Ultrastructural and molecular characteristics of *Kudoa crenimugilis* n. sp. infecting intestinal smooth muscle of fringelip mullet *Crenimugil crenilabis* in the Red Sea

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ABSTRACT: This study describes infection of intestinal smooth muscle in fringelip mullets *Crenimugil crenilabis* with *Kudoa crenimugilis* n. sp. Of 30 individuals sampled from the Red Sea off Saudi Arabia, 6 (20%) were infected. Ovoid plasmodia (279–412 × 157–295 µm) in the smooth muscle of the intestine were packed with only mature myxospores with 4 valves. Specifically, light and transmission electron microscopy revealed quadrate myxospores with 4 equal, rounded, spore valves uniting at thin delicate suture lines. The mature myxospores were 8 (7–9) µm long, 5.2 (5–6) µm thick and 7.8 (7–8) µm wide. The 4 polar capsules were equal-sized, elliptical to ovoid, and measured 5 (4–5) µm long and 2 (1.5–3) µm wide, possessing 2 filament coils. The sporoplasm was uninucleated and composed of a primary cell enveloping a secondary cell. The parasite had a significant histopathological impact since the developing plasmodia replaced normal muscle tissue and was associated with the myolysis of local muscle fibres and the inflammatory infiltration of lymphocytes and macrophages. The partial sequences of the 18S and 28S rDNA showed that *K. crenimugilis* n. sp. has the highest level of nucleotide similarity with *K. ciliatae* (98.46 and 94.11%, respectively) and *K. cookii* (97.51 and 92.11%, respectively), both of which have previously been reported from the intestines of their host fish. Phylogenetic analysis revealed that *K. crenimugilis* consistently clustered with these other 2 intestinal *Kudoa* species in a well-supported subclade, confirming the evaluative association between *Kudoa* species infecting the same organs.

KEY WORDS: Myxosporea · *Kudoa* · Histozoic · Intestine · TEM · SSU rRNA · LSU rRNA

INTRODUCTION

Myxozoans are a diverse group of microparasites widely dispersed among fishes and, more rarely, amphibians and reptiles (Lom & Dyková 2006). The genus *Kudoa* was established by Meglitsch (1947) to include histozoic myxozoans that are mostly parasitic within the skeletal muscle of fish, although they have also been found to infect other organs such as the heart, gills, kidney, brain, gall bladder, ovary and intestines (Moran et al. 1999, Eiras et al. 2014). *Kudoa* spp. are characterized by their production of radially

symmetrical quadrate or stellate myxospores with delicate spore membranes. These myxospores consist of 3 to 7 shell valves with often indistinct sutures between them and with each valve having a polar capsule (Whipps et al. 2004, Lom & Dyková 2006, Holzer et al. 2006).

Kudoa is currently comprised of 96 nominal species distributed throughout marine and estuarine fishes worldwide (Abdel-Baki et al. 2016). Several species of *Kudoa* exhibit a high degree of host specificity and have only been recovered from the type host (e.g. *K. atropi*, *K. hanchiata*). In contrast, other species have a worldwide distribution, such as *K. thyrsites*, which has been reported in more than 38 fish species (Kristmundsson & Freeman 2014). Recently, molecular phylogeny based on small subunit (SSU) rDNA sequences has revealed that *Kudoa* spp. with the same tissue tropism cluster with each other, regardless of fish host taxon and geographical location (Matsukane et al. 2010, Heiniger et al. 2013).

Mullets are ray-finned fish widely recognized as euryhaline species that can thrive in fresh or estuarine waters (Saleh 2008). Mullets are a valuable source of food in various parts of the world (Yurakhno & Ovcharenko 2014), and the growth of mullet aquaculture has led to an increased focus on the pathogenic potential of parasites in general and of Myxozoa in particular. To date, 64 myxosporean species belonging to 13 genera have been described in mullets worldwide, with 10 of these being representatives of *Kudoa* (Yurakhno & Ovcharenko 2014). Of all the myxosporean species infecting mullets, however, only *Myxobolus exiguus* Thelohan, 1895 has hitherto been reported in the fringelip mullet *Crenimugil crenilabis* (Forsskål) (see Pulsford & Matthews 1982). It is in this context that this paper describes a new species of *Kudoa* infecting the smooth muscle of the intestine of *C. crenilabis* from the Red Sea off Saudi Arabia.

MATERIALS AND METHODS

Samples

Thirty fringelip mullet were collected during April 2015 from the Red Sea off Yanbu (24° 05′ N 38° 00′ E) on the western coastline of Saudi Arabia. Muscles, visceral organs and brains were examined for the presence of myxosporean plasmodia. Fresh myxospores were photographed and measured according to the recommendations of Lom & Arthur (1989). Measurements were based on 50 myxospores and are expressed in µm. Schematic hand drawings were made from the photographs. For histology, 2 heavily infected parts of the intestine of 3 fish were fixed in 10% neutral-buffered formalin. The fixed tissues were then embedded in 3 paraffin blocks (1 for each fish), sectioned and stained with haematoxylin and eosin, and observed by light microscopy. For transmission electron microscopy, *Kudoa* plasmodia were fixed in 3% (v/v) glutaraldehyde in a 0.1 M sodium cacodylate buffer (pH 7.4) and post-fixed in 2.0% (v/v) osmium tetroxide in the cacodylate buffer. Samples were then dehydrated and embedded in Epon. Semi-thin sections were cut with a Leica ultracut UC7 and stained with toluidine blue. Ultra-thin sections were prepared and double stained with uranyl acetate and lead citrate and examined under a JEOL-JSM-1011 electron microscope operated at 80 kV.

Molecular analysis

The genomic DNA of the new species of *Kudoa* isolated in this study was extracted from plasmodia preserved in absolute ethanol using the QIAamp DNA Mini Kit (Qiagen) according to the manufacturer's instructions. Partial sequences of the 18S rRNA gene were amplified using the primers MyxF144 and MyxR1944, as reported by Mansour et al. (2014). The 28S fragment was amplified using the primers Kt28S1F and 28S1R (Whipps et al. 2004). Amplifications were performed in a final volume of 20 µl, containing 1 µl (50 ng) of genomic DNA, 1 µl of each primer (10 pmol), 6 µl of 5× FIREPol® Master Mix (Solis BioDyne) and 11 µl of nuclease-free water. Cycling conditions were as follows: 1 cycle of 94°C for 5 min; 30 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 2 min; and 1 cycle of 72°C for 10 min. The obtained PCR products were purified, and sequence reactions were conducted by Macrogen using the same primers as used for the PCR reactions.

For phylogenetic analysis based on the SSU rDNA gene, 49 sequences of related *Kudoa* species were retrieved from the GenBank database, including those reported earlier from the Arabian Gulf and Red Sea by Mansour et al. (2014, 2015) and Abdel-Baki et al. (2016). The sequence of *Ellipsomyxa gobii* was used as the outgroup. The species used for SSU rDNA gene analysis were also used for analysis based on the large subunit (LSU) rRNA gene, except for those lacking this sequence in GenBank. *Myxobolus cerebralis* was the outgroup species in this case. The sequences were aligned with ClustalX 2.1.0.12 software, applying the default parameters

(Larkin et al. 2007). The resulting alignment was exported as fasta and nexus files, edited and used for phylogenetic studies. Phylogenetic analysis was conducted with maximum likelihood (ML) and Bayesian inference (BI) methods. The tree inferred with the ML method was constructed using the Molecular Evolutionary Genetics Analysis (MEGA) software version 7.0.21 (Kumar et al. 2016) with bootstrap validation based on 1000 replicates with the modeltest GTR + I + G for nucleotide substitution. BI analysis was run using the program Mr-Bayes version v3.2.5 (Ronquist et al. 2012) for 1000000 generations for the SSU rRNA sequences and 6200000 generations for LSU rRNA sequences, respectively. The posterior probabilities were approximated using the Metropolis-Coupled Markov Chain Monte Carlo (MCMC) algorithm. Trees were sampled every 100 generations (samplefreq = 100). The first 25% of the trees sampled in each Bayesian run were discarded as burn-in to ensure sampling of the chain at stationarity. The consensus trees were obtained after an average standard deviation of split frequencies of 0.0044 for the SSU rRNA and 0.0009 for the LSU rRNA.

RESULTS

Plasmodia were seen in fresh preparations of intestinal wall fragments viewed under the light microscope; when squashed, large numbers of myxospores belonging to *Kudoa* were observed. Histological examination revealed intracellular ovoid plasmodia in the smooth muscle of the intestine, measuring 297−412 × 157−295 µm in size. The investigated plasmodia were packed with aggregates of numerous mature *Kudoa* myxospores and encompassed by a thin layer of fibrous tissue (Fig. 1). Regardless of their size, the plasmodia did not penetrate the submucosa or mucosa, and the intestinal lumen was never observed to be occluded or obstructed. The histopathological analysis of the parasitized tissue revealed replacement of muscle with developing plasmodia, myolysis of the local muscle fibres around the plasmodia and lymphocytic infiltrates and macrophages. Twenty percent (6/30) of the fish sampled in this survey were infected.

Description of the spores

Light microscopy

The observed myxospores are typical of *Kudoa*. Mature spores are tetramerous radially symmetric, quadrate to rounded in apical view, ovoid in lateral view with 4 equal valves, 8 (7−9) µm long, 5.2 (5−6) µm thick and 7.8 (7−8) µm wide (Figs. 2 & 3). Four polar capsules are situated at the anterior end of the spore, elliptical to ovoid and of roughly uniform size, 5 (4−5) µm long and 2 (1−3) µm wide (Figs. 2 & 3). The polar filament has 2 coils that are indistinct in fresh spores but can be counted in the electron micrographs as shown in the line drawing (Fig. 3).

Ultrastructure

Myxospores have 4 symmetrical shell valves uniting at delicate and thin suture lines (Fig. 4A,B). Myxospore walls are thin, smooth, enclosing 4 equal polar capsules, placed at the same level, with 1 positioned in each of the shell valves (Fig. 4A,B). The
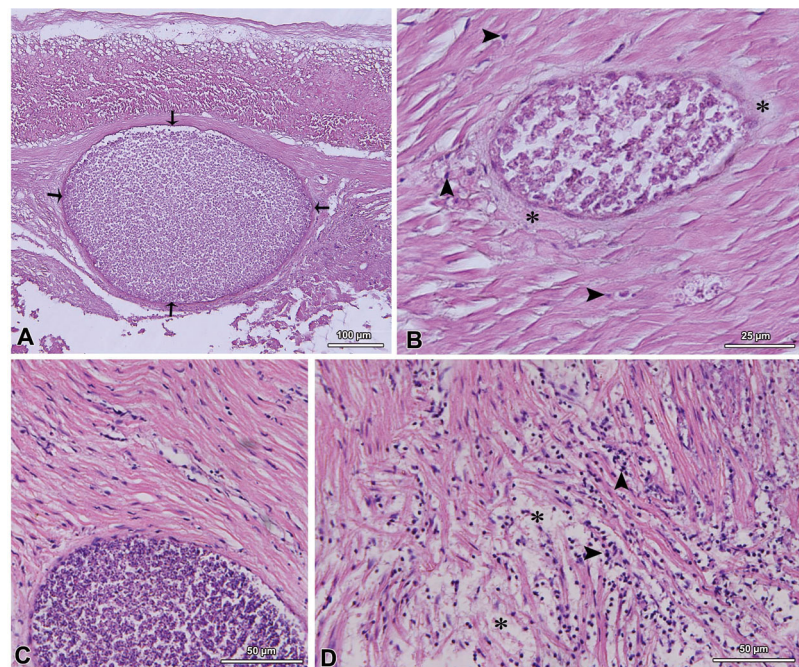

Fig. 1. *Kudoa crenimugilis* n. sp. infecting the intestinal smooth muscle of fringelip mullet *Crenimugil crenilabis*. (A−D) Plasmodia of *K. crenimugilis* in the smooth muscle of the intestine encompassed by a thin layer of fibrous tissue (arrows); H&E stain. Plasmodia were surrounded with local myolysis (asterisks) and inflammatory cellular infiltration (arrowheads)
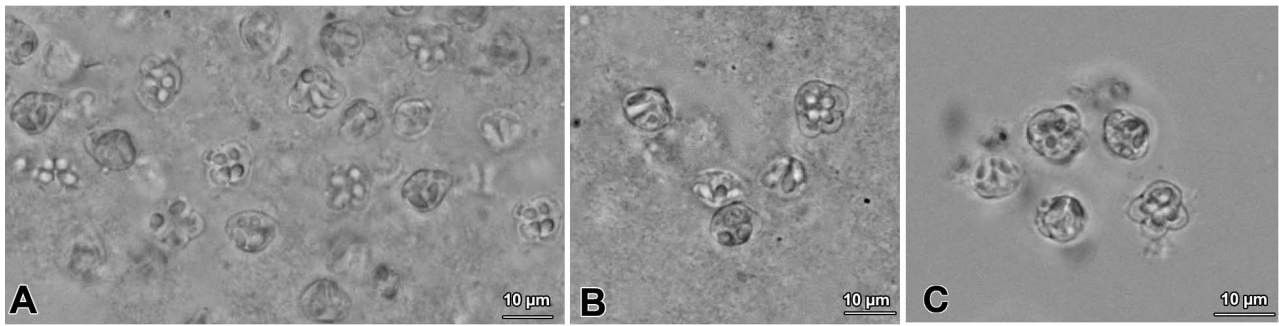
Fig. 2. Fresh myxospores of *Kudoa crenimugilis* n. sp. infecting the intestinal smooth muscle of fringelip mullet *Crenimugil crenilabis*
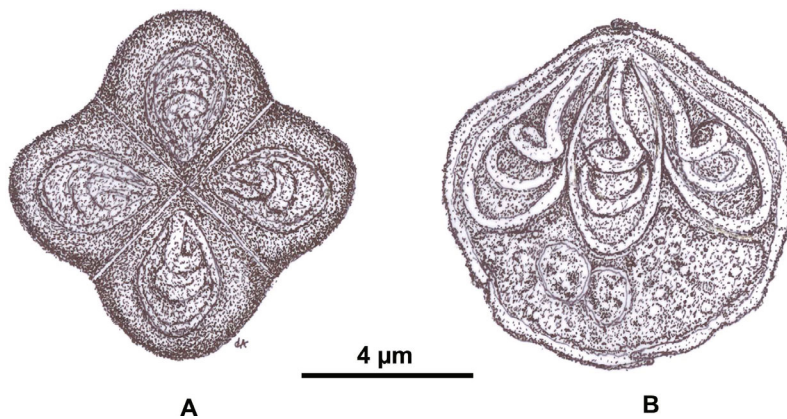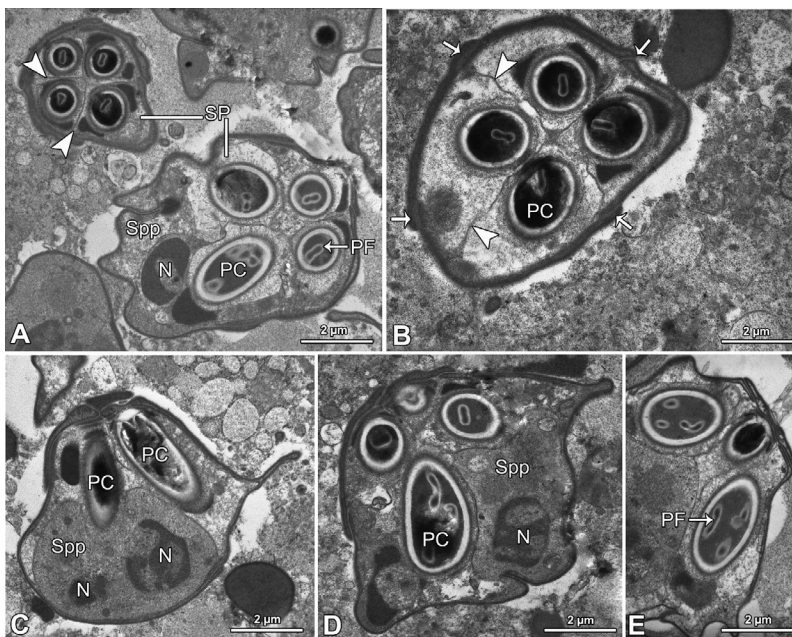

Fig. 3. Myxospores of *Kudoa crenimugilis* n. sp. infecting the intestinal smooth muscle of fringelip mullet *Crenimugil crenilabis*. (A) Top view, (B) lateral view

polar filament has 2 coils slightly oblique to the polar capsule's longitudinal axis. Sporoplasm is single with 2 uninucleated cells, i.e. a primary cell enveloping a secondary cell (Fig. 4C,D).

Taxonomic summary of *Kudoa crenimugilis* n. sp.

Type host: Fringelip mullet *Crenimugil crenilabis* (Forsskål, 1775) (Teleostei, Perciformes, Mugilidae).

Type locality: Red Sea, off Yanbu (24° 05′ N, 38° 00′ E) on the western coastline of Saudi Arabia.

Site of infection: Smooth muscle of the intestine.

Prevalence: 20 % (6/30).

Type material: One microscope slide of Giemsa-stained myxospores and another slide of H&E-stained plasmodia in smooth muscle of the intestine are deposited as syntypes in the

Fig. 4. *Kudoa crenimugilis* n. sp. infecting the intestinal smooth muscle of fringelip mullet *Crenimugil crenilabis*. (A,B) Ultrastructural aspects of some myxospores (SP), showing the position of the 4 polar capsules (PC) with polar filament (PF), sporoplasm (Spp) with nucleus (N) as well as the suture lines (arrowheads) and the points at which the valves unite (arrows). (C–E) Ultrastructure of myxospores in lateral position showing the PC in the apical region with PF and the sporoplasm of 2 uninucleated cells (N) in the posterior region

Parasitological Collection of the Hungarian Natural History Museum with the inventory number (HNHM-PAR-71846). In addition, the SSU rDNA gene and LSU rDNA gene sequences were deposited in GenBank under accession numbers MG592203 and MG592204, respectively.

Etymology: The specific epithet '*crenimugilis*' is given in reference to the fish host genus *Crenimugil*.

Phylogeny

Partial consensus sequences of the SSU rDNA gene (1480 bp) and LSU rDNA gene (733 bp) were obtained from *K. crenimugilis* n. sp. and deposited in GenBank under accession numbers MG592203 and MG592204), respectively. Pairwise alignments show that the most similar sequences were those of *K. ciliatae* (Lom et al. 1992) (98.5 and 94.1%, respectively) and *K. cookii* (Heiniger et al. 2013) (97.5 and 92.1%, respectively) for the SSU and LSU rRNA sequences. The lowest similarity was observed with *K. quraishii* (Mansour et al. 2014) (84.9%), which was reported infecting the skeletal muscle tissues of a scombrid, Indian mackerel *Rastrelliger kanagurta* from the Red Sea and Arabian Gulf. The trees derived using BI and ML were very similar, with only slight differences in some branch nodes. Both the SSU rRNA and LSU rRNA-based trees placed the new *Kudoa* species within the same subclade with 2 other *Kudoa* infecting intestinal organs: *K. ciliatae* and *K. cookii* (Figs. 5 & 6). In the BI tree, *K. orbicularis* (Azevedo et al. 2016), reported from the Amazon River, was placed at the base of this clade of intestinal infecting parasites. The posterior nodal support was 0.72, although this position was not supported by ML analysis.

DISCUSSION

More than 100 nominal *Kudoa* species have been identified thus far (Abdel-Baki et al. 2016, Eiras et al. 2016). Traditionally, the taxonomy of *Kudoa* has depended upon the myxospore morphology, but the pliability of mature myxospores, together with the fact that they are inornate and simple-shaped, often causes difficulty in determining the verity of morphologically similar species using only spore morphology (Fiala 2006). To reduce such confusion, some studies have suggested that biological characters such as host, geographical location and tissue tropism are important descriptive characters that can be useful alongside the myxospore morphology to assist in the

identification of *Kudoa* species (Burger et al. 2007, Heiniger et al. 2013, Azevedo et al. 2016). In addition, molecular markers are also indispensable for the identification and description of *Kudoa*, especially for species infecting the same or closely related hosts and developing in the same organs and tissues (Eszterbauer & Székely 2004, Liu et al. 2012).

A review of the available literature revealed 5 nominal *Kudoa* species previously described in smooth intestinal muscle of their hosts (Table 1). These species are: *Kudoa sphyraeni* Narasimhamurti & Kalavati, 1979 from *Sphyraena jello* Cuvier in India, *K. ciliatae* Lom, Rhode & Dyková, 1992 from *Sillago ciliata* Cuvier in Australia, *K. intestinalis* Maeno, Nagasawa & Sorimachi 1993 from *Mugil cephalus* Linnaeus in Japan, *K. valamugili* Kalavati & Anuradha 1993 from *Valamugil cunnesius* Valenciennes in India and *Liza aurata* (Risso) in Spain and *K. cookii* Heiniger, Cribb & Adlard 2013 from *Ostorhinchus cookii* (Macleay) in Australia (Narasimhamurti & Kalavati 1979; Lom et al. 1992, Kalavati & Anuradha 1993, Maeno et al. 1993, Yurakhno et al. 2007, Heiniger et al. 2013). *K. sphyraeni* is distinct in having larger spores with 7 polar filament coils. Similarly, *K. ciliatae* has shorter spores with shell valves extended in flat projections, and although it has 4 polar capsules, these are rather smaller and unequal. *K. intestinalis* can be differentiated from *K. crenimugilis* n. sp. by its smaller ellipsoidal subequal polar capsules, and the presence of a short projection on the top of the valves. Also, *K. valamugili* can be distinguished by its smaller, club-shaped spores with unequal valves, deep notches and unequal polar capsules. Finally, *K. cookii* can be differentiated by its thicker spores with smaller polar capsules.

To date, 10 *Kudoa* species have previously been described from hosts in the Family Mugilidae (Table 1). Two of these, *K. intestinalis* and *K. valamugili*, have already been compared above. The others include *K. bora* (Fujita, 1930) Nigrelli, 1946, which differs in having spores nearly double the thickness of *K. crenimugilis* n. sp., with 4 notches equidistantly arranged on the posterior margin of the valves (see Eiras et al. 2014); and *K. cascasia* Sarkar & Chaudhury, 1996, which is distinguished by its thicker, triangular or pyramidal spores with a truncate apex (see Sarkar & Chaudhury 1996). Similarly, *K. haridasae* Sarkar and Ghosh 1991 can be distinguished by its much thicker spores, which are nearly double the thickness of the present species, along with smaller polar capsules. *K. iwatai* Egusa & Shiomitsu, 1983 differs in having larger spores with 4 finger-like valve projections and subequal polar

Fig. 5. Maximum likelihood (ML) phylogenetic tree based on SSU rDNA sequences, showing the position of *Kudoa crenimugilis* n. sp. (in **bold**) within *Kudoa*. Accession numbers for each species are indicated within parentheses. Support values at branching points are listed as posterior probabilities from Bayesian analysis/bootstrap values from ML analysis. Values below 60% and non-supported nodes are indicated by a dash. Abbreviations of infected tissues: heart muscle (HM); intestinal smooth muscle (ISM); nervous system (NS); ovary (OV); skeletal muscles (SM); other organs (O). *Ellipsomyxa gobii* was used as the outgroup, and the scale bar shows the number of changes site^{-1}
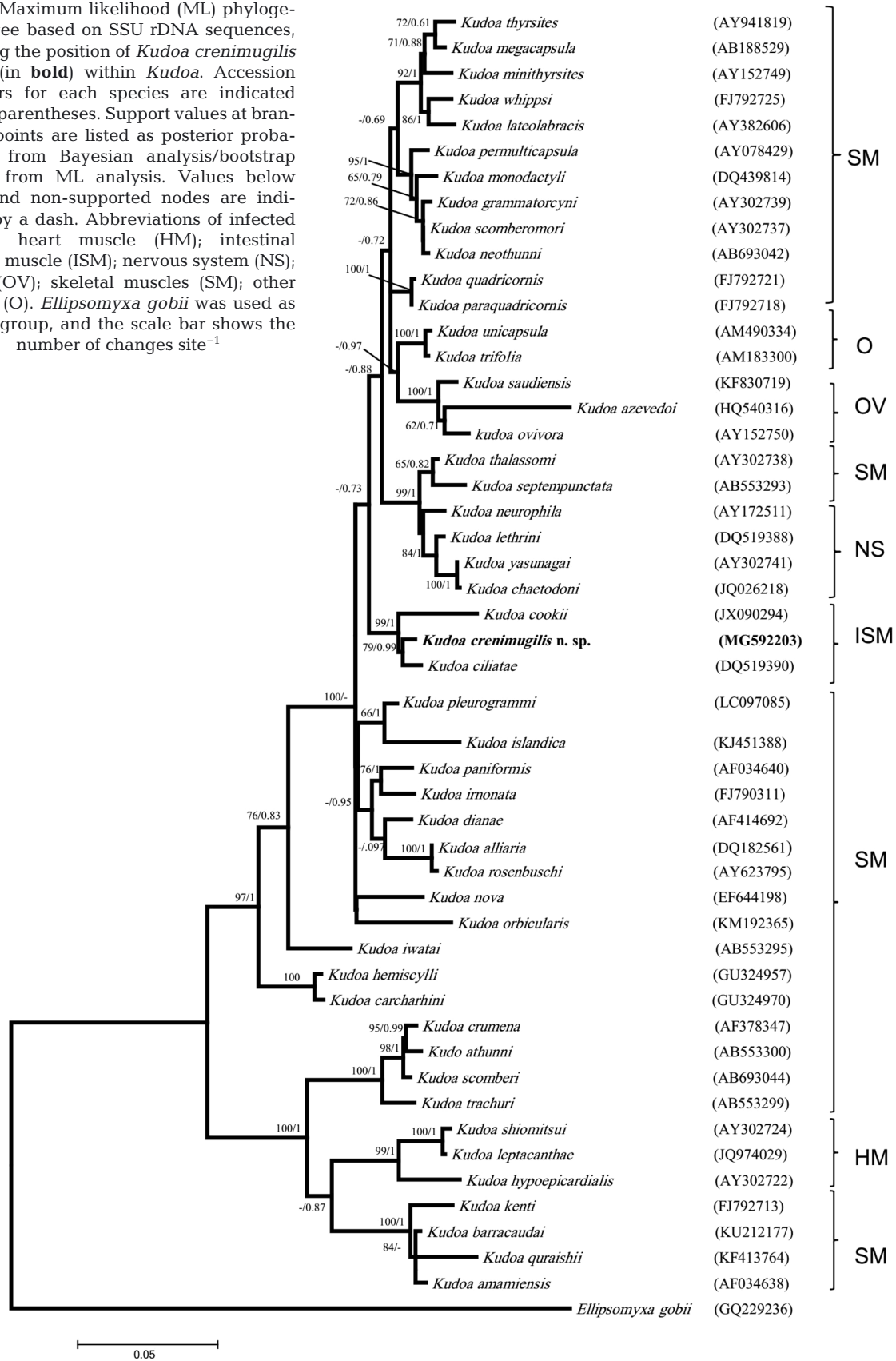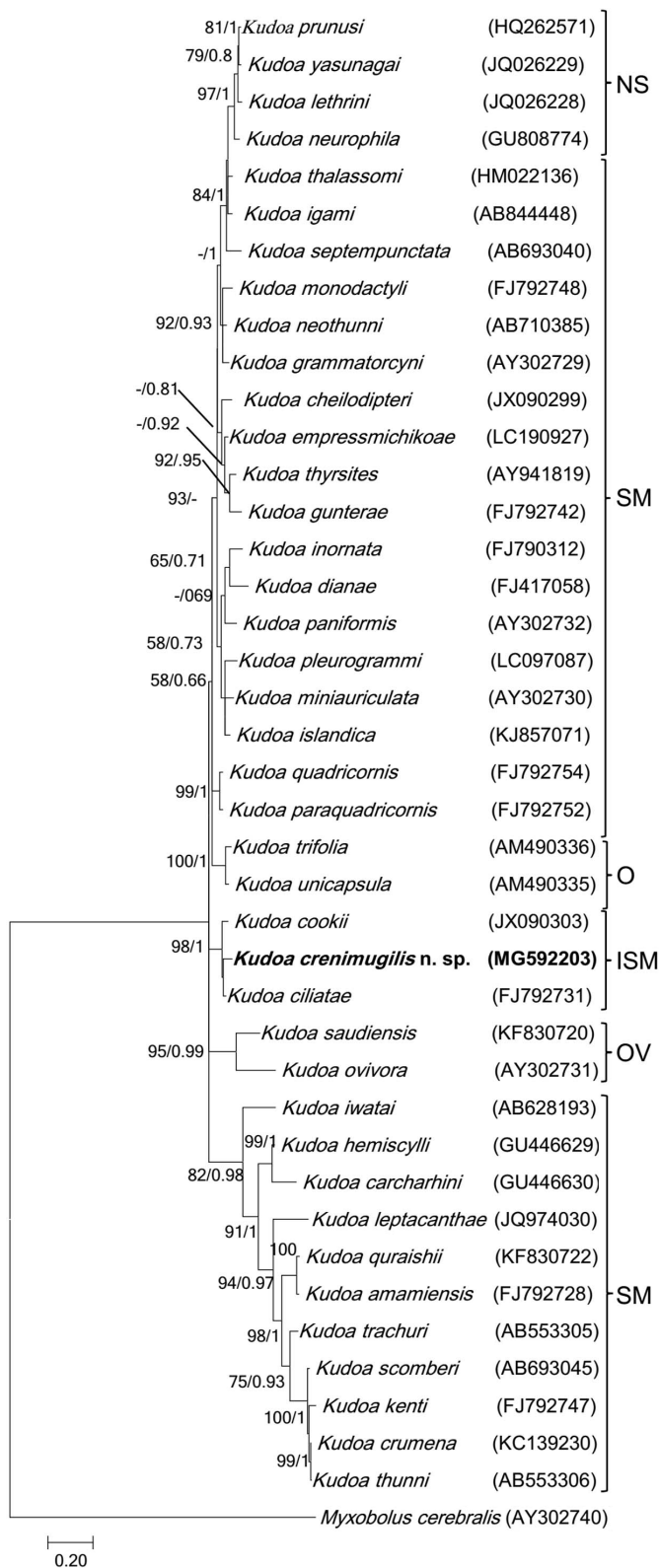
Fig. 6. Maximum likelihood phylogenetic tree based on LSU rDNA sequences, showing the position of *Kudoa crenimugilis* n. sp. (in **bold**) within *Kudoa*. *Myxobolus cerebralis* was used as the outgroup; other details as in Fig. 5

capsules (see Egusa & Shiomitsu 1983, Diamant et al. 2005). In the same way, *K. quadratum* (Thélohan, 1894) Meglitsch, 1947 has smaller spores with much smaller polar capsules less than half the size of the present species (see Iskov 1989). *K. trifolia* Holzer, Blasco-Costa, Sarabeev, Ovcharenko & Balbuena 2006 can be distinguished from the present form by its leaf-like and rather thicker spores with grape-like appendages (Holzer et al. 2006). Similarly, *K. unicapsula* Yurakhno, Ovcharenko, Holzer, Sarabeev & Balbuena, 2007 is distinct in having asymmetrical spores with unequal valves and 1 large polar capsule together with 3 degenerate polar capsules (see Yurakhno et al. 2007). Finally, *K. tetraspora* Narasimhamurti & Kalavati, 1979 differs in having much thicker spores with deep valvular notches.

Regarding the locality, *K. quraishii* Mansour, Harrath, Abd-Elkader, Alwasel, Abdel-Baki & Al Omar, 2014; *K. saudiensis* Mansour, Harrath, Abdel-Baki, Al-Quraishy, & Al Omar, 2015; and *K. barracudai* Abdel-Baki, Al-Quraishy, Al Omar & Mansour, 2016 are the only *Kudoa* species that have been described from the Red Sea off Saudi Arabia (Mansour et al. 2014, 2015, Abdel-Baki et al. 2016) (Table 1). All differ in having shorter spores with much smaller polar capsules nearly half the size of the present species.

Unfortunately, no molecular data are available for *K. sphyraeni. K. intestinalis*, *K. valamugili*, *K. cascasia*, *K. haridasae*, *K. quadratum* and *K. tetraspora*, thus not allowing for further comparison at this time. Phylogenetically, *K. crenimugilis* n. sp. is clustered with *K. ciliatae* and *K. cookii*. These species also infect the smooth muscle of the intestine but are otherwise quite distinct in terms of spore morphology, host family and geographical origin. Several authors have reported that the phylogenetic studies based on SSU rDNA sequences have revealed clustering of *Kudoa* spp. with the same tissue tropism with each other, regardless of host species or family, and geographical location (Whipps et al. 2003, Blaylock et al. 2004, Fiala 2006, Matsukane et al. 2010, Heiniger et al. 2013, Mansour et al. 2014, 2015, Azevedo et al. 2016). Overall, the phylogenetic analysis of the SSU rRNA gene of several different myxozoan parasites has demonstrated that the site of infection and the type of aquatic environment inhabited by the fish host are very important aspects influencing the evolution and differentiation of Myxozoa (Molnár 2002, Molnár & Eszterbauer 2015).

Histologically, the plasmodia of *K. crenimugilis* n. sp. were located in the smooth muscle of the intestine and the most common pathological change was the

Table 1. Comparison of *Kudoa crenimugilis* n. sp. with other *Kudoa* spp. that have 4 polar capsules reported infecting the smooth muscle of the intestine and/or encountered in the family Mugilidae and/or in the Red Sea off Saudi Arabia. Data are mean and range (in parentheses, when available)

Species	Host(s)	Site of infection	Locality	Spore (µm) Width	Thickness	Polar capsule (µm) Length	Width	Reference(s)
K. crenimugilis n. sp.	*Crenimugil crenilabis* (Forsskål)	Smooth muscle of intestine	Saudi Arabia	7.5 (8–9)	5.2 (5–6)	4.5 (4–5)	2 (1–3)	Present study
K. barracudai Abdel-Baki, Al-Quraishy, Al Omar & Mansour, 2016	*Sphyraena putnamae* Jordan & Seale	Trunk muscle	Saudi Arabia	5 (4.5–5.5)	5.5 (5–6)	2.5 (2–3)	1.5 (1–2)	Abdel-Baki et al. (2016)
K. bora (Fujita, 1930) Nigrelli, 1946	*Mugil japonicus* Temminck & Schlegel, *M. cephalus* (L.) and *Liza carinata* (Valenciennes)	Lateral muscles	Taiwan	8.0–8.5	11.0–12.0	5.5	1.8	Eiras et al. (2014)
K. cascasia Sarkar & Chaudhury, 1996	*Sicamugil cascasia* Hamilton	Mesentery	India	8.2 (7–9)	7.6 (7–8)	3.1 (2.5–3.5)	1.6 (1.2–2.0)	Sarkar & Chaudhury (1996)
K. ciliatae Lom, Rhode & Dyková, 1992	*Sillago ciliatae* Cuvier	Smooth muscle of intestine	Australia	5.4 (4.7–6.2)	6.6 (6.3–7.8)	2.4 (1.9–2.7)	1.4 (1.2–1.9)	Lom et al. (1992)
K. cookii Heiniger, Cribb & Adlard, 2013	*Ostorhinchus cookii* Macleay	Submucosa of intestine	Australia	9 (7.6–10)	8 (6.4–9.5)	2.3 (2.0–2.8)	1.6 (1.4–2.0)	Heiniger et al. (2013)
K. haridasae Sarkar & Ghosh, 1991	*Mugil persina* Hamilton	Gallbladder	India	5 (4–5.5)	10 (9–11)	2.7 (2–3)	1.1 (1–1.2)	Sarkar & Ghosh (1991)
K. intestinalis Maeno, Nagasawa & Sorimachi, 1993	*Mugil cephalus* Linnaeus	Smooth muscle of intestine	Japan	6.5 (6.3–7)	6.1 (5.8–6.5)	1.5 (1.3–1.5)	1.2 (1.0–1.5)	Maeno et al. (1993)
K. iwatai Egusa & Shiomitsu, 1983	*Mugil cephalus* Linnaeus	Trunk muscle	Israel	7.2 (6.7–8.0)	10.1 (9.7–10.7)	4.0 (3.8–4.5)	2.2 (2–2.4)	Egusa & Shiomitsu (1983), Diamant et al. (2005)
K. quadratum (Thélohan, 1894) Meglitsch, 1947	*Mugil cephalus* Linnaeus	Trunk muscle	Ukraine	5	5	2	–	Iskov (1989)
K. quraishii Mansour, Harrath, Abd-Elkader, Alwasel, Abdel-Baki & Al Omar, 2014	*Rastrelliger kanagurta* Cuvier	Trunk muscle	Saudi Arabia	6.1 (5.9–6.3)	5.5 (5.3–5.7)	2.1 (1.9–2.3)	1.3 (1.1–1.5)	Mansour et al. (2014)
K. saudiensis Mansour, Harrath, Abdel-Baki, Al-Quraishy, & Al Omar, 2015	*Rastrelliger kanagurta* Cuvier	Ovary	Saudi Arabia	4.7 (4.3–5.4)	3.8 (3.4–4.3)	1.6 (1.2–1.8)	1.3 (1.1–1.4)	Mansour et al. (2015)

Table 1 (continued)

Species	Host(s)	Site of infection	Locality	Spore (µm) Width	Thickness	Polar capsule (µm) Length	Width	Reference(s)
K. sphyraeni Narasimhamurti & Kalavati, 1979	*Sphyraena jello* Cuvier	Muscle of the intestinal wall	India	9.8 (9.5–10.5)	9.8 (9.5–10.5)	3.6	1.6	Narasimhamurti & Kalavati (1979)
K. trifolia Holzer, Blasco-Costa, Sarabeev, Ovcharenko & Balbuena, 2006	*Liza aurata* Risso, *L. ramada* Risso	Spleen, gills and wall of gallbladder	Spain	3.7 (3.3–4)	14.2 (13.6–15.5)	1.01 (0.96–1.07)	0.93 (0.82–0.99)	Holzer et al. (2006)
K. tetraspora Narasimhamurti & Kalavati, 1979	*Mugil cephalus* Linnaeus	Skeletal muscle	India	9 (8–11)	9 (8–12)	3.8 (3.4–4)	1.6 (1.5–1.8)	Narasimhamurti & Kalavati (1979)
K. unicapsula Yurakhno, Ovcharenko, Holzer, Sarabeev & Balbuena, 2007	*Liza ramada* Risso, *L. aurata* Risso	Mesenteries and pyloric caeca	Spain	7.7 (6.6–8.4)	5.5 (4.7–6.9)	2.8–3.4	2.5–2.9	Yurakhno et al. (2007)
K. valamugii Kalavati & Anuradha, 1993	*Valamugil cunnesius* Valenciennes	Smooth muscle of intestine	India	5.6 (5.2–6.6)	5.3 (5–5.4)	3.2 (3.0–3.8) 2.4 (2.0–3.2)	1.8 (1.6–2.0) 1.4 (1.2–1.6)	Kalavati & Anuradha (1993)

replacement of muscle tissue with developing plasmodia, similar to what has been reported in the case of *K. ciliatae* (Lom et al. 1992). The plasmodia were also surrounded by a thin fibrous layer with inflammatory infiltration composed of lymphocytes and macrophages, as has been observed for other species (Moran et al. 1999, Velasco et al. 2015, Marshall et al. 2016). Myolysis is not customary and is confined to the encompassing myofibrils, as has also been recorded for *K. quraishii* (Mansour et al. 2014).

Overall, the comprehensive analysis of both morphological characteristics and molecular data reinforce our suggestion that *K. crenimugilis* is a new member of the genus *Kudoa*.

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