

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

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## 26 **Abstract**

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

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41 **Keywords:** Fresh water fish; histopathology; inflammation; Myxosporidia; pacu

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## 43 **1. Introduction**

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

51 High stocking density and inadequate handling are responsible for increased stress that  
52 affect negatively the pacu health causing increased disease susceptibility (Belo et al., 2005;  
53 2012; Manrique et al., 2015a). On the other hand, members of the class Myxosporea use not  
54 only wild and cultured fish (Capodifoglio et al., 2016) but also amphibians, reptiles (Eiras,  
55 2005), aquatic birds (Bartholomew et al., 2008) and terrestrial mammals (Friedrich et al.,  
56 2000) as hosts. These parasites have been recognized as a key limiting factor in the  
57 development of aquaculture because they infect a large variety of commercially important  
58 fishes, and these parasites may develop intra- and intercellularly (histozoic) or may be located  
59 in the organs and body cavity (celozoic) (Lom and Dyková, 2006).

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

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

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

## 73 **2. Materials and methods**

### 74 *2.1. Fish samples*

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

## 78 2.2. *Experimental procedures*

79 The living fish were euthanized by fish immersion in an alcoholic solution of benzocaine  
80 1:500 v/v anesthesia/water (0.1 g benzocaine per mL of ethanol) according to the ethical  
81 procedures approved by Ethics Committee (CEUA-UNESP protocol n° 020092/09) for  
82 posterior blood collection from the caudal vein using syringes containing 10% EDTA to make  
83 the blood smears, that were stained with Giemsa to evaluation of structures examined in  
84 optical microscope. Then necropsy was performed for collection of the posterior kidney for  
85 histopathology and a small fragment for analysis in fresh mounts.

## 86 2.3. *Morphological analyses of myxospores*

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

## 94 2.3. *Histopathology analyses*

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

## 98 3. **Results**

### 99 3.1. *Myxobolus* sp. and *Henneguya* sp. myxospores

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

### 105 3.2. Histopathology

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

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

## 121 4. Discussion

122 The kidney of freshwater fishes is a complex organ with two different functions. The trunk  
123 kidney and the hind kidney have excretory function, while the head kidney has a  
124 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

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

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

### 183 **Conflicts of interest**

184 The authors have no conflicts of interest to declare.

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### 190 **References**

191

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

196 Adriano, E.A., Arana, S., Cordeiro, N.S., 2005a. An ultrastructural and histopathological  
197 study of *Henneguya pellucida* n. sp. (Myxosporea: Myxobolidae) infecting *Piaractus*  
198 *mesopotamicus* (Characidae) cultivated in Brazil. Parasite 12, 221–227.  
199 <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 trivalent 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 Paralichthyidae). 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>

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

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

279 Manrique, W.G., Claudiano, G.S., Figueiredo, M.A.P., Petrillo, T.R., Moraes, J.R.E., Moraes,  
280 F.R., 2012. Myxosporidiosis in intensively-reared *Piaractus mesopotamicus*:  
281 Histopathological diagnosis by means of Ziehl-Neelsen staining. *Pesq. Vet. Bras.* 32,  
282 1133-1137. <http://dx.doi.org/10.1590/S0100-736X2012001100010>

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

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

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

295 Manrique, W.G., Figueiredo, M.A.P., Claudiano, G.S., Martins, M.L., Mores, F.R., 2013.  
296 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: Myxosporidia) 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-](https://dx.doi.org/10.1007/978-3-319-14753-6)  
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

322 the internal organs of rudd, *Scardinius erythrophthalmus* (L.), and bleak, *Alburnus*  
323 *alburnus* (L.). J. Fish Dis. 32, 219-231. [https://dx.doi.org/10.1111/j.1365-](https://dx.doi.org/10.1111/j.1365-2761.2008.00976.x)  
324 [2761.2008.00976.x](https://dx.doi.org/10.1111/j.1365-2761.2008.00976.x)

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

328 Molnár, K., Székely, C., 1999. *Myxobolus* infection of the gills of common bream (*Abramis*  
329 *brama* L.) in Lake Balaton and in the Kis-Balaton reservoir. Hungary. Acta Vet. Hung.  
330 47, 419-432. <https://dx.doi.org/10.1556/AVet.47.1999.4.3>

331 Molnár, K., Székely, C., 2014. Tissue preference of some myxobolids (Myxozoa:  
332 Myxosporidia) from the musculature of European freshwater fishes. Dis. Aquat. Org. 107,  
333 191-198. <https://dx.doi.org/10.3354/dao02688>

334 Müller, M.I., Adriano, E.A., Ceccarelli, P.S., Silva, M.M.R., Maia, A.A.M., Ueta, M.T., 2013.  
335 Prevalence, intensity, and phylogenetic analysis of *Henneguya piaractus* and *Myxobolus*  
336 *cf. colossomatis* from farmed *Piaractus mesopotamicus* in Brazil. Dis. Aquat. Org. 107,  
337 129-139. <http://dx.doi.org/10.3354/dao02668>

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

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

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

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

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### Figure legends

354

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

357

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

360

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

365

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

369

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

372 epithelium of the tubule melanomacrophage (arrowheads) cells are seen. Glomeruli (G) and  
373 the Bowman capsule are also damaged (dashed line, arrow). Inside the blood vessel (star) red  
374 blood cells and a mononucleate cell is seen. H & E staining. Scale bar = 20  $\mu\text{m}$ .

375

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