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Observations on the Infection by *Kudoa* sp. (Myxozoa, Multivalvulida) in fishes caught off Rio Grande, Rio Grande do Sul State, Brazil

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ABSTRACT. It is reported the parasitization of *Kudoa* sp. (Myxozoa, Multivalvulida) within the somatic muscles of the fish *Odontesthes bonariensis* (Valenciennes, 1835), *Micropogonias furnieri* (Desmarest, 1823) and *Mugil liza* Valenciennes, 1836, captured off Rio Grande, Rio Grande do Sul State, Brazil. Other species of fish caught at the same place were not infected. The parasites formed elongated plasmodia inside the muscle fibres. Myoliquefaction or any host tissue reaction were not observed. The morphological and morphometric features of the spores are described, the parasites are compared with other *Kudoa* species, and the importance of the infection is discussed.

Keywords: cnidaria, myxosporea, fish, muscles, myoliquefaction.

Observações sobre a infecção por *Kudoa* sp. (Myxozoa, Multivalvulida) em peixes capturados em Rio Grande, Estado do Rio Grande do Sul, Brasil

RESUMO. Descreve-se a infecção de *Kudoa* sp. (Myxozoa, Multivalvulida) nos músculos somáticos dos peixes *O. bonariensis* (Valenciennes, 1835), *M. furnieri* (Desmarest, 1823) e *M. liza* (Valenciennes, 1836), capturados em Rio Grande, Estado do Rio Grande do Sul, Brasil. Outras espécies de peixes capturados no mesmo local não estavam parasitadas. Os parasitas formavam plasmódios alongados no interior das fibras musculares. Não se observou liquefação do músculo do hospedeiro ou qualquer outra reação dos tecidos. Descrevem-se as características morfológicas e morfométricas dos esporos, os parasitas são comparados com outras espécies de *Kudoa*, e a importância da parasitose é discutida.

Palavras-chave: cnidaria, myxosporea, peixes, músculo, mioliquefacção.

Introduction

Kudoa spp. (Myxozoa, Multivalvulida) are frequent parasites of marine and estuarine fish. A recent synopsis of the species reported the existence of 95 nominal species (Eiras, Saraiva, & Cruz, 2014b) infecting a wide range of hosts all over the world. Since then, at least 6 new species were described, one of them infecting a freshwater host (Azevedo et al., 2015).

A little more than half of the parasites locate within the skeletal muscle fibers, and a number of species are especially important once they cause liquefaction of the host muscle within a short period after the death of the host which becomes useless for consumption (Cruz, Silva, & Saraiva, 2011; Gilman & Eiras 1998; Henning, Hoffman, & Manley, 2013; Moran, Whitaker, & Kent, 1999; Romero & Burgos 1996; Yokoyama & Itoh 2005; Yokoyama, Whipps, Kent, Mizuno, & Kawakami, 2004). This can occur in both wild and cultured fish, and the infection may be decisive for the economic success of fisheries and farming.

In Brazil there are only a few records of these parasites: *K. aequidens* infecting *Aequidens plagiozonatus* (Casal, Matos, Matos, & Azevedo, 2008), *K. sciaenae* from *Stellifer minor* (Oliva, Luque, Terán, & Llicán, 1992), *K. crumena* from *Thunnus albacares*, *K. orbicularis* described from the freshwater host *Chaetobranchopsis orbicularis* (Azevedo et al. 2015), and several not identified species parasitizing *M. liza*, *Trichiurus lepturus*, *Plagioscion squamosissimus*, *M. platanus*, and *Scomberomorus brasiliensis* (Eiras, Lima, Cruz, & Saraiva, 2014a). From the above mentioned species, only *K. crumena* and *K. orbibularis* were subjected to molecular studies. *K. aequidens* was described by light and transmission electron microscopic observations, and the remaining forms were characterized by light microscopy.

In this paper we report *Kudoa* sp. infecting *O. bonariensis* (Valenciennes, 1835), *M. furnieri* (Desmarest, 1823) and *M. liza* Valenciennes, 1836, captured off Rio Grande, Rio Grande do Sul State, Brazil.

Material and methods

Fresh fish specimens were acquired from fishermen at Rio Grande fish market. In the laboratory they were identified, measured (total length) and inspected for *Kudoa* infection. Small samples of muscle, collected from both sides of the specimens, were crashed between two glass slides and inspected at the stereomicroscope. When detected, the plasmodia were preserved in formalin at 4%, and latter 15 to 25 spores were examined at the microscope and measured according to Burger and Adlard (2010). The spores dimensions were compared using Kruskall-Wallis test, followed by multiple comparisons when significant differences ($p < 0.05$) were detected. The eventual myoliquefactive action of the parasites was assessed by visual inspection of the muscle to evaluate the integrity of the muscle fibers, and by palpation of the fish to detect softening of the muscle.

Results

The fish species examined, number and total length of the specimens are indicated in Table 1.

Three fish species were infected by *Kudoa* sp.: *M. liza* (2 specimens), *M. furnieri* (2 specimens), and *O. bonariensis* (4 specimens, 23.7 - 29.0 cm in total length). The intensity of the infection was low – several muscle samples were required to detect a plasmodium.

The parasites were located inside the skeletal muscles fibers in the anterior part of both sides of the body forming small intracellular elongate plasmodia, with both extremities round or slightly tapering. The dimensions of the plasmodia were similar in all the hosts, measuring 0.72 - 1.0 mm in length by about 0.07 mm wide. All the plasmodia were in the same developmental stage containing only mature spores.

Table 1. Fish species examined for *Kudoa* infection, number and total length (cm) of the specimens.

Fish species and Brazilian common name	Number of specimens	Total length (cm)
Genidens barbus (Lacépède, 1803) - Bagre Marinho	1	45.3
Genidens planifrons (Higuchi, Reis & Araújo, 1982) - Bagre marinho	1	53.5
Macrodon atricauda (Günther, 1880) - Pescada	2	28.2, 35.6
Menticirrhus americanus (Linnaeus, 1758) - Papa-Terra	2	29.5, 30.5
Menticirrhus litorallis (Holbrook, 1847) - Papa-Terra	3	30.3 - 31.6
Micropogonias furnieri (Desmarest, 1823) - Corvina	2	42.8, 48.9
Mugil liza Valenciennes, 1836 - Taínha	2	42.4, 43.2
Odontesthes bonariensis (Valenciennes, 1835) - Peixe-Rei	7	21.2 - 29.0
Parona signata (Jenyns, 1841) - Viúva	2	30.1, 33.9
Peprilus paru (Linnaeus, 1758) - Gordinho	1	33.1
Pomatomus saltatrix (Linnaeus, 1766) - Anchova	2	33.6, 40.4
Seriola lalandi Valenciennes, 1833 - Olhete	1	45.4

The spores were also morphologically similar in all the hosts. They were bell-like shaped in lateral view. In apical view they were stellate, quadrate, having four equal radiating shell valves with margins smoothly curved, without projections or extensions. The four polar capsules were slightly pyriform, elongated, equally sized with pointed anterior extremities. The number of coils of the polar filament was not visible in optic microscopy.

Despite the similar morphology of the spores of the three different hosts there were consistent differences concerning the morphometry of the spores. Considering the three forms observed the specimens from *O. bonariensis* presented the highest dimensions and those of *M. furnieri* were smaller than *M. liza* for spore width and spore thickness. *M. furnieri* spores were clearly smaller than those from *O. bonariensis* and *M. liza*, and those from *M. liza* presented intermediate values – (Table 2).

Table 2. *Kudoa* sp. spores measures in μm, from different hosts (mean ± s.d; (range) and N). Significant differences detected among spores measures by Kruskal-Wallis test are indicated (similar letters mean no significant differences). a.v. - apical view; l.v.- lateral view.

Species	Spore Width (a.v.)	Spore Thickness (a.v.)	Spore Length (l.v.)	Capsule Length (a.v.)	Capsule Width (a.v.)	Capsule Length (l.v.)	Capsule Width (l.v.)
Odontesthes bonariensis	9.2 ± 0.7 (6.7 - 10.1) 25 c	7.7 ± 0.6 (6.0 - 9.4) 25 c	6.1 ± 0.4 (5.4 - 7.6) 25 b	1.9 ± 0.2 (1.3 - 2.3) 25 b	1.3 ± 0.2 (1.0 - 1.7) 25 b	1.8 ± 0.3 (1.3 - 2.7) 25 b	1.4 ± 0.2 (1.0 - 2.0) 25 b
Mugil liza	8.1 ± 0.5 (7.4 - 8.7) 25 b	6.6 ± 0.8 (6.0 - 9.4) 25 b	5.2 ± 0.3 (4.7 - 5.7) 15 a	1.5 ± 0.2 (1.3 - 1.7) 25 a	1.2 ± 0.2 (1.0 - 1.7) 15 a	1.4 ± 0.2 (1.3 - 1.7) 25 a	1.2 ± 0,2 (1.0 - 1.3) 15 a
Micropogonias furnieri	6.9 ± 0.7 (5.7 - 8.7) 20 a	5.3 ± 0.6 (4.7 - 6.7) 20 a	5.1 ± 0.4 (4.4 - 6.0) 15 a	1.5 ± 0.2 (1.3 - 2.0) 20 a	1.1 ± 0.2 (1.0 - 1.7) 20 a	1.8 ± 0.3 (1.3 - 2.3) 15 b	1.2 ± 0.2 (0.7 - 1.3) 15 ab
Significant differences	p = 0.000	p = 0.000	p = 0.000	p = 0.000	p = 0.002	p = 0.000	p = 0.017

Discussion

The morphometry of the spores and statistical data lead us to the conclusion that the parasites from the three hosts correspond at least to two different species, one infecting *M. furnieri,* and another infecting *O. bonariensis* and *M. liza*. However, the existence of three different species can not be ruled out, and a molecular study of the specimens would be necessary to clarify this question.

Our specimens were compared with all the *Kudoa* spp. described for Brazilian hosts, muscle parasites from hosts from other sampling places, and parasites with other location than the fish muscles.

From the comparison with species from Brazilian hosts it is obvious that our specimens belong to different species due to differences in the size of the spores and polar capsules.

From the about 100 nominal species described so far a little more than half of the species parasitize the muscles of the hosts. The comparison of the present material with all the species strongly suggests they correspond also to different species.

The specimens from *O. bonariensis* are similar to *K. petala* from *Sillago sihama* in China, though a little smaller in length and a little bigger in width. The polar capsules of *K. petala* are drop-like, while in our material they are elongated, and *K. petala* is coelozoic in the gall bladder (Zhou & Zhao, 2008). Therefore the identity of both species is unlikely. Our specimens are also similar to *K. scomberi* in the somatic muscles of *Scomber japonicus* in Japan, forming plasmodia seen as minute white cysts, having spores longer (6.4) and thicker (8.1) than our specimens and larger drop-like polar capsules (Li et al. 2013). For these reasons the two species are considered different. Some similarities exist with *K. shiomitsui* detected in the pericardial cavity and heart of *Takifugu rubripes* (Egusa & Shiomitsu 1983). However, the spores from Brazilian hosts are not so wide, and the different location within the hosts separates both forms. Interestingly, they are very similar in dimensions to *K. permulticapsula* (6.0 x 9.1 x 7.7) infecting the somatic muscles of *S. commerson* in Australia (Whipps, Adlard, Bryant, & Kent, 2003). However, *K. permulticapsula* has 13 polar capsules (occasionally 14, rarely 15), which distinguishes immediately the two species without further considerations.

Concerning the specimens infecting *M. liza*, they do not match any species, namely those infecting *Mugil* spp.: the dimensions of the spores and polar capsules are higher than those of *K. bora* infecting the muscles of *M. japonicus* (Nigrelli, 1946). They are smaller than *K. haridase* in the gall bladder of *M. persina* (Sarkar & Gosh 1991), and larger than *K. intestinalis* from the muscular layer of intestine of *M. cephalus* (Maeno, Nagasawa, & Sorimachi, 1993), and smaller than *K. tetraspora* located around the optic lobes of *M. cephalus* (Narasimhamurti & Kalavati 1979). Other differences relate to the size and shape of the polar capsules and the shape and dimensions of the plasmodia. As a consequence our forms can not be identified with any of the species infecting *Mugil* spp., conclusion which is re-inforced by the different location of the parasites within the hosts, excepting *K. bora* which infects also the muscles. Comparison with other species failed to show forms identical to the present material.

The parasites of *M. furnieri* are distinct from all the other species. The most identical one is *K. azoni* infecting *Pleurogrammus azonus* in the Sea of Japan (Amur Bay and Ussuri Bay). However it locates between, and not inside the muscle fibres, and causes post-mortem myoliquefaction (Aseeva, 2004), and can not be identified to the parasites from *M. furnieri*.

It is concluded that the *Kudoa* spp. observed infecting *O. bonariensis*, *M. liza* and *M. furnieri* are most probably species not described hitherto, and correspond to two or three different species. However, and due to lack of molecular studies, we refrain for the moment further considerations about their identity.

Considering the high diversity of marine fish from Brazil, estimated at around 1,300 species, it is surprising that so few species of *Kudoa* were reported so far. The same situation occurs generally in South America. As far as we are aware only 6 species of *Kudoa* were reported for South American countries besides Brazil (Argentina, Chile, Peru and Uruguai), and *Kudoa* sp. were found in Brazil, Chile and Peru, infecting in all only a total of 14 different host species. Clearly more research is needed on these parasites. It is important to emphasize that, besides the scientific interest of the parasites, the study of its biology is important due to the host myoliquefaction caused by a number of species which may impair the commercialization and the farming of some host species.

Conclusion

The *Kudoa* spp. observed in fish caught off Rio Grande increase the knowledge about these parasites infecting marine fish in Brazil. Taking into account the low number of species described for the country it is emphasized the importance of the study of these parasites. Furthermore, the fish infection by *Kudoa* may have important economic consequences

concerning the exploitation of wild and farmed fish due to the dramatic changes which some species induce in the host muscle. This is one more and important reason for the study of these parasites in Brazil.

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