ISSN 1984-2961 (Electronic) www.cbpv.org.br/rbpv

Braz. J. Vet. Parasitol., Jaboticabal, v. 27, n. 3, p. 289-294, july.-sept. 2018 Doi: https://doi.org/10.1590/S1984-296120180040

# Parasitic helminths of the non-native serrasalmid fish Metynnis lippincottianus from the Três Marias Reservoir, Southeast Brazil

Helmintos parasitos do peixe serrasalmídeo não-nativo *Metynnis lippincottianus* do Reservatório de Três Marias, Sudeste do Brasil

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Received January 17, 2018 Accepted May 3, 2018

#### **Abstract**

*Metynnis lippincottianus* is a freshwater native fish to the Guiana basin and Amazon basin and was recently introduced into the São Francisco River in Brazil. This study aimed to determine the helminths parasites of *M. lippincottianus* from the Três Marias Reservoir, upper São Francisco River, State of Minas Gerais, Southeast Brazil. From 67 hosts examined, 63 were parasitized by 9,257 helminths represented by five species: *Dadayius* sp. [prevalence (P) = 1.49%, mean abundance (MA) = 0.01]; proteocephalid plerocercoids (P = 43.28%, MA = 5.04); *Procamallanus* (*Spirocamallanus*) *inopinatus* (P = 29.85%, MA = 0.54); *Spinitectus rodolphiheringi* (P = 2.99%, MA = 0.03); *Spinoxyuris* sp. (P = 85.07%, MA = 132.54). These findings show that *M. lippincottianus* is an additional host to the helminths infecting fish native to the São Francisco River (plerocercoids, *P.* (*S.*) *inopinatus* and *S. rodolphiheringi*), and highlight the fact that *Dadayius* sp. and *Spinoxyuris* sp., which were typically found in native *Metynnis* spp. in other basins, also parasitizing the non-native serrasalmid *M. lippincottianus* in São Francisco River. These two helminth species have thrived in this freshwater ecosystem with the expansion of the geographical distribution of the host species to the São Francisco River.

Keywords: Allochthonous fish, Dadayius, plerocercoids, Procamallanus, Spinitectus, Spinoxyuris.

#### Resumo

Metynnis lippincottianus é uma espécie de peixe de água doce nativa da bacia da Guiana e bacia amazônica que foi recentemente introduzida no rio São Francisco, Brasil. O presente estudo objetivou determinar quais espécies de helmintos parasitam M. lippincottianus do Reservatório de Três Marias, alto rio São Francisco, Minas Gerais, Sudeste do Brasil. Dos 67 espécimes de peixes examinados, 63 estavam parasitados pelo total de 9.257 helmintos representados por cinco espécies: Dadayius sp. [prevalência (P) = 1,49% e abundância média (AM) = 0,01)]; plerocercoides proteocefalídeos (P = 43,28%, AM = 5,04); Procamallanus (Spirocamallanus) inopinatus (P = 29,85%, AM = 0,54); Spinitectus rodolphiheringi (P = 2,99%, AM = 0,03); Spinoxyuris sp. (P = 85,07%, AM = 132,54). Estes resultados mostram que M. lippincottianus é um hospedeiro adicional para os helmintos que infectam peixes nativos do rio São Francisco (plerocercoides, P. (S.) inopinatus e S. rodophiheringi) e destacam o fato de que Dadayius sp. e Spinoxyuris sp., geralmente encontradas em Metynnis spp. nativas em outras bacias, também parasitam o serrasalmídeo não-nativo M. lippincottianus no rio São Francisco. Estas duas espécies se estabeleceram neste ecossistema de água doce com a expansão da distribuição geográfica do hospedeiro para o rio São Francisco.

Palavras-chave: Peixe alóctone, Dadayius, plerocercoides, Procamallanus, Spinitectus, Spinoxyuris.

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#### Introduction

The natural distribution of Metynnis lippincottianus (Cope, 1870), common name in Brazil: "pacu" or "pacu-marreca", includes the Amazon River basin and the Guiana shield in the Northeast of the continent (FROESE & PAULY, 2017); however, it has expanded its geographic range to other South American watersheds (OTA et al., 2016). Since 2004 M. lippincottianus has been fished at the Três Marias Reservoir, upper São Francisco River basin, State of Minas Gerais, Southeast Brazil (SATO & SAMPAIO, 2006; OTA et al., 2016) as well as in the middle (LUZ et al., 2009) and lower São Francisco River (ASSIS et al., 2017). Most of the fish allochthonous species that occur in Brazilian aquatic ecosystems were introduced via anthropogenic action such as fish farming, pest control, and fishkeeping, and has as a consequence the inadvertent, accidental introduction of parasites in these freshwater environments (AGOSTINHO et al., 2005). There is no previous study on the helminth species that parasitize M. lippincottianus since it was introduced into the upper São Francisco River. In this sense, this paper aimed to determine which helminths parasitizing M. lippincottianus from the Três Marias Reservoir, upper São Francisco River, State of Minas Gerais, Southeast Brazil.

#### Materials and Methods

A total of 67 specimens of the serrasalmid *M. lippincottianus*, popularly known as "pacu", were purchased from artisanal fishermen at the Três Marias Reservoir, upstream of the Três Marias Dam (18° 12' 59" S; 45° 17' 34" W), upper São Francisco River, 43 specimens in June 2016 and the others in October 2016. The gender and the total body length of each fish were recorded [24 males (total length: 12.8 ± 1.74; 11.0-17.0 cm) and 43 females (total length:  $14.5 \pm 2.14$ ; 9.0-19.0 cm)], and samples of the digestive system organs were sent to the Laboratory of Biology and Ecology of Parasites (LABEPAR) at the Universidade Federal Rural do Rio de Janeiro (UFRRJ) in the city of Seropédica, State of Rio de Janeiro (RJ), Southeast Brazil, for parasitological analysis. During necropsy, each of the organs was examined individually and the helminths found in each of them were collected. Helminths were prepared in temporary or permanent mounts according to standard procedures of fish parasitology (AMATO et al., 1991), and were examined by light microscopy (Olympus BX 41). Plerocercoid larvae (Onchoproteocephalidea, Proteocephalidae - Platyhelminthes) (CHERVY, 2002; CAIRA et al., 2014) and the juvenile digenean Cladorchiidae (JONES, 2005; GIBSON et al., 2002), were fixed in AFA (93 parts of ethanol 70° GL, 5 parts of commercial formalin, and 2 parts of glacial acetic acid). Plerocercoids were stained with Semichon carmine, and the specimen of *Dadayius* was stained with Mayer acetic carmine. Specimens were mounted on microscopic glass slides (permanent preparations). Nematodes, Camallanidae and Cystidicolidae (MORAVEC, 1998) and Pharyngodonidae (MORAVEC, 1998; MORAVEC & THATCHER, 2001), were cleared in Amann lactophenol as temporary preparations or mounted in Canada balsam as permanent preparations. Unmounted specimens of

plerocercoids and nematodes were stored in 70% ethanol and 70% ethanol with 5% glycerin, respectively. Parasites were identified, and representative specimens of each species were deposited in the Helminthological Collection of the Oswaldo Cruz Institute (CHIOC-Fiocruz) in Rio de Janeiro, Brazil. Parasite indexes as prevalence (P), mean intensity (MI), and mean abundance (MA) were determined according to the guidelines published by Bush et al. (1997) and the two last were followed by standard error of mean (SEM). The dispersion index (DI) was used to detect the distribution pattern of the parasite infracommunities in the hosts from each infracommunity, and DI significance was tested using the statistic-d according to Rózsa et al. (2000) and Ludwig & Reynolds (1988), respectively. The Chi-square test ( $\chi^2$ ) with Yate's correction and the Mann-Whitney (U') test were used to determine the effects of the host's gender on the prevalence, and on the intensity and abundance of each parasite species, respectively. The Pearson's correlation coefficient (*r*) used to assess the possible correlation between the total host length divided into classes (STURGES, 1926) and the prevalence (arcsine transformation) of parasite infection. The Spearman's rank correlation coefficient (rs) was used to evaluate the possible influence of total host length on parasites intensity and abundance (ZAR, 2000). The Chi-square test  $(\chi^2)$  was used to determine the occurrence of associations between species of parasites, and the Spearman's rank correlation coefficient (rs) and Tukey-Kramer method (q) were applied to assess correlations between the intensity and abundance of parasite species that were associated to each other, respectively (LUDWIG & REYNOLDS, 1988). The Shannon-Wiener index (*H* ) was used to determine community diversity. Species richness, Berger-Parker dominance index, Simpson's index, and Pielou evenness index were calculated for each parasite infracommunity using the DE4 Ecological software DivEs - "Diversidade de Espécies (Species Diversity)", version 4.0 (RODRIGUES, 2017). Statistical test results were considered significant at  $p \le 0.05$ , and these tests were performed only for those parasite species with prevalence over 10% (BUSH et al., 1990).

#### Results

The parasitic analysis was performed in sample from 67 specimens of M. lippincottianus, of which 63 specimens (94.02%) were infected with one or more parasite species. The helminth fauna of M. lippincottianus was comprised of five taxa which included two species of Platyhelminthes: a juvenile specimen of Digenea, Dadayius sp., Cladorchiidae, (CHIOC 39001), and plerocercoid larvae of an unidentified species of Cestoda, Proteocephalidae (CHIOC 39002); and three species of Nematoda: Procamallanus (Spirocamallanus) inopinatus Travassos, Artigas & Pereira, 1928, Camallanidae (CHIOC 39004-male, 39003-female); Spinitectus rodolphiheringi Vaz & Pereira, 1934, Cystidicolidae (CHIOC 39005-female); and an unindentified species of the nematode genus Spinoxyuris Petter, 1994, Pharyngodonidae (CHIOC 39006a-male, 39006b-female). A total of 9,257 parasite specimens were found in the 67 necropsied fish, among these 9,254 helminth specimens belonging to three species with a prevalence greater than 10% (Table 1) and with an aggregate pattern of distribution in

the infracommunities: plerocercoids (P = 43.28%; DI = 28.91, d = 50.33); P. (S.) *inopinatus* (P = 29.85%; DI = 2.39, d = 6.31) and *Spinoxyuris* sp. (P = 85.07%; DI = 237.28, d = 165.53). Besides the high prevalence of *Spinoxyuris* sp., this parasite also had the highest intensity and mean abundance in the community (Table 1).

The prevalence, intensity and abundance of *P.* (*S.*) *inopinatus* and *Spinoxyuris* sp. were not influenced by gender or total length of the hosts (Tables 2 and 3).

The high prevalence of *Spinoxyuris* sp. and plerocercoids resulted in a large number of fish parasitized by both helminths (26 records), and some analyzed hosts were infected by both *Spinoxyuris* sp. and *P.* (*S.*) *inopinatus* (17 records), whose co-occurrence in the same

fish host was statistically significant, with exception to the pair plerocercoids and *P.* (*S.*) *inopinatus* (nine records). Differences in parasitism intensity among species from each infracommunity reflect the high number of specimens in the infrapopulations of *Spinoxyuris* sp. in comparison with plerocercoids and *P.* (*S.*) *inopinatus* infrapopulations (Table 4).

The specific features and findings in each parasite infracommunity are shown in Table 5. There was a significant relative dominance of *Spinoxyuris* sp. on other species in the infracommunities. Although the intensity and abundance values in the infracommunities were higher for *Spinoxyuris* sp., *P.* (*S.*) *inopinatus* was more evenly distributed in the infracommunities.

**Table 1.** Prevalence, mean intensity (MI) and mean abundance (MA), with corresponding standard error of mean (SEM), parasitized fish number (PN), examined fish number (EN), confidence limit (CL) and Collection number (Helminthological Collection of the Oswaldo Cruz Institute (CHIOC) of helminths of *Metynnis lippincottianus* from the Três Marias Reservoir, upper São Francisco River, State of Minas Gerais, Southeast Brazil.

Parasites	Prevalence	MI ± SEM	DNI.	CL 95%		MA ± SEM	EN-	CL 95%		Dance	Sites of
rarasites	(%)	IVII ± SEIVI	FIN	Lower	Upper MIA ± SEM	Lower		Upper	Range	infection	
Platyhelminthes											
Cladorchiidae											
Dadayius sp.	1.49	1.00	1	1.00	1.00	$0.01 \pm 0.01$	67	0.01	0.04	1	PI
CHIOC 39001 – juvenile specimen											
Proteocephalidae											
Plerocercoids	43.28	$11.66 \pm 3.02$	29	5.48	17.83	$5.04 \pm 1.47$	67	2.10	7.99	1-65	S, AI, I, PI
CHIOC 39002 – larvae											
Nematoda											
Camallanidae											
Procamallanus	29.85	$1.80 \pm 0.32$	20	1.13	2.47	$0.54 \pm 0.14$	67	0.26	0.81	1-7	S, PI
(Spirocamallanus) inopinatus											
CHIOC 39004 – male specimen											
CHIOC 39003 – female specimen											
Cystidicolidae											
Spinitectus rodolphiheringi	2.99	1.00	2	1.00	1.00	$0.03 \pm 0.02$	67	0.01	0.07	1-1	S
CHIOC 39005 - female specimen											
Pharyngodonidae											
Spinoxyuris sp.	85.07	155.79 ± 24.20	57	107.29	204.29	132.54 ± 21.66	67	89.25	175.82	4-808	PI
CHIOC 39006a – male specimen											
CHIOC 39006b – female specimen											

AI = anterior intestine; I = medium intestine; PI = posterior intestine; S = stomach.

**Table 2.** Prevalence of parasites according to gender and fish size classes, *Metynnis lippincottianus* from the Três Marias Reservoir, upper São Francisco River, State of Minas Gerais, Southeast Brazil.

Prevalence x host parameters	Plerocercoi	ds (larvae)		nallanus inus) inopinatus	Spinoxyuris sp.	
•	Value	p	value	P	Value	p
Prevalence x Gender						
x <sup>2</sup> Yates	0.04	0.84	1.69	0.19	0.01	0.95
Prevalence x Size classes (total length)						
Pearson correlation (r)	-0.06	0.85	-0.05	0.86	-0.04	0.89
Prevalence x Gender x Size classes (total length)						
Pearson correlation (r)	0.04	0.89	-0.16	0.59	0.14	0.63

Significant values:  $p \le 0.05$ ;  $x^2$  Yates: chi-square with Yates correction.

**Table 3.** Intensity and abundance of endoparasites according to gender and total length of *Metynnis lippincottianus* from the Três Marias Reservoir, upper São Francisco River, State of Minas Gerais, Southeast Brazil.

Parasites	Intensity				Abundance				
	Gender		Total Length		Gender		Total Length		
	U'	p	rs	p	U'	p	Rs	p	
Platyhelminthes									
Plerocercoids (larvae)	97.50	0.93	0.002	0.99	526.50	0.89	0.11	0.33	
Nematoda									
Procamallanus (Spirocamallanus) inopinatus	62.50	0.30	-0.30	0.20	623.50	0.08	-0.19	0.13	
Spinoxyuris sp.	450.00	0.24	0.36	0.47	568.50	0.50	-0.12	0.34	

Significant values:  $p \le 0.05$ ; rs: Spearman's rank correlation coefficient; U': Mann-Whitney test.

**Table 4.** Analysis of parasite descriptors of helminth coinfections in 67 specimens of *Metynnis lippincottianus* from the Três Marias Reservoir, upper São Francisco River, State of Minas Gerais, Southeast Brazil.

Pair of species	Prev	alence	Inte	nsity	Abundance	
Endoparasite adults	$\chi^2$	p	rs	p	q	P
Procamallanus (Spirocamallanus) inopinatus - Spinoxyuris sp.	17.01	< 0.0001	0.27	0.29	10.53	< 0.001
Endoparasite larvae and adults						
Plerocercoids (larvae) - Procamallanus (S.) inopinatus	0.48	0.47	-0.09	0.81	0.36	>0.05
Plerocercoids (larvae) - Spinoxyuris sp.	14.88	0.0001	-0.14	0.48	10.17	< 0.001

Significant values: ρ ≤ 0.05; χ²: chi-square; rs: Spearman's rank correlation coefficient; q: Tukey-Kramer multiple comparisons test.

**Table 5.** Specific features and findings in the parasite infracommunities from *Metynnis lippincottianus* at the Três Marias Reservoir, upper São Francisco River, State of Minas Gerais, Southeast Brazil.

Parameters	Metynnis
	lippincottianus
Number of specimens collected	67
Number of parasitized specimens	63
Percentage of parasitism	94.03%
Total number of specimens	9,257
Total number of species	5
Platyhelminthes species	2
Nematoda species	3
Diversity of Shannon-Wiener	3.84
Mean Relative Dominance	
Plerocercoids (larvae)	0.08
Procamallanus (Spirocamallanus) inopinatus	0.03
Spinoxyuris sp.	0.81
Berger-Parker Dominance Index	
Plerocercoids	0.19
Procamallanus (S.) inopinatus	0.19
Spinoxyuris sp.	0.09
Simpson's Index	
Plerocercoids	0.10
Procamallanus (S.) inopinatus	0.05
Spinoxyuris sp.	0.04
Pielou Evennes Index	
Plerocercoids	0.81
Procamallanus (S.) inopinatus	0.93
Spinoxyuris sp.	0.88

The value of community diversity (H' = 3.84) reflects the presence of infracommunities formed by one dominant species and a well-distributed number of specimens among their infrapopulations (Table 5).

#### Discussion

In this study a juvenile specimen of Dadayius sp. was found in the small intestine of a M. lippincottianus. Probably this is not an incidental finding but a sign that digenean parasites are thriving in a new ecosystem in which gastropods play a role in the transmission of this parasite to fish. In the literature four valid species of Dadayius Fukui, 1929 were found in fish species from South America, two of which were reported in Metynnis spp.: Dadayius pacuensis Thatcher, Sey & Jégu, 1996 was found infecting the serrasalmids Myloplus rubripinnis (Müller & Troschel, 1844) (cited also in Myloplus asterias (Müller & Troschel, 1844), a junior synonym of M. rubripinnis, (see FROESE & PAULY, 2017), from the Jatapu River, Brazilian Amazon, and Guaporé River, State of Rondônia, Brazil (THATCHER et al., 1996), and Metynnis hypsauchen (Müller & Troschel, 1844) at the Jari River, Brazilian Amazon (OLIVEIRA et al., 2015); and Dadayius pacupeva Lacerda, Takemoto & Pavanelli, 2003 was found in the serrasalmid Metynnis maculatus (Kner, 1858) from the upper Paraná River, Brazil, by Lacerda et al. (2003), in M. lippincottianus at the Paraná River, Brazil, by Moreira et al. (2009), and in the same host species from Igarapé da Fortaleza, a tributary of the Amazon River, Eastern Amazon, Brazil, by Hoshino & Tavares-Dias (2014). The records of mature specimens of *Dadayius* in the definitive host Metynnis spp. in South America with prevalence varying from 61 to 100%, mean intensity and mean abundance varying from 11.0 to 132.1 and from 9.1 to 81.1, respectively (MOREIRA et al., 2009; HOSHINO & TAVARES-DIAS, 2014; OLIVEIRA et al., 2015) indicates the development of a very close host-parasite interaction between Dadayius sp. and M. lippincottianus also at the São Francisco River in combination with other local factors including the presence of an intermediate host.

During the necropsies we found the plerocercoids forming small masses within the peritoneal cavity and attached to the mesentery of the affected fish. In the São Francisco River basin, several species of fish are parasitized by these plerocercoid larvae (BRASIL-SATO, 2003; SABAS & BRASIL-SATO, 2014; ALBUQUERQUE et al., 2016; DUARTE et al., 2016; VIEIRA-MENEZES et al., 2017). Based on these results, we suggest that *M. lippincottianus* harbors generalist plerocercoids. This finding expands the list of hosts of these cestodes. The development of these plerocercoids inside their definitive host perpetuate the life cycle of the parasite at the São Francisco basin. This is the first record of plerocercoids in a species of *Metynnis* Cope, 1878.

Procamallanus (S.) inopinatus is a generalist nematode that has been found in a number of fish species from the São Francisco River basin (BRASIL-SATO, 2003; SANTOS-CLAPP & BRASIL-SATO, 2014) and in several freshwater ecosystems as well (MORAVEC, 1998). This helminth species parasitizes *M. maculatus* from the Paraná River, *M. lippincottianus* from the Paraná River and a tributary of the Amazon River basin (MOREIRA et al., 2009; HOSHINO & TAVARES-DIAS, 2014), and *M. hypsauchen* from the Jari River, Brazilian Amazon (OLIVEIRA et al., 2015).

Spinitectus rodolphiheringi is part of the parasite fauna of fish from São Francisco basin, and occurs in the doradid Franciscodoras marmoratus (Reinhardt, 1874) (SANTOS & BRASIL-SATO, 2004) and in the characids Tetragonopterus chalceus Spix & Agassiz, 1829 and Astyanax fasciatus (Cuvier, 1819) (ALBUQUERQUE et al., 2016; VIEIRA-MENEZES et al., 2017, respectively). Their parasitic indexes were low in three parasite communities.

Oxyurid nematodes have a monoxenous (= homoxenous), direct life cycle (MORAVEC & THATCHER, 2001; MORAVEC & LAOPRASERT, 2008) in which all developmental stages of the parasite occur in the same host. In the present study, larval and adult specimens of *Spinoxyuris* sp. were found in the posterior intestine of the fish host. These findings show that Spinoxyuris sp. has thrived in fish from the São Francisco basin where it will eventually infect other hosts including serrasalmids or doradids. The occurrence of Spinoxyuris oxydoras Petter, 1994 in doradid Oxydoras kneri Bleeker, 1862 from the Paraná River, Paraguay (PETTER, 1994) in a serrasalmid M. lippincottianus from the Paraná and Amazon Rivers, Brazil (MOREIRA et al., 2009; HOSHINO & TAVARES-DIAS, 2014, respectively) and in M. hypsauchen from the Jari River, Brazilian Amazon (OLIVEIRA et al., 2015) from South America, with significant parasitic indexes; and the occurrence of Spinoxyuris annulata Moravec & Thatcher, 2001 in the serrasalmid Myleus ternetzi (Norman, 1929), a junior synonymy of Myloplus ternetzi (Norman, 1929), at the Sinnamary River of the French Guyana (MORAVEC & THATCHER, 2001), show that there is a close parasite-host association possibly related to the direct life cycle of this helminth.

Endoparasitic indexes were not influenced by gender or total body length of the "pacus" in our study. Though according to Moreira et al. (2009), Hoshino & Tavares-Dias (2014) and Oliveira et al. (2015), prevalence and abundance of helminth infection in fish, mainly *Dadayius* spp. and *S. oxydoras* infections, are influenced to some degree by the host's body size. In this study, *P.* (*S.*) *inopinatus* showed lower parasite indexes, and *Spinoxyuris* sp. had higher infection rates compared to *M. lippincottinaus* from the Paraná River, Brazil (MOREIRA et al., 2009) and from the

tributary of the Amazon River (HOSHINO & TAVARES-DIAS, 2014), and to *M. hypsauchen* from the Jari River basin, Brazilian Amazon (OLIVEIRA et al., 2015).

*Procamallanus* (*S.*) *inopinatus* showed an interspecific relationship in parasite infracommunities of *M. lippincottianus* in this study. There were records of coinfection of *P.* (*S.*) *inopinatus* with plerocercoids or with *Spinoxyuris* sp. which mean intensity of infection and mean abundance were both high resulting in significant positive covariation of the abundance between these helminths.

According to literature survey, five species of helminths were also recorded in Metynnis spp. from the Paraná River, Brazil (MOREIRA et al., 2009), and from Brazilian Amazon basin (HOSHINO & TAVARES-DIAS, 2014; OLIVEIRA et al., 2015). Some parasites were found in "pacus" in these basins, but not in fishes from São Francisco River: Contracaecum sp. larval stages in "pacus" from Paraná River and Amazon basins (MOREIRA et al., 2009; HOSHINO & TAVARES-DIAS, 2014; OLIVEIRA et al., 2015); adult specimens of Raphidascaris mahnerti (Petter & Cassone, 1984) in "pacus" from Paraná River (MOREIRA et al., 2009); and unidentified acanthocephalan specimens have been reported in "pacus" from the Amazon basin (OLIVEIRA et al., 2015). In contrast, two species were found only in "pacus" from São Francisco River in this study: an unidentified species of larval cestode (plerocercoid stage) and the nematode S. rodolphiheringi. These represent new findings, increase current knowledge in the field of fish parasitology by expanding the list of host fish for these helminths in the São Francisco basin.

Our results indicates that, since at least 2004, the non-native "pacu" *M. lippincottianus*, has been increasingly parasitized by helminth species of the parasites community of native fish at the São Francisco River basin. These include an undetermined species of proteocephalidean cestode, *P.* (*S.*) *inopinatus*, and *S. rodolphiheringi*. The generalist species *P.* (*S.*) *inopinatus*, the rare *Dadayius* sp., and the dominant species *Spinoxyuris* sp. from "pacu" of the São Francisco River, constitute a set of species commonly reported as part of the parasitic fauna of *Metynnis* spp. from other basins. *Dadayius* sp. and *S. rodolphiheringi* are more specific relationship with the serrasalmid fish, and are reported for the first time at the São Francisco basin. The results of our survey show that both *Dadayius* sp. and *Spinoxyuris* sp. have thrived in the Três Marias Reservoir, and indicates that these two parasites are following the *M. lippincottianus* host in its geographic expansion to the São Francisco River.

## Acknowledgements

We would like to thank Dr Yoshimi Sato from the Centro Integrado de Pesca e Aquicultura, Três Marias, MG, Brazil, for providing us his laboratory facilities and literature for this research, the "ad doc" reviewers by the important suggestions at the manuscript, and Dr Marcelo Knoff, CHIOC curator, for specimen deposition at his institution's collection.

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