Acta Scientiarum

http://periodicos.uem.br/ojs/acta
ISSN on-line: 1807-863X
Doi: 10.4025/actascibiolsci.v40i1.41280

ZOOLOGY

Metacercariae of *Tylodelphys* sp. (Trematoda: Diplostomidae) parasite of *Brochis multiradiatus* and *Corydoras splendens* (Siluriformes: Callichthyidae) from the Peruvian Amazon

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ABSTRACT. *Corydoras splendens* (Castelnau, 1855) and *Brochis multiradiatus* (Orcés V., 1960) are highly demanded for exporters in the Peruvian Amazon and importers all over the world. The exportation of this fish species may represent a gateway for endoparasites to enter in a new environment with serious effects, so, the present study reports the presence of *Tylodelphys* sp. infecting *C. splendens* and *B. multiradiatus*. Fish were acquired from fishermen of the city of Iquitos, Peru. After the necropsy and analyses of the fish, metacercariae of *Tylodelphys* sp. were found in the liver and intestine. This finding represents the first report of *Tylodelphys* Diesing, 1850 infecting a fish in the Peruvian Amazon. Considering the possible damages caused by species of *Tylodelphys,* highlighting the nutritional and respiratory ones, prophylactic measures must be taken into consideration for exporters to avoid some damages in the fish that can deteriorate their health and also to avoid the introduction of this parasite to other countries.

Keywords: callichthyids; digenean; ornamental fish; parasite.

Metacercárias de *Tylodelphys* sp. (Trematoda: Diplostomidae) parasito de *Brochis multiradiatus* e *Corydoras splendens* (Siluriformes: Callichthyidae) da Amazônia peruana

RESUMO. *Corydoras splendens* (Castelnau, 1855) e *Brochis multiradiatus* (Orcés V., 1960) são altamente demandados por exportadores da Amazônia peruana e importadores em todo o mundo. A exportação dessas espécies de peixes pode representar uma entrada de endoparasitas a um novo ambiente com efeitos graves. Assim, o presente estudo relata a presença de *Tylodelphys* sp. infectando *C. splendens* e *B. multiradiatus*. Os peixes foram adquiridos de pescadores da cidade de Iquitos, Peru. Após a necropsia e análise dos peixes, metacercarias de *Tylodelphys* sp. foram encontrados no fígado e no intestino, representando o primeiro registro de *Tylodelphys* Diesing, 1850 infectando um peixe na Amazônia peruana. Considerando os possíveis danos causados por espécies de *Tylodelphys*, destacando-se os danos nutricionais e respiratórios; medidas profiláticas devem ser levadas em consideração pelos exportadores para evitar alguns danos nos peixes que podem comprometer a saúde e também a introdução deste parasita em outros países.

Palavras-chave: callictídeos; digenéticos; peixes ornamentais; parasita.

Introduction

The ornamental fishery is a major activity in the Amazon Region and constitutes an important economic resource, since these fish are exported to other countries in large quantities (Chao, 2001). Species of Callichthyidae have benthic habits and are considered ornamental fish with high appreciation in the international market due to its rusticity and color patterns. In the Amazon Region, they are captured in rivers and streams (Sánchez, García, Vásquez, & Alcántara, 2011). Within this group of fishes, *Corydoras splendens* and *Brochis multiradiatus* are highly demanded, being exported for many aquarists of the Peruvian Amazon (Sánchez et al., 2011).

It is well known that exportation of ornamental fish may represent a gateway for both ecto and endoparasites to enter a new environment. Their introduction may have serious effects, especially when parasites come into contact with new hosts (Fujimoto, Barros, Marinho-Filho, Diniz, & Eiras, 2013). Several cases of introduced parasites, sometimes with devastating consequences, can be found in the literature (Moravec, Wolter, & Körting, 1999; Evans & Lester, 2001).

Members of the compound genus *Diplostomum* Dubois, 1970 include three subgenera, often elevated to genus level, *Diplostomum*, *Tylodelphys* and *Dolichorchis* (Dubois, 1970). *Tylodelphys* (Diplostomidae) parasitize mainly the eyes, brains and cranial cavities of

freshwater fishes (Buchmann & Uldal, 1994; Blasco-Costa, Poulin, & Presswell, 2016) and is considered as major pathogen (Chappell, 1995). Their life cycle involve three hosts: snails as first intermediate hosts, fish as second intermediate hosts and fish-eating birds as final hosts (Chappell, 1995).

Studies on *Tylodelphys* have attracted attention because of their pathogenic metacercariae in fish (Chappell, Hardie, & Secombes, 1994). Fish heavily infected with the metacercariae may experience loss of vision, reduced growth, emaciation (Chappell et al., 1994) or deformation of the vertebral column, brain tumour, cellular necrosis and finally death (Machado, Takemoto, & Pavanelli, 2005).

In the present study, metacercariae of *Tylodelphys* sp. of *C. splendens* and *B. multiradiatus* captured in the Peruvian Amazon are reported infecting different organs. It is important to mention that parasites of these fish species have never previously been studied in this Region.

Material and methods

Adult specimens of *B. multiradiatus* (n = 20) and *C. splendens* (n = 20) were collected between July and August 2017 from local fishermen of the city of Iquitos, Peru. According to the fishermen, fish were captured in the River Tapiche.

Parasitological analysis was conducted in the *Laboratorio de Hidrobiología* of the *Universidad Nacional de la Amazonía Peruana* (UNAP) located in the city of Iquitos, Peru. In the laboratory, fishes were quickly immersed in a 75-mg clove oil·L^{-1} solution and euthanized (*Conselho Nacional de Controle de Experimentação Animal* [CONCEA], 2013). Posteriorly, fishes were measured and weighed. Looking for digeneas, internal organs were removed and examined under stereoscope. Metacercariae found were counted, fixed in hot water (65ºC) and conserved in 95% ethanol. Specimens were stained with Langeron´s alcoholic carmine, cleared in Eugenol and mounted in Canada balsam as permanent slides. Voucher specimens were deposited in the zoological collection of the *Instituto Nacional de Pesquisas da Amazônia* (INPA), Manaus, Brazil.

Taxonomical identification was according to Gibson, Jones, and Bray (2001). Illustrations were prepared with the aid of a microprojector. Parasites were photographed with a digital camera (Olympus Qcolor 5) and the images were used to obtain the measurements of the body and internal organs. Measurements are given in micrometers (μm), except the total body length that is given in millimeters (mm). Average measurements are followed by ranges in parentheses. The ecological terms in parasitology follow Bush, Lafferty, Lotz, and Shostak (1997).

Results

Tylodelphys sp.

Host: *Corydoras splendens* (Castelnau, 1855) and *Brochis multiradiatus* (Orcés V., 1960) (Siluriformes: Callichthyidae).

Locality: River Tapiche, Iquitos, Peru.

Site of infection: liver and intestine

During the study, metacercaria of *Tylodelphys* were collected from liver and intestine of *C. splendens* and *B. multiradiatus*. Parasites recovered from both sites resembles in their morphology.

They were identified on the basis of large and elongate body, 1.41 (1.20 – 1.62) length, 505.55 (442.64 – 568.49) wide, which consists of oval shaped fore body and bluntly pointed hind body; pseudo-suckers indistinct; oral sucker, 74.03 (64.14 – 82.73) length, 78.92 (60.29 – 96.94) wide; ventral sucker, 173.46 (139.98 – 214.60) length, 136.45 (101.41 – 152.07) wide; elliptical holdfast organ, 284.15 (235.81 – 344.52) length, 157.37 (138.13 – 168.44) wide; oval pharynx; intestinal caeca extending up to the posterior region of the body and excretory pore present at the terminal portion of the body; vitellaria distributed throughout the body. The distance between oral and ventral sucker, 538.22 (488.48 – 579.91); distance between ventral sucker and holdfast organ, 358.52 (253.99 – 442.62). (Figure 1).

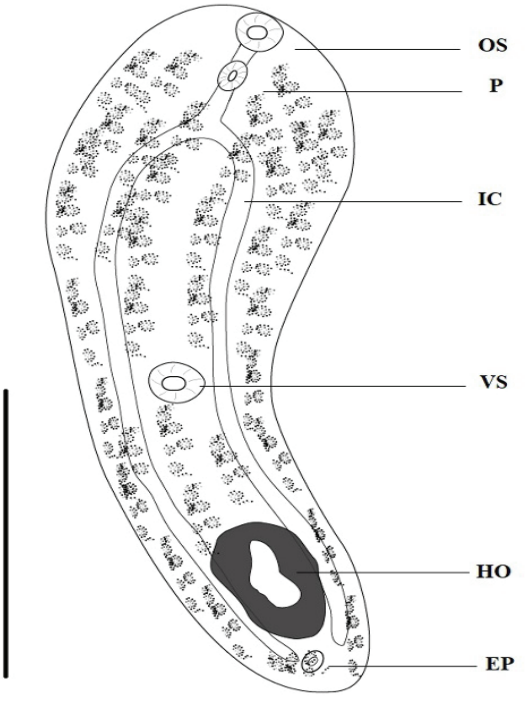

Figure 1. Metacercariae of *Tylodelphys* sp. collected from the liver and intestine of *Corydoras splendens* (Castelnau, 1855) and *Brochis multiradiatus* (Orcés V., 1960). OS = oral sucker, P = pharynx, IC = intestinal caecum, VS = ventral sucker, HO = holdfast organ, EP = excretor pore. Bar scale = 500 μm.

Higher parasitic indexes were reported for *B. splendens* (Table 1). Some parasites were found free, others in pairs and in groups varying in number, between 7-15 individuals inside of a circular membrane (Figure 2).

Discussion

Currently the genus *Tylodelphys* has 16 described species, distributed around the world. Of these, only 12 species were reported in the Americas, of which 7 were described only from metacercariae: *Tylodelphys destructor* Szidat & Nani, 1951; *T. barilochensis* Quaggiotto & Valverde, 1992; *T. crubensis* Quaggiotto & Valverde, 1992; *T. argentinus* Quaggiotto & Valverde , *T. jenynsiae* Szidat, 1969, *T. cardiophilus* Szidat, 1969 and *T. scheuringi* Hughes, 1929 (Hughes, 1929; Lunaschi & Drago, 2004; Muzzal & Kilroy 2007; Drago & Lunaschi 2008). The other 5 species are *T. adult*, parasite of *Podiceps major* Boddaert, 1783 from Argentina; *T. brevis*, parasite of *Mycteria americana* Linnaeus, 1758, also from Argentina; *T. elongata*, parasite of *Podiceps dominicus* Linnaeus, 1766 from Cuba, Venezuela and Brazil; *T. americana* parasite of *M. americana*, and *Podilymbus podiceps* from Brazil, Venezuela and Mexico and *Tylodelphys aztecae* García-Varella, Sereno-Uribe, Pinacho-Pinacho, Hernández-Cruz, and de León, (2016). In the present study, the genus *Tylodelphys* is cited for the first time parasitizing a fish from Peru, expanding its geographic distribution to the Peruvian Amazon Region.

Some helminths like digeneans infecting fish can induce cellular responses, (Richards, Hoole, Lewis, Ewens, & Arme, 1994a). Immature stages of parasites may be encapsulated with eosinophils, neutrophils and monocytes, there is, in addition, an increase in leucocyte numbers in lymphoid tissue due to infection (Richards, Hoole, Lewis, Evans, & Arme, 1994b). In the present study, some *Tylodelphys* individuals were found free and others were found in pairs or in multiple numbers inside of a membrane. Apparently, this can be a mechanism of immune response of fish against the infection of this parasite.

The differentiation of metacercariae, which are frequently encountered in fish, is especially problematic (Chappell, 1995; Niewiadomska, 1996; Niewiadomska & Laskowski, 2002). Identification of metacercariae is made more difficult by the lack of a key devoted to this stage in the life cycle. All present keys and monographs are based on adult stages (Niewiadomska, 2002). Additionally, identification of species of *Tylodelphys* is difficult due to striking morphological similarity, and especially at the metacercariae stage (Niewiadomska, 1996). In this study, it was not possible to identify the species, due to the stage of the parasite and due to the lack of taxonomic keys for this genus. This study represents a contribution to the study of this group of parasites. Parasitological studies on piscivorous birds that feed on callichthyids would be necessary to identify the parasite species.

The over dispersion of metacercariae in fish host populations has been attributed to heterogeneity in susceptibility, and uneven exposure of fish to infection that is a result of distribution of cercariae in the habitat caused by distribution of snail intermediate hosts (Bush, Fernandez, Esch, & Seed, 2001). According to Eiras (1994), cercariae penetrated the fishes through any part of their bodies. In the present study, the presence of *Tylodelphys* sp. in *C. splendens* and *B. multiradiatus* indicates the presence of snails and piscivorous birds, coexisting in the same habitat. In this way, it can be assumed that this parasite uses these fish as second intermediate hosts, being snails the first intermediate hosts and piscivorous birds the final ones.

Species of Callichthyidae are obligate air-breathers that use the intestine as an accessory respiratory organ: air is taken in through the mouth, passed through the gut and let out from the anus. (Gee & Graham, 1978). Kramer and Graham (1976) suggested synchronous air-breathing may reduce susceptibility to attack from aquatic and terrestrial predators and likened its effect to schooling which reduces the probability of contact between predator and prey.

Table 1. Parasitic indexes of *Tylodelphys* sp. infecting *Corydoras splendens* (Castelnau, 1855) and *Brochis multiradiatus* (Orcés V., 1960). AF = analyzed fish, PF = parasitized fish, P% = prevalence, N = number of parasites recorded, I = amplitude of intensity of infection, mI = mean intensity of infection, mA = mean abundance of infection.

Host	Site of Infection	AF	PF	P%	N	I	mI	mA
Corydoras splendens	Liver	20	5	25	34	4-8	6.8 ± 1.78	1.7 ± 3.13
	Intestine	20	4	20	27	4-9	6.75 ± 2.21	1.35 ± 2.90
Brochis multiradiatus	Liver	20	7	35	67	5-20	9.57 ± 5.19	3.35 ± 5.51
	Intestine	20	8	40	167	2-89	20.88 ± 29.38	8.35 ± 20.69

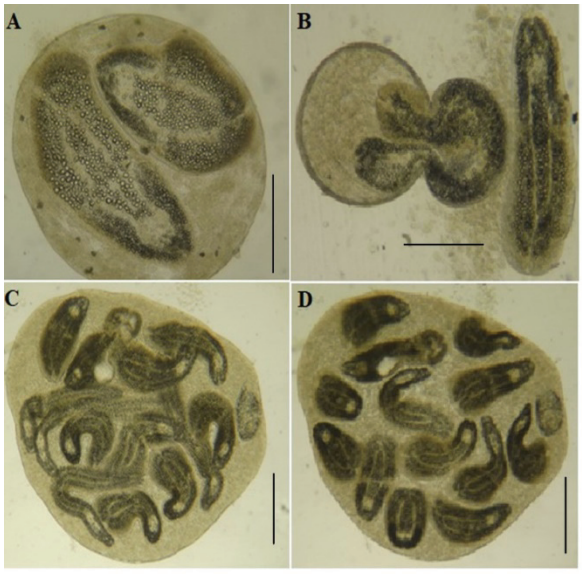

Figure 2. Metacercaria of *Tylodelphys* sp. collected form the liver and intestine of *Corydoras splendens* (Castelnau, 1855) and *Brochis multiradiatus* (Orcés V., 1960). A. two parasites inside a circular membrane, B. metacercariae leaving the membrane, C and D. multiple metacercariae inside a membrane. Bar scale = 500 μm.

Parasites extract energy, in the form of nutrition, from their hosts. If hosts become starved for nutrients, their behavior might change. If energy drain impairs their physiology, they might become more sluggish or display lower physical performance. Alternatively, if a host is drained of energy, it might become more active and increase foraging rate. Either change in behavior could benefit the parasite responsible for the energy drain. (Lafferty & Shaw, 2013). Energy drain might also influence habitat selection. Taking risks to forage might place the host in situations where it is more likely to be eaten by a predator that acts as the final host for the parasite (Lafferty & Shaw, 2013). In the present study, the presence of *Tylodelphys* metacercariae in the intestine of *C. splendens* and *B. multiradiatus* may be a strategy to affect physiology of the fish or to alter their behavior. Probably the parasite not only extracts energy from the hosts, affecting their nutrition, but also affects the complementary respiratory function of the intestine, causing the rise of the fish to the surface to take oxygen, and thus, increasing the chances of being consumed by piscivorous birds. Additionally, energy taken by parasites might affect foraging decisions. A hungry host might be more likely to avoid shelter to increase foraging rates, and consequently, allowing the closing of the parasite's biological cycle.

The finding of *Tylodelphys* sp. in *C. splendens* and *B. multiradiatus* could be very important, because they can harm the hosts' health. According to Eiras (1994), cercariae penetrated the fishes through any part of their bodies, and metacercariae of *Tylodelphys* sp. have similar behavior as *Diplostomum* spp., causing formation of cataracts and even death of the fish.

Conclusion

Considering possible nutritional and respiratory problems caused by the infection of *Tylodelphys* sp., prophylactic measures must be taken into consideration for exporters to avoid damages in the fish that can deteriorate their health (quality) and avoid the introduction of this parasite to other countries through ornamental fish exports.

References

Blasco-Costa I., Poulin R., & Presswell, B. (2016). Morphological description and molecular analyses of *Tylodelphys* sp. (Trematoda: Diplostomidae) newly recorded from the freshwater fish *Gobiomorphus cotidianus* (common bully) in New Zealand. *Journal of Helminthology*, *91*(3), 1-14. doi: 10.1017/S0022149X16000298

Buchmann, K., & Uldal, A. (1994). Effects of eyefluke infections on growth of rainbow trout (*Oncorhynchus mykiss*) in a mariculture system. *Bulletin of the European Association of Fish Pathologists*, *14*(3), 104-107.

Bush, A. O., Fernandez, J. C, Esch, G. W, & Seed, J. R. (2001) *Parasitism: The diversity and ecology of animal parasites*. Cambridge, UK: Cambridge University Press.

Bush, A. O., Lafferty, K. D., Lotz, J. M., & Shostak, A. W. (1997). Parasitology meets ecology on its own terms: Margolis et al. revisited. *The Journal of Parasitology*, *83*(4), 575-583.

Chao, N. L. (2001). The fishery diversity and conservation of ornamental fishes in the Rio Negro Basin, Brasil: A review of Project Piaba (1989-1999). In N. L. Chao, P. Petry, G. Prang, L. Sonnenschein, & M. T. Tlusty (Ed.), *Conservation and management of ornamental fish resources of the Rio Negro Basin, Amazonia Brasil* (p. 161-204), Manaus, AM: Universidade do Amazonas.

Chappell, L. H. (1995). The biology of diplostomatid eyeflukes of fishes. *Journal of Helminthology*, *69*(2), 97-101. doi: 10.1017/S0022149X00013961

Chappell, L. H., Hardie, L. J., & Secombes, C. J. (1994). Diplostomiasis: the disease and host–parasite interactions. In A. W., Pike, & J. W., Lewis (Ed.), *Parasitic diseases of fish* (p. 59-86). Dyfed, UK: Samara Publishing Limited.

Conselho Nacional de Controle de Experimentação Animal [CONCEA]. (2013). *Diretrizes da Prática de Eutanásia do Conselho Nacional de Controle de Experimentação Animal - CONCEA*. Brasília, DF: Ministério da Ciência, Tecnologia e Inovação.

Drago, F., & Lunaschi, L. (2008). Description of a new species of *Tylodelphys* (Digenea, Diplostomidae) in the

wood stork, *Mycteria americana* (Aves, Ciconiidae) from Argentina. *Acta Parasitologica*, *53*(3), 263-267. doi: 10.2478/s11686-008-0038-3

Dubois, G. (1970). Synopsis des Strigeidae et des Diplostomatidae (Trematoda). *Memoires de Societe Neuchateloise des Sciences Naturelles*, *10*(2), 288-376.

Eiras, J. C. (1994). *Elementos de Ictioparasitologia.* Porto, PT: Fundação Eng. Antônio de Almeida.

Evans, B. B., & Lester, R. J. (2001). Parasites of ornamental fish imported into Australia. *EAFP Bulletin*, *21*(2), 51-55.

Fujimoto, R. Y., Barros, Z. M. N. D., Marinho-Filho, A. N., Diniz, D. G., & Eiras, J. C. (2013). Parasites of four ornamental fish from the Chumucuí River (Bragança, Pará, Brazil). *Revista Brasileira de Parasitologia Veterinária*, *22*(1), 34-38.

García-Varela, M., Sereno-Uribe, A. L., Pinacho-Pinacho, C. D., Hernández-Cruz, E., & de León, G. P. P. (2016). An integrative taxonomic study reveals a new species of *Tylodelphys* Diesing, 1950 (Digenea: Diplostomidae) in central and northern Mexico. *Journal of helminthology*, *90*(6), 668-679. doi: 10.1017/S0022149X15000917

Gee, J. H., & Graham, J. B. (1978). Respiratory and hydrostatic functions of the intestine of the catfishes *Hoplosternum thoracatum* and *Brochis splendens* (Callichthyidae). *Journal of Experimental Biology*, *74*(1), 1-16.

Gibson, D. I., Jones, A., & Bray, R. A. (2001). *Keys to the Trematoda*, London, UK: CABI Publishing.

Hughes, R. C. (1929). Studies on the trematode family Strigeidae (Holostomidae), No. XIV: Two new species of *Diplostomula*. *Journal of Parasitology*, *15*(4), 267-271

Kramer, D. L. & Graham, J. B. (1976). Synchronous air breathing, a social component of respiration in fishes. *Copeia*, *1*(4), 689-697

Lafferty, K. D., & Shaw, J. C. (2013). Comparing mechanisms of host manipulation across host and parasite taxa. *Journal of Experimental Biology*, *216*(1), 56-66. doi: 10.1242/jeb.073668.

Lunaschi, L. I., & Drago, F. B. (2004). Descripción de una especie nueva de *Tylodelphys* (Digenea: Diplostomidae) parásita de *Podiceps major* (Aves: Podicepedidae) de Argentina. *Anales del Instituto de Biología. Serie Zoología*, *75*(2), 245-252.

Machado, P. M., Takemoto, R. M., & Pavanelli, G. C. (2005) *Diplostomum* (*Austrodiplostomum*) *compactum* (Lutz, 1928) (Platyhelminthes, Digenea) metacercariae in fish from the floodplain of the Upper Parana River, Brazil. *Parasitology Research*, *97*(6), 436-444.

Moravec, F., Wolter, J., & Körting, W. (1999). Some nematodes and acanthocephalans from exotic ornamental freshwater fishes imported into Germany. *Folia Parasitologica*, *46*(4), 296-310. doi: 10.1007/s004 36-005-1483-7

Muzzall, P. M., & Kilroy, A. L. (2007). *Tylodelphys scheuringi* (Diplostomidae) infecting the brain of the central mudminnow, *Umbra limi*, in Silver Creek, Michigan, USA. *Comparative parasitology*, *74*(1), 164-166. doi: 10.1654/4278.1

Niewiadomska, K. (1996) The genus *Diplostomum* – taxonomy, morphology and biology. *Acta Parasitologica*, *41*(2), 55-66.

Niewiadomska, K. (2002). Family Diplostomidae Poirier, 1886. In D. I. Gibson, A. Jones, R. A. Bray (Ed.), *Keys to the Trematoda* (p. 167-196). Oxon, UK: Cabi Publishing.

Niewiadomska, K., & Laskowski, Z. (2002). Systematic relationships among six species of *Diplostomum* Nordmann, 1832 (Digenea) based on morphological and molecular data. *Acta Parasitologica*, *47*(1), 20-28.

Richards, D. T., Hoole, D., Lewis, J. W., Evans, E., Arme, C. (1994b). Changes in the cellular composition of the spleen and pronephros of carp *Cyprinus carpio* infected with the blood fluke *Sanguinicola inermis* (Trematoda: Sanguinicolidae). *Diseases of Aquatic Organisms*, *19*(3), 173-179.

Richards, D. T., Hoole, D., Lewis, J. W., Ewens. E., Arme, C. (1994a). Ultrastructural observations on the cellular response of carp (*Cyprinus carpio* L.) to eggs of the blood fluke *Sanguinicola inermis* Plehn, 1905 (Digenea: Sanguinicolidae). *Journal of Fish Disease*, *17*(5), 439-446.

Sánchez, H., García, A., Vásquez, J., & Alcántara, F. (2011). *Peces ornamentales amazónicos*. Iquitos, AM: Instituto de Investigaciones de la Amazonía Peruana.

Received on October 17, 2017.
Accepted on September 10, 2018.

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