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Original Article

New occurrence of *Kudoa orbicularis* parasitizing the freshwater catfish *Trachelyopterus galeatus* (Siluriformes: Auchenipteridae) in the Brazlian Amazon region

Nova ocorrência de *Kudoa orbicularis* parasitando peixe de água doce *Trachelyopterus galeatus* (Siluriformes: Auchenipteridae) na região Amazônica brasileira

Weverton John Pinheiro dos Santos[1,2]; Diehgo Tuloza da Silva[2,3]; Patrícia de Fátima Saco dos Santos[2]; Edilson Rodrigues Matos[1,2,3]*; Igor Guerreiro Hamoy[4]

[1] Programa de Pós-graduação em Aquicultura e Recursos Aquáticos Tropicais - AqRAT, Universidade Federal Rural da Amazônia – UFRA, Belém, PA, Brasil

[2] Laboratório de Pesquisa Carlos Azevedo, Universidade Federal Rural da Amazônia – UFRA, Belém, PA, Brasil

[3] Programa de Pós-graduação em Biologia de Agentes Infecciosos e Parasitários – BAIP, Universidade Federal do Pará – UFPA, Belém, PA, Brasil

[4] Laboratório de Genética Aplicada, Universidade Federal Rural da Amazônia – UFRA, Belém, PA, Brasil

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Abstract

The aim of this was describe an infection by *Kudoa orbicularis* in freshwater catfish *Trachelyopterus galeatus*. A sample of 80 specimens of *T. galeatus* was collected in the municipality of Cachoeira do Arari, Marajó Island, in the state of Pará, Brazil. Pseudocysts were found in the muscle fibers of the epaxial and hypaxial regions of 85.0% of the specimens analyzed, reflecting a high infection rate. The pseudocysts contained spores that were pseudo-square in shape, with a mean length of 4.65 µm (range: 4.04–5.54) and mean width of 1.53 µm (1.56–1.74). Analyses on the morphology of the spores and a partial 934-bp sequence of the SSU rDNA gene confirmed that the microparasite was *Kudoa orbicularis*. This is the second record of this microparasite in a siluriform host in the Brazilian Amazon region.

Keywords: Freshwater fish, infection, molecular biology, Myxozoa, microparasite.

Resumo

O objetivo deste estudo foi descrever a infecção por *Kudoa orbicularis* em *Trachelyopterus galeatus.* Foram analisados 80 espécimes de *T. galeatus* capturados no município de Cachoeira do Arari, ilha de Marajó, estado do Pará, Brasil. A presença de pseudocistos nas fibras musculares das regiões epiaxial e hipoaxial em 85,0% dos exemplares analisados, mostra alto grau de infecção. Os pseudocistos continham esporos de formato pseudoquadrado, medindo 4,65 (4,04-5,54) µm de comprimento e 5,25 (4,78-5,98) µm de largura, com quatro cápsulas polares de tamanho iguais medindo 2,22 (2,05-2,32) µm de comprimento e 1,53 (1,56-1,74) µm de largura. Através das análises morfológicas dos esporos e molecular de uma sequência parcial de 934bps do gene SSU rDNA, confirma que o microparasito é *Kudoa orbicularis*, sendo este o segundo registro desse microparasito em hospedeiro da ordem Siluriformes da Amazônia brasileira.

Palavras-chave: Peixe de água doce, biologia molecular, infecção, Myxozoa, microparasito.

Introduction

The microscopic cnidarians of the subphylum Myxozoa are an important group of parasites of aquatic organisms that infect marine and freshwater vertebrates and invertebrates. They include the majority of the microorganisms that cause diseases in fish (KENT et al., 2001; LOM & DYKOVÁ, 2006).

The myxozoans of the genus *Kudoa* are typically star-shaped, square, pseudo-square or rounded in apical view, with four or more valves and polar capsules. They are found primarily in the muscle tissue of the host, and can cause postmortem myoliquefaction. These parasites may also infect other types of tissue, such as the brain, integument, kidney, fins, peritoneum, and mesentery (MORAN et al., 1999; SWEARER & ROBERTSON, 1999; WHIPPS et al., 2004; LOM & DYKOVÁ, 2006; CASAL, 2009; KRISTMUNDSSON & FREEMAN, 2014).

***Corresponding author:** Edilson Rodrigues Matos. Universidade Federal Rural da Amazônia – UFRA, Av. Tancredo Neves, 2501, Terra Firme, CEP 66077-830, Belém, PA, Brasil. e-mail: edilson.matos9@gmail.com

The morphological and morphometric analyses that are traditionally used to identify most myxozoan species may not be sufficient to confirm all taxa reliably. This highlights the need for complementary techniques: in particular, molecular biology, which has been widely used to identify fish parasites at species level (CLARK, 2006; MENEZES et al., 2010).

The fisheries of the Amazon region of Brazil are distinct from those of other regions of the country, in terms of the diversity of species harvested, the volume of the catches and the dependence of the local populations on this economic activity (BARTHEM & FABRÉ, 2004). One of the target species in the Amazon basin is the anujá catfish, *Trachelyopterus galeatus* Linnaeus, 1766, which is found in swamps and under rafts of floating vegetation. This economically valuable siluriform species, also known as the cangati or "cachorrinho de padre", is an omnivorous fish found throughout South America, where it is an important source of food and income for many riverine communities (BORGES et al., 1999; COSTA-NETO, 2000; SANTOS et al., 2004; FERRARIS JR, 2003; SANTIM et al., 2015; SOUSA et al., 2016).

Trachelyopterus galeatus may be a host for an enormous diversity of pathogens. Parasites are relatively common in freshwater fish, and many cause tissue lesions that not only may be fatal to the fish, but also may reduce the quality and value of the fishery product, as well as being a potential risk to human health (FERRE, 2001; EIRAS et al., 2004; WOO, 2006).

The present report confirms the occurrence of infection by *Kudoa orbicularis* in *T. galeatus* from the in the Marajó Island, northern Brazil,. This parasite was found infecting the musculature of the fish, and was identified through a combination of morphological and molecular analyses.

Materials and Methods

A total of 80 specimens of *Trachelyopterus galeatus* were collected in the region of Cachoeira do Arari, in the Marajó archipelago, state of Pará, northern Brazil (01°00' S, 48°57' W) between January 2016 and December 2017. These specimens had a mean total length of 13.00 ± 2.01 cm (range: 10.5–16.5 cm) and mean weight of 50.24 ± 15.64 g (27.97–80.3 g). Specimen collection had previously been authorized by the Brazilian Institute for the Environment and Renewable Natural Resources (IBAMA; through SISBIO, license number 27119-1) and by the Committee for the Ethical Use of Animals in Research of the Federal Rural University of Amazonia (under the number CEUA 013/2014).

After capture, the specimens were transported alive in aerated water from their natural habitat to the Carlos Azevedo Research Laboratory (LPCA) at UFRA, in Belém, state of Pará. In the laboratory, the specimens were maintained in aquaria, in water at a temperature of 28–30 °C. Before necropsy, they were anesthetized using tricaine methanesulfonate (MS-222) diluted in water at a concentration of 50 mg/L.

Following euthanasia, the specimens were first examined under a stereomicroscope to verify the presence of cysts, which were then observed by means of light microscopy. Once the parasitism had been confirmed, small (0.5 cm) samples of muscle tissue were obtained from infected epaxial and hypaxial tissue for common light microscopy and processing for molecular analyses.

For common light microscopy, the parasitized samples were fixed in 95% Davidson solution (ethanol, formaldehyde, acetic acid and distilled water) for 24 hours, embedded in paraffin, and stained with hematoxylin-eosin (HE) and Ziehl-Neelsen (LUNA, 1968). The stained slides and fresh spores were then photographed using a Zeiss Primo Star microscope with an attached Zeiss AxioCam ERc 5s camera, and the AxioVision 5.1 software. Some tissue samples containing cysts were analyzed by means of the differential interference contrast (DIC) technique, using a Zeiss AxioScope A1 microscope with a Zeiss AxioCam 512 color camera.

The fresh spores were measured in micrometers (μm), and the measurements were presented as means with minimum and maximum values between parentheses (LOM & DYKOVÁ, 1992). The prevalence of infection was determined as described by Bush et al. (1997).

For the molecular analysis, the myxozoan spores were collected and fixed in 80% ethanol. The DNA of the spores was extracted using the PureLink® Genomic DNA mini kit (Invitrogen, USA), following the protocol for extraction of "mammalian tissue and mouse/rat tail lysate" provided by the manufacturer. The DNA samples were quantified in a Biodrop Duo spectrophotometer (Biodrop).

Polymerase chain reactions (PCRs) were run to obtain small subunit ribosomal DNA (SSU rDNA), initially using the universal eukaryotic forward primer 18E (HILLIS & DIXON, 1991) and the reverse primer 18R (WHIPPS et al., 2003b). The PCR was run in a final volume of 25 μl, containing 1 x ReddyMix PCR master mix (Thermo Scientific, USA), 75 mM of Tris-HCl (pH 8.8), 20 mM of KCl, 0.1 (v/v) of Nonidet P40, 1.5 mM of $MgCl_2$, 0.2 mM of each nucleotide triphosphate (Thermo Scientific, USA), 10 pmol of each primer, 1.25 U of *Taq* DNA polymerase (Thermo Scientific, USA) and the DNA template (10-50 ng/μl). The reaction protocol for the primers 18E and 18R consisted of 95 °C for 5 minutes, followed by 35 cycles of 95 °C for 60 seconds, 48 °C (annealing temperature) for 60 seconds and 72 °C for 120 seconds, with a final extension of 72 °C for 10 min.

Subsequently, 3 μl of the PCR mix was subjected to electrophoresis on 1% agarose gel with 1X tris-borate-EDTA (TBE), stained with SYBR® Safe (Invitrogen, USA). The result was viewed under blue light. The PCR products were purified using GFX™ PCR DNA and a gel band purification kit (GE Healthcare, UK), in accordance with the manufacturer's instructions. The sequencing reactions were conducted using the Big Dye Terminator v3.1 cycle sequencing kit (Applied Biosystems, USA), following the manufacturer's instructions, in an ABI 3100 genetic analyzer (Applied Biosystems, USA).

The sequences obtained through this procedure were aligned in the BioEdit software (HALL, 1999) and ambiguous bases were clarified using the respective chromatograms. Sequences of the SSU rDNA gene from the myxozoan species deposited in GenBank were aligned in Clustal X 1.8 (THOMPSON et al., 1997), at the default setting, to determine their phylogenetic relationships with the new species described here. High similarity scores in the basic local alignment search tool (BLAST) were used as the criterion for selecting the GenBank sequences for inclusion in the analysis.

The jModelTest 0.1.1 software (GUINDON & GASCUEL, 2003; POSADA, 2008) was used to identify the optimal nucleotide substitution model for the dataset.

Bayesian inference was implemented through MrBayes, version 3.1.2 (RONQUIST & HUELSENBECK, 2003) using Markov chain Monte Carlo searches in two simultaneous runs of four chains of 5,000,000 generations, from which every 500th tree was sampled. The first thousand trees were discarded as burn-in, and the posterior probability of each node was calculated from the remaining trees, examined initially in TreeView X (PAGE, 1996). Genetic distances were computed in PAUP* 4.0b1 (SWOFFORD, 2003), using the default *p* parameter for the SSU rDNA gene.

Results

Morphological description

Common light microscopy revealed the presence of pseudocysts in the skeletal muscle fiber of the epaxial and hypaxial regions of the host specimens analyzed (Figure 1a). The pseudocysts, formed by agglomerations of *Kudoa* spores, were pseudo-square in shape with rounded edges in the apical view, and had four piriform polar capsules of equal size (Figures 1b-c), although it was not possible to confirm the number of coils in the polar filament. The spores were 4.65 µm in length (range: 4.04–5.54) and 5.25 µm in width (4.78–5.98), with polar capsules 2.22 µm long (2.05–2.32) and 1.53 µm wide (1.56–1.74) (Table 1). Overall, 68 (85.0%) of the 80 *T. galeatus* specimens examined were infected by *Kudoa orbicularis*.

Histology

The histological analysis indicated that the pseudocysts had developed in the intracellular region of the muscle fibers. They had replaced the sarcoplasm of the fiber segments completely with *Kudoa orbicularis* spores, thus causing deformation of these structures, as well as some adjacent muscle fibers (Figures 2-c). The spores were enveloped in a fine layer of sarcolemma (Figure 2c). The pseudocysts were located in the cytoplasm of the host fibers (Figure 2a), although no inflammatory response to the infection was observed in the host. Nor were any clinical symptoms confirmed, including myoliquefaction.

Taxonomic Summary

Phylum Cnidaria Hatschek, 1888
Class Myxosporea Bütschli, 1881
Order Multivalvulida Shulman, 1959
Family Kudoidae Meglitsch, 1960
Genus *Kudoa* Meglitsch, 1947
Species: *Kudoa orbicularis* Azevedo et al., 2016
Type host: *Trachelyopterus galeatus* Linnaeus, 1766
Infection site: Striated skeletal musculature.
Type locality: Brazil, state of Pará, municipality of Cachoeira do Arari, Marajó Island (01°00' S, 48°57' W).
Prevalence: 85.0% (68/80) of the hosts examined were infected.

Phylogenetic analysis

A partial, 934 base-pair sequence of the SSU rDNA gene was obtained from the *K. orbicularis* spores found in the musculature of *T. galeatus*. This sequence was deposited in GenBank under accession number MK204656. Two clades, named A and B, were identified in the phylogenetic analysis (Figure 2). Clade A was divided into two subclades formed exclusively by marine *Kudoa* species, with the exception of *K. orbicularis* (AZEVEDO et al., 2016) and *Kudoa amazonica* (VELASCO et al., 2019), which clustered together with the other *Kudoa* species analyzed, with high nodal (posterior probability) support. In the phylogenetic analyzes *Kudoa amazonica* was a sister taxon of *K.orbicularis*.

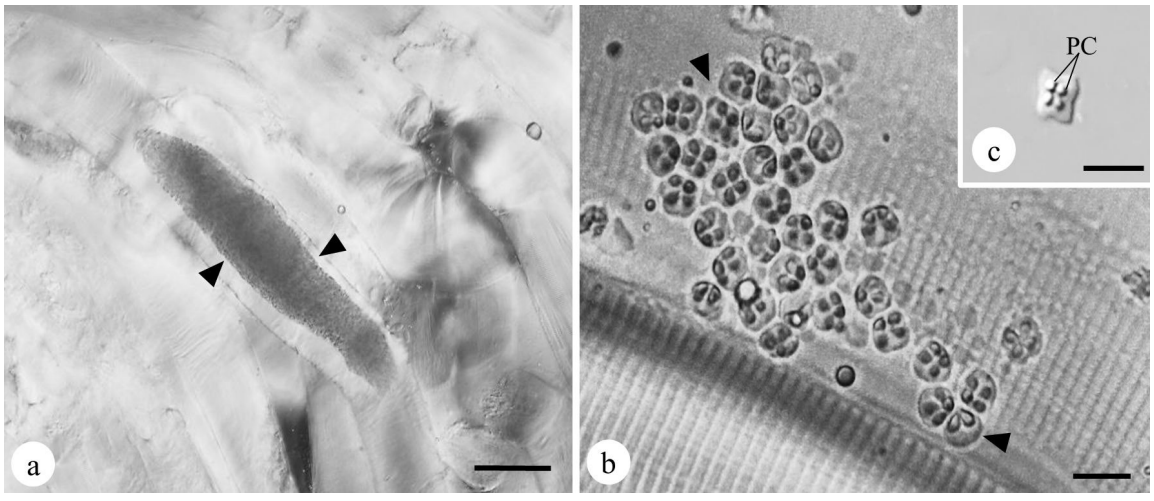

Figure 1. Photomicrographs of *Kudoa orbicularis* in *Trachelyopterus galeatus*: a) Pseudocyst (arrowhead) in the striated skeletal muscle fiber seen using DIC. Scale bar: 100 µm; b) Fresh spores (arrowhead). Scale bar: 5 µm; c) Detail of the spore (apical view) showing the four polar capsules (PC) seen using DIC. Scale bar: 5 µm.

Table 1. Comparative descriptive measurements (means in µm, and ranges in parentheses) of *Kudoa orbicularis* and other species that parasitize the musculature of their fish hosts.

Species	Host	Site of infection	Spore morphology	Spore length	Spore width	PC length	PC width	Locality	Reference
Kudoa orbicularis	*Trachelyopterus galeatus*	Muscle	Pseudo-square	4.65 (4.04-5.54)	5.25 (4.78-5.98)	2.22 (2.05-2.32)	1.53 (1.56-1.74)	Brazil	Present study
Kudoa orbicularis	*Chaetobranchopsis orbicularis*	Muscle	Pseudo-square	4.4-4.8	5.0-5.6	1.4-2.0	1.2-1.6	Brazil	Sindeaux-Neto et al. (2017)
Kudoa orbicularis	*Chaetobranchopsis orbicularis*	Muscle	Rounded square	4.3 (3.6-5.0)	5.1 (4.2-5.8)	2.1 (1.7-2.6)	1.3 (0.9-1.7)	Brazil	Azevedo et al. (2016)
Kudoa amazonica	*Hypophthalmus marginatus*	Esophageal musculature	Rounded square	5.6 (5.0-5.9)	6.7 (6.3-7.4)	1.8	1.2	Brazil	Velasco et al. (2019)
Kudoa pleurogrammi	*Pleurogrammus monopterygius*	Muscle	Sub-square	6.3 (5.6-8.8)	8.6 (8.2-9.1)	2.8 (2.7-2.8)	1.6 (1.4-2.0)	USA	Kasai et al. 2016
Kudoa inornata	*Cynoscion nebulosus*	Skeletal muscles	Rounded square	5.4 (5.3-5.5)	5.9 (5.8-6.0)	2.7	-	USA	Dyková et al. (2009)
Kudoa islandica	*Cyclopterus lumpus*	Skeletal muscles	Rounded square	4.8 (4.1-5.1)	7.4 (6.5-8.6)	1.7 (1.4-1.9)	1.5 (1.2-1.8)	Iceland	Kristmundsson & Freeman (2014).
Kudoa ogawai	*Hyperoglyphe japonica*	muscle tissue	Pseudo-square	8.93 (8.3-9.6)	13.29 (12.0-14.2)	2.45 (1.9-3.2)	2.48 (1.7-3.0)	Japan	Yokoyama et al. (2012)
Kudoa aequidens	*Aequidens plagiozonatus*	Sub-opercular Musculature	Square or pseudo-square	3.2 (2.9-3.5)	6.8 (6.2-7.1)	2.2 (2.0-2.6)	1.2 (1.1-1.5)	Brazil	Casal et al. (2008)

PC – Polar capsule

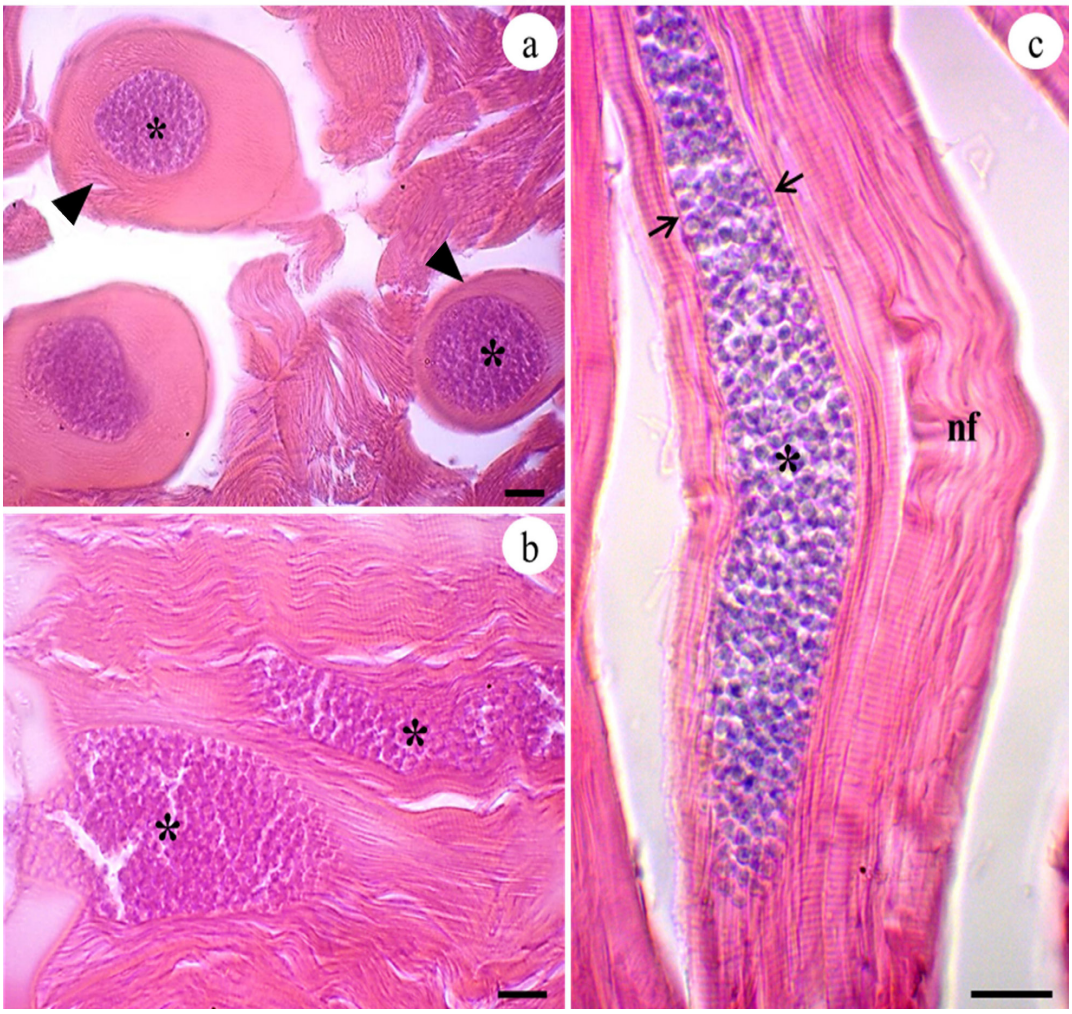

Figure 2. Photomicrographs of *Kudoa orbicularis* infecting *Trachelyopterus galeatus*: a) Pseudocysts (*) located in the cytoplasm (arrowheads) of the muscle fibers stained with HE. Scale bar: 20 µm; b) Histological section of the pseudocysts stained with HE (*). Scale bar: 20 µm; c) Pseudocyst (*) deforming the neighboring fibers (nf) and pseudocyst enveloped by a fine layer of sarcolemma (arrows) stained with Ziehl Neelsen. Scale bar: 20 µm.

The *Kudoa* species of clade A are found almost exclusively in the muscle tissues of their hosts, which are predominantly fish of the order Cichliformes, with tissue tropism in the musculature. However, some *Kudoa* species parasitize other types of tissue, such as the organs of the digestive system (esophagus and intestine), which is parasitized by *Kudoa dianae* (AF414692) and *Kudoa cookii* (JX090294); and the central nervous system (neurons and brain), which is infected by *Kudoa neurophila* (AY172511), *Kudoa chaetodoni* (DQ519387), *Kudoa prunusi* (AB573715), *Kudoa lemniscati* (JQ026222), and *Kudoa lethrini* (DQ519388). The *Kudoa* species that infect the digestive system were allocated to subclade A1 in the present analysis, together with the *Kudoa* species that infect the muscle tissue of host species belonging to a variety of fish orders. The *Kudoa* species that parasitize the nervous system present tropism that is characteristic of this type of tissue, and cluster in subclade A2, together with *Kudoa igami* (AB844444) and *Kudoa thalassomi* (AB844443), which infect the musculature. All the species in this subclade parasitize fish of the Cichliformes, and thus appear to have specialized in hosts of this order.

Clade B is formed by species of the genus *Unicapsula* Davis, 1924, a member of the order Multivalvulida Shulman, 1959. Most of these species infect the skeletal musculature of their hosts, except for *Unicapsula fatimae* (KT894108), which is a parasite of the esophagus. Once again, however, all the host species of this clade are fish of the order Cichliformes (Figure 3).

The sequences were realigned for pairwise comparison of a subset of the *Kudoa* species that parasitize the musculature of their hosts. The minimum p distance recorded in the present study (Table 2) was 3.5% between the *K. orbicularis* analyzed here and *K. orbicularis* (KM192365). All other distances were over 4.0%, reaching a maximum of 6.0%, in the case of *Kudoa whippsi* and *Kudoa empressmichikoae*.

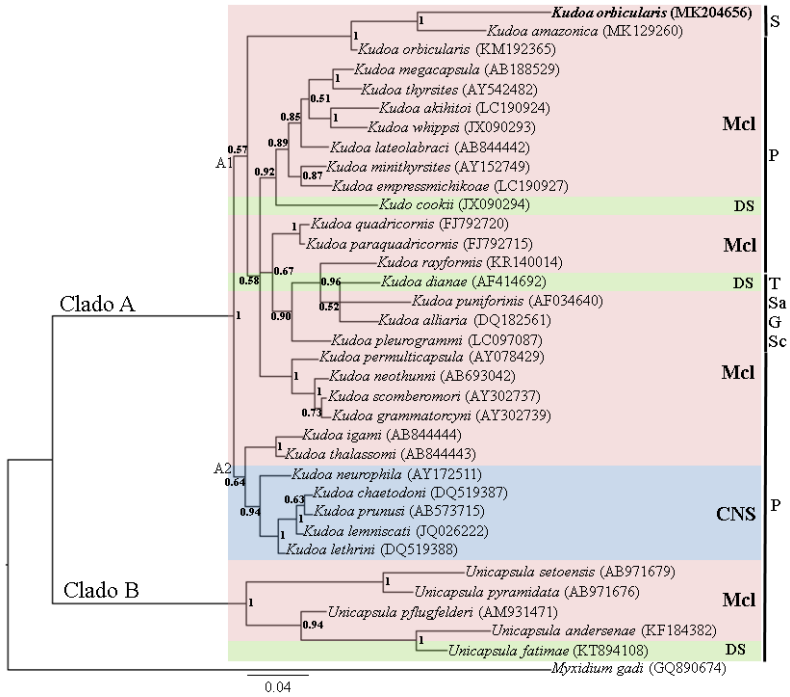

Figure 3. Phylogenetic tree generated through Bayesian inference (BI) performed on the partial sequences of the SSU rDNA gene of Kudoa orbicularis retrieved from *Trachelyopterus galeatus* in the present study and from other closely-related myxozoans. The GenBank accession numbers are shown next to the species names. The numbers at each node are the posterior probabilities calculated through BI. The species analyzed in the present study is highlighted in bold type. Abbreviations: Mcl = musculature; DS = digestive system; CNS = central nervous system; S = Siluriformes; P = Cichliformes; T = Tetraodontiformes; G = Gadiformes; Sa = Salmoniformes; Sc = Scorpaeniformes.

Table 2. Pairwise *p* distances among the *Kudoa* species that parasitize the muscle tissues of their hosts.

Species	1	2	3	4	5	6	7	8	9
(1) *Kudoa orbicularis* (XX006660)	-								
(2) *Kudoa orbicularis* (KM192365)	0.0348	-							
(3) *Kudoa amazonica* (MK129260)	0.0474	0.0291	-						
(4) *Kudoa megacapsula* (AB188529)	0.0497	0.0428	0.0720	-					
(5) *Kudoa thyrsites* (AY542482)	0.0509	0.0416	0.0720	0.0111	-				
(6) *Kudoa akihitoi* (LC190924)	0.0567	0.0497	0.0791	0.0246	0.0246	-			
(7) *Kudoa whippsi* (JX090293)	0.0602	0.0555	0.0803	0.0268	0.0291	0.0246	-		
(8) *Kudoa lateolabraci* (AB844442)	0.0520	0.0461	0.0744	0.0178	0.0200	0.0291	0.0212	-	
(9) *Kudoa empressmichikoae* (LC190927)	0.0602	0.0463	0.0732	0.0234	0.0212	0.0257	0.0280	0.0212	-

Discussion

Kudoa orbicularis was described from the musculature of specimens of the cichlid *Chaetobranchopsis orbicularis,* a Cichliformes collected from the Arari River, in Marajó Island, Brazil (AZEVEDO et al., 2016). This is same locality from which the *T. galeatus* specimens analyzed in the present study were collected. The morphological features of the *K. orbicularis* spores and polar capsules found in *T. galeatus* were closely similar to those described by Azevedo et al. (2016) and Sindeaux-Neto et al. (2017) in the *K. orbicularis* specimens obtained from the host fish *C. orbicularis*. Despite being the same species, minor morphological differences in the size of myxozoan spores are typically found in different hosts (KOVALEVA et al., 1979; YANAGIDA et al., 2004). The *K. orbicularis* spores are much smaller than those of *K.amazonica*, *Kudoa islandica*, *Kudoa inornata* and *Kudoa ogawai*, although the polar capsules are larger than those of *K. islandica* (Table 1).

The spores of *Kudoa quadricornis*, infecting *Carangoides fulvoguttatus* (WHIPPS et al., 2003b), and *Kudoa minithyrsites* in *Pempheris ypsilychnus* (WHIPPS et al., 2003a), were observed developing intracellularly in the microfibrils of the host, as observed with *K. orbicularis* in the present study. The histology of *K. orbicularis* in the present study was similar to that of *K. inornata* observed in the skeletal musculature of *Cynoscion nebulosus* by Dyková et al. (2009), who also described a fine layer of sarcolemma enveloping the mature spores within the fibers. Similar configurations were described by Dyková et al. (2002) in the case of *K. dianae* infecting the esophageal musculature of

Sphoeroides annulatus, and by Shirakashi et al. (2014) in *Kudoa igami* infecting the muscle tissue of *Calotomus japonicus*. In both cases, no evidence of inflammation or any other response to the presence of the parasite was observed in the host. However, Azevedo et al. (2016) and Sindeaux-Neto et al. (2017) did observe inflammation in the muscle tissue infected by *K. orbicularis*, as well as lethargic behavior in the host fish, including irregular tail movements and immobility, although these patterns were not observed in *T. galeatus* in the present study.

The similarities in the morphological characteristics of the *K. orbicularis* specimens observed in the present study, in comparison with the original description of this species (AZEVEDO et al., 2016), together with the muscle tissue tropism, the freshwater habit of the host and the geographical location of the two cases, all confirm the species identification. In addition to identification of *Kudoa* species based on morphological and morphometric parameters, a number of recent descriptions have also included molecular comparisons, which not only provide a more reliable diagnosis, but also enable analysis of phylogenetic patterns (EIRAS et al., 2014).

The phylogenetic tree derived from the molecular analyses that were applied in the present study (Figure 3) also confirmed the proximity of the *K. orbicularis* found in *T. galeatus* to the *K. orbicularis* sequence described by Azevedo et al. (2016), with maximum (100%) branch support and *K. amazonica* as a sister taxon, corroborated by the findings of Velasco et al. (2019). In addition to this significant genetic similarity, this is the only *Kudoa* species of clade A known to parasitize a freshwater host, in contrast with all the other *Kudoa* species included in this clade, which infect marine hosts. Hervio et al. (1997) observed that *Kudoa* species tend to group according to geographical location, rather than the morphological similarity of the spores, as confirmed in the present study.

In the other subclades, some *Kudoa* species were grouped according to host specificity, tissue tropism and/or geographical region. The second cluster of subclade A1, for example, groups 07 *Kudoa* species that parasitize the muscle tissue of perciform fish, but are from distinct localities. The third cluster is formed by two species (*K. quadricornis* and *K. paraquadricornis*) that parasitize the muscle tissue of perciform fish: in this case, from the same locality, Heron Island in Australia. The first of these clusters had 92% branch support, and the second had 100% support.

The findings from the present study thus support the hypothesis that the tissue tropism found in some *Kudoa* species has a genetic component, although there are also significant deviations (BURGER et al., 2007). In the fourth cluster, for example, which includes the *Kudoa* species not grouped through any of these factors, the parasites infect different types of tissue, and are found in a broad diversity of fish orders and geographical localities, with branch support of only 90%. However, what these species do have in common is the marine habitat of their hosts. Subclade A2, which has branch support of 64%, includes *Kudoa* species that infect different types of tissue, but is supported by the specificity of its hosts, such that all these parasites are found in perciform fish.

The present study confirmed that *K. orbicularis* may infect fish hosts belonging to different orders, which indicates that this microparasite is not host-specific. Although many *Kudoa* species are host-specific, a number of species are known to infect different types of host. These species include *Kudoa hypoepicardialis*, which has been found infecting seven different host species belonging to seven different genera and families (BLAYLOCK et al., 2004), while *Kudoa thyrsites* is known to infect fish of different families and is thought to have cosmopolitan distribution (WHIPPS & KENT, 2006; JONES et al., 2016).

The relationships among *Kudoa* species, based on their morphology, tissue tropism, habitat (freshwater *vs.* saltwater) and geographical region, and the results from the phylogenetic analysis of the present study conclusively support the conclusion that *T. galeatus* was infected by *K. orbicularis*, a species described previously in *C. orbicularis* (AZEVEDO et al., 2016). This is the second record of the occurrence of a *Kudoa* parasite in a (Siluriformes host in the Brazilian Amazon region.

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