Myxozoans *Myxobolus* sp. and *Henneguya* sp. co-infection in kidney of *Piaractus mesopotamicus* (Characiformes: Serrasalmidae)

Wilson G. Manrique[a*], Mayra A.P. Figueiredo[b], Marco A.A. Belo[a,c], Maurício L. Martins[d] Kálmán Molnár[e]

[a]Department of Veterinary Pathology, Brazil University - Descalvado Campus, Brazil

[b]Department of Parasitology, Institute for Biomedical Sciences, University of São Paulo - São Paulo, Brazil.

[c]Department of Preventive Veterinary Medicine, State University of São Paulo - Jaboticabal, Brazil.

[d]AQUOS-Aquatic Organisms Health Laboratory, Aquaculture Department, Federal University of Santa Catarina, Florianópolis, SC, Brazil.

[e]Veterinary Medical Research Institute, Hungarian Academy of Sciences, Budapest, Hungary.

*Corresponding author: Tel.: + 55 16 98137-0089 Departamento de Patologia Veterinária, Universidade Brasil – Câmpus de Descalvado. Av. Hilário da Silva Passos, 950 – Parque Universitário Descalvado, CEP: 13690-970, São Paulo - Brazil

E-mail address: wilsongomezmanrique@yahoo.es

Abstract

This study evaluated the myxozoan infection and histopathology of the kidney of the freshwater fish *Piaractus mesopotamicus* from intensive fish farming in Brazil. A total of fifty-five fish were examined and the organs processed according to usual histological methods by staining with haematoxylin-eosin and Ziehl-Neelsen. In renal tissue free myxospores of *Myxobolus* sp. (85.5% prevalence) and *Henneguya* sp. (56.4% prevalence) were observed. The presence of myxospores was associated with histological alterations in both stromal and renal parenchyma. Myxospores were found mostly in the peritubular interstitial tissue and in low intensity in the glomerulus which caused nuclear hypertrophy and loss of Bowman space. An increase in the glomerular tuft and a reduction in the lumen of the collector tubules was also observed, besides high number of melanomacrophage cells in the glomerulus. This study reports for the first time detection of mixed infection by myxozoans in just one organ of pacu and discuss on the possible transport of myxospores in the circulating blood.

Keywords: Fresh water fish; histopathology; inflammation; Myxosporea; pacu

1. Introduction

Piaractus mesopotamicus Holmberg, 1887, popularly known in Brazil as "pacu", belongs to the Family Serrasalmidae, is a teleost fish native to the Paraná-Paraguay Basin. It is an emergent species in the world aquaculture, and presents great economic importance in the South America (Belo et al., 2014; Valladão et al., 2016), China (Lin et al., 2015) and United States (Witmer and Fuller, 2011). This species has proven to be a good bioindicator of water quality (Farias et al., 2016), and in accordance with Castro et al. (2014) the pacu has been used in ecotoxicity studies for registration of chemicals in Brazil.

High stocking density and inadequate handling are responsible for increased stress that affect negatively the pacu health causing increased disease susceptibility (Belo et al., 2005; 2012; Manrique et al., 2015a). On the other hand, members of the class Myxosporea use not only wild and cultured fish (Capodifoglio et al., 2016) but also amphibians, reptiles (Eiras, 2005), aquatic birds (Bartholomew et al., 2008) and terrestrial mammals (Friedrich et al., 2000) as hosts. These parasites have been recognized as a key limiting factor in the development of aquaculture because they infect a large variety of commercially important fishes, and these parasites may develop intra- and intercellularly (histozoic) or may be located in the organs and body cavity (celozoic) (Lom and Dyková, 2006).

Myxosporean parasites are known to be responsible for several forms of damage, including myoliquefaction of the host (Eiras et al., 2007), reduction of the capacity of respiration (Molnár and Székely, 1999), damage to the ovaries (Mansour et al., 2013) changes in meat quality (Manrique et al., 2015b) and changes in the renal tissue (Molnár, 2007; Manrique et al., 2012; Abdel-Baki et al., 2015).

So far, the occurrence of two *Myxobolus* species in pacu, *M. cuneus* infecting the connective tissue (Adriano et al., 2006) and the skeletal muscle (Manrique at al., 2016), and *M. colossomatis* in branchial arches and gill (Müller et al., 2013), and two *Henneguya* species *H. pellucida* in swim bladder (Adriano et al., 2005a) and *H. piaractus* in gill lamellae (Adriano et al., 2005b; Azevedo et al., 2010; Müller et al., 2013). .

In this paper, we report on a mixed infection with myxospores of a *Myxobolus* sp. and a *Henneguya* sp. in the posterior kidney of *P. mesopotamicus* and on histopathological changes in the renal tissue caused by these parasites.

2. Materials and methods

2.1. Fish samples

Fifty-five live young fish of *P. mesopotamicus* with 124.0 ± 3.7 g mean weight and standard length 19.9 ± 2.7 cm were captured during August 2014 from a pond of intensive fish farming in Southeast Brazil, São Paulo State.

2.2. Experimental procedures

The living fish were euthanized by fish immersion in an alcoholic solution of benzocaine 1:500 v/v anesthesia/water (0.1 g benzocaine per mL of ethanol) according to the ethical procedures approved by Ethics Committee (CEUA-UNESP protocol nº 020092/09) for posterior blood collection from the caudal vein using syringes containing 10% EDTA to make the blood smears, that were stained with Giemsa to evaluation of structures examined in optical microscope. Then necropsy was performed for collection of the posterior kidney for histopathology and a small fragment for analysis in fresh mounts.

2.3. Morphological analyses of myxospores

The samples of organs were placed in a petri dish, moistened with saline solution (0.65%) and macerated with scalpel blades and placed between a glass and a coverslip for myxospore measurements in fresh (Burger and Adlard, 2010), only in the caudal kidney were observed myxospores. A total of 173 myxospores were measured from the histological sections (107 *Myxobolus* sp. and 66 *Henneguya* sp.). All analyses were performed in an Olympus BX51 light microscope with image capture in a DP73 camera and morphometry using the cellSens v.1.5 Software (Olympus).

2.3. Histopathology analyses

The posterior kidney was fixed in Bouin solution for 6 h and submitted to routine procedures in order to obtain cross sections of 5 µm thickness in paraffin and stained with hematoxylin-eosin (H&E) and Ziehl-Neelsen (ZN) for microscopical examination.

3. Results

3.1. Myxobolus sp. and Henneguya sp. myxospores

In fresh mounts of the kidney, myxospores of *Myxobolus* sp. (Fig. 1) and *Henneguya* sp. (Fig. 2) were identified. The myxospores were measured from the histological sections stained with ZN and compared with others *Myxobolus* and *Henneguya* species of Brazilian native Characiformes fish (Table 1), and showed characteristics similar to those reported in the literature. However, no myxospores were recorded in blood smears or in other organs.

3.2. Histopathology

The analysis of histological sections stained with ZN showed that neither plasmodial nor sporogonic stages of the above species were found in the kidney. Nevertheless, disseminated mature myxospores were located in the renal interstitium, in the wall and the lumen of the glomeruli, and in the tubules. The prevalence of *Myxobolus* sp. was 85.5% (47/55) and *Henneguy*a sp. was 56.4% (31/55).

Most of the myxospores seemed to be intact, and their sporoplasm, polar capsules and the spore wall stained intensely (Fig. 3 and 4), some other damaged myxospores, however, were surrounded and incorporated into melanomacrophage cells. Melanomacrophage cells were regularly found inside the malpighian corpuscle, in the lumen and among epithelial cells of the convoluted channels or free in the renal interstitium. In some of the slides stained with hematoxylin and eosin, the debris of the decayed myxospores was also observed (Fig. 5 and 6). A special feature of the infection was that melanomacrophage centers were not found in the renal interstitium, but agglomerated melanomacrophage cells were located inside the Bowman capsules and tubules (Fig. 5 and 6). The cellular infiltration in the renal parenchyma (Fig. 5 and 6) was in the form of aggregation of mononuclear cells.

4. Discussion

The kidney of freshwater fishes is a complex organ with two different functions. The trunk kidney and the hind kidney have excretory function, while the head kidney has a haematopoietic function. The structure of the hind kidney is similar to those of mammals and

125 birds, having glomeruli in Bowman capsule, convoluted tubules and urinary ducts surrounded
126 by the renal interstitium (Harder, 1975). The large number of myxosporean parasites located
127 in different parts of the kidney, mainly in the trunk kidney, and they can develop in several
128 ways (Molnár, 2007). Some species, like *M. erythrophthalmi* of *Scardinius erythrophthalmus*
129 form large plasmodia in the renal interstitium (Molnár et al., 2009), while others develop in
130 the epithelium and the lumen of the urinary channels or in the renal glomeruli (Molnár and
131 Eszterbauer, 2015).

132 Csaba et al. (1984) described that *Sphaerospora renicola*, a sphaerosporid type
133 myxosporean completes its presporogonic development circulating in the blood and arrives at
134 the lumen of renal tubules for finishing its sporogonic development, where it performs spore
135 production. The pathogenic effect of myxosporeans shows also a great variation.
136 Capodifoglio et al. (2016) have observed that the infection by *M. hilarii* in the kidney of
137 *Brycon hilarii* caused compression, deformation and destruction of the tubular cells and
138 adjacent tissue. Myxospores of several species develop in organs (muscles, liver, connective
139 tissue, abdominal cavity) from where their mature myxospores have been carried by the blood
140 stream to the organs (gills, skin, kidney) (Molnár and Eszterbauer, 2015). Apart from these,
141 spores are stuck, engulfed by macrophages and destroyed. We suppose that both, *Myxobolus*
142 and *Henneguya* myxospores, found by us free in the kidney tissues or engulfed by
143 macrophages, belong this type of species.

144 Myxosporean species infecting the pacu have different site and tissue affinities. From the
145 two *Henneguya* species, *H. piaractus* is a parasite of the gills, while *H. pellucida* infects
146 serous membranes in the abdominal cavity (Adriano et al., 2005a). Of the two *Myxobolus* spp.
147 found in pacu, both *M. cuneus* and *M.* cf. *colossomatis* are found to be parasites of the
148 connective tissue and develop in the internal organs (Adriano et al., 2006; Müller et al., 2013).
149 However, a third *Myxobolus* species mentioned by Manrique et al. (2015b; 2016) seems to

150 infect the skeletal muscle. Of the above species, *Henneguya* sp. releases its spores directly to
151 the outside from its gill cysts, spores of some other species among them those developing in
152 the muscle, however, could leave the living host via blood stream, a part of which enter the
153 kidney (Molnár and Székely, 2014).

154 We agree with authors (McGeorge et al., 1996; Belem and Pote, 2001; Molnár et al., 2009;
155 Bjork and Bartholomew, 2010) that myxospores of most *Myxobolus* spp. developing in
156 internal organs, and first of all in the skeletal muscle can reach the kidney via the circulating
157 blood, and myxospores found by us free in the renal tissues and captured by
158 melanomacrophage cells belong to these species. By the shape and measurements spores
159 found in the kidney we cannot exclude that myxospores of the muscle species were also
160 among them.

161 At a similar way we think that *Henneguya* sp. myxospores found in the kidney belong to
162 *H. pellucida*. It is well known (Molnár and Kovács-Gayer, 1985; Holzer and Schachner,
163 2001; Molnár, 2007) that melanomacrophage centers of the kidney and some other organs are
164 the major place for destroying spore stages, larvae and eggs of parasites and through innate
165 and non-specific immune responses, as well as by cellular host activity they eliminate
166 pathogens (Manrique et al., 2014; Sitja-bobadilla et al., 2015). It is rather curious that in our
167 case instead macrophage centers myxospores were damaged and eliminated in solitary
168 macrophages or groups of macrophages accumulated in the Bowman capsule or in the
169 convoluted tubules.

170 Besides macrophage activity around myxospores, cellular infiltration in the renal
171 parenchyma (Fig. 5 and 6) with mononuclear cells were recorded; we could not, however
172 relate this infiltration with cellular host answer against myxospores. In our study the
173 myxosporean infection in the kidney cannot be regarded as fatal, but histological changes
174 found show that due to these disseminated myxospores remarkable local damages can develop

in the kidney. Studies made on *Myxobolus cyprini* by Molnár and Kovács-Gayer (1985) call attention that myxospores of some myxosporean species developing in inner organs and in the muscle, leave the host body through the kidney but a part of these myxospores are captured and eliminated by macrophages.

The findings of this investigation demonstrated that further studies should focus their attention to find the exact place of plasmodial development, and how myxospores were carried to the kidney, leading as a consequence to changes in fish health, as well in order to eliminate the pathogen.

Conflicts of interest

The authors have no conflicts of interest to declare.

Acknowledgements

The authors thanks Nacional Council of Scientific and Technological Development (CNPq) for grant to M.L. Martins (CNPq 305869-2014-8).

References

Abdel-Baki A.A.S., Abdel-Haleem H.M., Sakran T., Zayed E., Ibrahim, K.E., Al-Quraishy, S., 2015. Two *Myxobolus* spp. infecting the kidney of Nile tilapia (*Oreochromis niloticus*) in the River Nile at Beni-Suef governorate, Egypt, and the associated renal changes. Parasitol. Res. 114, 1107–1112. http://dx.doi.org/10.1007/s00436-014-4282-1

Adriano, E.A., Arana, S., Cordeiro, N.S., 2005a. An ultrastructural and histopathological study of *Henneguya pellucida* n. sp. (Myxosporea: Myxobolidae) infecting *Piaractus mesopotamicus* (Characidae) cultivated in Brazil. Parasite 12, 221–227. http://dx.doi.org/10.1051/parasite/2005123221

200 Adriano, E.A., Arana, S., Cordeiro, N.S., 2005b. Histology, ultrastructure and prevalence of
201 *Henneguya piaractus* (Myxosporea) infecting the gills of *Piaractus mesopotamicus*
202 (Characidae) cultivated in Brazil. Dis. Aquat. Org. 64, 229-35.
203 https://dx.doi.org/10.3354/dao064229
204 Adriano, E.A., Arana, S., Cordeiro, N.S., 2006. *Myxobolus cuneus* n. sp. (Myxosporea)
205 infecting the connective tissue of *Piaractus mesopotamicus* (Pisces: Characidae) in Brazil:
206 histopathology and ultrastructure. Parasite 13, 137-142.
207 http://dx.doi.org/10.1051/parasite/2006132137
208 Azevedo, C., Amaral, C.M.C., Casal, C., Marques, D.K.S., Matos, E., Matos, P., Silva, E.V.,
209 2010. Ultrastructural Re-description of *Henneguya piaractus* (Myxozoa), a parasite of the
210 freshwater fish *Piaractus mesopotamicus* (Teleostei, Characidae) from the Paraguai River,
211 Brazil. Acta Protozool. 49, 115-120.
212 Bartholomew, J.L., Atkinson, S.D., Hallett, S.L., Lowenstine, L.J., Garner, M.M., Gardiner,
213 C.H., Rideout, B.H., Keel, B.H., Brown, J.D., 2008. Myxozoan parasitism in waterfowl.
214 Int. J. Parasitol. 38, 1199–1207. https://dx.doi.org/10.1016/j.ijpara.2008.01.008
215 Belem, A.M.G., Pote, L.M., 2001. Portals of entry and systemic localization of proliferative
216 gill disease organisms in channel catfish *Ictalurus punctatus*. Dis. Aquat. Org. 48, 37–42.
217 https://dx.doi.org/10.3354/dao048037
218 Belo, M.A.A., Moraes, F.R., Yoshida, L., Prado, E.J.R., Moraes, J.R.E., Soares, V.E., Silva,
219 M.G., 2014. Deleterious effects of low level of vitamin E and high stocking density on the
220 hematology response of pacus, during chronic inflammatory reaction. Aquaculture. 422-
221 423, 124-128. http://dx.doi.org/10.1016/j.aquaculture.2013.12.013
222 Belo, M.A.A., Moraes, J.R.E., Soares, V.E., Martins, M.L., Brum, C.D., Moraes, F.R., 2012.
223 Vitamin C and endogenous cortisol in foreign-body inflammatory response in pacus. Pesq.
224 Agropec. Bras. 47, 1015-1021. http://dx.doi.org/10.1590/S0100-204X2012000700019

225 Belo, M.A.A., Schalch, S.H.C., Moraes, F.R., Soares, V.E., Otoboni, A.M., Moraes, J.E.,
226 2005. Effect of dietary supplementation with vitamin E and stocking density on
227 macrophage recruitment and giant cell formation in the teleost fish, *Piaractus*
228 *mesopotamicus.* J. Comp. Pathol. 133, 146-154.
229 https://dx.doi.org/10.1016/j.jcpa.2005.04.004

230 Bjork, S.J., Bartholomew, J.L., 2010. Invasion of *Ceratomyxa shasta* (Myxozoa) and
231 comparison of migration to the intestine between susceptible and resistant fish hosts. Int. J.
232 Parasitol. 40, 1087–1095. https://dx.doi.org/10.1016/j.ijpara.2010.03.005

233 Burger, M.A.A., Adlard, R.D., 2010. Four new species of *Kudoa* Meglitsch, 1947
234 (Myxosporea: Multivalvulida) from Australia with recommendations for species
235 descriptions in the Kudoidae. Parasitology. 137, 793–814.
236 https://dx.doi.org/10.1017/S0031182009991557

237 Capodifoglio, K.R.H., Adriano, E.A., Milanin. T., Silva, M.R.M., Maia, A.A.M., 2016.
238 Morphological, ultrastructural and phylogenetic analyses of *Myxobolus hilarii* n. sp.
239 (Myxozoa, Myxosporea), a renal parasite of farmed *Brycon hilarii* in Brazil. Parasitol. Int.
240 65, 184–190. https://dx.doi.org/10.1016/j.parint.2015.12.006

241 Carriero, M.M., Adriano, E.A., Silva, M.R.M., Ceccarelli, P.S., Maia, A.A., 2013. Molecular
242 phylogeny of the *Myxobolus* and *Henneguya* genera with several new South American
243 species. PLoS ONE 8(9), e73713. http://dx.doi.org/10.1371/journal.pone.0073713

244 Castro, M.P., Moraes, F.R., Fujimoto, R.Y., da Cruz C., Belo, M.A., de Moraes, J.R., 2014.
245 Acute toxicity by water containing hexavalent or trivalente chromium in native Brazilian
246 fish, *Piaractus mesopotamicus*: Anatomopathological alterations and mortality. Bull.
247 Envir. Cont. Toxicol. 92, 213-219. https://dx.doi.org/10.1007/s00128-013-1174-5

248 Csaba, G., Kovács-Gayer, E., Békési, L., Bucsek, M., Szakolczai, J., Molnár, K., 1984.
249 Studies into the possible protozoan aetiology of swimbladder inflammation in carp fry. J.
250 Fish Dis. 7, 39–56. http://dx.doi.org/10.1111/j.1365-2761.1984.tb00905.x
251 Eiras, J.C., 2005. An overview of myxosporean parasites in amphibians and reptiles. Acta
252 Parasitol. 50, 267–275.
253 Eiras, J.C., Júnior, J.P., Sampaio, L.A., Robaldo, R. Abreu, P.C., 2007. *Myxobolus* sp. can
254 cause *in vivo* myoliquefaction in the host *Paralichthys orbignyanus* (Osteichthyes,
255 Paralichthydae). Dis. Aquat. Organ. 77, 255–258. https://dx.doi.org/10.3354/dao01852
256 Farias, T.H.V., Levy-Pereira, N., Alves, L.O., Dias, D.C., Tachibana, L., Pilarski, F., Belo,
257 M.A.A., Ranzani-Paiva, M.J.T., 2016. Probiotic feeding improves the immunity of pacus,
258 *Piaractus mesopotamicus*, during *Aeromonas hydrophila* infection. Anim. Feed. Sci.
259 Technol. 211, 137-144. http://dx.doi.org/10.1016/j.anifeedsci.2015.11.004
260 Friedrich, C., Ingolic, E., Freitag, B., Kastberger, G., Hohmann, V., Skofitsch, G.,
261 Neumeister, U., Kepka, O., 2000. A myxozoan-like parasite causing xenomas in the brain
262 of the mole, *Talpa europaea* L., 1758 (Vertebrata, Mammalia). Parasitology. 121, 483–
263 492. http://dx.doi.org/10.1017/S0031182099006769
264 Harder, W., 1975. Anatomy of fishes. Parts I, II. E. Schweizerbart'sche Verlagsbuchhandlung
265 (Nägele u. Obermiller), Stuttgart.
266 Holzer, A., Schachner, O., 2001. Myxosporea and macrophage centers in chub (*Leuciscus*
267 *cephalus*) quantitative interaction seems to be focused on *Myxobolus cyprini*.
268 Parasitology. 122, 53–62. https://doi.org/10.1017/S003118200000706X
269 Lin, Y., Gao, Z., Zhan, A., 2015. Introduction and use of non native species for aquaculture in
270 China: status, risks and management solutions. Rev. Aquac. 7, 28-58.
271 http://dx.doi.org/10.1111/raq.12052

Lom, J., Dyková, I., 2006. Myxozoan genera: definition and notes on taxonomy, life cycle terminology and pathogenic species. Folia Parasitol. 53, 1–36. http://dx.doi.org/10.14411/fp.2006.001

Manrique, W.G., Claudiano, G.S., Castro, M.P., Petrillo, T.R., Figueiredo, M.A.P., Belo, M.A.A., Berdeal, M.I.Q., Moraes, J.R.E., Moraes, F.R., 2015a. Expression of cellular components in granulomatous inflammatory response in *Piaractus mesopotamicus* model. PLoS ONE 10(3), e0121625. http://dx.doi.org/10.1371/journal.pone.0121625

Manrique, W.G., Claudiano, G.S., Figueiredo, M.A.P., Petrillo, T.R., Moraes, J.R.E., Moraes, F.R., 2012. Myxosporidiosis in intensively-reared *Piaractus mesopotamicus*: Histopathological diagnosis by means of Ziehl-Neelsen staining. Pesq. Vet. Bras. 32, 1133-1137. http://dx.doi.org/10.1590/S0100-736X2012001100010

Manrique, W.G., Claudiano, G.S., Petrillo, T.R., Castro, M.P., Figueiredo, M.P., Belo, M.A.A., Moraes J.R.E., Moraes, F.R., 2014. Response of splenic melanomacrophage centers of *Oreochromis niloticus* (Linnaeus, 1758) to inflammatory stimuli by BCG and foreign bodies. J. Appl. Ichthyol. 30, 1001–1006. http://dx.doi.org/10.1111/jai.12445

Manrique, W.G., Figueiredo, M.A.P., Belo, M.A.A., Martins, M.L., Moraes, F.R., 2015b. First report of *Myxobolus* sp. infection in the skeletal muscle of Neotropical freshwater fish *Piaractus mesopotamicus*. Parasitol. Res. 114, 2041–2044. https://dx.doi.org/10.1007/s00436-015-4454-7

Manrique, W.G., Figueiredo, M.A.P., Belo, M.A.A., Martins, M.L., Azevedo, C., 2016. Ultrastructural description of *Myxobolus cuneus* (Myxosporea) in the skeletal muscle and kidney of tropical farmed fish *Piaractus mesopotamicus* (Characiformes: Characidae). Parasitol. Res. 115, 2505–2510. https://dx.doi.org/10.1007/s00436-016-5026-1

Manrique, W.G., Figueiredo, M.A.P., Claudiano, G.S., Martins, M.L., Mores, F.R., 2013. Extraction technique and recovery of myxozoan parasites from the paraffin-embedded

297 kidney of *Piaractus mesopotamicus*. Biotemas. 26, 265-268.
298 http://dx.doi.org/10.5007/2175-7925.2013v26n4p263
299 Mansour, L., Thabet, A., Chourabi, K., Harrath, A.H., Gtari, M., Al Omar, S.Y., Ben,
300 Hassine, O.K., 2013. *Kudoa azevedoi* n. sp. (Myxozoa, Multivalvulida) from the oocytes
301 of the Atlantic horse mackerel *Trachurus trachurus* (Perciformes, Carangidae) in Tunisian
302 coasts. Parasitol. Res. 112, 1737–1747. https://dx.doi.org/10.1007/s00436-013-3332-4
303 McGeorge, J., Sommerville, C., Wootten, R., 1996. Epizootiology of *Sphaerospora truttae*
304 (Myxozoa: Myxosporea) infections of Atlantic salmon *Salmo salar* at freshwater smolt
305 producing hatcheries in Scotland. Dis. Aquat. Org. 26, 33–41.
306 http://dx.doi.org/10.3354/dao026033
307 Milanin, T., Maia, A.A.M., Silva, M.R.M., Carriero, M.M., Adriano, E.A., 2015. Molecular
308 phylogeny and ultrastructure of *Myxobolus* cf. *cuneus*, a parasite of patinga hybrid and
309 *Henneguya pseudoplatystoma*, a parasite of pintado hybrid. Acta Parasitol. 60, 442–450.
310 https://dx.doi.org/10.1515/ap-2015-0061
311 Molnár, K., 2007. Site preference of myxozoans in the kidneys of Hungarian fishes. Dis.
312 Aquat. Org. 78, 45–53. https://dx.doi.org/10.3354/dao01827
313 Molnár, K., Eszterbauer, E., 2015. Specificity of infection sites in vertebrate hosts. In: B.
314 Okamura B, Gruhl A, Bartholomew JL. (eds). Myxozoan evolution, ecology and
315 Development. Springer, Switzerland, 441 p. https://dx.doi.org/10.1007/978-3-319-14753-
316 6
317 Molnár, K., Békési, L., 1993. Description of a new *Myxobolus* species, *M. colossomatis* n. sp.
318 from the teleost *Colossoma macropomum* of the Amazon River basin. J. Appl. Ichthyol. 9,
319 57-63. http://dx.doi.org/10.1111/j.1439-0426.1993.tb00388.x
320 Molnár, K., Eszterbauer, E., Marton, S., Cech, G., Székely, C., 2009. *Myxobolus*
321 *erythrophthalmi* sp. n. and *Myxobolus shaharomae* sp. n. (Myxozoa: Myxobolidae) from

the internal organs of rudd, *Scardinius erythrophthalmus* (L.), and bleak, *Alburnus alburnus* (L.). J. Fish Dis. 32, 219-231. https://dx.doi.org/10.1111/j.1365-2761.2008.00976.x

Molnár, K., Kovács-Gayer, E., 1985. The pathogenicity and development within the host fish of *Myxobolus cyprini* Doflein, 1898. Parasitology 90, 549–555. http://dx.doi.org/10.1017/S0031182000055530

Molnár, K., Székely, C., 1999. *Myxobolus* infection of the gills of common bream (*Abramis brama* L.) in Lake Balaton and in the Kis-Balaton reservoir. Hungary. Acta Vet. Hung. 47, 419-432. https://dx.doi.org/10.1556/AVet.47.1999.4.3

Molnár, K., Székely, C., 2014. Tissue preference of some myxobolids (Myxozoa: Myxosporea) from the musculature of European freshwater fishes. Dis. Aquat. Org. 107, 191-198. https://dx.doi.org/10.3354/dao02688

Müller, M.I., Adriano, E.A., Ceccarelli, P.S., Silva, M.M.R., Maia, A.A.M., Ueta, M.T., 2013. Prevalence, intensity, and phylogenetic analysis of *Henneguya piaractus* and *Myxobolus* cf. *colossomatis* from farmed *Piaractus mesopotamicus* in Brazil. Dis. Aquat. Org. 107, 129-139. http://dx.doi.org/10.3354/dao02668

Sitja-Bobadilla, A., Schmidt-Posthaus, H., Wahli, T., Holland, J. W., Secombes, C. J., 2015. Fish immune responses to Myxozoa. in: B. Okamura et al. (eds.), Myxozoan Evolution, Ecology and Development, Springer International Publishing Switzerland.

Valladão, G.M.R., Gallani, S.U., Pilarski, F., 2016. South American fish for continental aquaculture. Rev. Aquac. http://dx.doi.org/10.1111/raq.12164.

Walliker, D., 1969. Myxosporidea of some Brazilian freshwater fishes. J. Parasitol. 55, 942-948. http://dx.doi.org/10.2307/3277155

Witmer, G., Fuller, P.L., 2011. Vertebrate species introductions in the United States and its territories. Curr. Zool. 5, 559-567. http://dx.doi.org/10.1093/czoolo/57.5.559

Figure legends

Fig. 1. Photomicrography of the isolated fresh myxospores of the myxosporean *Myxobolus* sp. infecting the kidney of *Piaractus mesopotamicus*. Scale bar = 5 µm. (B).

Fig. 2. Photomicrography of the isolated fresh myxospores of the myxosporean *Henneguya* sp. infecting the kidney of *P. mesopotamicus*. Scale bar = 5 µm.

Fig. 3. Photomicrography of the posterior kidney of *Piaractus mesopotamicus*. In one of the renal tubules (star) relatively intact myxospores of *Myxobolus* sp. (arrowhead) and *Henneguya* sp. (arrow) are seen. Some free myxospores in the renal parenchyma around tubules are also seen. ZN staining. Scale bar = 20 µm.

Fig. 4. Enlarged picture of the posterior kidney of *Piaractus mesopotamicus*. Note the mature myxospores of *Myxobolus* sp. (arrowhead), mature spore of *Henneguya* sp. (arrow) free, melanomacrophages (MM) and macrophages (MØ). ZN staining. Scale bar = 10 µm.

Fig. 5. Inflammatory infiltrate (I), predominantly with mononuclear cells, in the renal parenchyma around a damaged tubule and glomerulus. In the lumen and the damaged

epithelium of the tubule melanomacrophage (arrowheads) cells are seen. Glomeruli (G) and the Bowman capsule are also damaged (dashed line, arrow). Inside the blood vessel (star) red blood cells and a mononucleate cell is seen. H & E staining. Scale bar = 20 μm.

Fig. 6. A part of the kidney with renal tubules (star) and glomerulus. Renal interstitium surrounding an intact glomerulus is infiltrated by inflammatory, predominantly mononuclear (I) cells. An infected, damaged glomerulus (G) is filled by melanomacrophage centers (MMC). The wall of the Bowman capsule (dashed line, arrow) is also damaged. Some free melanomacrophages (arrowhead) are located. H & E staining. Scale bar = 20 μm.