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FISH PARASITOLOGY - ORIGINAL PAPER

Description of a new myxozoan *Kudoa eugerres* n. sp. and reclassification of two *Sphaerospora sensu lato* species

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Abstract

Ultrastructural and phylogenetic description of a fish-infecting myxosporean found infecting the gallbladder wall of the teleostean *Eugerres brasilianus* Cuvier, 1830, collected from the Atlantic coast near the city of Maceió (Alagoas State), Brazil. Groups of mature pseudo-conical myxospores, agglutinated forming pseudocyst structures, occurring in the mucosa of gallbladder were 5.2 ± 0.8 μm (4.5–6.0) ($n = 30$) long, 4.3 ± 0.6 μm (3.8–4.7) ($n = 25$) thick, and 2.9 ± 0.2 μm (2.7–3.2) ($n = 25$) wide. The two ellipsoidal polar capsules, 1.8 ± 0.4 × 1.2 ± 0.4 μm ($n = 25$), opened close to the sutural line, each containing an isofilar polar tubule. The latter consisted of a single coil with five to six turns, arranged obliquely to the axis of the polar capsule. This myxosporean parasite, while being morphologically similar to *Sphaerospora* spp., displays tissue tropism and phylogenetic relationships distinct from the latter. Bayesian inference (BI) and maximum likelihood (ML) analyses showed the parasite and two other related species clustering within the marine clade, more specifically within a subclade of the larger *Kudoa* (Multivalvulida) clade. Consequently, this atypical new myxozoan species was classified as *Kudoa eugerres* n. sp. and two other histozoic *Sphaerospora* spp. sensu *lato* were transferred to the genus *Kudoa*.

Keywords Electron microscopy · Phylogenetic analysis · 18S rDNA gene · Myxosporean · Teleostean

Introduction

Myxosporeans are metazoan parasites belonging to the phylum Cnidaria Hatschek, 1888, characterized by a complex life cycle, in which there is an alternation of stages between an invertebrate definitive host (Polychaeta or Oligochaeta), and a poikilothermic vertebrate as intermediate host (usually freshwater, brackish, or marine fishes, but also amphibians or reptiles) (Lom and Dyková 2006). Presently, it is widely recognized that the main evolutionary trends are the definitive and intermediate hosts and the tissue tropism (Carriero et al. 2013; Holzer et al. 2018).

Numerous myxosporeans have been reported from freshwater and marine habitats in different geographic regions. The genus *Sphaerospora* Thélohan, 1892 accounts for at least 102 species described from Eurasia and North America, being most of them coelozoic parasites of the urinary system (Lom and Dyková 2006; Jirků et al. 2007; Patra et al. 2018) and, more rarely, of the gallbladder (Gb) (Su and White 1994; Zhao et al. 2015). Some occurrences in other organs which involve histozoic development have also been reported (Lom and Dyková 1992). *Sphaerospora molnari* infects the gill filaments and skin of the common carp *Cyprinus carpio* (Lom et al. 1983; Eszterbauer et al. 2013), *S. testicularis* infects testicular tissue and intestine of wild and cultured of *Dicentrarchus labrax* (Sitjà-Bobadilla and Alvarez-Pellitero 1990; Fioravanti et al. 2004), *S. ovophila* was found in

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Canada in the ovary of the teleostean *Lepomis gibbosus* (Xiao and Desser 1997), and *S. dicentrarchi* is a systemic parasite of European seabass, *D. labrax* (Sitjà-Bobadilla and Alvarez-Pellitero 1992; Fioravanti et al. 2004) having been described from the tissues of several organs, predominantly the Gb and intestine, with a prevalence of 100% and 70.5% in wild and cultured fishes, respectively (Sitjà-Bobadilla and Alvarez-Pellitero 1993). The latter species was also reported from spotted seabass, *D. punctatus*, caught in the estuary of the Alvor River (southern Portugal) (Xavier et al. 2013).

Phylogenetic analyses show the genus *Sphaerospora* as one the most polyphyletic groups among myxozoans. *Sphaerospora* (sensu *stricto*) (*s.s.*), also known as "true Sphaerosporids," form a basal clade in which most species are included, while *Sphaerospora* (sensu *lato*) (*s.l.*) appeared dispersed among several subclades of marine and freshwater clades (Bartošová et al. 2013; Holzer et al. 2013). Previous phylogenetic studies have shown that *Sphaerospora s.l.* are not true Sphaerosporids and, therefore, should be taxonomically reclassified (Diamant et al. 2005; Bartošová et al. 2011; Jones et al. 2011).

This study describes the occurrence of a *Sphaerospora*-like myxosporean from a South American fish of the genus *Eugerres* (Family Gerreidae). This genus accounts for 8 species, from which no myxozoan parasitosis was ever reported. The Brazilian fish *E. brasilianus* is distributed along the Western Central Atlantic: from South Carolina (USA) to the South of Brazil, being broadly caught along the northeastern region of Brazil and in the lower São Francisco River. In the latter region, this fish represents one of the most important marine species, much appreciated and of great commercial value, namely as a potential species to be introduced in aquaculture (Soares et al. 2016). In the present study, a new species, *Kudoa eugerres* n. sp., is described from the gallbladder wall (GbW) of *E. brasilianus*. The morphological characteristics and phylogenetic positioning of marine histozoic bivalvulids are discussed, resulting in the emendation of the diagnosis of the order Multivalvulida, Family Kudoidae and genus *Kudoa* in order to accommodate the parasite in the study and two other known species.

Materials and methods

Fish sampling

Nineteen specimens of the teleost fish *Eugerres brasilianus* Cuvier, 1830 (Teleostei, Gerreidae) (Brazilian common name "carapeba" or "mojarra") (about 20 to 30 cm long, and about 150 to 250 g), were collected between July and December 2017, from the Atlantic coast (09° 44′ S/35° 49′ W), near the city of Maceió (Alagoas State), Brazil. Fishes were transported alive to the laboratory and maintained for 3–6 days in an aquarium with aerated seawater, following the abiotic parameters calculated at the site of fish collection: salinity 3.0–3.5‰, pH 6.0–6.5, temperature ~ 25 °C. All specimens were periodically observed and later anesthetized with MS 222 before being dissected.

Light microscopy and transmission electron microscopy

For LM, small fragments of tissues of different organs were checked under a microscope for a parasitological survey. Small fragments of infected GbW were removed and photographed using a light microscope.

For TEM, small fragments of infected GbW were fixed in 4–5% glutaraldehyde in 0.2 M sodium cacodylate buffer (pH 7.2–7.4) for 20–24 h at 4 °C and then washed overnight in the same buffer at 4 °C, and post-fixed with 2% osmium tetroxide in the same buffer for 3 h at the same temperature, dehydrated through an ascending ethanol and propylene oxide series, and embedded in Epon. The semi-thin sections were stained with methylene blue-Azur II for LM observations. Ultrathin sections were double stained with uranyl acetate and lead citrate before observation in a JEOL 100CXII TEM (JEOL Optical, Tokyo, Japan), operated at 60 kV.

DNA extraction, PCR amplification, and DNA sequencing

Small fragments of infected GbW were collected from three specimens and preserved in 80% ethanol at 4 °C before genomic DNA extraction, which was performed using a GenElute™ Mammalian Genomic DNA Miniprep Kit for each sample, following the manufacturer's instructions. The DNA was stored in 50 μL of TE buffer at − 20 °C until further use. Amplification of the SSU rDNA gene was achieved using both universal and specific myxosporean primers: the 5′-end with the primers 18e (5′CTG GTT GAT CCT GCCAGT3′) (Hillis and Dixon 1991)/MYX4r (5′CTGACAGATCACTC CACGAAC3 ′) (Hallett and Diamant 2001), and Kud6r (5′ CGTTCATTCCGATAGTGA3′) (Whipps et al. 2003); and the 3′-end with the primers MyxospecF (5′TTCTGCCCTATCAA CTTGTTG3′) (Fiala 2006), and MYX4f (5′GTTCGTGG AGTGATCTGTCAG3 ′) (Rocha et al. 2015)/18r (5′ CTACGGAAACCTTGTTACG3′) (Whipps et al. 2003). PCR was carried out in 50-μL reactions using 10 pmol of each primer, 10 nmol of each dNTP, 2.5 mM $MgCl_2$, 5 μL 10 × Taq polymerase buffer, 1.5 units Taq DNA polymerase (Nzytech, Lisbon, Portugal), and approximately 50–100 ng of genomic DNA. The reactions were run on a Hybaid P × E Thermocycler, with initial denaturation at 95 °C for 3 mins, followed by 35 cycles of 94 °C for 45 s, 53 °C for 45 s, and 72 °C for 90 s. The final elongation step was performed at 72 °C for 7 mins. Five-microliter aliquots of the PCR products

were electrophoresed through a 1% agarose 1 × tris–acetate–EDTA buffer (TAE) gel stained with ethidium bromide. The purification of PCR products was achieved using a single-step enzymatic cleanup that eliminates unincorporated primers and dNTPs by means of the ExoFast method. Purified PCR products were directly sequenced using a Big Dye Terminator v.1.1 from the Applied Biosystems Kit and were run on an ABI3700 DNA analyzer (Perkin-Elmer, Applied Biosystems, Stabvida, Caparica, Portugal).

Distance and phylogenetic analysis

The obtained forward and reverse sequence segments were manually aligned with ClustalW (Thompson et al. 1994) in MEGA 7.0.9 software (Kumar et al. 2016), and ambiguous bases were clarified using corresponding ABI chromatograms. To evaluate the relationship of this species to other myxozoans, a homology search was performed using BLAST software, resulting in the selection of 78 SSU rDNA sequences from GenBank namely several *Kudoa* species, *Sphaerospora* species (excluding replicate sequences), as well as some representatives of coelozoic myxozoans genera (*Chloromyxum*, *Gadimyx*a, *Myxidium*, *Myxobilatus*, *Sphaeromyxa*, *Parvicapsula*, and *Zschokkella*), and histozoic myxozoans of the genera *Enteromyxum*, *Gastromyxum*, *Monomyxum*, and *Unicapsula*. *Tetracapsuloides bryosalmonae* (U70623) and *Buddenbrockia plumatellae* (AY074915) were selected as the outgroup. The sequences were aligned using the software multiple alignments using fast Fourier transform version 7 (MAFFT v.7) available online, and subsequent phylogenetic and molecular evolutionary analyses were conducted in MEGA 7.0.9.

For inferring phylogenetic relationships, Bayesian inference (BI) and maximum likelihood (ML) methods were used. BI analyses were performed using MrBayes v.3.2.6 (Ronquist and Huelsenbeck 2003). The general time reversible model with gamma-shaped rate variations across sites (Invgamma) (GTR + I + Γ) was used, in accordance with the model test algorithm of the software. Posterior probability distributions were generated using the Markov chain Monte Carlo (MCMC) method, with four chains running simultaneously for 1,000,000 generations, and every 100th tree sampled. ML analyses were conducted in MEGA 7.0.9, using the general time reversible substitution model with four gamma-distributed rate variation among sites, with bootstrap confidence values calculated from 500 replicates.

Distance estimation was carried out for some sequences of the main marine clade (*Kudoa* subclade, 2 *Sphaerospora* spp., and the myxosporean described here), which was first aligned with the software MAFFT version 7, and then, the distance calculated using a *p* distance model distance matrix for transitions and transversions in MEGA 7.0.9. All positions containing gaps and missing data were eliminated; all ambiguous positions removed for each sequence pair.

Results

All internal organs were checked under a microscope for a parasitological survey of a total of 19 specimens, of which 11 specimens (7 females and 4 males) were infected by myxospores located in the GbW connective tissues. Myxospores were morphologically similar to the bivalvulids of the genus *Sphaerospora* Thélohan, 1892. The myxospores were agglutinated forming pseudocyst structures (Pcls) with a variable number of myxospores located in the mucosa of GbW (Fig. 1a–c). These groups of myxospores formed Pcls with variable dimensions were in direct contact with the host tissues (Fig. 1b, c), some of which are open contacting with the Gb lumen content (Fig. 1a and inset). LM observations of isolated mature myxospores show its morphological aspects, mainly the position of the two polar capsules located side by side (Fig. 1d).

Systematic position

Phylum Cnidaria Hatschek, 1888

Sub-phylum Myxozoa Grassé, 1970;

Class Myxosporea Bütschli, 1881;

Order Multivalvulida Shulman, 1959

Diagnosis: Amended (bold): Spores are radially symmetrical, the posterior face is flat or semispherical. Spores' wall comprising **2–13 soft valves** (**frequently 4**) **that overlap**, **making** the suture line often indistinct. One polar capsule per valve situated at the apex of spore; their tips are covered by the valve and they discharge apically. Plasmodia are histozoic and develop, usually, in the muscle tissues of marine fishes. The pansporoblast formation has not been observed.

Family Kudoidae Meglitsch, 1960

Diagnosis: Amended (bold) after Whipps et al. 2004: Spores with 4–13 valves (**rarely 2**) and 4–13 polar capsules (**rarely 2**). Two sporoplasm cells, one inside the other, **or a single binucleated sporoplasm**.

Genus *Kudoa* Meglitsch, 1947

Diagnosis: Amended (bold) after Whipps et al. 2004: Spores stellate, quadrate, or subpherical to ovoid in apical view. Four or more (**rarely 2**) ellipsoids to pyriform polar capsules, usually of equal-size, corresponding to the number of valves.

Species *Kudoa eugerres* n. sp.

Description of the species

Type host: *Eugerres brasilianus* Cuvier, 1830 (Teleostei, Gerreidae).

Type locality: The Atlantic coast (09° 44′ S/35° 49′ W), near the city of Maceió (Alagoas State), Brazil.

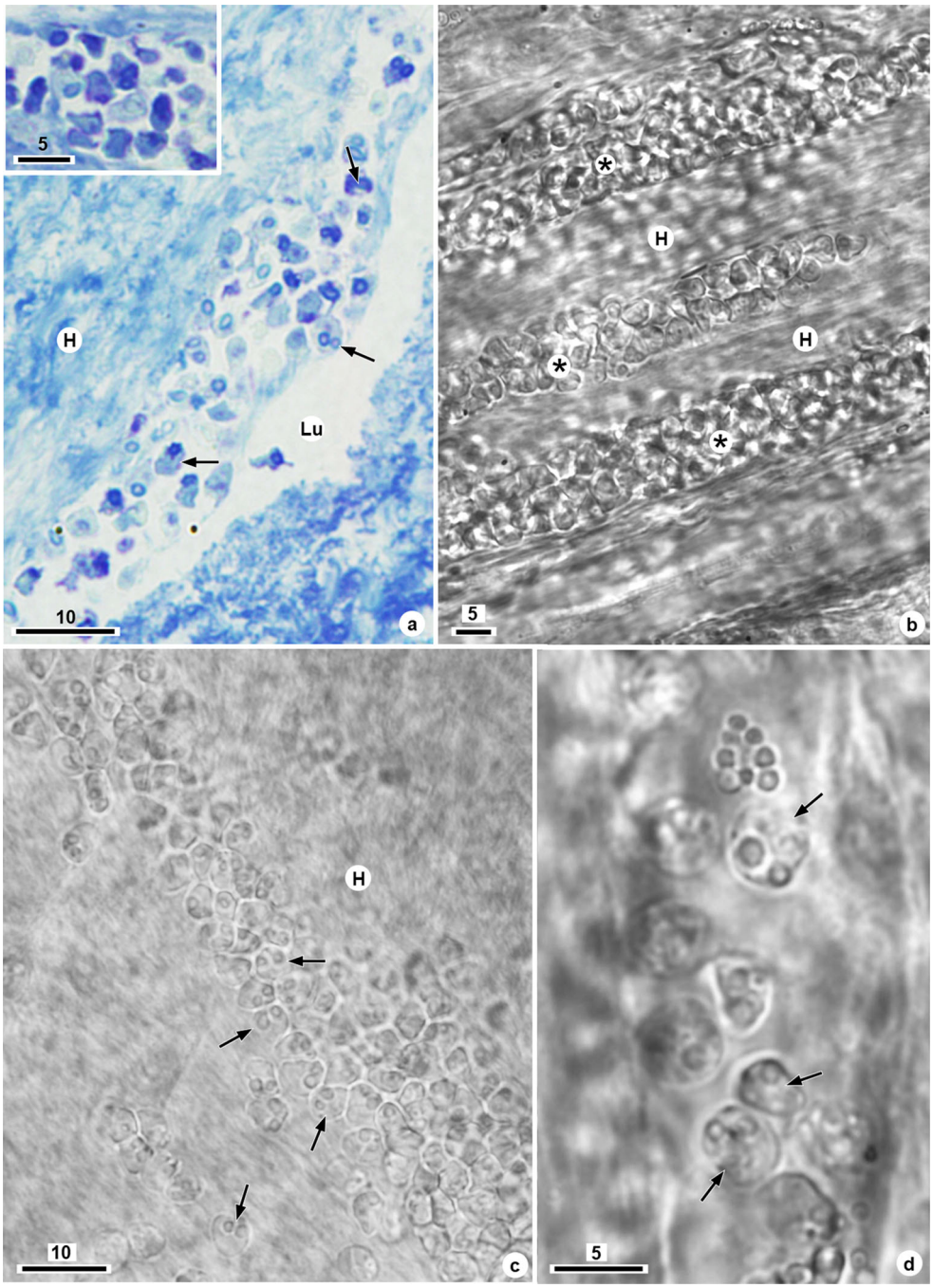


Fig. 1 Light micrographs of the infected gallbladder wall (GbW) of the teleostean *Eugerres brasilianus* from Brazil showing several myxospores of *Kudoa eugerres* n. sp. located in pseudocyst structures within the internal GbW tissues near the lumen. **a** Semi-thin section (stained with toluidine blue-Azur II) of a pseudocyst located in the GbW tissues (H), near the lumen (Lu), showing several myxospores (arrows), sectioned at different levels. Inset: detail of the different myxospore sections. **b** Fresh portion of the GbW showing some pseudocyst structures containing myxospores (*), organized among the Gb tissue layers (H). **c** Details of some myxospores (arrows) among host tissues (H), showing the two polar capsules. **d** Group of myxospores (arrows) randomly distributed in the host tissue

Site of infection: The myxospores were agglutinated forming groups with a variable number of myxospores located among the GbW tissues.

Prevalence of infection: Eleven out of 19 fish examined (~ 47%): 7 females (7/19) (~ 37%) and 4 males (4/19) (~ 21%).

Type of specimens: One glass slide with semi-thin sections of the infected tissue of a Gb, containing myxospores of the hapantotype, was deposited in the Myxozoa (Cnidaria)-type collection at the "Instituto Nacional de Pesquisa da Amazônia" ("INPA"), Manaus, Brazil, under the acquisition number (46/2019). The SSU rDNA gene was deposited in GenBank under the accession number MH581487.

Etymology: The specific epithet "*eugerres*" is derived from the generic name of the host species name.

Description of the myxospores of *Kudoa eugerres* n. sp.

Some myxospores contained within the Pcls were in close contact with the thick collagen layer that surrounded these

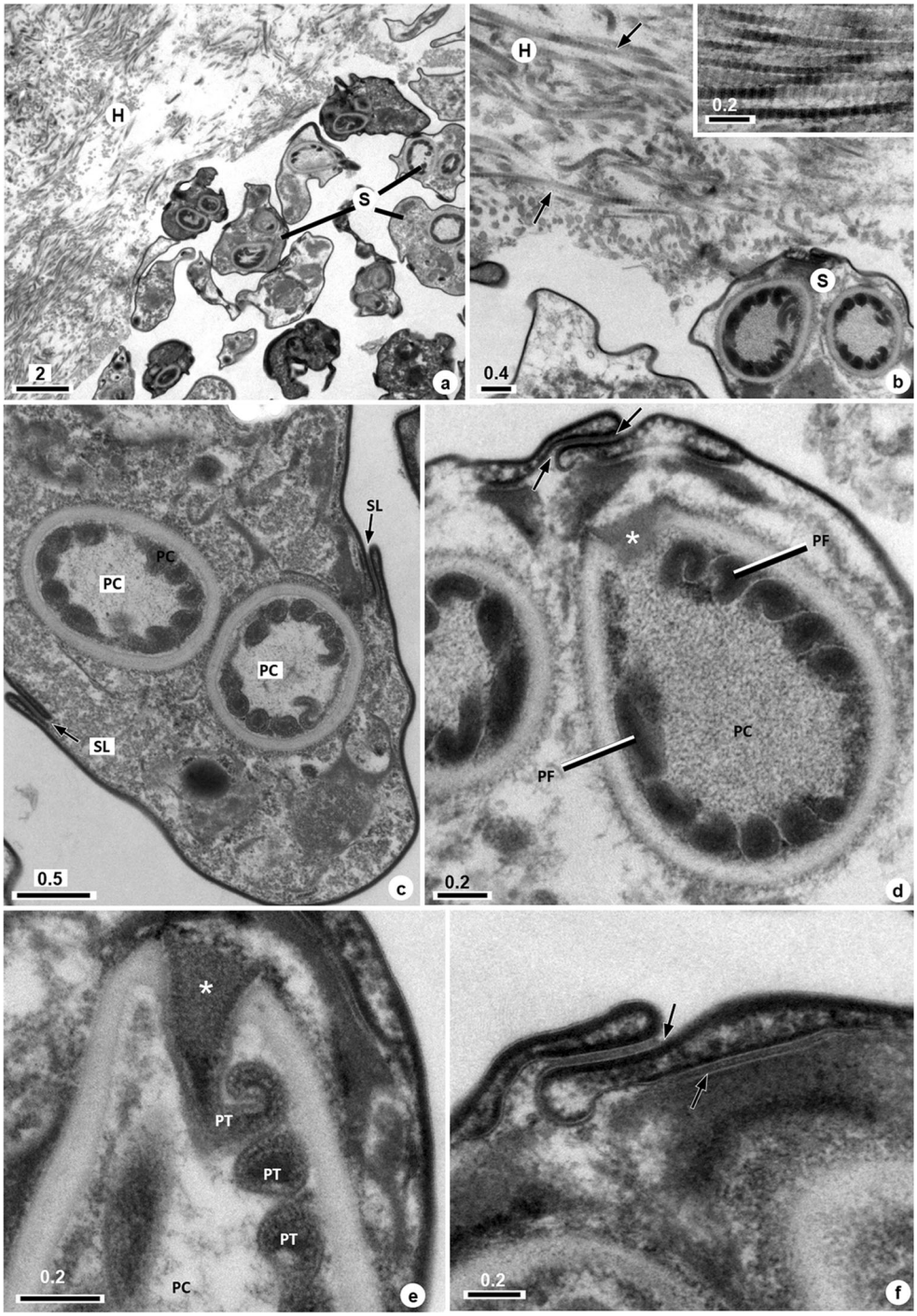


Fig. 2 Ultrastructural aspects of the myxospores of *Kudoa eugerres* n. sp. infecting the GbW of the teleostean *Eugerres brasilianus* in Brazil. **a** Pseudocyst structure composed mainly by numerous disorganized collagen fibers (H) and showing different sections of the myxospores (S). **b** Detail of a similar aspect of the anterior figure showing a myxospore (S) in close contact with the collagen fibers (arrows). Inset: detail of longitudinal sections of collagen fibers. **c** Transverse section of a myxospore showing the wall united along a suture line (SL), the two polar capsules (PC), and the cross-sections of its polar tubule. **d** Longitudinal section of a polar capsule (PC) showing the polar capsule wall, with the orifice for extrusion of the polar tubule (*) located close to the suture line (arrows). **e** Detail of a longitudinal section of the apical region of the polar capsule (PC) showing the polar tubule (PT) and the orifice (*) through which it extrudes. **f** Detail of a cross-section of the valve wall showing the ultrastructural organization of the suture line (arrows)

structures. At these sites, aspects of the degradation of the collagen fibers were evident (Fig. 1a, inset and Fig. 2a, b). Myxospores were pseudo-conical, measuring 5.2 ± 0.8 μm (4.5–6.0) ($n = 30$) in length, 4.3 ± 0.6 μm (3.8–4.7) ($n = 25$) in thickness, and 2.9 ± 0.2 μm (2.7–3.2) ($n = 25$) in width. The two ellipsoidal polar capsules (PC) were located obliquely side by side and opened close to the apical sutural line, each being 1.8 ± 0.4 × 1.2 ± 0.4 μm ($n = 15$) long and wide, respectively (Figs. 1c, d, and 2c, d). In the apical portion of the myxospores, the cap-like structure formed conical structures in continuity with the polar tubules (Fig. 2e). The polar tubule was isofilar and consisted of a single coil with five to six turns, organized obliquely to the PC axis (Fig. 2d, e). The two valves were thin and formed a prominent sutural line with extensive overlap (Fig. 2d, f). Internally, there was a binucleated sporoplasm localized in the basal region of myxospore. Diagrammatic illustration of the myxospore, based on ML and TEM observations, is present in Fig. 3.

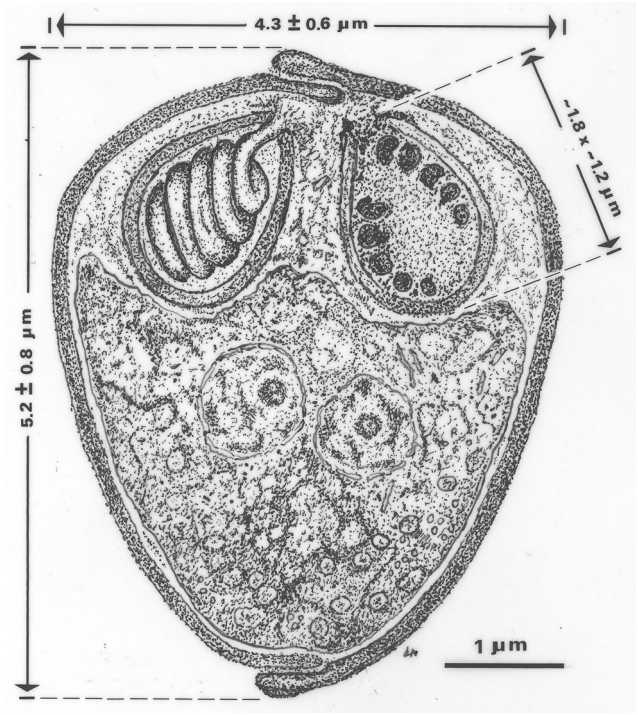


Fig. 3 Schematic drawing of the ultrastructural morphology of a mature myxospore of *Kudoa eugerres* n. sp. showing the different aspects observed in serial ultrathin sections

Reclassification of two species

Sphaerospora dicentrarchi Sitjà-Bobadilla and Alvarez-Pellitero, 1992 is transferred to *Kudoa dicentrarchi* n. comb.

Sphaerospora sp. (*Mugil curema*) Fiala, 2006 is transferred to *Kudoa* sp. (*Mugil curema*) n. comb.

Molecular phylogenetic analysis

Purified PCR products for the SSU rDNA gene with an approximate size of 1450 nt (18e/MXF4R), 460 bp (18e/Kud6r), 1500 bp (MXF/18r), and 550 bp (MXF4F/18r) were directly sequenced, and then assembled into a sequence of consensus of 1676 bp, with a GC content of 47.1%, corresponding almost to the complete SSU rDNA gene. The assembled sequence was deposited in the GenBank database under the accession number MH581487.

A total of 80 rDNA sequences, including those with the highest BLAST scores, were aligned with *Kudoa eugerres* n. sp., resulting in 5013 positions after trimming of the 3′ ends. All BI and ML phylogenetic trees constructed for the selected SSU rDNA sequences revealed very similar topology and showed that *K. eugerres* n. sp. is sister to *K. dicentrarchi* (KT970638), as well as to the *Kudoa* sp. (DQ3777695) found in *Mugil curema*. This clustering was well supported by the posterior probability of 1.00 for BI and bootstrap values of 98% for ML. These three species clustered to form a subclade of the main marine clade, closely related to histozoic species belonging to the genus *Kudoa*, with support of posterior probability/bootstrap values of 1.00/100% (BI/ML) (Fig. 4). For pairwise comparisons, a second alignment was performed with some SSU rDNA gene sequences of species of *Kudoa*, including the two species transferred to genus *Kudoa* and *K. eugerres* n. sp. All ambiguous positions were removed, resulting in a total of 1824 positions in the final dataset. The minimum genetic distance (*p* distance) was 1.9% to the partial sequence (772 nt) of *Kudoa* sp. (DQ377695) and 2.9% to *K. dicentrarchi* (KT970639), corresponding to almost complete sequence (1666 nt). All other analyzed sequences presented genetic distances greater than 5.1% (Table 1). Comparing the SSU rDNA sequences with the sequence obtained in this study, there are a total of 15 and 48 different nucleotides to *Kudoa* sp. (DQ377695) and *K. dicentrarchi* (KT970639), respectively.

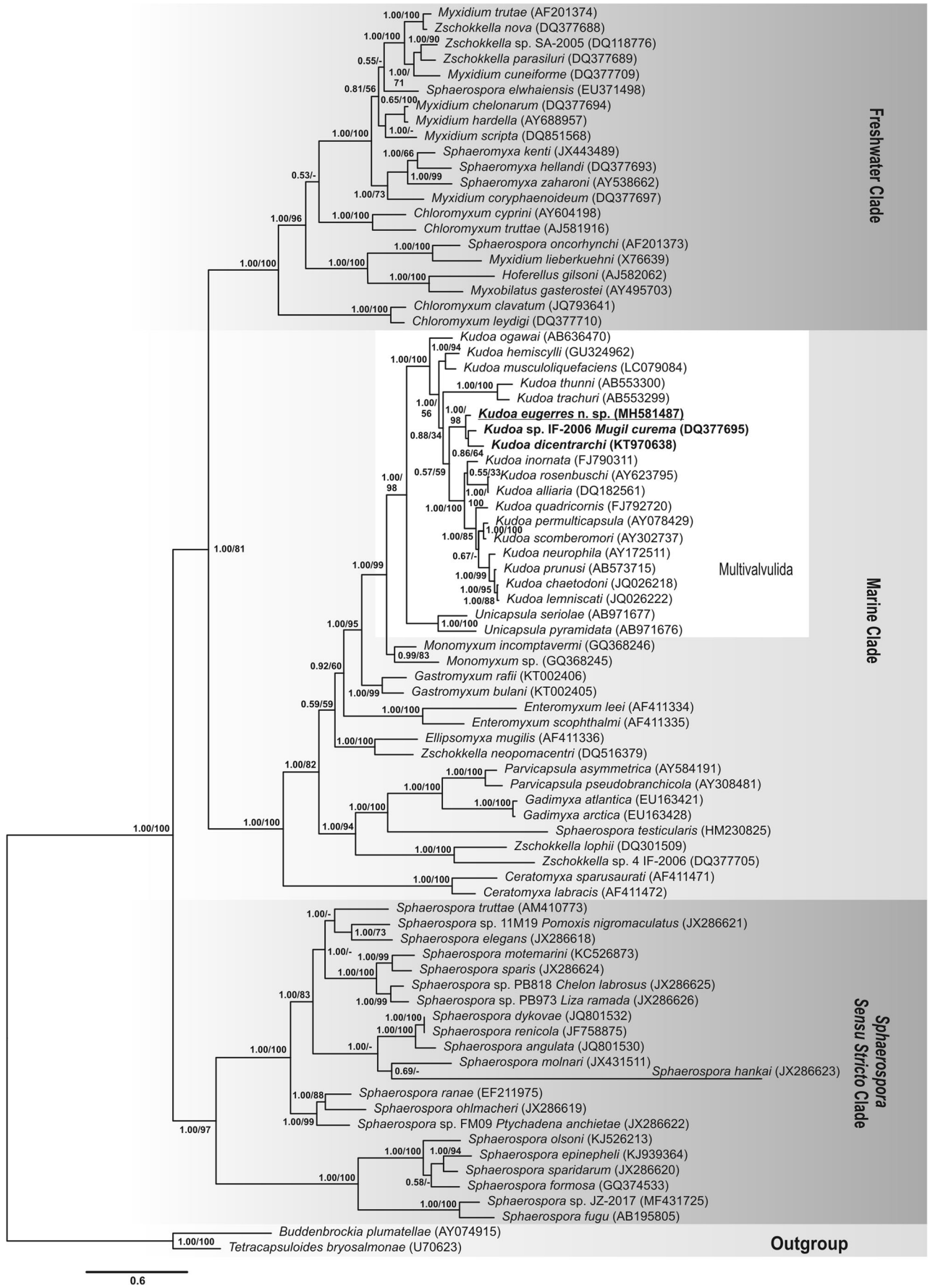
Histopathology

Some free mature myxospores were found floating in the bile. The Pcls located in the mucosa of the GbW, and containing mature myxospores, were formed by a thick layer of collagen fibers, among which no host cell was observed (Fig. 2a, b, and inset). Several ultrastructural aspects of the collagen fibers contacting directly to the myxospores were observed (Fig. 2a–f). The surface of the collagen fibers contacting the myxospores appeared disorganized and degraded, suggesting that the myxospores were later released into the Gb lumen.

Remarks

Comparison of the site of infection, morphology, and morphometry of the myxospores to other species previously described shows the new parasite presenting some similarity to two histozoic *Kudoa* spp.: more specifically, *K. dicentrarchi* (Stijà Bobadilla and Alvarez Pellitero 1992) and *Kudoa* sp. (*Mugil curema*) Fiala, 2006. The myxospores of *Kudoa eugerres* n. sp., *K. dicentrarchi*, and *Kudoa* sp. (*Mugil curema*) exhibit reduced dimensions, probably the smallest among Myxozoa. However, the specificity of some morphometric features, combined with the molecular data acquired, demonstrates that the parasite described here is a new species (Table 2). The main differences are the host (genus and family), as well as the geographic localization; the myxospore shape, namely because of *K. eugerres* n. sp. has a ratio length/thickness of 1.2, while *K. dicentrarchi* and *Kudoa* sp. (*Mugil curema*) have a ratio length/thickness of 0.85 and 0.80,

Fig. 4 Phylogenetic tree obtained from Bayesian analysis of the SSU rDNA gene sequence of *Kudoa eugerres* n. sp. and related myxozoans. Numbers at the nodes represent Bayesian posterior probabilities/maximum likelihood bootstrap values; dashes represent a different branching for the maximum likelihood tree


Myxidium trutae (AF201374)
Zschokkella nova (DQ377688)
Zschokkella sp. SA-2005 (DQ118776)
Zschokkella parasiluri (DQ377689)
Myxidium cuneiforme (DQ377709)
Sphaerospora elwhaiensis (EU371498)
Myxidium chelonarum (DQ377694)
Myxidium hardella (AY688957)
Myxidium scripta (DQ851568)
Sphaeromyxa kenti (JX443489)
Sphaeromyxa hellandi (DQ377693)
Sphaeromyxa zaharoni (AY538662)
Myxidium coryphaenoideum (DQ377697)
Chloromyxum cyprini (AY604198)
Chloromyxum truttae (AJ581916)
Sphaerospora oncorhynchi (AF201373)
Myxidium lieberkuehni (X76639)
Hoferellus gilsoni (AJ582062)
Myxobilatus gasterostei (AY495703)
Chloromyxum clavatum (JQ793641)
Chloromyxum leydigi (DQ377710)
Freshwater Clade
Kudoa ogawai (AB636470)
Kudoa hemiscylli (GU324962)
Kudoa musculoliquefaciens (LC079084)
Kudoa thunni (AB553300)
Kudoa trachuri (AB553299)
Kudoa eugerres n. sp. (MH581487)
Kudoa sp. IF-2006 Mugil curema (DQ377695)
Kudoa dicentrarchi (KT970638)
Kudoa inornata (FJ790311)
Kudoa rosenbuschi (AY623795)
Kudoa alliaria (DQ182561)
Kudoa quadricornis (FJ792720)
Kudoa permulticapsula (AY078429)
Kudoa scomberomori (AY302737)
Kudoa neurophila (AY172511)
Kudoa prunusi (AB573715)
Kudoa chaetodoni (JQ026218)
Kudoa lemniscati (JQ026222)
Multivalvulida
Unicapsula seriolae (AB971677)
Unicapsula pyramidata (AB971676)
Monomyxum incomptavermi (GQ368246)
Monomyxum sp. (GQ368245)
Gastromyxum rafii (KT002406)
Gastromyxum bulani (KT002405)
Enteromyxum leei (AF411334)
Enteromyxum scophthalmi (AF411335)
Ellipsomyxa mugilis (AF411336)
Zschokkella neopomacentri (DQ516379)
Parvicapsula asymmetrica (AY584191)
Parvicapsula pseudobranchicola (AY308481)
Gadimyxa atlantica (EU163421)
Gadimyxa arctica (EU163428)
Sphaerospora testicularis (HM230825)
Zschokkella lophii (DQ301509)
Zschokkella sp. 4 IF-2006 (DQ377705)
Ceratomyxa sparusaurati (AF411471)
Ceratomyxa labracis (AF411472)
Marine Clade
Sphaerospora truttae (AM410773)
Sphaerospora sp. 11M19 Pomoxis nigromaculatus (JX286621)
Sphaerospora elegans (JX286618)
Sphaerospora motemarini (KC526873)
Sphaerospora sparis (JX286624)
Sphaerospora sp. PB818 Chelon labrosus (JX286625)
Sphaerospora sp. PB973 Liza ramada (JX286626)
Sphaerospora dykovae (JQ801532)
Sphaerospora renicola (JF758875)
Sphaerospora angulata (JQ801530)
Sphaerospora molnari (JX431511)
Sphaerospora hankai (JX286623)
Sphaerospora ranae (EF211975)
Sphaerospora ohlmacheri (JX286619)
Sphaerospora sp. FM09 Ptychadena anchietae (JX286622)
Sphaerospora olsoni (KJ526213)
Sphaerospora epinepheli (KJ939364)
Sphaerospora sparidarum (JX286620)
Sphaerospora formosa (GQ374533)
Sphaerospora sp. JZ-2017 (MF431725)
Sphaerospora fugu (AB195805)
Sphaerospora Sensu Stricto Clade
Buddenbrockia plumatellae (AY074915)
Tetracapsuloides bryosalmonae (U70623)
Outgroup
0.6

respectively; and different number of polar tubule coils in relation to *K. dicentrarchi*. Despite these 3 species having the wall of the Gb as the site of infection, *K. dicentrarchi* infects predominantly the muscularis of the Gb and intestine (Sitjà-Bobadilla and Alvarez-Pellitero 1992), while *K. eugerres* n. sp. develops in the mucosa, close to the epithelial tissue. Histological observations were not performed for the *Kudoa* sp. (*Mugil curema*) reported by Fiala (2006), so it is not possible to accurately indicate the tissue of infection.

Discussion

According to the literature, *Sphaerospora* spp. are a group of parasites infecting marine, brackish, and freshwater fishes, typically displaying coelozoic development in the urinary system (Lom and Dyková 2006), such as in the renal corpuscles (Dyková and Lom 1997), renal tubules (El-Matbouli and Hoffman 1996; Dyková and Lom 1997; Liu et al. 2018), urinary bladder, and ureters (Lom and Dyková 2006; Gunter and Adlard 2010). Occurrences as histozoic parasites in the gills (Lom et al. 1983), testis (Sitjà-Bobadilla and Alvarez-Pellitero 1990), ovary (Xiao and Desser 1997), intestine, and Gb (Sitjà-Bobadilla and Alvarez-Pellitero 1990, 1992) have been also reported, although in a much smaller number. In the case of Gb infections, there are 2 species of the genus *Sphaerospora* known to have coelozoic development, both from marine fishes, and without molecular information. In Tasmania, *S. aldrichetta* was found in the teleostean *Aldrichetta forsteri* (Su and White 1994), and in the Yellow Sea of China; *S. sebasta* was described in *Sebastes schlegelii* (Zhao et al. 2015).

The singularity of the systemic parasite *K. dicentrarchi* has been reported in several studies that establish the parasite having histozoic development, usually developing in the muscularis of the gallbladder and intestine, and less frequently in the epithelial tissues of the gallbladder, intestine and trunk kidney, and the connective tissues of the gonads, kidney, pancreas, spleen, and swim bladder. This species has been reported from marine fishes of high commercial value: European seabass *D. labrax* (Sitjà-Bobadilla and Alvarez-Pellitero 1992, 1993; Fioravanti et al. 2004) and *D. punctatus* (Xavier et al. 2013). Posteriorly, in the Gb of *Mugil curema* from the Caribbean Sea, another histozoic parasite of the genus *Kudoa* was reported (Fiala 2006). Recently, the SSU rDNA sequence of an actinospore classified as a new tetractinomyxon and found in the Polychaete of the genus *Capitella* allowed to molecularly infer the life cycle of *K. dicentrarchi* (Rangel et al. 2016).

Despite these three parasites displaying similar morphology to *Sphaerospora* spp., namely by having 2 polar capsules positioned perpendicularly to the sutural line formed by 2 valves, they are significantly smaller than all others and have histozoic development in internal tissues of marine fishes. In turn, true Sphaerosporids are widely reported as being predominately freshwater coelozoic species displaying affinity to the excretory system (Patra et al. 2018). Furthermore, ultrastructural analyses show that both *K. eugerres* n. sp. and *K. dicentrarchi* have thin valves that overlap at the sutural line (Sitjà-Bobadilla and Alvarez-Pellitero 1992), following what has been commonly reported for *Kudoa* and *Unicapsula* spp. (Diamant et al. 2005; Al-Jufaili et al. 2015). On the other hand, the sporoplasm cell of these two uncommon *Kudoa* spp. is binucleated, contrarily to what has been reported for *Sphaerospora* spp., which have two or more uninucleated sporoplasm cells, and also *Kudoa* spp., which are characterized by two uninucleated sporoplasm cells, one being located within the other.

Several aspects of GbW rupture were observed, showing a possible direct contact of the myxospores with the bile, and consequent liberation of the myxospores, which also appeared floating in the bile. In more advanced stages of the disease, enteric *Sphaerospora* sp. infecting groupers of the genus *Epinephelus* in the South China Sea presented similar aspects, with complete degradation of the intestinal mucosa and submucosa resulting in the release of sporogonic stages into the organ cavity (Liu et al. 2018).

Analyzing the genetic distance between these 3 histozoic parasites, the new species described in this paper presents a significant number of different nucleotides, even to the small partial sequenced region of *Kudoa* sp. IF-2006 (DQ377695). Considering the genetic distances between *Kudoa* spp. differences of less than 1% have been reported between species, such as *K. permulticapsula* (AY078429), *K. scomberomori* (AY302737), *K. alliaria* (DQ182561), and *K. rosenbuschi* (AY623795) (Table 1).

The molecular and phylogenetic analysis performed here show *K. eugerres* n. sp., *K. dicentrarchi*, and *Kudoa* sp. IF-2006 clustering together to form a histozoic subclade of the largest marine monophyletic histozoic clade of multivalvulids species (the *Kudoa* clade). Thus, these 3 *Kudoa* spp. are phylogenetically distant to all other Sphaerosporids, including those that are also considered as *Sphaerospora* spp. *s.l.* The main freshwater clade houses 2 *Sphaerospora* spp. of the urinary system: *S. elwhaiensis* (Jones et al. 2011) and *S. oncorhynchi* (Kent et al. 1998). Finally, *S. testicularis* (Bartošová et al. 2011) is located within the main marine clade, being sister to the genera *Gadimyxa* and *Parvicapsula*. Like previous phylogenetic studies that focused on the evolution of *Sphaerospora* spp. (Fiala 2006; Bartošová et al. 2011, 2013), we also showed a very close relationship of histozoic Gb (*K. dicentrarchi* and *Kudoa* sp. IF-2006) and *K. eugerres* n. sp. with the great monophyletic clade of *Kudoa* spp. being in common the habitat and the proliferation in tissues.

Table 1 Comparison of some SSU rRNA gene sequences of marine clade composed by histozoic species of *Kudoa* clade: pairwise distance (bottom diagonal) obtained by *p* distance. MEGA 7. The number of base differences per site between sequences is shown. All ambiguous positions removed for each sequence pair. (Nt) Number of nucleotides of the sequences

Genus *Kudoa*	Nt	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
(1) *K. eugerres* n. sp. (MH581487)	1676	–																	
(2) *Kudoa* sp. (DQ377695)	772	0.019	–																
(3) *K. dicentrarchi* (KT970638)	1666	0.029	0.027	–															
(4) *K. musculoliquefaciens* (LC079084)	5082	0.051	0.049	0.058	–														
(5) *K. hemiscylli* (GU324962)	1722	0.056	0.052	0.066	0.029	–													
(6) *K. neurophila* (AY172511)	1552	0.057	0.052	0.069	0.059	0.066	–												
(7) *K. inornata* (FJ790311)	1732	0.059	0.047	0.069	0.055	0.055	0.036	–											
(8) *K. quadricornis* (FJ792720)	1683	0.062	0.048	0.073	0.063	0.062	0.029	0.039	–										
(9) *K. chaetodoni* (JQ026218)	1599	0.063	0.051	0.073	0.070	0.069	0.015	0.043	0.033	–									
(10) *K. ogawai* (AB636470)	1671	0.065	0.059	0.071	0.052	0.057	0.061	0.069	0.070	0.069	–								
(11) *K. prunusi* (AB573715)	1679	0.065	0.051	0.074	0.070	0070	0.016	0.043	0.035	0.004	0.073	–							
(12) *K. alliaria* (DQ182561)	1680	0.065	0.046	0.071	0.063	0.063	0.036	0.040	0.054	0.046	0.072	0.049	–						
(13) *K. permulticapsula* (AY078429)	1679	0.066	0.051	0.077	0.063	0.064	0.028	0.038	0.026	0.033	0.072	0.032	0.048	–					
(14) *K. scomberomori* (AY302737)	1680	0.066	0.047	0.076	0.062	0.064	0.023	0.039	0.025	0.029	0.069	0.029	0.047	0.008	–				
(15) *K. rosenbuschi* (AY623795)	1740	0.066	0.048	0.072	0.062	0.064	0.036	0.040	0.055	0.046	0.072	0.050	0.001	0.048	0.048	–			
(16) *K. lemniscati* (JQ026222)	1592	0.067	0.051	0.074	0.076	0.072	0.015	0.047	0.039	0.001	0.075	0.007	0.052	0.035	0.031	0.052	–		
(17) *K. trachuri* (AB553299)	1776	0.085	0.074	0.093	0.087	0.086	0,096	0.094	0.101	0.106	0.096	0.106	0.098	0.103	0.102	0.098	0.111	–	
(18) *K. thunni* (AB553300)	1763	0.090	0.079	0.099	0.095	0.092	0.098	0.096	0.100	0.107	0.099	0.110	0.102	0.103	0.101	0.102	0.111	0.041	–

Table 2 Morphological characteristics of the most similar species of the genus *Kudoa* (all measures in μm)

Genus *Kudoa*	Host—Family	Local of infection	Country	SpL	SpT	SpW	PCL × W	PTc	Authors
K. dicentrarchi	*Dicentrarchus labrax*—Fam. Serranidae	Muscularis wall of gallbladder and intestine	Spain (a)	3.5–6.0 4.5 ± 0.5	4.6–8.0 5.3 ± 0.5	3.5–4.0 3.9 ± 0.2	1.8 ± 0.3 × 1.4 ± 0.3	4–5	Stija-Bobadilla and Alvarez-Pellitero 1992
Kudoa sp.	*Mugil curema*—Fam. Mugilidae	Wall of gallbladder	Caribbean Sea	6.9 (6–8)	8.6 (8–9)	–	4.0 × 2.5	5–6	Fiala 2006
K. eugerres n. sp.	*Eugerres brasilianus*—Fam. Gerreidae	Mucosa wall of gallbladder	Brazil (b)	5.2 ± 0.8	4.3 ± 0.6	2.9 ± 0.2	1.8 ± 0.4 × 1.2 ± 0.4	5–6	Present study

SpL, myxospore length; *SpT*, myxospore thickness; *SpW*, myxospore width; *PCL* × *W*, polar capsule length × width; *PTc*, polar tubule coils; (a), Mediterranean sea coast; (b), Brazilian Atlantic coast

Several phylogenetic studies have shown that the species considered to be true Sphaerosporids, the *Sphaerospora s.s.*, cluster among each other to form a well-supported clade that is basal to all other Myxosporea. In turn, the species comprising the so-called *Sphaerospora s.l.* are scattered across several other clades of the Myxosporea, acknowledging the necessity of revising the taxonomy of this genus, either through the erection of new genera or through the transference of species to other more appropriate genera.

In the last years, as a result of the increasing availability of genetic information, it has been possible to verify that several species are poorly classified because taxonomy was established exclusively on the basis of morphology. Consequently, several taxonomic revisions of the Myxosporea have been performed in recent years. In 2014, Atkinson et al. created the genus *Ceratonova* to include the well-known freshwater species *Ceratomyxa shasta* and another one *C. gasterosteus* n. sp. Members of *Ceratomyxa* constitute one of the main monophyletic coelozoic group of the marine clade and parasitize the Gb or urinary system of marine teleosts and elasmobranchs. In turn, *Ceratonova* spp. infects the intestine of the freshwater fish *Gasterosteus aculeatus* and is phylogenetically distant from all other *Ceratomyxa* spp. (Fiala 2006; Atkinson et al. 2014; Fiala et al. 2015). On the other hand, the genus *Polysporoplasma* was extinguished and its 2 species transferred to the genus *Sphaerospora* since they group within the basal clade formed by *Sphaerospora s.s.*, differing only by having multiple sporoplasms (Bartošová et al. 2013). Extinction of genera has also occurred within the order Multivalvulida. Based on phylogenetic analyses, the genera *Pentacapsula*, *Hexacapsula*, and *Septemcapsula* were synonymized with *Kudoa*, turning the latter into the largest monophyletic genus composed of marine and histozoic species (Whipps et al. 2004). Considering the great plasticity of the order Multivalvulida, which envelops representatives with 3 to 13 valves, each one with a polar capsule, it is justifiable the inclusion of some species that present some morphological similarities with bivalvulids (myxospores formed by 2 valves). In the future, there should be an attempt to reassess the taxonomic classification of the remaining 3 *Sphaerospora s.l.* (2 freshwater and 1 marine).

Conclusion

In this study, the morphology and ultrastructural aspects of the myxospore, associated with the 18S rDNA gene sequence analysis, confirm that this parasite constitutes a new myxosporean species. Considering that this species displayed mixed morphological characters of a histozoic Bivalvulida and Multivalvulida species, inhabits the marine environment and is phylogenetically closely related to the *Kudoa* clade, it was classified as *Kudoa eugerres* n. sp. Consequently, the 2

Sphaerospora s.l., *Sphaerospora dicentrarchi*, and *Sphaerospora* sp. (*Mugil curema*) should be transferred to *Kudoa dicentrarchi* n. comb. and *Kudoa* sp. (*Mugil curema*) n. comb. Finally, the diagnosis of the order Multivalvulida Shulman, 1959; Family Kudoidae Meglitsch, 1960; and genus *Kudoa* Meglitsch, 1947 was amended in order to include myxospores of 2–13 (mostly 4) polar capsules and valves, valves that are very thin and overlapping at the sutural line.

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Compliance with ethical standards

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. This study carried out according to Brazilian laws (MMA-ICMbio, license MMA—56475-1 to LAQUA—UFAL).

Conflict of interest All authors declare that they have no conflict of interest.

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