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First record of *Rhipidocotyle santanaensis* (Digenea) parasitizing *Acestrorhynchus lacustris* from Batalha River, Brazil

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(With 3 figures)

Abstract

This paper describes the occurrence of digenetic *Rhipidocotyle santanaensis* Lunaschi, 2004 in Brazil. This parasite was found in the liver, heart, gonads, intestine, pyloric caeca, stomach, swim bladder and cavity of *Acestrorhynchus lacustris* (Lütken, 1875) from the Batalha River, São Paulo State, with higher incidence in gonads and digestive tract. The parasite specimens found in this study showed morphological characteristics very similar to specimens from Argentina, with differences only in relation to the ejaculatory duct and seminal vesicle. However, Brazilian specimens presented great morphometrical differences between specimens described in Argentina, with much higher measures. This is the first record of this metazoan parasite species in Brazil and in this host fish.

Keywords: Acestrorhynchidae, Bucephalidae, morphometrical differences, freshwater.

Primeiro registro de *Rhipidocotyle santanaensis* (Digenea) parasitando *Acestrorhynchus lacustris* do rio Batalha, Brasil

Resumo

O presente trabalho descreve a ocorrência do digenético *Rhipidocotyle santanaensis* Lunaschi, 2004 no Brasil. Estes parasitos foram encontrados no fígado, coração, gônadas, intestino, cecos pilóricos, estômago, bexiga natatória e cavidade do peixe *Acestrorhynchus lacustris* (Lütken, 1875) provenientes do rio Batalha, Estado de São Paulo, com maior abundância nas gônadas e trato digestório. Os espécimes dos parasitos encontrados neste trabalho apresentaram características morfológicas muito semelhantes aos espécimes provenientes da Argentina, com diferenças apenas em relação ao ducto ejaculatório e vesícula seminal. Porém, os exemplares brasileiros apresentaram grandes diferenças morfométricas entre os espécimes descritos na Argentina, com medidas muito superiores. Este é o primeiro registro desta espécie de parasito no Brasil e neste hospedeiro.

Palavras-chave: Acestrorhynchidae, Bucephalidae, diferenças morfométricas, água doce.

1. Introduction

Parasites can be effective tools for comparative studies in ecology and biogeography. Because they are host-dependent regarding the essential features of survival (nutrient acquisition and reside on or within the host's body for long periods of time), its distribution is invariably linked to the presence of that particular host on the environment. In case generalists parasitic species (low host specificity), the geographical range will be much larger in relation to specialists, since it will have the ability to explore different hosts, which in turn present migration patterns that will influence the dispersal of these parasites in the environment (Poulin et al., 2011).

Bucephalidae Poche, 1907 is a major family of Digenea, with a wide host and geographic distribution (Derbel et al., 2011). These digeneans are characterized by the presence of a rhynchus, when the oral sucker is absent (Travassos et al., 1969). Five genera were previously known from freshwater fishes of South America: *Rhipidocotyle* (Diesing, 1858), *Prosorhynchus* (Odhner, 1905), *Prosorhynchoides* (Dollfus, 1929), *Bellumcorpus* (Kohn, 1962) and *Glandulorhynchus* (Thatcher, 1999) (Lunaschi, 2004).

Rhipidocotyle has a large number of species that parasitize marine fish and just some are found in freshwater fish. In South America, are described four species of marine

fish parasites: *R. adbaculum* Keep, 1940 (Tantalean et al., 1992), *R. angusticolle* Chandler, 1941, *R. fluminensis* Vicente and Santos, 1973 and *R. quadriculata* Kohn, 1961, while *R. santanaensis* Lunaschi, 2004, *R. gibsoni* Kohn and Fernandes, 1994 and *R. jeffersoni* (Kohn, 1970) Overstreet & Curran, 2002 are described for neotropical freshwater fishes (Kohn and Fernandes, 1994; Lunaschi, 2004).

Rhipidocotyle santanaensis was described by Lunaschi (2004) parasitizing the pyloric caeca of *Acestrorhynchus pantaneiro* (Menezes, 1992) on Paraná River in Santa Ana, Corrientes province, Argentina. This species differs from the congenerics by presenting a rhynchus with two lateral projections, the testes arranged in diagonal and the vitelline follicles forming an arch in the pre-oral region (Lunaschi, 2004). Until the present time, only *R. gibsoni* (Kohn and Fernandes, 1994) was reported to *A. lacustris* (Lütken, 1875).

Acestrorhynchus lacustris is a fish belonging to Acestrorhynchidae and it is often found in the basins of the Tietê-Paraná and São Francisco River, mainly in lentic environments. It is a carnivorous species and its predominant feed items are forage fishes such as *Astyanax altiparanae* Garutti and Britski, 2000, *Moenkhausia intermedia* (Eigenmann, 1908) and *Steindachnerina insculpta* (Fernández-Yépez, 1948) (Hahn et al., 2000). According to Agostinho et al. (2004), it is considered a non-migratory or short-distance migratory species with external fertilization and no parental care. In this paper, we provide new information about morphometrical and morphological characteristics of adult specimens of *R. santanaensis* in this freshwater fish from São Paulo State, Brazil.

2. Material and Methods

Thirty-two specimens of *A. lacustris* were collected from the Batalha river (22° 22' 56" S 49° 06' 54" W) in a helminthological research carried out from May to September, 2013 (Figure 1). This river flows through the cities of Agudos, Bauru, Piratininga, Avaí, Duartina, Gália,

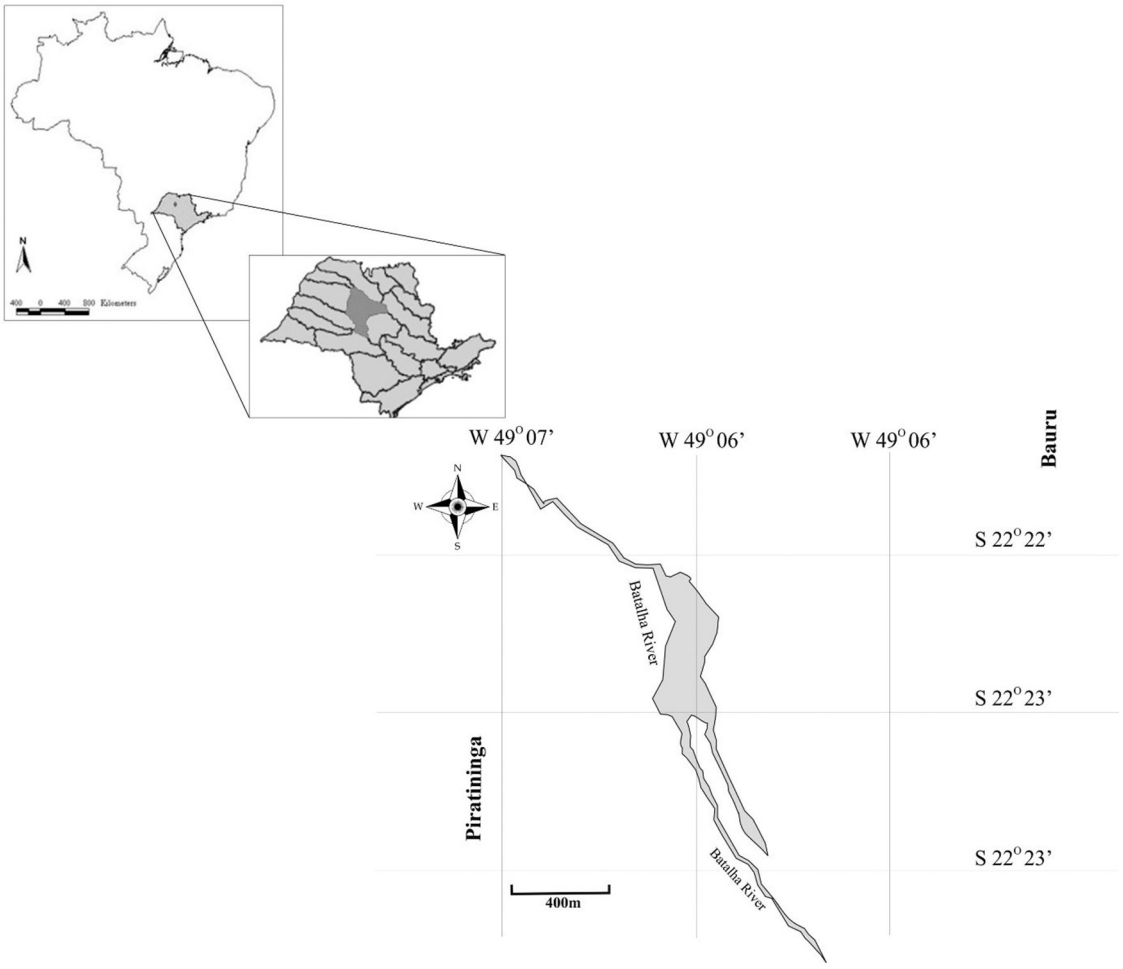

Figure 1. Map of the study area, showing details of Batalha River with your geographic location coordinates and highlighting the Hydrographic Basin of Tietê-Batalha on Sao Paulo State map, where is located the Batalha River.

Presidente Alves, Reginópolis and Uru. It has a great importance for the region, being responsible for supplying 45% of Bauru population (Santos and Heubel, 2008).

The fishes were collected by using gillnets with different meshes. In collect, fishes were packed in individual plastic bags and transported in a refrigerated cooler box to the Laboratório de Ictioparasitologia in the Central de Laboratórios de Ciência e Tecnologia Ambiental at USC (Universidade do Sagrado Coração) in Bauru, where they were kept in a freezer until necropsy. All internal organs were analyzed individually in stereomicroscope.

The digeneans found were counted and stored in alcohol 70° GL. The specimens were stained with Mayer's Carmalúmen and mounted in Canada balsam according to Eiras et al. (2006). The ecological parasitism descriptors were obtained by Bush et al. (1997). Trinocular microscopy (Nikon E200) was employed for the morphologic examination. The measured samples were randomly selected among the various hosts organs where they were found. Measurements were obtained by using a computerized image analysis system (Motic, Moticam 5.0MP). Measures are given in micrometers and presented as the mean followed by the minimum and maximum values in parentheses. Specimens were deposited in Invertebrates Collection of Instituto Nacional de Pesquisas da Amazônia (INPA), Manaus, AM, Brazil, with number 642.

3. Results

From the 32 fishes analyzed, 24 were parasitized with adults specimens of *R. santanaensis* (Figure 2a-d) (prevalence = 78.12%, abundance = 29.84 and intensity = 38.2). Were collected 955 specimens being found parasitizing the liver, heart, gonads (Figure 3), intestine, pyloric caeca, stomach, bladder and swim cavity of the hosts. The gonads, stomach and intestine were the most infected organs.

The specimens registered in this paper showed much higher measures to samples recorded by Lunaschi (2004), only the acetabulum of the organisms in current study were much smaller compared to specimens from Argentina and the eggs morphometry of both are similar (Table 1). We observed morphometrical variation among collected samples within the same organ, and this variation was similar on all different infection sites.

Proportionally, the parasites found in this study showed morphologic characteristics similar to organisms of Argentina described by Lunaschi (2004), with differences only in relation to the ejaculatory duct and seminal vesicles. In this study, the vesicle showed tighter when compared to Lunaschi (2004) specimens, which presented vesicle with oval shape. The samples reported in this paper presented an ejaculatory duct occupying a larger space within the cirrus sac, whereas the Argentina specimens showed a lower proportion (Figure 2d).

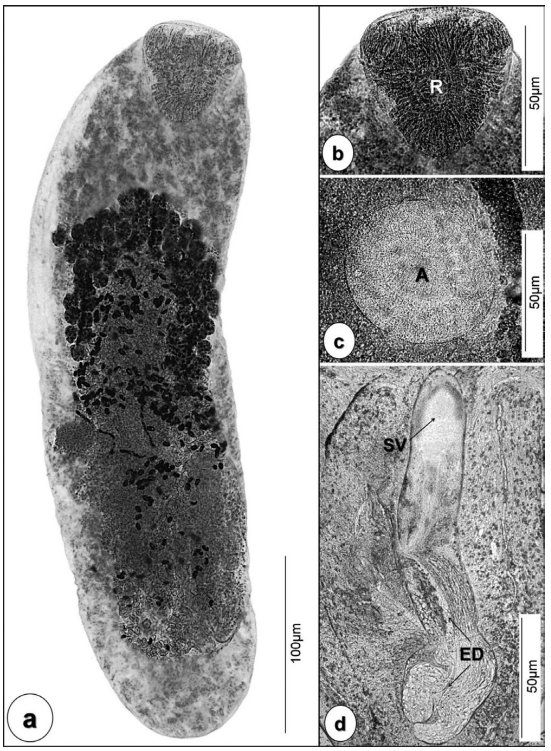

Figure 2. *Rhipidocotyle santanaensis* Lunaschi, 2004. (a) Complete specimen, ventral view (10x); (b) Rhynchus (R) (40x); (c) Acetabulum (A) (40x); (d) Seminal Vesicle (SD) and Ejaculatory Duct (ED) (40x).

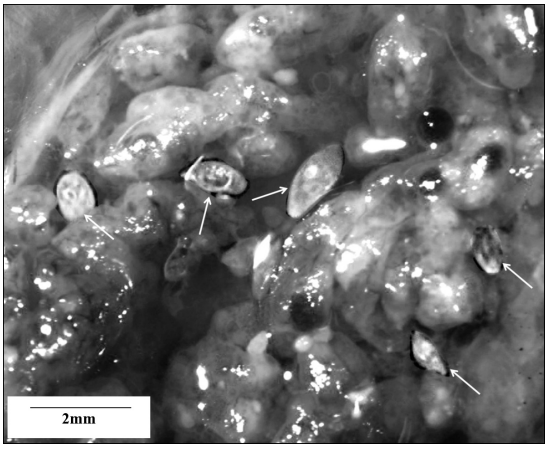

Figure 3. Gonads of *Acestrorhynchus lacustris* parasitized by *Rhipidocotyle santanaensis* (indicated by white arrows).

4. Discussion

Lunaschi (2004) does not comment anything on the prevalence, abundance and intensity of the *R. santanaensis* in *A. pantaneiro*, which hinders any kind of comparison with present study data. Furthermore, in description of *R. santanaensis* conducted by Lunaschi (2004), only three specimens were measured collected from pyloric caeca, which reduces variation range of the measures

Table 1. Comparison between the measures of *Rhipidocotyle santanaensis* Lunaschi, 2004 in *Acestrorhynchus pantaneiro* (Menezes, 1992) from Argentina and *Acestrorhynchus lacustris* (Lütken, 1875) from Brazil (all measures in µm and representing length and width respectively).

Structures	***Rhipidocotyle santanaensis* in *Acestrorhynchus pantaneiro* Lunaschi, 2004 n=3**	***Rhipidocotyle santanaensis in Acestrorhynchus lacustris* Present study n=30**
Body	618 × 283 (432-797 × 177-336)	1185 × 456 (923-2133 × 362-713)
Rhynchus	97 × 84 (63-115 × 70-103)	173 × 214 (96-373 × 98-377)
Acetabulum	178 × 373	112 × 84 (71-182 × 59-118)
Previous testicle	94 × 102 (60-115 × 73-122)	182 × 203 (138-266 × 126-305)
Posterior testicle	84 × 86 (63-102 × 65-102)	196 × 241 (132-391 × 145-316)
Ovary	79 × 75 (31-104 × 32-126)	119 × 167 (91-223 × 89-213)
Cirrus	270 × 65 (150-340 × 33-92)	538 × 109 (410-1157 × 85-177)
Pre-oral region	204 (115-258)	365 (201-735)
Post-oral region	379 (266-494)	651 (278-914)
Eggs	18 × 13 (16-18 × 12-13)	19 × 12 (17-21 × 10-15)

and moreover, different fixatives were used which could influence the parasites distension. However Carvalho et al. (2003) studying the parasitic fauna of *A. lacustris* collected in the Paraná river floodplain found *R. gibsoni* with much lower prevalence and intensity values (prevalence = 17.6% and intensity = 3.6) to those found in this study.

Parasites provide an ideal model for testing ecological constraints, such as the size of the host, on the evolution of body size in a group of organisms (Harvey and Keymer, 1991; Poulin, 1995). Changes in body size related to evolution towards parasitism follow diverse trends depending on the parasite group (Morand et al., 1996). The intraspecific morphometric and morphologic changes are much common in parasites and some authors comment that these changes may be related to the different times of infection and/or intense competition for space and other host resources (Dobson, 1986; Shostak and Dick, 1987; Szalai and Dick, 1989). Physiological differences found in different hosts may affect the establishment, grow and sexual maturity of the parasites; to specific polymorphism of the parasites; to parasites different stages of development and to environmental variability (Watson and Pike, 1993; Willis, 2002; Francisco et al., 2011; González et al., 2013).

Fish endoparasites adults feed either on the digested contents of the host's intestine or the host's own tissues (including blood). They might also feed by means of osmotic absorption (Markov, 1946). The structure of various digestive tract parts, the histology of each organ and the spatial relationship between the organs are factors that also determine the degree of endohelminth infection (Dogiel et al., 1970). In this work, the infection sites with higher parasites incidence and abundance were the gonads and the digestive tract, that is, sites with high energy content available for the parasitic absorption. Isaac et al. (2004) studying the parasitic fauna of *Gymnotus* spp. found the digenetics *Crocodilicola* sp. 1, *Crocodilicola* sp. 2 and *Herpetodiplostomum* sp. 1 parasitizing gonads of this host. They comment that the use of gonads as microhabitat can interfere in host reproduction or even lead to parasitic castration and this can be a total or partial castration. In connection with this topic, Paperna (1974) recorded nematode larvae *Eustrongylides* sp. in gonads of *Haplochromis* spp. and remarked that a large number of cysts can deform the ovaries, making them increasingly irregular, leading to their degeneration and the formation of a large cystic mass.

Digenetic are parasites commonly found on different sites (microhabitats) within the hosts. It is rare for the initial encounter with a host individual to happen exactly where the parasite's microhabitat will be. The invading parasite must detect signals and move to reach the microhabitat. When a parasite penetrates a host it enters an environment that has numerous highly predictable characteristics. Certain parasites also show intermicrohabitat migrations, and this may be related to the period of feeding, digestion, migration patterns or reproducing of the host for example. To identify the appropriate microhabitat, parasites have surface molecules that recognize host molecules. There are powerful selective pressures for the discovery of the correct habitat, because parasites that localize outside the normal microhabitat would likely not transmit their genes,

either they die because the resources are not correct or they survive but cannot correctly disperse their offspring (Stunkard, 1974; Combes, 2001).

Beyond the present study, there is only one record of *R. santanaensis* parasitizing the congeneric species *A. pantaneiro* in Argentina. Due to the few records, it is difficult to consider this species as a specialist or generalist. It would be more appropriate to classify it as stenoxenus species (or with stenoxenus tendency), which according to Euzet and Combes (1980), are parasites found in a small group of related host species, usually in the same genus or family. Finally, we would like to comment that the fishes collected by Lunaschi (2004) came from the Parana River, which belongs to the same hydrographic basin of studied fish (Basin of Paraná-Tietê), since the Batalha River is a tributary of Tietê River, thus concluding that such basins have connectivity. This is the first occurrence of *R. santanaensis* in Brazil and also in *A. lacustris*.

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References

AGOSTINHO, A.A., GOMES, L.C., VERÍSSIMO, S. and OKADA, E.K., 2004. Flood regime, dam regulation and fish in the Upper Paraná River: effects on assemblage attributes, reproduction and recruitment. *Reviews in Fish Biology and Fisheries*, vol. 14, pp. 11-19. http://dx.doi.org/10.1007/s11160-004-3551-y.

BUSH, A.O., LAFFERTY, K.D., LOTZ, J.M. and SHOSTAK, A.W., 1997. Parasitology meets ecology on its own terms: Margolis et al. revisited. *The Journal of Parasitology*, vol. 83, no. 4, pp. 575-583. http://dx.doi.org/10.2307/3284227. PMid:9267395.

CARVALHO, S., GUIDELLI, G.M., TAKEMOTO, R.M. and PAVANELLI, G.C., 2003. Ecological aspects of endoparasite fauna of *Acestrorhynchus lacustris* (Lütken, 1875) (Characiformes, Acestrorhynchidae) on the Upper Paraná River floodplain, Brazil. *Acta Scientiarum: Biological Sciences*, vol. 25, pp. 479-483.

COMBES, C., 2001. *Parasitism: the ecology and evolution of intimate interactions*. 1st ed. Chicago: The University of Chicago Press. 728 p.

DERBEL, H., CHÂARI, M. and NEIFAR, L., 2011. Redescription of *Rhipidocotyle galeata* (Rudolphi, 1819) (Digenea, Bucephalidae), the type species of *Rhipidocotyle* Diesing, 1907. *Zoosystema*, vol. 33, no. 2, pp. 133-139. http://dx.doi.org/10.5252/z2011n2a1.

DOBSON, A.P., 1986. Inequalities in the individual reproductive success of parasites. *Parasitology*, vol. 92, no. Pt 3, pp. 675-682. http://dx.doi.org/10.1017/S0031182000065537. PMid:3737247.

DOGIEL, V.A., PETRUSHEVSKI, G.K. and POLYANSKI, Y.I., 1970. *Parasitology of fishes*. London: Olivier & Boyd. 384 p.

EIRAS, J.C., TAKEMOTO, R.M. and PAVANELLI, G.C., 2006. *Métodos de estudo e técnicas laboratoriais em parasitologia de peixes*. 2nd ed. Maringá: Universidade Estadual de Maringá. 199 p.

EUZET, L. and COMBES, C., 1980. Les problèmes de l'espèce chez les animaux parasites. *Mémoires de la Société Zoologique Française*, vol. 40, no. 3, pp. 239-285.

FRANCISCO, C.J., ALMEIDA, A., CASTRO, A.M., PINA, S., RUSSELL-PINTO, F., RODRIGUES, P. and SANTOS, M.J., 2011. Morphological and molecular analysis of metacercariae of *Diphtherostomum brusinae* (Stossich, 1888) Stossich, 1903 from a new bivalve host *Mytilus galloprovincialis*. *Journal of Helminthology*, vol. 85, no. 2, pp. 179-184. http://dx.doi.org/10.1017/S0022149X10000428. PMid:20701832.

GONZÁLEZ, M.T., HENRÍQUEZ, V. and LÓPEZ, Z., 2013. Variations in the fecundity and body size of digenean (Opecoelidae) species parasitizing fishes from Northern Chile. *Revista de Biología Marina y Oceanografía*, vol. 48, no. 3, pp. 421-429. http://dx.doi.org/10.4067/S0718-19572013000300002.

HAHN, N.S., DELARIVA, R.L. and LOUREIRO, V.E., 2000. Feeding of *Acestrorhynchus lacustris* (Characidae): a post impoundment studies on Itaipu reservoir, upper Paraná river. *Brazilian Archives of Biology and Technology*, vol. 43, no. 2, pp. 207-213. http://dx.doi.org/10.1590/S1516-89132000000200010.

HARVEY, P.H. and KEYMER, A.E., 1991. Comparing life histories using phylogenies. *Philosophical Transactions of the Royal Society of London*, vol. 332, no. 1262, pp. 31-39. http://dx.doi.org/10.1098/rstb.1991.0030.

ISAAC, A., GUIDELLI, G.M., FRANÇA, J.G. and PAVANELLI, G.C., 2004. Composição e estrutura das infracomunidades endoparasitárias de *Gymnotus spp.* (Pisces: Gymnotidae) do rio Baía, Mato Grosso do Sul, Brasil. *Acta Scientiarum Biological Sciences*, vol. 26, no. 4, pp. 453-462. http://dx.doi.org/10.4025/actascibiolsci.v26i4.1527.

KOHN, A. and FERNANDES, B.M.M., 1994. *Rhipidocotyle gibsoni* n. sp. from a Brazilian freshwater fish and *Rhipidocotyle froesi* n. sp. for *R. baculum* (Linton, 1905) of Eckmann (1932) (Bucephalidae; Digenea). *Memórias do Instituto Oswaldo Cruz*, vol. 89, no. 4, pp. 567-570. http://dx.doi.org/10.1590/S0074-02761994000400011.

LUNASCHI, L.I., 2004. Two news species of bucephalids (Digenea: Bucephalidae) parasitic in freshwater fishes of Argentina. *Parasitology International*, vol. 53, no. 3, pp. 229-234. http://dx.doi.org/10.1016/j.parint.2004.01.014. PMid:15468530.

MARKOV, G.S., 1946. Modes of feeding of parasitic worms. *Priroda*, vol. 12.

MORAND, S., LEGENDRE, P., GARDNER, S.L. and HUGOT, J.P., 1996. Body size evolution of oxyurid (Nematoda) parasites: the role of hosts. *Oecologia*, vol. 107, no. 2, pp. 274-282. http://dx.doi.org/10.1007/BF00327912.

PAPERNA, H., 1974. Hosts, distribution and pathology of infections with larvae of Eustrungylides (Dioctophymidae, Nematoda) in fishes from East African lakest. *Journal of Fish Biology*, vol. 6, pp. 67-76. http://dx.doi.org/10.1111/j.1095-8649.1974.tb04523.x.

POULIN, R., 1995. Phylogeny, ecology, and the richness of parasite communities in vertebrates. *Ecological Monographs*, vol. 65, no. 3, pp. 283-302. http://dx.doi.org/10.2307/2937061.

POULIN, R., KRASNOV, B.R., MOUILLOT, D. and THIELTGES, D.W., 2011. The comparative ecology and biogeografy of parasites. *Philosophical Transactions of the Royal Society*, vol. 366, no. 1576, pp. 2379-2390. http://dx.doi.org/10.1098/rstb.2011.0048. PMid:21768153.

SANTOS, F.S. and HEUBEL, M.T.C.D., 2008. General composição da comunidade ictilógica e biometria taxológica na lagoa de captação de água do DAE no rio Batalha (Bauru-SP). *Salusvita*, vol. 27, pp. 29-44.

SHOSTAK, A.W. and DICK, T.A., 1987. Individual variability in reproductive success of *Triaenophorus crassus* Forel (Cestoda: Pseudophyllidea), with comments on use of the Lorenz curve and Gini coefficient. *Canadian Journal of Zoology*, vol. 65, no. 12, pp. 2878-2885. http://dx.doi.org/10.1139/z87-437.

STUNKARD, H.W., 1974. The trematode family Bucephalidae: problems of morphology, development and systematics: description of *Rudolphinus* gen. nov. *Transactions of the New York Academy of Sciences. Series II*, vol. 36, no. 2, pp. 143-170. http://dx.doi.org/10.1111/j.2164-0947.1974.tb01563.x. PMid:4593638.

SZALAI, A.J. and DICK, T.A., 1989. Differences in numbers and inequalities in mass and fecundity during the egg-producing period for *Raphidascaris acus* (Nematoda: Anisakidae). *Parasitology*, vol. 98, no. 03, pp. 489-495. http://dx.doi.org/10.1017/S0031182000061588.

TANTALÉAN, M.V., SARMIENTO, L.B. and HUIZA, A.F., 1992. Digeneos (Trematoda) del Perú. *Boletín de Lima*, vol. 80, pp. 47-84.

TRAVASSOS, L., TEIXEIRA DE FREITAS, J.F. and KOHN, A., 1969. Trematódeos do Brasil. *Memórias do Instituto Oswaldo Cruz*, vol. 67, no. 1, pp. 1-886. PMid:5397756.

WATSON, J.J. and PIKE, A.W., 1993. Variation in the morphology of adult *Apatemon gracilis* Rudolphi, 1819 (Digenea: Strigeidae) reared in different avian hosts. *Systematic Parasitology*, vol. 26, no. 1, pp. 33-38. http://dx.doi.org/10.1007/BF00009645.

WILLIS, S., 2002. Morphological variation of *Allocreadium lobatum* (Digenes: Allocreadiidae) in the creek chub, *Semotilus atromaculatus* (Osteichthyes: Cyprinidae), in Nebraska, USA. *Transactions of the Nebraska Academy of Sciences*, vol. 28, pp. 21-27.