An Acad Bras Cienc (2020) 92(2): e20181388 DOI 10.1590/0001-3765202020181388
Anais da Academia Brasileira de Ciências | *Annals of the Brazilian Academy of Sciences*
Printed ISSN 0001-3765 I Online ISSN 1678-2690
www.scielo.br/aabc | www.fb.com/aabcjournal

BIOLOGICAL SCIENCES

Molecular characterization of the progenetic metacercariae *Crocodilicola pseudostoma* parasitizing *Rhamdia quelen* (Siluriformes, Heptapteridae) in Brazil

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Abstract: The trematodes have developed several adaptations and strategies to complete their life cycle in the intermediate host, without even reaching the definitive host. Thus, metacercariae through progeny can produce viable eggs by self-fertilization in the second intermediate host. We analyzed 30 specimens of *Rhamdia quelen* Quoy & Gaimard 1824 (Siluriformes, Heptapteridae) collected in the Jacaré-Pepira River, Ibitinga. Among the specimens analyzed, only one host was parasitized by the progenetic metacercariae of *Crocodilicola pseudostoma* Willemoes-Suhm 1870 (Digenea: Proterodiplostomidae) presenting prevalence of 3.3%, mean intensity of 68.0 ± 12.4 and mean abundance of 2.3 ± 0.4. This is the first record of progenesis in the metacercariae of *C. pseudostoma* in the Jacaré-Pepira River, as well as the first partial sequence of COI gene obtained from this species in Brazil.

Key words: Biodiversity, Digenea, Proterodiplostomidae, Siluriformes.

INTRODUCTION

Rhamdia quelen Quoy & Gaimard 1824 belongs to the Siluriform order and Heptapteridae family. Popularly known as "Jundiá", has nocturnal habit and occupy calm and deep places of the rivers, having an omnivorous habit with piscivorous tendency. This species can be found in Central and South America, as well as in southern Brazil and is of great importance for consumption (Gomes et al. 2000, Eschmeyer et al. 2017, Froese & Pauly 2017).

The Jacaré-Pepira River is one of the main rivers that composes the Tietê-Jacaré Hydrographic Basin, located in the center of the state of São Paulo and it is considered one of the cleanest rivers in the state. This area is known as "Pantaninho", presenting an ecosystem with characteristics similar to those of Pantanal Matogrossense (Estado de São Paulo & Secretaria do Meio Ambiente 2013).

According to Mouritsen & Poulin (2002) parasites may interfere in the natural animal communities and the level of impact of the parasites on the hosts will depend of the prevalence and intensity of infection or infestation. Trematodes usually have a life cycle involving three hosts. Eggs produced by adults parasites are firstly released into the environment through the feces of the definitive host. After hatching, a free-living larvae is release and reach the first intermediate host, a mollusk, where develops into a free-living cercariae. They must find a suitable second intermediate host,

developing in a metacercariae. The life cycle is complete when the definitive host ingest the metacercariae together with the second intermediate host (Lefebvre & Poulin 2005a).

Due to the need for a high predation rate among hosts to complete the life cycle, trematodes have developed various adaptations and strategies for reaching the full cycle in the intermediate host, without even reaching the definitive host (Lagrue & Poulin 2009). Thus, metacercariae through progeny can produce viable eggs by self-fertilization in the second intermediate host, usually these eggs are released into the environment only after the host's death (Poulin 2001, Lagrue & Poulin 2009, Herrmann & Poulin 2011). According to Poulin & Cribb (2002) the greatest challenge in progenesis is the releasing of eggs into the environment. Progenetic species expect its host to die naturally by decay or predation to release the eggs (Herrmann & Poulin 2011).

The aim of this study was to analyze morphologically and molecularly the progenetic metacercariae of *Crocodilicola pseudostoma* Willemoes-Suhm 1870 (Digenea: Proterodiplostomidae) collected in *R. quelen*.

MATERIALS AND METHODS

A total number of 30 specimens of *R. quelen* were collected in the Jacaré-Pepira River, in the city of Ibitinga (21°53'30.8"S 48°48'46.0"W) between January and May 2017, under authorization to capture (SISBio, number 55914-1) and under the Ethics Commission in the Use of Animals (CEUA No. 9530230816). Fish were captured with a simple mesh fishing net and were packed in individual plastic bags to avoid any alteration of the parasitic fauna. At the time of necropsy, data on collection date, standard length (cm), weight (g) and host sex were recorded. After necropsy, the organs were separated and observed in stereomicroscope (Bel Photonics) for the collection of the parasites. A portion of the collected metacercariae were stored in 70°GL ethanol until the staining procedure and another part of the metacercariae were fixed in absolute ethanol until the molecular biology procedure.

For identification, specimens were stained with Mayer's Carmalumen and diaphanized using Eugenol, later were mounted in permanent blade using Canada Balsam (Eiras et al. 2006) and analyzed with the aid of a microscope (Nikon E200). The image software (Moticam 5.0MP) was used to perform morphometric analysis and the bibliographies of Armas de Conroy (1986) and Ferrari-Hoeinghaus et al. (2007) were used to verify if the species of this study is the same studied by these authors. Parasitological indices of prevalence, mean intensity and mean abundance were calculated according to Bush et al. (1997). A representative specimen of *C. pseudostoma* collected from the intestine of *R. quelen* was deposited in the Helminthological Collection of the Institute of Biosciences of Botucatu (CHIBB), at the Universidade Estadual Paulista "Julio de Mesquita Filho", Botucatu campus, state of São Paulo, Brazil, under deposit number: 357 L.

For molecular analysis, The DNeasy Blood & Tissue Kit (Qiagen®, Germany) was used for the extraction of deoxyribonucleic acid (DNA) from one specimen of *C. pseudostoma* found from the liver of the fish, following the animal tissue protocol. The COI (Cytochrome C Oxidase Subunit 1) gene was amplified using the primers MplatCOX1dF (5'-TGTAAAACGACGGC CAGTTTWCITTRGATCATAAG-3') and MplatCOX1dR (5'-CAGGAAACAGCTATGACTGAAAYAAYAIIGGATCI CCACC-3') (Moszczynska et al. 2009). Polymerase chain reaction (PCR) was performed using Ready-to-GoPCRbeads(PureTaq™Ready-to-Go™

beads, GE Healthcare, Chicago, USA) which consists of buffers BSA, dATP, dCTP, dGTP, Dttp and ±2.5 units of puReTaq DNA polymerase. Were added 3 µl DNA extraction, 1 µl of each primer and deionized water to complete the final volume of 25 µl. Amplification reactions were performed using Bio-Rad Mycycler (Bio-Rad Laboratories Pty Ltd., Gladesville, Australia) thermo cycler, with initial denaturation at 94 °C for 3 min, followed by 5 cycles of 94 °C for 40 s, 45 °C for 40 s for annealing temperature, 72 °C for 1 min. After, 35 cycles were performed with 94 °C for 40 s, 51 °C for annealing temperature, 72 °C for 1 min and a final extension at 72 °C for 5 min. Results of the amplifications of the DNA were analyzed in agarose gel at 1% in TAE buffer by electrophoresis. PCR product was purified using QIAquick PCR Purification Kit (Qiagen®, CA, USA).

Sequence was run using Applied Biosystems ABI 3500 DNA genetic analyzer. The sequence obtained from parasite was edited in Sequencher™ v. 5.2.4 (Gene Codes, Ann Arbor, MI) and to confirm the identity, this sequence was subjected to BLAST analyse (http://blast.ncbi.nlm.nih.gov). The partial sequence obtained from the COI gene (GenBank Acc. Num. MN516738) was aligned with related sequences previously obtained from species of trematodes recorded in Genbank. *Mesostephanus microbursa* Caballero, Grocott & Zerecero 1953 (MF398316) was used as an "outgroup".

The Geneious v. 7.1.3 (Kearse et al. 2012) with ClustalW (Larkin et al. 2007) and default settings were used to align the sequences. The analysis was performed using only positions that were unambiguously alignable across all taxa. MrBayes v. 3.1.2 (Ronquist & Huelsenbeck 2003) was used to perform bayesian inference. The nucleotide substitution model used was GTR+I+G. The Markov Chain Monte Carlo (MCMC) chains were run for 10 million generations and the log-likelihood scores plotted. The 'burn in' was set to 25%. Phylogenetic tree was generated and edited in FigTree v. 1.4 (Rambaut 2012).

RESULTS AND DISCUSSION

Fish presented an average weight of 63.0 ± 76.3 g and standard length of 14.9 ± 4.7 cm. Sixty-eight metacercariae of *C. pseudostoma* (Figure 1) were collected, some specimens were found encysted in the liver and others free in the swimming bladder, cavity, intestine and stomach

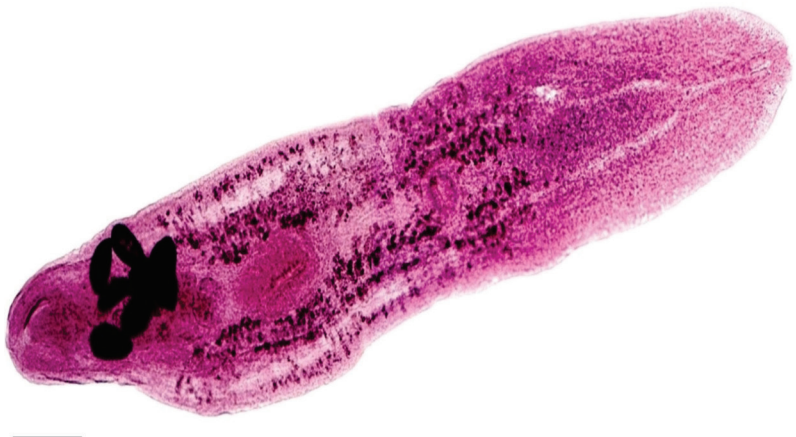

Figure 1. Progenetic metacercariae of *Crocodilicola pseudostoma* Willemoes-Suhm 1870 found encysted in the liver and free in the swimming bladder, cavity, intestine and stomach of *Rhamdia quelen* Quoy & Gaimard 1824. Scale bar: 200 µm.

of a single female host. According to Armas de Conroy (1986), the infection caused by progenetic metacercariae of *C. pseudostoma* can cause sterility in female catfish species, because the eggs that are eliminated by these parasites can change the normal functioning of the gonads. Parasitological indices of *C. pseudostoma* presented a prevalence of 3.3%, mean intensity 68.0 ± 12.4 and mean abundance 2.3 ± 0.4.

Morphometric analysis of progenetic metacercariae of *C. pseudostoma* (based on 13 specimens) found in *R. quelen* are

Table I. Comparative morphometric data of the progenetic metacercariae of *Crocodilicola pseudostoma* Willemoes-Suhm 1870 found in *Rhamdia quelen* Quoy & Gaimard 1824, *Loricariichthys platymetopon* Isbrücker & Nijssen 1979 and *Rhamdia hilarii* Valenciennes 1840 from Brazil.

		Present study	Ferrari-Hoeinghaus et al. (2007)	Armas de Conroy (1986)
Site of infection		Encysted in the liver and free in the swimming bladder, cavity, intestine and stomach	Digestive tract	Body cavity
Host		*Rhamdia quelen*	*Loricariichthys platymetopon*	*Rhamdia hilarii*
Locality		Jacaré-Pepira River, Brazil	Upper Paraná River floodplain, Brazil	Instituto de Pesca, Brazil
Body	Length	1250.0 – 3000.0 (2000.0) µm	2075.0 – 2575.0 (2364.0) µm	1336.0 – 1721.0 µm
	Width	250.0 – 580.0 (490.0) µm	500.0 – 800.0 (662.0) µm	722.0 – 845.0 µm
Oral sucker	Length	60.0 – 89.0 (71.0) µm	60.0 – 70.0 (64.0) µm	61.8 – 148.0 µm
	Width	54.0 – 86.0 (74.0) µm	50.0 – 70.0 (58.0) µm	63.0 – 80.0 µm
Pharynx	Length	61.0 – 112.0 (80.0) µm	50.0 – 70.0 (56.0) µm	54.8 – 64.8 µm
	Width	36.0 – 60.0 (48.0) µm	50.0 – 60.0 (55.0) µm	43.8 – 53.8 µm
Acetabulum	Length	100.0 – 150.0 (122.0) µm	110.0 – 150.0 (124.0) µm	102.4 – 112.8 µm
	Width	116.0 – 170.0 (136.0) µm	110.0 – 190.0 (145.0) µm	132.2 – 185.6 µm
Tribocytic organ	Length	140.0 – 252.0 (212.0) µm	170.0 – 260.0 (206.0) µm	50.4 – 78.0 µm
	Width	93.0 – 189.0 (136.0) µm	150.0 – 230.0 (182.0) µm	98.0 – 147.2 µm
Eggs	Length	99.0 – 131.0 (116.0) µm	80.0 – 120.0 (96.0) µm	111.2 – 124.0 µm
	Width	43.0 – 74.0 (60.0) µm	50.0 – 80.0 (74.0) µm	54.4 – 68.0 µm

presented in Table I, as well as are approaches the morphometric values obtained in the description by Armas de Conroy (1986), which was studied *C. pseudostoma* in the host *Rhamdia hilarii* Valenciennes 1840 from the Instituto de Pesca, state of São Paulo, Brazil and also, the morphometric values in the description by Ferrari-Hoeinghaus et al. (2007), which was studied *C. pseudostoma* collected in the intermediate host *Loricariichthys platymetopon* Isbrücker & Nijssen 1979 from the Upper Paraná River floodplain, Brazil.

The primers used successfully amplified a COI gene partial sequence of 440 base pairs. The partial sequence obtained, after edited and aligned, coincided and showed 95.5% similarity in the COI gene with the partial sequence of the diplostomidae *C. pseudostoma* available in Genbank and is the first partial sequence of the COI gene obtained from this species in Brazil (Table II). The small difference in relation to the sequence similarity obtained with the already deposited sequence of the COI gene of the *C. pseudostoma* species in Genbank by Hernández-Mena et al. (2017) possibly is related to the fact that the parasite was collected in another host species (*Rhamdia guatemalensis* Günther 1864) and also in another country (Mexico).

Phylogenetic analysis with COI gene showed the formation of three major clades, divided among families within the Diplostomoidea superfamily. In the clade of the family Proterodiplostomidae only the sequences referring to species *C. pseudostoma* was observed (Figure 2). Our study corroborated with Hernández-Mena et al. (2017) which showed that Proterodiplostomidae is the sister group of the Diplostomidae, within the superfamily Diplostomoidea. Mitochondrial genes are recognized for their usefulness in solving phylogenies at a deeper level, especially for flatworms (Littlewood et al. 2015).

The authors Armas de Conroy (1986), Pérez-Ponce de León et al. (1992) and Guidelli et al. (2003) have already registered progenetic metacercariae of *C. pseudostoma* in fish species representatives of the Siluriformes. Guidelli et al. (2003) also analyzed a prevalence of 84.6%, mean intensity of 6.5 and mean abundance of 5.5 of *C. pseudostoma* in the host *Hemisorubim platyrhynchos* Valenciennes 1840, but the values obtained were different regarding the present study due to the number of analyzed (136) and parasitized (115) fish and also the number of parasites collected (742).

According to Herrmann & Poulin (2011) the metacercariae are considered progenetic when they mature and reproduce by self-fertilization within the second intermediate host before normal time, that is, these metacercariae become an adult parasite in an early stage and in this case no need of a definitive host, therefore the life cycle of this parasite is determined to be incomplete. According to Poulin (2001), progenetic metacercariae present a lower level of infection compared to normal metacercariae, in order to keep the intermediate host alive longer. There are four factors that can cause this change in the life cycle of the parasite: 1) internal resources of the host, 2) environmental instability, 3) unavailability of the definitive host and 4) time of development of the parasite. The first factor is related to the production of eggs that depends on the feeding of the hosts or the organs in which the metacercariae are present. The second factor determines that progenesis development is related to habitat characteristics, such as water levels with unpredictable increases and decreases, salinity and water temperature. According to the third factor, progenetic metacercariae are common when definitive hosts are absent or temporarily unavailable in the study areas, or even when definitive hosts are present, but have low

Table II. Species of Proterodiplostomidae, Diplostomidae and Strigeidae presented in the molecular phylogenetic analysis with details of host, locality and reference.

Parasite	Host	Locality	Reference
(MF398317) *Crocodilicola pseudostoma*	*Rhamdia guatemalensis*	Mexico	Hernández-Mena et al. 2017
(MF398318) *Crocodilicola pseudostoma*	*Rhamdia guatemalensis*	Mexico	Hernández-Mena et al. 2017
(GQ292490) *Diplostomum huronense*	Multiple species	Canada	Locke et al. 2010a
(FJ477196) *Diplostomum indistinctum*	-	Canada	Moszczynska et al. 2009
(HM064679) *Diplostomum* sp.	Multiple species	Canada	Locke et al. 2010b
(JQ639170) *Diplostomum pseudospathaceum*	*Perca fluviatilis*	Germany	Behrmann-Godel 2013
(JX986909) *Tylodelphys clavata*	*Perca fluviatilis*	Germany	Georgieva et al. 2013
(KC685329) *Tylodelphys mashonensis*	*Clarias gariepinus*	Tanzania	Chibwana et al. 2013
(KM115882) *Austrodiplostomum ostrowskiae*	*Cichlasoma trimaculatum*	Mexico	García-Varela et al. 2015
(KR271494) *Tylodelphys jenynsiae*	*Cnesterodon decemmaculatus*	South America	Locke et al. 2015
(FJ477223) *Tylodelphys scheuringi*	-	Canada	Moszczynska et al. 2009
(KR271481) *Tylodelphys immer*	Multiple species	South America	Locke et al. 2015
(JX977780) *Apharyngostrigea cornu*	*Nyctycorax nyctycorax*	Mexico	Hernández-Mena et al. 2014
(HM064887) *Apaharyngostrigea pipientis*	-	Canada	Locke et al. 2010b
(JX977731) *Parastrigea diovadena*	*Eudocimus albus*	Mexico	Hernández-Mena et al. 2014
(JX977776) *Parastrigea plataleae*	*Platalea ajaja*	Mexico	Hernández-Mena et al. 2014
(JX977760) *Parastrigea cincta*	*Eudocimus albus*	Mexico	Hernández-Mena et al. 2014
(JX977781) *Cotylurus gallinulae*	*Aythya affinis*	Mexico	Hernández-Mena et al. 2014
(KY513232) *Cotylurus cornutus*	*Gyraulus acronicus*	Norway	Soldánová et al. 2017
(KY513232) *Cotylurus cornutus*	*Radix balthica*	Norway	Soldánová et al. 2017
(KT831347) *Cotylurus gallinulae*	*Physella gyrina*	Canada	Gordy et al. 2016
(JX977784) *Cardiocephaloides* sp.	*Larus occidentalis*	Mexico	Hernández-Mena et al. 2014
(JX977782n) *Cardiocephaloides medioconiger*	*Larus* sp.	Mexico	Hernández-Mena et al. 2014
(JX977782) *Cardiocephaloides medioconiger*	*Larus* sp.	Mexico	Hernández-Mena et al. 2014

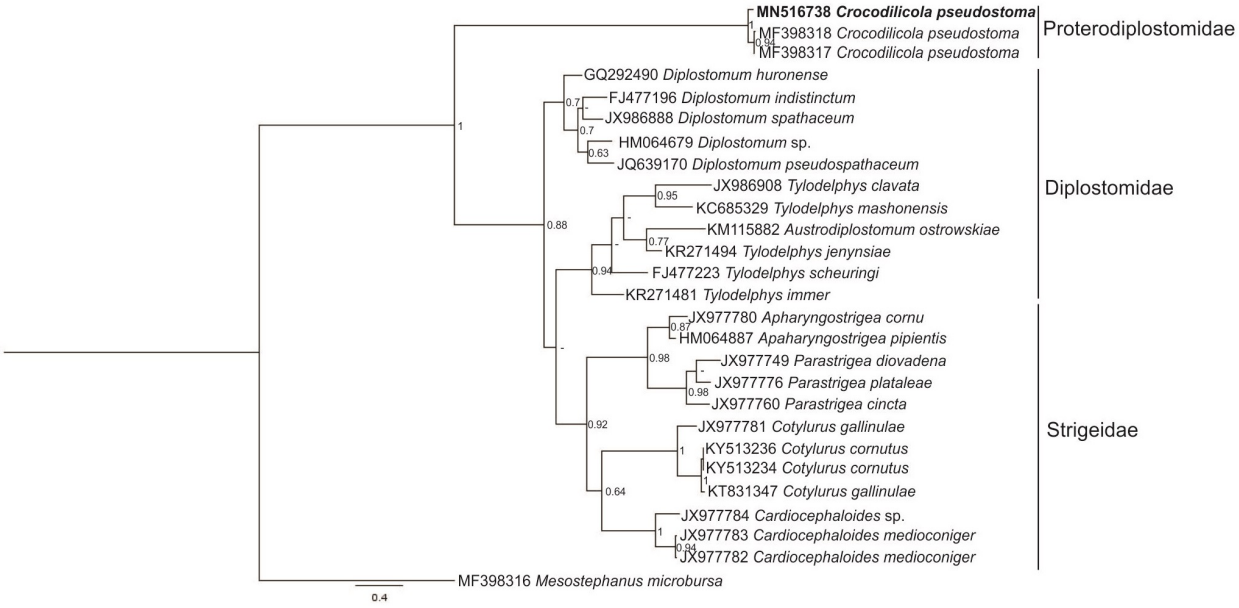

Figure 2. Bayesian inference analysis on partial COI sequences showing the position of *Crocodilicola pseudostoma* Willemoes-Suhm 1870 among other Digenea. Numbers at the nodes represent Bayesian posterior probability gaining more than 0.6 posterior probability. Values lower than 0.6 are represented by dashes. Scale bar is given under the tree.

abundance, which ends up exhibiting a low predation rate. The fourth factor is related to the prolonged duration of the metacercaria stage in the intermediate hosts (Lefebvre & Poulin 2005b).

The alligators and crocodiles are definitive hosts of *C. pseudostoma* and according to Lefebvre & Poulin (2005b) the possible cause of the presence of the progenetic metacercariae may be the extinction or reduction the definitive hosts. In this study the progenesis may be related due reduction of alligator specimens in the Jacaré-Pepira River. Similarly, the development time of the parasite in the intermediate host is a crucial factor for the development of the progenesis.

According to information on conservation of the Estado de São Paulo & Secretaria do Meio Ambiente (2013), the Jacaré-Pepira River houses species of alligators that are threatened with extinction. One of the reasons for this reduction may be related to socioeconomic activities, due to the extensive livestock and local agriculture activity, which makes the intensive use of agrochemicals, as well as the deforestation of ciliary forest for ranch construction. Another factor may be related with the population present in this region, which may be hunting the alligator for consumption.

CONCLUSION

Firstly, it is difficult to determine the cause of progenesis in this study, but with environmental changes in ecosystems, it is likely that organisms will adapt by creating some life strategies. The progenetic metacercariae shows how the parasites can adapt to a shorter life cycle due to environmental conditions, not needing the definitive host to develop. The metacercariae can also mature early and produce eggs due to

some alteration of their own development as a form of adaptation, thus obtaining the flexibility needed to adjust for changes, related or not to the absence of the host.

Although there is no certainty about the development of this progenetic metacercaria of *C. pseudostoma* in *Rhamdia quelen*, this is a study that will contribute to other morphological and phylogenetic studies, as well, it is collaborating with the study of biodiversity in the Jacaré-Pepira River, being that *C. pseudostoma* a new record for this river.

Acknowledgments

We thank to the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP - Process num. 2016/01587-3 and 2015/24901-2) for the scientific and financial support; and the Laboratório de Parasitologia de Animais Silvestres (LAPAS), Department of Parasitology, UNESP, for the support in the carrying out of the molecular biology.

REFERENCES

ARMAS DE CONROY G. 1986. *Crocodilicola pseudostoma* (Willemoes-Suhm, 1870) Poche, 1925 (Trematoda: Proterodiplostomatidae), endoparásito del bagre pimelódido *Rhamdia hilarii* Val., 1840 del Estado de São Paulo, Brasil. Rev Ibér Parasitol 46: 35-38.

BEHRMANN-GODEL J. 2013. Parasite identification, succession and infection pathways in perch fry (*Perca fluviatilis*): new insights through a combined morphological and genetic approach. Parasitology 140: 509-520.

BUSH AO, LAFFERTY KD, LOTZ JM & SHOSTAK AW. 1997. Parasitology meets ecology on its own terms: Margolis et al. revisited. J Parasitol 83: 575-583.

CHIBWANA FD, BLASCO-COSTA I, GEORGIEVA S, HOSEA KM, NKWENGULILA G, SCHOLZ T & KOSTADINVOA A. 2013. A first insight into the barcodes for African diplostomids (Digenea: Diplostomidae): Brain parasites in *Clarias gariepinus* (Siluriformes: Clariidae). Infect Genet Evol 17: 62-70.

EIRAS JC, TAKEMOTO RM & PAVANELLI GC. 2006. Métodos de estudo e técnicas laboratoriais em parasitologia de peixes, 2ª ed., Maringá, PR:UEM.

ESCHMEYER W, FRICKE R & LAAN RV. 2017. Catalog of Fishes: Genera, Species, References Available at: http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp. Accessed on 14/12/2017.

ESTADO DE SÃO PAULO & SECRETARIA DO MEIO AMBIENTE. 2013. Fundação para a Conservação e Produção Florestal do Estado de São Paulo. APA Ibitinga: Pantanal Paulista Patrimônio Socioambiental do Interior do Estado de São Paulo. 1ª ed., São Paulo: EPG - Editoração e Produção Grafica LTDA, 28 p.

FERRARI-HOEINGHAUS A, TAKEMOTO RM & PAVANELLI GC. 2007. Digenetic trematode parasites of *Loricariichthys platymetopon* (Loricariidae, Siluriformes) of the upper Paraná river floodplain, Brazil. Acta Sci Biol Sci 29: 327-329.

FROESE R & PAULY D. 2017. FishBase. World Wide Web electronic publication. Available at: http://www.fishbase.org. Accessed on 14/12/2017.

GARCÍA-VARELA M, SERENO-URIBE AL, PINACHO-PINACHO CD, DOMÍNGUEZ-DOMÍNGUEZ O & PÉREZ-PONCE DE LEÓN G. 2015. Molecular and morphological characterization of *Austrodiplostomum ostrowskiae* Dronen, (Digenea: Diplostomatidae), a parasite of cormorants in the Americas. J Helminthol 90: 174-185.

GEORGIEVA S, SOLDÁNOVÁ M, PÉREZ-DEL-OLMO A, DANGEL RD, SITKO J, SURES B & KOSTADINOVA A. 2013. Molecular prospecting for European *Diplostomum* (Digenea: Diplostomidae) reveals cryptic diversity. Int J Parasitol 43: 57-72.

GOMES LDC, GOLOMBIESKI JI, GOMES ARC & BALDISSEROTTO B. 2000. Biologia do jundiá *Rhamdia quelen* (Teleostei, Pimelodidae). Cienc Rural 30: 179-185.

GORDY MA, KISH L, TARRABAIN M & HANINGTON PC. 2016. A comprehensive survey of larval digenean trematodes and their snail hosts in central Alberta, Canada. Parasitol Res 115: 3867-3880.

GUIDELLI GM, ISAAC A, TAKEMOTO RM & PAVANELLI GC. 2003. Endoparasite infracommunities of *Hemisorubim platyrhynchos* (Valenciennes, 1840) (Pisces: Pimelodidae) of the Baía River, upper Paraná River floodplain, Brazil: specific composition and ecological aspects. Braz J Biol 63: 261-268.

HERNÁNDEZ-MENA DI, GARCÍA-PRIETO, L & GARCÍA-VARELA M. 2014. Morphological and molecular differentiation of *Parastrigea* (Trematoda: Strigeidae) from Mexico, with the description of a new species. Parasitol Int 63: 315-323.

HERNÁNDEZ-MENA DI, GARCÍA-VARELA M & LEÓN GPP. 2017. Filling the gaps in the classification of the Digenea Carus,

1863: systematic position of the Proterodiplostomidae Dubois, 1936 within the superfamily Diplostomoidea Poirier, 1886, inferred from nuclear and mitochondrial DNA sequences. Syst Parasitol 94: 833-848.

HERRMANN KK & POULIN R. 2011. Encystment site affects the reproductive strategy of a progenetic trematode in its fish intermediate host: is host spawning an exit for parasite eggs? Parasitology 138: 1183-1192

KEARSE M ET AL. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28: 1647-1649.

LAGRUE C & POULIN R. 2009. Life cycle abbreviation in trematode parasites and the developmental time hypothesis: is the clock ticking? J Evol Biol 22: 1727-1738.

LARKIN MA ET AL. 2007. Clustal W and Clustal X version 2.0. Bioinformatics 23: 2947-2948.

LEFEBVRE F & POULIN R. 2005a. Life history constraints on the evolution of abbreviated life cycles in parasitic trematodes. J Helminthol 79: 47-53.

LEFEBVRE F & POULIN R. 2005b. Progenesis in digenean trematodes: a taxonomic and synthetic overview of species reproducing in their second intermediate hosts. Parasitology 130: 587-605.

LITTLEWOOD DTJ, BRAY RA & WAESCHENBACH A. 2015. Phylogenetic patterns of diversity in the cestodes and trematodes. In: Morand S et al. (Eds). Parasite diversity and diversification: Evolutionary ecology meets phylogenetics. Cambridge: Cambridge University Press, p. 304-319.

LOCKE AS ET AL. 2015. Diversity, specificity and speciation in larval Diplostomidae (Platyhelminthes: Digenea) in the eyes of freshwater fish, as revealed by DNA barcodes. Int J Parasitol 45: 841-855.

LOCKE SA, MCLAUGHLIN JD, DAYANANDAN S & MARCOGLIESE DJ. 2010a. Diversity and specificity in *Diplostomum* spp. metacercariae in freshwater fishes revealed by cytochrome c oxidase I and internal transcribed spacer sequences. Int J Parasitol 40: 333-343.

LOCKE SA, MCLAUGHLIN JD & MARCOGLIESE DJ. 2010b. DNA barcodes show cryptic diversity and a potential physiological basis for host specificity among Diplostomoidea (Platyhelminthes: Digenea) parasitizing freshwater fishes in the St. Lawrence River, Canada. Mol Ecol 19: 2813-2827.

MOSZCZYNSKA A, LOCKE SA, MCLAUGHLIN JD, MARCOGLIESE DJ & CREASE TJ. 2009. Development of primers for the mitochondrial cytochrome c oxidase I gene in digenetic trematodes (Platyhelminthes) illustrates the challenge of barcoding parasitic helminths. Mol Ecol Resour 9: 75-82.

MOURITSEN KN & POULIN R. 2002. Parasitism, community structure and biodiversity in intertidal ecosystems. Parasitology 124: 101-117.

PÉREZ-PONCE DE LEÓN G, OSORIO-SARABIA D & GARCÍA-PRIETO L. 1992. Helmintofauna del juile *Rhamdia guatemalensis* (Pisces: Pimelodidae), del lago de Catemaco, Veracruz. Rev Soc Mex Hist Nat 43: 25-31.

POULIN R. 2001. Progenesis and reduced virulence as an alternative transmission strategy in a parasitic trematode. Parasitology 123: 623-630.

POULIN R & CRIBB TH. 2002. Trematode life cycles: short is sweet? Trends Parasitol 18: 176-183.

RAMBAUT A. 2012. FigTree v1.4. Molecular evolution, phylogenetics and epidemiology. Available at: http://tree.bio.ed.ac.uk/.

RONQUIST F & HUELSENBECK JP. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572-1574.

SOLDÁNOVÁ M ET AL. 2017. Molecular analyses reveal high species diversity of trematodes in a sub-Arctic lake. Int J Parasitol 47: 327-345.

How to cite

NEGRELLI DC, VIEIRA DHMD, ABDALLAH VD & AZEVEDO RK. 2020. Molecular characterization of the progenetic metacercariae *Crocodilicola pseudostoma* parasitizing *Rhamdia quelen* (Siluriformes, Heptapteridae) in Brazil. An Acad Bras Cienc 92: e20181388. DOI 10.1590/0001-3765202020181388.

Manuscript received on December 26, 2018;
accepted for publication on October 17, 2019

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Negrelli DC performed sampling analysis, study design, analyzed data, including morphometric data. Vieira DHMD performed the analysis and writing corresponding to molecular biology and phylogenetic analysis. Abdallah VD & Azevedo RK identified the parasite specimen, collaborated throughout the study, reviewed and were responsible for the development of the manuscript. All authors contributed to the writing and all stages of this manuscript.