studies have shown that Characiformes are potential second intermediate hosts of diplostomids in Brazil (Abdallah *et al.* 2005, Guidelli *et al.* 2006, Ramos *et al.* 2013, Ramos *et al.* 2016). In the present study, this metacercariae species was recorded in four new intermediate hosts

Table I. Ecological descriptors of *Diplostomum lunaschiae* Locke, Drago, Núñez, Rangel e Souza & Takemoto, 2020 collected in five fish species from Carás stream, municipality of Crato, Ceará state, Brazil.

Host species	N	NP	P (%)	MA ± SE	MI ± SE
Prochilodontidae					
<i>Prochilodus brevis</i>	22	67	55	3.35 ± 1.1	6.09 ± 1.59
Anostomidae					
<i>Leporinus piau</i>	1	151	-	-	-
Characidae					
<i>Astyanax bimaculatus</i>	50	651	52	13.02 ± 2.31	25.03 ± 2.84
<i>Psalidodon fasciatus</i>	6	47	83.33	7.17 ± 2.07	8.6 ± 1.83
Callichthyidae					
<i>Hoplosternum littorale</i>	43	152	34.88	3.51 ± 1.05	10.78 ± 2.2

(N) number of analyzed fish; (NP) total number of parasites; (P%) prevalence; (MA) mean abundance; (MI) mean intensity; (SE) standard error.

Table II. Comparative morphometry (µm) of *Diplostomum lunaschiae* Locke, Drago, Núñez, Rangel e Souza & Takemoto, 2020 collected in five fish species from Carás stream, municipality of Crato, Ceará state, Brazil, and parasitizing Siluriformes fish from the Tietê-Batalha basin, state of São Paulo, Brazil. *Present study.

Measurements	<i>A. bimaculatus</i> *	<i>P. fasciatus</i> *	<i>H. littorale</i> *	<i>L. piau</i> *	<i>P. brevis</i> *	Siluriformes
						Pelegri et al. (2019)
Body (W)	163.9 ± 3.1 (144.4–193.4)	205.5 ± 6.5 (143.7–261.41)	205.5 ± 7.3 (136.2–252.8)	232.9 ± 7.1 (167.1–285.5)	190.8 ± 8.1 (140.9–287.9)	176.8 ± 29.4 (86.5–224.7)
Body (L)	702.3 ± 14.8 (537.9–821)	696.3 ± 23.1 (505.6–894.4)	639.8 ± 16.2 (507.8–770.9)	627.9 ± 15.9 (447.6–819.5)	731.9 ± 14.9 (622.7–826.3)	654.4 ± 88.6 (543.7–1017.4)
Forebody (W)	163.9 ± 3.1 (144.4–193.4)	205.5 ± 6.5 (143.7–261.4)	205.5 ± 7.3 (136.2–252.8)	232.9 ± 7.1 (167.1–285.5)	190.8 ± 8.1 (140.9–287.9)	177.0 ± 29.3 (92.7–230)
Forebody (L)	557.9 ± 9.2 (497.4–643.5)	547.1 ± 17.9 (395.4–699.4)	525.6 ± 13.1 (415–601.5)	481.2 ± 13.7 (376.8–691)	590.3 ± 7.9 (535.1–647.3)	535.2 ± 72.6 (417.3–810.2)
Hindbody (W)	92 ± 2.4 (73.8–112)	97.1 ± 4.1 (59.01–131.4)	92.9 ± 2.8 (59.9–120.8)	108.7 ± 4 (65.1–136.6)	94 ± 4.2 (51.2–118.7)	99.7 ± 20.5 (39.2–133.7)
Hindbody (L)	168 ± 5.5 (115.1–218.5)	155 ± 5.7 (113.9–197.2)	159.6 ± 6.7 (102.9–204.9)	150.2 ± 6.1 (67.2–191.5)	166.5 ± 7.9 (108.7–227.1)	120.4 ± 23.8 (87–203.1)
Oral sucker (W)	38.8 ± 1.1 (30–49)	39.9 ± 1.6 (28.6–55.7)	40.1 ± 0.9 (34.3–47.8)	36.8 ± 0.8 (28.1–40.4)	41.3 ± 1.2 (30.3–50.9)	36.1 ± 7.7 (11.7–48)
Oral sucker (L)	27.4 ± 1.5 (17.2–44.5)	33.6 ± 1.7 (21.21–47.4)	36.6 ± 2 (21.2–47.6)	46.5 ± 1.4 (34.1–56.6)	38.2 ± 1.3 (26.2–45.4)	37.8 ± 11 (13.2–59.6)
Ventral sucker (W)	41.2 ± 2.4 (4.2–64.5)	42.5 ± 1.3 (31.2–53.5)	45.6 ± 1.3 (36.3–56.2)	47.3 ± 1.4 (36.6–63)	46.9 ± 1.7 (27.9–61.7)	41.3 ± 11.7 (11.9–65.2)
Ventral sucker (L)	42.5 ± 1.2 (29.6–49.5)	42.282 ± 1.6 (29.7–61.9)	44.8 ± 1.1 (37.2–55.4)	44.7 ± 1 (33.8–51)	47.7 ± 2 (33–81.2)	42.8 ± 10.6 (20.7–76.3)
Pseudosuckers (W)	25.7 ± 1.4 (20.1–46.8)	21.4 ± 0.9 (14–31.7)	24.2 ± 1 (18.8–35.4)	26 ± 0.9 (16.6–33.8)	23.7 ± 1.5 (15.1–46.8)	17.7 ± 4.8 (7.7–28.9)
Pseudosuckers (L)	74.4 ± 2 (62–89.8)	79.3 ± 3.4 (55.8–112.6)	78.7 ± 1.5 (66.2–94.5)	75.8 ± 2 (56.7–97.7)	81.1 ± 2.3 (58.4–100.3)	68.8 ± 10.1 (55.3–104.6)
Holdfast organ (W)	55.3 ± 1.3 (47.1–67.3)	59.1 ± 1.5 (42–77.8)	55.9 ± 3.1 (26.7–79.8)	54.9 ± 2 (39.1–81.2)	56.6 ± 2.3 (36.9–76.3)	59.6 ± 11.8 (24.9–94.9)
Holdfast organ (L)	64.2 ± 2.3 (44.3–)	68.4 ± 2.5 ()	60.2 ± 2.7 (36.2–)	64.8 ± 2 (46.1–)	74 ± 2.9 (42.6–)	65.3 ± 10.7 (44.1–)

Measurements	Siluriformes					
	<i>A. bimaculatus</i> *	<i>P. fasciatus</i> *	<i>H. littorale</i> *	<i>L. piau</i> *	<i>P. brevis</i> *	Pelegrini <i>et al.</i> (2019)
Pharynx (W)	82.5) 26.2 ± 1.3 (15.6–36.6)	(49.21–89.7) 23.8 ± 0.8 (18.1–30.7)	78.4) 28.2 ± 3 (18.8–70.6)	80.5) (15.1–28.8)	96.7) 27.5 ± 1 (21.3–35.4)	86.7) 22.3 ± 4.7 (14–30.4)
Pharynx (L)	30 ± 1.4 (9–37.8)	30.5 ± 1.1 (21.83–43.4)	38.4 ± 3.5 (24.9–95.9)	35.1 ± 0.8 (30.2–46)	32.5 ± 0.7 (26.4–40.4)	26.8 ± 4.8 (17.7–35.3)
Dist. ventral sucker–anterior end	396.5 ± 6.4 (352.1–452)	384.8 ± 14 (261.2–499.3)	351.5 ± 21.1 (103.9–434.5)	317.8 ± 17.3 (73.2–526.2)	405.4 ± 8.6 (317.1–471.1)	417.3 ± 67.3 (303.5–653.9)
Dist. ventral sucker–holdfast organ	33.9 ± 3.4 (12.2–72.2)	46 ± 6.7 (4.93–103.5)	72.3 ± 6.6 (7.4–117.2)	16.4 ± 1.9 (5–32.3)	35.6 ± 1.8 (19.2–49.2)	139 ± 23.7 (101.3–206.9)
Hindbody(W)/forebody (W)	0.6 ± 0.01 (0.4–0.7)	0.5 ± 0.2 (0.29–0.64)	0.5 ± 0.02 (0.3–0.7)	0.5 ± 0.01 (0.4–0.6)	0.5 ± 0.02 (0.3–0.7)	0.6 ± 0.1 (0.4–0.7)
Hindbody(L)/forebody (L)	0.3 ± 0.01 (0.2–0.4)	0.3 ± 0.01 (0.2–0.4)	0.3 ± 0.02 (0.2–0.4)	0.3 ± 0.01 (0.2–0.4)	0.3 ± 0.01 (0.2–0.4)	0.2 ± 0.1 (0.2–0.3)
Body(W)/pseudosuckers (W)	6.6 ± 0.25 (4.1–8)	9.8 ± 0.4 (6.8–13.6)	8.7 ± 0.5 (5.7–13.4)	9.1 ± 0.3 (6.7–12.6)	8.5 ± 0.5 (4.1–14.4)	10.5 ± 2.3 (6.8–17.7)
Body(L)/pseudosuckers (L)	9.6 ± 0.3 (7–11.6)	8.9 ± 0.4 (6.7–11.9)	8.2 ± 0.2 (5.9–10)	8.3 ± 0.2 (6.9–9.7)	9.2 ± 0.3 (7–13.3)	9.6 ± 0.9 (6.8–11.3)
Pseudosuckers (W)/oral sucker (W)	0.7 ± 0.04 (0.4–1.4)	0.5 ± 0.02 (0.3–0.7)	0.6 ± 0.03 (0.4–0.9)	0.7 ± 0.03 (0.4–1)	0.6 ± 0.05 (0.4–1.4)	0.5 ± 0.1 (0.3–0.8)
Pseudosuckers (L)/oral sucker (L)	2.9 ± 0.16 (1.5–4.2)	2.4 ± 0.17 (1.57–3.8)	2.3 ± 0.1 (1.6–3.7)	1.7 ± 0.1 (1.3–2.2)	2.2 ± 0.1 (1.3–3.3)	2.0 ± 0.9 (1.0–4.8)
Pseudosuckers (W)/pharynx (W)	1 ± 0.07 (0.6–1.5)	0.9 ± 0.04 (0.5–1.3)	1 ± 0.1 (0.3–1.3)	1.2 ± 0.1 (0.7–1.8)	0.9 ± 0.1 (0.5–1.5)	0.8 ± 0.2 (0.4–1.4)
Pseudosuckers (L)/pharynx (L)	1.2 ± 0.05 (0.8–1.5)	1.2 ± 0.05 (0.8–1.6)	1.4 ± 0.1 (0.9–2)	1.2 ± 0.03 (0.9–1.4)	1.1 ± 0.1 (0.8–1.9)	2.5 ± 0.5 (1.8–4.1)
Body (W)/holdfast organ (W)	3 ± 0.05 (2.7–3.6)	3.5 ± 0.15 (2.4–4.9)	3.9 ± 0.2 (2.4–6.7)	4.3 ± 0.2 (2.1–5.1)	3.4 ± 0.1 (2.2–4.4)	3.0 ± 0.6 (1.9–4.7)
Body (L)/holdfast organ (C)	11.2 ± 0.44 (8.2–16)	10.2 ± 0.3 (8.4–13.7)	11 ± 0.5 (8.1–16.3)	9.8 ± 0.2 (8.5–12.7)	10.3 ± 0.6 (6.5–18.8)	10.1 ± 1.8 (7.1–15.7)
Forebody(W)/holdfast organ (W)	3 ± 0.05 (2.7–3.6)	3.5 ± 0.15 (2.45–4.93)	3.9 ± 0.2 (2.4–6.7)	4.3 ± 0.2 (2.1–5.1)	3.4 ± 0.1 (2.2–4.4)	3.0 ± 0.6 (1.9–4.7)
Forebody(L)/holdfast organ (L)	8.9 ± 0.32 (7–12.3)	8 ± 0.3 (6.6–11.3)	9.1 ± 0.5 (6–13.9)	7.5 ± 0.1 (6.2–9)	8.2 ± 0.4 (6.4–13.8)	8.3 ± 1.5 (5.9–12.3)
Oral sucker	1.6 ± 0.11 (0.8–2.4)	1.71 ± 0.1 (1.2–)	1.6 ± 0.1 (0.6–)	1.7 ± 0.1 (1.3–)	1.5 ± 0.1 (1–)	1.8 ± 0.5 (1.2–3.3)

						Siluriformes
Measurements	<i>A. bimaculatus</i> *	<i>P. fasciatus</i> *	<i>H. littorale</i> *	<i>L. piau</i> *	<i>P. brevis</i> *	Pelegri et al. (2019)
(W)/pharynx (W)		2.6)	2.1)	2.3)	2.1)	
Oral sucker			1 ± 0.1 (0.5–1.4)			1.6 ± 0.4 (0.8–2.3)
(L)/pharynx (C)	1 ± 0.15 (0.5–3.6)	1.1 ± 0.1 (0.6–1.6)		1.3 ± 0.04 (0.9–1.6)	1.2 ± 0.1 (0.7–1.6)	

(W) width; (L) length; (SE) standard error.

of the order Characiformes (*A. bimaculatus*, *P. fasciatus*, *L. piau* and *P. brevis*), in addition to extending its geographic distribution to the northeastern semiarid region. Diplostomids trematodes when infected the eyes of the fish, can increase the opportunities of infected fish being preyed upon by piscivorous birds. Affonso *et al.* (2017) demonstrated the movement of *Austrodiplostomum compactum* (Lutz, 1928) in eyes of *Satanoperca pappaterra* (Heckel, 1840), and found that this parasite behavior prevents that the fish are devoured by other unwanted predators, and that the moving depending on the environment luminosity. In conclusion, this study extended the record of geographic distribution of *D. lunaschiae*, as well as added five hosts species as secondary host, contributing to the knowledge of the biodiversity of fish parasites in the Caatinga domain, in Brazil.

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