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Morphological and Molecular Characteristics of *Kudoa viseuensis* n. sp. (Myxosporea: Multivalvulida), Found in the Muscle of *Batrachoides surinamensis* (Teleostei: Batrachoididae) in the Brazilian Amazon Region

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Abstract. A new species of myxozoan, *Kudoa viseuensis* n. sp. (Myxosporea: Multivalvulida), is described based on specimens extracted from the musculature of the Pacuma toadfish, *Batrachoides surinamensis*, collected in the municipality of Viseu, in the northern Brazilian state of Pará. A total of 60 specimens of *B. surinamensis* were examined, of which 52 (86%) presented whitish pseudocysts containing numerous rounded spores (7.2±0.2 µm in length and 5.2±0.2 µm in width). These spores have four polar capsules of equal size, measuring 1.8±0.2 µm x 1.3±0.1 µm in the apical view, and 2.7±0.2 µm x 1.3±0.1 µm in the lateral view. A partial sequence (1400 bps) of the small subunit ribosomal RNA gene was obtained and deposited in GenBank (access number: MK256272). The comparison of the morphological and molecular data with those of other *Kudoa* species supported the description of a new species of mixosporean from the Amazon region, which is denominated here as *Kudoa viseuensis* n. sp.

Key words: Myxozoa, molecular characterisation, morphology.

INTRODUCTION

The subphyla Myxozoa, part of the phylum Cnidaria (Hatschek, 1888), is composed of endoparasites that occur in both marine and freshwater environments (Fiala *et al*. 2015), affecting wild and farmed fish populations. The diseases caused by these parasites can cause considerable economic losses in fishery and aquaculture operations (Okamura *et al*. 2015).

The myxozoan of this group often cause only innocuous infections that have little impact on the host organism (Shul'man 1990, Lom and Dyková 1992), but in some cases, they proliferate rapidly and may provoke grave epidermic lesions and epidemics (Saha and Ban-

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dyopadhyay, 2017b). In general, myxozoans infest two hosts during their life cycle, typically an intermediate vertebrate host, and a definitive invertebrate host (Okamura *et al*. 2015).

Marine fish are an economically important fishery resource (Rosa and Lima, 2008), and one example is the Pacuma toadfish, *Batrachoides surinamensis* (Bloch and Schneider, 1801), known locally as the pacamão (Espírito-Santo *et al*. 2005) in the Bragança region of northeastern Pará, at the eastern extreme of the Brazilian Amazon region, where it is a popular food fish in local markets (Freire *et al*. 2011).

The myxozoan genus *Kudoa* presents tropism towards the skeletal musculature, and is known to be responsible for postmortem myoliquefaction in the host (Kristmundsson and Freeman 2014), although these parasites have also been described in other types of tissue and organs, such as the intestine (Yurakhno *et al*. 2007), ovary (Mansour *et al*. 2015), oesophagus (Velasco *et al*. 2015b), and heart (Abdel-Ghaffar *et al*. 2016). The spores of this genus may be star-shaped, square or rounded quadrangular (Casal 2009), and are generally made up of four or more shell valves and polar capsules of equal number (Whipps *et al*. 2004).

In the Amazon region, mixosporean of the genus *Kudoa* have been found infecting the muscle of *Aequidens plagionatus* (Casal *et al*. 2008), *Chaetobranchopsis orbicularis* (Azevedo *et al*. 2016, Sindeaux Neto *et al*. 2017) and *Plagioscion squamosissimus* (Oliveira *et al*. 2015), while in *Hypophthalmus marginatus*, Velasco *et al.* (2015b) found this parasite in the muscle layer of the intestine. In the present study, we describe a new species, *Kudoa viseuensis* n. sp., which was observed infecting the musculature of *B. surinamensis* specimens collected in the municipality of Viseu, northeastern Pará.

MATERIALS AND METHODS

Sampling

A total of 60 *B. surinamensis* specimens (42 females and 18 males) were collected from the Gurupi River, on the eastern margin of the municipality of Viseu, Pará (1°08' S, 46°05' W) between August 2016 and February 2018. The specimens were transported alive in aerated plastic bags filled with river water to the Carlos Azevedo Research Laboratory (LPCA) at the Federal Rural University of Amazonia (UFRA) in Belém, northern Brazil, where they were maintained in the same water in 20 L glass aquaria, at a temperature of 26–28°C, pH of 7, with 4.73–6.8 mg/L of dissolved oxygen, and 15.3‰ salinity. The specimens had a mean length of 10.9 cm (range: 7.0 cm–14.0 cm) and a mean weight of 15.6 g (6.1 g–31.5 g).

For analysis, the specimens were anaesthetised with 50 g/L tricaine metanosulfonate (MS-222 SIGMA), and then necropsied under a stereomicroscope for the identification of mixosporean lesions or pseudocysts on the external surface of the body, and in the gills and internal organs. The experimental procedures were approved by the UFRA Committee for Ethics in Animal Research (CEUA – UFRA 013/2014) and authorised by the Brazilian Institute for the Environment and Renewable Natural Resources, IBAMA (SISBIO / ICMBIO licence number 27119-1).

Processing for histology

Whitish pseudocysts were found in the epi- and hypo-axial musculature of the host. Small fragments (0.5 cm) of these pseudocysts were collected and fixed in Davidson solution (neutral buffered formalin, glacial acetic acid, 95% ethyl alcohol, and distilled water) for 24 hours, dehydrated in an increasing alcohol series (70%, 80%, 90%, Absolute I, II, and III), diaphanised in xylol, and then embedded in paraffin. Sections of 5 µm thickness were then obtained, deparaffinised, and stained first in Haematoxylin and Eosin (HE) to facilitate the identification of the presence of pseudocysts, and then in Ziehl-Neelsen (ZN) to highlight the pseudocysts, spores, and polar capsules in contrast with the muscle fibers (Luna 1968). The parasite and the histological alterations associated with the infection were also examined by light microscopy, and photographed using a Zeiss Primo Star microscope attached by a Zeiss AxiocaCAm Erc 5 camera equipped with AxioVision LE software.

The fresh spores were also observed under a microscope equipped with differential interference contrast (DIC) and photographed. Morphometrics were obtained from 30 fresh spores, with the means and standard deviations being calculated for each parameter, presented together with the range (minimum-maximum) of values (Lom and Arthur, 1989).

Processing for molecular biology

For the DNA analysis, samples of the muscle tissue infected with spores were collected and preserved in 80% ethanol. The total DNA of these samples was extracted using a PureLink® Genomic DNA mini kit (Invitrogen, USA), following the manifacture's instructions.

The concentration of DNA in the samples was calculated in a Biodrop Duo (Biodrop) spectrophotometer. A sequence of the small subunit ribosomal DNA (SSU rDNA) was obtained by Polymerase Chain Reaction (PCR), initially using the universal Eukaryote 18E primer (Hillis and Dixon, 1991) with the 18R reverse primer (Whipps et al. 2003a), followed by a nested PCR with the Kudf/Kudr primers (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 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 was run in an Applied Biosystems Simple Amp™ thermocycler, based on the protocol for the 18E and 18R primers, that is: an initial denaturation for 5 minutes at 95°C, followed by 35 cycles of 2 min at 95°C, 2 min at 48°C (annealing temperature), and

4 min at 72°C, with a final extension of 10 minutes at 72°C. For the Kudf/Kudr primers, the reaction protocol was 5 minutes at 95°C, followed by 35 cycles of 30 seconds at 95°C, 30 seconds at 53°C (annealing temperature), and 60 seconds at 72°C, with a final extension of 10 minutes at 72°C. The size and quality of the amplified DNA were verified by the electrophoresis of 3 µl of the product in 1% agarose gel with 1X Tris-borate-EDTA (TBE), stained with SYBR® Safe (Invitrogen, EUA) and visualised under blue light. The PCR products were purified with PCR GFX™ DNA and a Gel Banding purification kit (GE Healthcare, UK), following the manifacture's protocol. The PCR products of the 18E, Kudf and 18R primers were sequenced separately. The sequencing reactions were conducted using the Big Dye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, EUA), based on the manifacture's instructions, and run in an ABI 3100 Genetic Analyser (Applied Biosystems, EUA).

The sequences obtained by this procedure were aligned in the BioEdit software (Hall 1999) and ambiguous bases were clarified using the respective chromatograms. The sequences of the SSU rDNA gene of the myxozoan species deposited in the 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 to select the GenBank sequences for inclusion in the analysis. The jModelTest software, version 0.1.1 (Guindon and Gascuel 2003; Posada 2008) was used to identify the best nucleotide substitution model for the dataset. Bayesian Inference was implemented in MrBayes, version 3.1.2 (Ronquist and Huelsenbeck 2003), using Markov Chain Monte Carlo searches of two simultaneous runs of four chains of 5,000,000 generations, with every 500th tree being sampled. The first thousand trees were discarded as burn-in, and the posterior probability of each node were calculated from the remaining trees, examined initially in TreeView X (Page 1996). Genetic distances computed in PAUP* 4.0b1 (Swofford 2003) using the default p parameter for the SSU rDNA gene.

RESULTS

Morphological analysis

Whitish ovoid pseudocysts were observed in the skeletal musculature of the *B. surinamensis* specimens (Figure 1A), and when pressed between slide and coverslip, these pseudocysts released a number of spores with four piriform, symmetrical polar capsules, with slightly rounded valves lacking any projection (Figure 1B and 1C), typical of the genus *Kudoa*. It was not possible to verify the number of coils in the polar filament.

The histological sections of the muscle tissue indicated that the parasite developed intracellularly in the myofibers (Figure 2A), located centrally, and enveloped in a fine membrane that separates the mature spores from the muscle of the host. Individual infections were found when the cystic formation was observed within a single muscle fiber (Figure 2B), although multiple infections were also found, where two or more pseudocysts developed within the same fiber (Figure 2C).

Kudoa viseuensis n. sp.

ZooBank: lsid:zoobank.org:act:AF886D76-EC95-4EA7-8F0D-DA2D4B956A60

Host: *Batrachoides surinamensis* (Bloch and Schneider, 1801).

Infection site: Pseudocysts in the somatic musculature.

Type locality: Brazil, state of Pará, municipality of Viseu (1°08' S, 46°05' W).

Prevalence: 86% (52/60) of the examined hosts were infected.

Etymology: the specific name, *viseuensis*, refers to the municipality of Viseu, where the specimens were captured.

Type specimen: A glass slide with a 5 µm-thick histological section stained in Haematoxylin and Eosin, containing the spores of the new species was deposited in the Zoology Museum of the National Institute of Amazonian Research (INPA) in Manaus, Amazonas, Brazil, under catalog number CNIDARIA – INPA 038.

Description of the spores: The spores of *Kudoa viseuensis* n. sp. were 7.2±0.2 µm in length and 5.2±0.2 µm in width (Figure 3). In the apical view, the polar capsules were 1.8±0.2 µm in length and 1.3±0.1 µm in width. When observed laterally, the mean length was 2.7±0.2 µm and the width was 1.3±0.1 µm (Table 1). Table 1 compares the dimensions of the spores and polar capsules (and the shape of the spores) of other *Kudoa* species with *Kudoa viseuensis* n. sp. (Cnidaria: Myxozoa: Myxosporea: Multivalvulida: Kudoidae). *Kudoa viseuensis* n. sp. was restricted to the skeletal musculature of the host, and was not found in any other organ of the host fish.

The morphological comparisons indicated that *Kudoa viseuensis* n. sp. is most similar to *K. orbicularis* (Azevedo *et al*. 2016) in terms of the width of the spore and the polar capsule, although other dimensions are clearly distinct from those of this species, and other *Kudos* species, studies previously. These findings, together with the molecular data (see below) lend support to the present description of the new species.

Phylogenetic analyses

A partial sequence of 1400 base pairs (bps) of the SSU rDNA gene was obtained from the spores of *Kudoa viseuensis* n. sp., found in the musculature of *B. suri-*

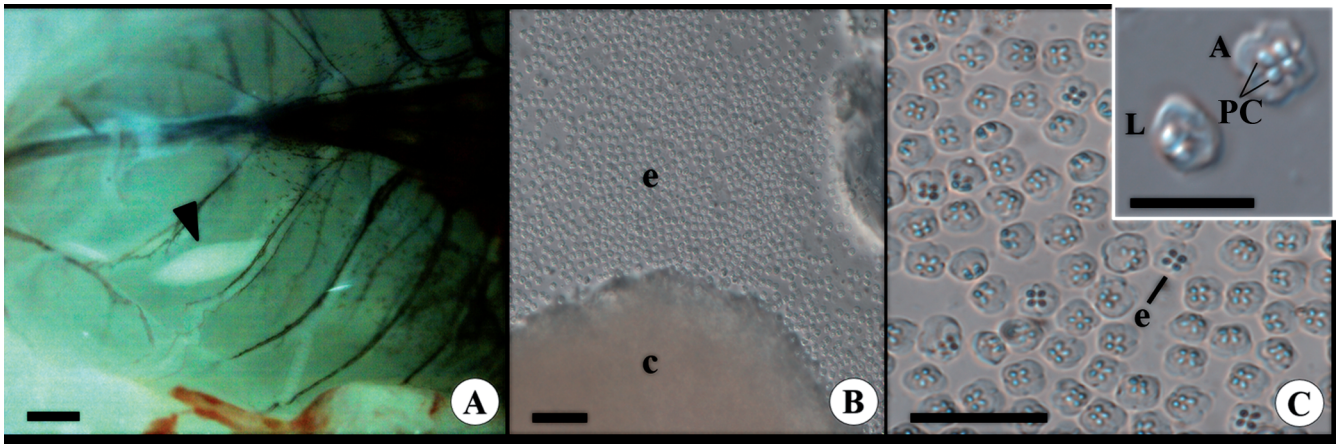

Figure 1. Light photomicrograph: (A) Whitish pseudocyst (arrowhead) found in the musculature of *B. surinamensis*. Scale bar: 1000 µm; (B) pseudocyst (c) and numerous mature spores (e) observed following the rupture of the pseudocyst. Scale bar: 100 µm; (C) Fresh, pseudo-square spores (e) of *Kudoa viseuensis* n. sp. Scale bar: 20 µm; Inset: polar capsules (PC) in lateral (L) and apical (A) views (DIC). Scale bar: 10 µm.

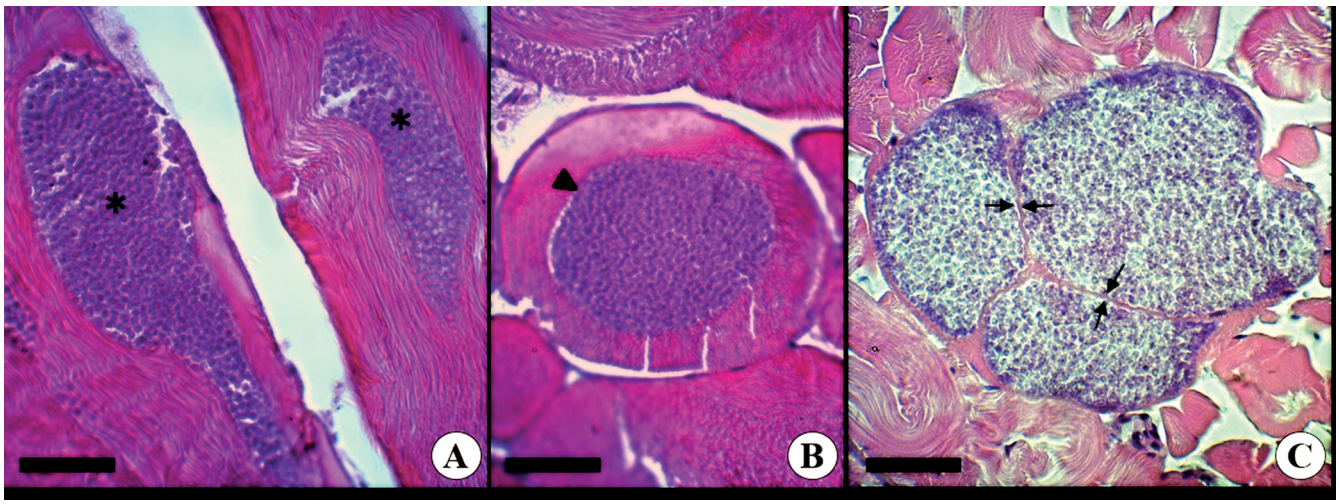

Figure 2. Light photomicrograph: (A) longitudinal histology section of the skeletal musculature of *B. surinamensis* containing a pseudocyst (*), along the axis of the muscle, showing the substitution of the fiber by the parasite; (B) transversal section showing the pseudocyst of the mixosporean occupying the central portion of the muscle fiber (arrowhead), typical of an individual infection; (C) Multiple infection of pseudocysts within a single muscle fiber, separated from one another and the muscle tissue by a fine conjunctive membrane (arrows). Scale bars: 40 µm.

namensis. This sequence was deposited in GenBank under accession number MK256272. The phylogenetic tree generated by Bayesian Inference defined a major clade, denominated clade A, composed of species of the genus *Kudoa* (Figure 4), which is subdivided into two clades, denominated A1 and A2, with high support (posterior probabilities). Each of these clades was influenced strongly by the tissue tropism of the parasites, with *Kudoa viseuensis* n. sp. being included in subclade A2. Subclade A1 is composed of *Kudoa* species that parasitise the musculature, brain, and intestine of fishes. *Kudoa viseuensis* n. sp. is included in subclade A2, which is basal to A1. *Kudoa viseuensis* n. sp. parasitizes the musculature of the marine fish *B. surinamensis*, and clusters with *Kudoa orbicularis* (Azevedo *et al*. 2016), a parasite of the musculature of the freshwater fish, *Chaetobranchopsis orbicularis*. In this arrangement, *K. orbicularis* is the sister species of *Kudoa viseuensis* n. sp., and the two species not only share the infection site, but are also found in the same geographic region.

Table 1. Morphometric measurements (µm) of the spores of *Kudoa viseuensis* n. sp., and closely-related species. The data area presented as means with their respective standard deviations (with the range of values within parentheses).

Species (Reference)	Host	Spore (µm)		Polar capsule (µm)				Spore form
		Width	Length	Width (L*)	Length (L*)	Width (A*)	Length (A*)	
This study	*Batrachoides surinamensis*	5.2±0.2 (5.1–5.4)	7.2±0.2 (6.7–7.4)	1.3±0.1 (1.1–1.4)	2.7±0.2 (2.4–2.9)	1.3±0.1 (1.2–1.5)	1.8±0.2 (1.6–2.0)	Pseudo-square
K. orbicularis (Azevedo *et al*. 2016)	*Chaetobranchopsis orbicularis*	5.1 (4.2–5.8)	4.3 (3.6–5.0)	ND	2.1 (1.7–2.6)	1.3 (0.9–1.7)	ND	Rounded quadrate
K. pleurogrammi (Kasai *et al*. 2016)	*Pleurogrammus monopterygius*	8.6 (8.2–9.1)	6.3 (5.6–6.8)	ND	2.8 (2.7–2.8)	1.6 (1.4–2.0)	ND	Subquadrate
K. rayformis (Shin *et al*. 2016)	*Scomberomorus sierra*	5.0 ± 0.3 (4.6–5.7)	5.4 ± 0.2 (5.0–5.7)	1.7 ± 0.2 (1.4–2.0)	2.3 ± 0.2 (1.9–2.6)	1.9 ± 0.2 (1.5–2.5)	1.9 ± 0.3 (1.5–2.6)	Subquadrate
K. islandica (Kristmundsson and Freeman, 2014)	*Cyclopterus lumpus*	7.4 (6.5–8.6)	4.8 (4.1–5.1)	ND	1.7 (1.4–1.9)	1.5 (1.2–1.8)	ND	Pseudo-square
K. paraquadricornis (Burger and Adlard, 2010)	*Caranx ignobilis*	7.79±0.22 (7.3–8.2)	8.03± 0.24 (7.6–8.5)	1.57±0.09 (1.3–1.7)	4.17±0.21 (3.7–4.5)	1.7± 0.06 (1.5–1.8)	1.98± 0.05 (1.9–2.1)	Subquadrate
K. quadricornis (Burger and Adlard, 2010)	*Carangoides fulvoguttatus*	7.84±0.28 (7.2–8.3)	8.38± 0.27 (7.9–9.0)	1.55± 0.1 (1.4–1.8)	4.26±0.2 (3.8–4.6)	1.67± 0.08 (1.5–1.8)	2.01± 0.08 (1.9–2.2)	Subquadrate
K. inornata (Dyková *et al*. 2009)	*Cynoscion nebulosus*	5.9 (5.8–6.0)	5.4 (5.3–5.5)	ND	2.7	ND	ND	Pseudo-square
K. alliaria (Whipps and Diggles, 2006)	*Macruronus magellanicus*	8.51 (7.84–9.25)	6.35 (5.84–6.86)	ND	2.60 (2.27–2.82)	1.61 (1.40–1.87)	ND	Pseudo-square
K. rosenbuschi (Abollo *et al*. 2005)	*Merluccius hubbsi*	4.47 (4.2–4.8)	3.21 (2.8–3.6)	ND	ND	ND	ND	Pseudo-square
K. paniformis (Hervio *et al.* 1997)	*Merluccius productus*	5.9 (5.0–6.5)	5.0 (4.5–6.0)	ND	2.0 (1.9–2.4)	1.6 (1.4–1.9)	ND	Pseudo-square

ND – Not available; L* – Lateral; A* – Apic

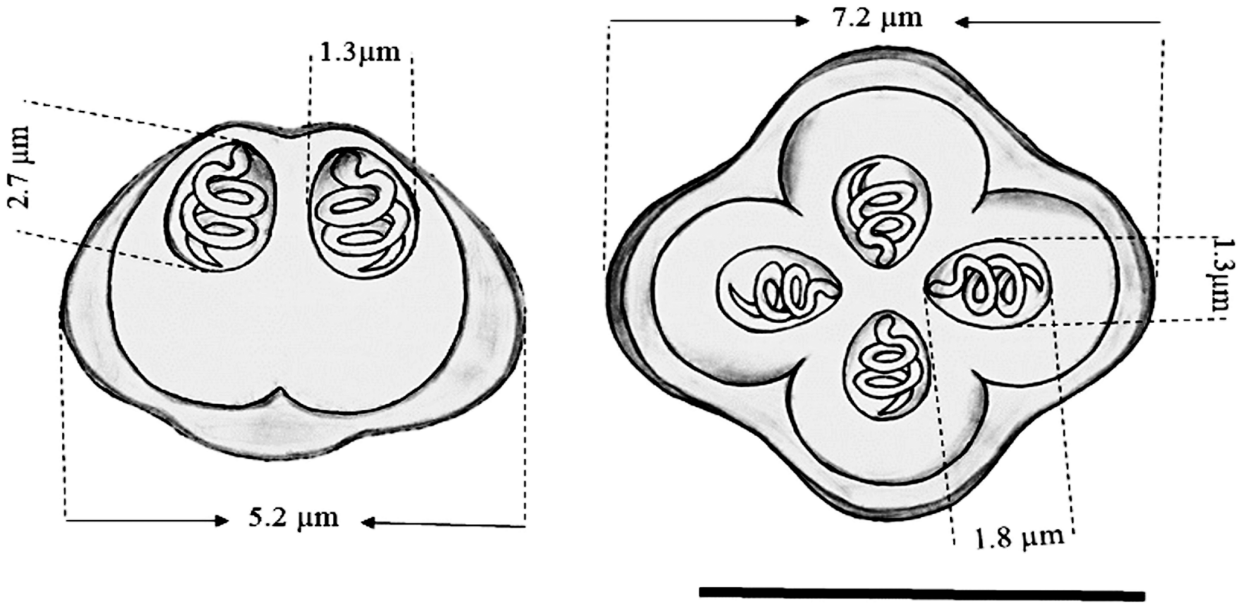

Figure 3. Schematic drawing of a spore of *Kudoa viseuensis* n. sp. in apical view (right) and side view (left). Scale bar = 5 µm

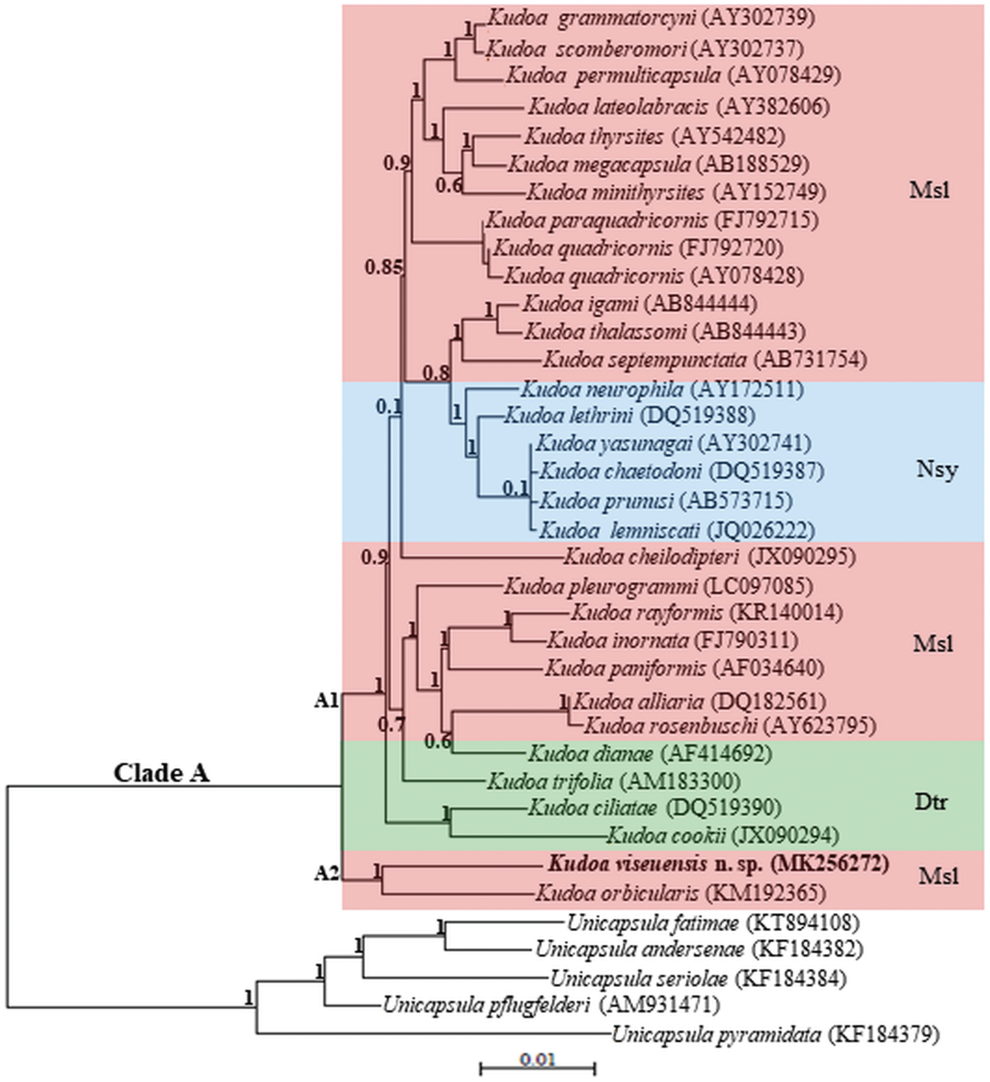

Figure 4. Phylogenetic tree derived from Bayesian Inference (BI), based on the partial sequences of the SSU rDNA gene of *Kudoa viseuensis* n. sp. and closely-related myxosporans. The GenBank access numbers are shown next to the species names, and the numbers at each node are the BI posterior probabilities. The new species is highlighted in bold type. Abbreviations: Msl – muscle; Dtr – digestive tract; Nsy – nervous system.

Table 2. Genetic distances (p) between *Kudoa* species that infect muscle tissue, with the respective GenBank access numbers of the samples.

Species (acess number)	1	2	3	4	5	6	7
1 – *K. viseuensis* n. sp. (MK256272)	–						
2 – *K. orbicularis* (KM192365)	0.0360	–					
3 – *K. pleurogrammi* (LC097085)	0.0360	0.0360	–				
4 – *K. paraquadricornis* (FJ792715)	0.0360	0.0382	0.0211	–			
5 – *K. quadricornis* (AY078428)	0.0382	0.0405	0.0234	0.0022	–		
6 – *K. rayformis* (KR140014)	0.0420	0.0390	0.0271	0.0315	0.0338	–	
7 – *K. inornata* (FJ790311)	0.0420	0.0427	0.0241	0.0278	0.0300	0.0116	–
8 – *K. rosenbuschi* (AY623795)	0.0495	0.0480	0.0293	0.0352	0.0375	0.0352	0.0315

The outgroup is composed of species of the genus *Unicapsula* Davis, 1924, a member of the order Multivalvulida Schulman, 1959, which infects the musculature of its hosts.

A new alignment was run for the pairwise comparison of key *Kudoa* species with *Kudoa viseuensis* n. sp. (Table 2). The smallest pairwise genetic distance (p) found in this analysis between *Kudoa viseuensis* n. sp. and the other *Kudoa* species was 3.9% in the case of *K. orbicularis* (KM192365), while the greatest distance was 4.9% for *K. rosenbuschi* (AY623795).

DISCUSSION

All the analyses presented here support the description of *Kudoa viseuensis* n. sp. as a distinct new taxon of the genus *Kudoa*. The new parasite is clearly aligned with other *Kudoa* species, although this is the first kudoid species from a Brazilian estuary for which molecular data have been obtained. Infection by this new parasite is characterised by the formation of a pseudocyst in the musculature of its host (Yokoyama *et al*. 2012; Kasai *et al*. 2016, Kasai *et al*. 2017; Sakai *et al*. 2018). The infection of *Kudoa* species presents muscle tissue tropism, as observed in the present study, with *Kudoa viseuensis* n. sp. being found only in the skeletal muscle of *B. surinamensis*, with no infection being found in any other organ, a scenario also found in the Pacific barrelfish, *Hyperoglyphe japonica*, in which infection by *Kudoa ogawai* was also limited to the muscle tissue (Yokoyama et al. 2012).

In some cases, the pseudocysts are relatively easy to detect, as in the case of *Kudoa trachuri*, found in the musculature of the Japanese horse mackerel, *Trachurus japonicus*, and *Kudoa thunni* in that of the albacore, *Thunnus alalunga* (Matsukane *et al.* 2011). Abdel-Ghaffar *et al.* (2016) also observed macrocsopic pseudocysts of *Kudoa pagrusi* in the heart muscle of the sea bream, *Pagrus pagrus*. In most cases, however, the infection is subclinical, and the pseudocysts are indiscernible to the naked eye, which means that the infected fish may be consumed by humans who are unable to perceive the presence of the spores (Yokoyama and Itoh 2005, Whipps and Kent 2006, Schmidt-Posthaus *et al*. 2012). In the present study, the pseudocysts in the skeletal muscle of *B. surinamensis* were not immediately visible, but were discovered under light microscopy, as observed by Shirakashi *et al*. (2014) in the Japanese parrotfish, *Calotomus japonicus*.

The pseudo-square spores of *Kudoa viseuensis* n. sp. are distinctly smaller than those of *Kudoa pleurogrammi*, *Kudoa paraquadricornis*, *Kudoa quadricornis*, and *Kudoa alliaria* (Whipps and Diggles 2006, Burger and Adlard 2010, Kasai *et al*. 2016), even though the spores of some other species, such as *K. orbicularis* and *Kudoa rayformis* (Azevedo *et al*. 2016, Shin *et al*. 2016) are more similar in size. The new species has larger spores than those of *K. rosenbuschi* (Abollo *et al*. 2005).

The prevalence of the new species of parasite was 86%, a rate lower than those recorded in the silver

croaker, *Plagioscion squamosissimus*, in which 100% of the specimens analysed by Oliveira *et al*. (2015) were infected, or the spotted seatrout, *Cynoscion nebulosus*, in which 91% of the specimens examined by Dyková *et al*. (2009) were infected with *Kudoa inornata*.

Histological analyses have revealed that a large proportion of the musculature may be replaced by *Kudoa* pseudocysts, forming a ring around the fish (Moran *et al*. 1999b, Kristmundsson and Freeman 2014). Multiple infections in a single muscle fiber have also been described by Lom *et al*. (1983) and Moran *et al*. (1999c). In the present study, while localised pathological alterations were found, no clear impact on the physiology or behaviour of the host fish was detected. The response of the host varies considerably among different *Kudoa* species, including substantial infiltration of the inflamed cells and the formation of granulomas, although in many other cases, no response is observed in the host (Whitaker *et al*. 1996, Casal *et al*. 2008, Dyková *et al*. 2009).

Some kudoid species are important pathogenic agents in fisheries and aquaculture, due to the pathology caused in the host. *Kudoa yasunagai*, for example, infects the brain of its host, causing deformations of the vertebral column. *Kudoa thyrsites* (Whipps and Kent, 2006) and *Kudoa lateolabracis* (Yokoyama *et al*. 2004) cause extensive postmortem necrosis of the host, inducing the degradation of the infected tissue. *Kudoa amamiensis* (Burger *et al.* 2008) and *K. islandica* (Kristmundsson and Freeman, 2014) cause nonspecific pseudocysts in fish fillets. *Kudoa septempunctata* causes food poisoning (Kawai *et al.* 2012). These pathologies are responsible for serious economic losses in the fishery industry (Davies *et al*. 1998, Whipps *et al*. 2003a, Hadfield 2014).

The molecular analysis of sequences of the SSU rDNA gene provides important complementary evidence for the interpretation of the diversity of myxozoans. Although the morphology of the spores is essential for the taxonomic classification of species, the phenotypic similarities among kudoid species and the intraspecific variation found in many cases hampers the reliable diagnosis of species based solely on morphology (Urawa *et al.* 2009, Burger and Adlard 2010, Heiniger and Adlard 2012, Heiniger *et al*. 2013). Phylogenetic analyses have provided a better understanding of the diversity and biogeographic relationships among these parasites and their hosts (Urawa *et al.* 2011, Kasai *et al.* 2017, Sakai *et al.* 2018).

In the phylogenetic analysis of the myxosporeans, tissue tropism is evident in the kudoids (Fiala 2006). The phylogenetic analysis of the partial SSU rDNA sequences produced by the Bayesian Inference (BI) revealed a close relationship between the *Kudoa* species that infect musculature and those that infect the nervous system, such as *Kudoa neurophila* (Grossel *et al.* 2005), *Kudoa yasunagai* (Whipps et al. 2004), *Kudoa prunusi* (Meng *et al*. 2011), *Kudoa lemniscati* (Miller and Adlard, 2012), *Kudoa chaetodoni*, and *Kudoa lethrini* (Burger *et al*. 2007), and those that infect the digestive tract, such as *Kudoa trifolia* (Holzer *et al*. 2006), *Kudoa ciliatae* (Burger *et al*. 2007), *Kudoa cookii* (Heiniger *et al*. 2013), and *Kudoa dianae* (Dyková *et al*. 2002). However, the *Kudoa* species that infect only the skeletal musculature form a group distinct from the principal *Kudoa* clades.

The results of the present study support the potential relationship between the biogeographic and morphological similarities of the different *Kudoa* species. In particular, *K. orbicularis*, which is phylogenetically closest to *Kudoa viseuensis* n. sp., not only infects the same type of tissue, but also occurs in the same geographic region, being found in the Amazon basin.

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