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Four carangid fish species as new host records for *Kudoa trachuri* Matsukane, Sato, Tanaka, Kamata et Sugita-Konishi, 2011 (Myxozoa: Multivalvulida), and description of a new species, *Kudoa longichorda* sp. n., forming pseudocysts in the muscle of *Decapterus tabl* Berry

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Abstract: Multivalvulid myxosporeans of the genera *Kudoa* Meglitsch, 1947 and *Unicapsula* Davis, 1924 (Cnidaria: Myxozoa) are often the cause of unsightly cyst formation or *postmortem* myoliquefaction in the trunk muscle of commercial marine fish, which reduces the market value of infected individuals. Twenty species (18 *Kudoa* spp. and two *Unicapsula* spp.) have been recorded from carangid fish, although the majority of them, excluding polyxenous species, such as *K. amamiensis* Egusa et Nakajima, 1980, *K. iwatai* Egusa et Shiomitsu, 1983, *K. nova* Naidenova, 1975, *K. quadratum* (Thélohan, 1895) and *K. yasunagai* (Hsieh et Chen, 1984), are limited to a single or a few fish species. We report the occurrence of macroscopic cysts of *Kudoa trachuri* Matsukane, Sato, Tanaka, Kamata et Sugita-Konishi, 2011 in the trunk muscle of four new host fish species, i.e., *Pseudocaranx dentex* (Bloch et Schneider), *Decapterus akaadsi* Abe, *D. muroadsi* (Temminck et Schlegel) and *Decapterus tabl* Berry, fished from the Philippine Sea (Northwest Pacific Ocean), off southwestern of Japan. Myxospore morphology and genetic characteristics of the ribosomal RNA gene (rDNA) of these isolates were consistent with previous records of *K. trachuri* from *Trachurus japonicus* (Temminck et Schlegel) from around Japan. In addition, a new species of *Kudoa* that forms long filamentous pseudocysts in trunk myofibres was found in four of the six *D. tabl* collected in this study. We describe *Kudoa longichorda* sp. n. for this new isolate, based on its morphology of subquadrate myxospores with four shell valves and polar capsules and with small dimensions (length 4.3–5.5 µm, width 6.0–6.8 µm, thickness 4.8–6.3 µm, polar capsule length 2.3–3.1 µm, polar capsule width 1.1–1.7 µm), as well as 18S and 28S rDNA sequences distinct from those of known species.

Keywords: myxospore, morphology, rDNA, new host record, Carangidae, Japan

Unsightly cyst formation or *postmortem* myoliquefaction in the trunk muscle of commercial marine fish are often ascribed to species of the genera *Kudoa* Meglitsch, 1947 and *Unicapsula* Davis, 1924 (Cnidaria: Myxozoa: Myxosporea: Multivalvulida). These conditions negatively impact product quality and market value of fish, and are of concern to aquaculture and the wild fish fisheries (Davis 1924, Egusa 1986, Moran et al. 1999). More than 120 nominal *Kudoa* spp. and 16 *Unicapsula* spp. have been recorded to date (Eiras et al. 2014, Li et al. 2020a–c, Inoue et al. 2021, Yunus et al. 2021), and twenty multivalvulid species (18 *Kudoa* spp. and two *Unicapsula* spp.) have been recorded from carangid fish (Actinopteri: Carangiformes: Carangidae) (Table 1), although host records for each multivalvulid species are limited to a single fish species except for polyxenous species, such as *Kudoa amamiensis* Egusa et Nakajima, 1980, *Kudoa iwatai* Egusa et Shiomitsu, 1983, *Kudoa nova* Naidenova, 1975, *Kudoa quadratum* (Thélohan, 1895), *Kudoa shiomitsui* Egusa et Shiomitsu, 1983, *Kudoa yasunagai* (Hsieh et Chen, 1984), and *Unicapsula seriolae* Lester, 1982.

Few macroscopic cysts were detected in the musculature of pomacentrid damselfishes, which serve as the natural hosts of *Kudoa amamiensis* (see Egusa and Nakajima 1980, Burger et al. 2008). However, farmed Japanese amberjack *Seriola quinqueradiata* Temminck et Schlegel, introduced around Amami-Oshima and the Okinawa Islands, Japan, were heavily parasitised by *K. amamiensis* leading to a loss in their market values as edible fish (Egusa and Nakajima 1980, Sugiyama et al. 1999). *Unicapsula serio-*

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Table 1. Multivalvulid myxosporean species recorded in carangid fish

Myxosporean species	Locality	Host species	Reference
**Kudoa amamiensis* Egusa et Nakajima, 1980	Philippine Sea (Japan); Coral Sea (Australia)	***Seriola quinqueradiata*** Temminck et Schlegel, 1845 *Abudefduf bengalensis* (Bloch, 1787) [Pomacentridae] *Abudefduf sexfasciatus* (Lacepède, 1801) [Pomacentridae] *Abudefduf vaigiensis* (Quoy et Gaimard, 1825) [Pomacentridae] *Abudefduf whitleyi* Allen et Robertson, 1974 [Pomacentridae] ***Caranx sexfasciatus*** Quoy et Gaimard, 1825 *Chromis chrysura* (Bliss, 1883) [Pomacentridae] *Chromis notatus* (Temminck et Schlegel, 1843) [Pomacentridae] *Chrysiptera cyanea* (Quoy et Gaimard, 1825) [Pomacentridae] *Pempheris ypsilychnus* Mooi et Jubb, 1996 [Pempheridae] ***Seriola dumerili*** (Risso, 1810)	Egusa and Nakajima (1980); Sugiyama et al. (1999); Whipps et al. (2003b); Burger et al. (2008); Burger and Adlard (2011)
**Kudoa atropi* Sandeep, Kalavati et Narasimhamurti, 1986	Indian Ocean	***Atropus atropus*** (Bloch et Schneider, 1801)	Sandeep et al. (1986)
**Kudoa azevedoi* Mansour, Thabet, Chourabi, Harrath, Gtari, Al Omar et Ben Hassine, 2013	Mediterranean Sea (Tunisia)	***Trachurus trachurus*** (Linnaeus, 1758)	Mansour et al. (2013)
**Kudoa decaptera* (Xie et Chen, 1988)	South China Sea	***Decapterus maruadsi*** (Temminck et Schlegel, 1843)	Xie and Chen (1988)
**Kudoa insolita* Schul'man et Kovaleva, 1979	Atlantic Ocean	***Seriola dumerili*** (Risso, 1810)	Eiras et al. (2014)
Kudoa iwatai Egusa et Shiomitsu, 1983	Seawater around Japan; Red Sea	*Pagrus major* (Temminck et Schlegel, 1843) [Sparidae] *Acanthopagrus latus* (Houttuyn, 1782) [Sparidae] *Acanthopagrus schlegelii* (Bleeker, 1854) [Sparidae] *Argyrops filamentosus* (Valenciennes, 1830) [Sparidae] *Chaetodon paucifasciatus* Ahl, 1923 [Chaetodontidae] *Dascyllus trimaculatus* (Rüppell, 1829) [Pomacentridae] *Dicentrarchus labrax* (Linnaeus, 1758) [Moronidae] *Hyporhamphus gamberur* (Rüppell, 1837) [Hemiramphidae] *Lateolabrax japonicus* (Cuvier 1828) [Lateolabracidae] *Lethrinus nebulosus* (Forsskål, 1775) [Lethrinidae] *Lethrinus variegatus* Valenciennes, 1830 [Lethrinidae] *Mugil cephalus* Linnaeus, 1758 [Mugilidae] *Neopomacentrus miryae* Dor et Allen, 1977 [Pomacentridae] *Oplegnathus fasciatus* (Temminck et Schlegel, 1844) [Oplegnathidae] *Oplegnathus punctatus* (Temminck et Schlegel, 1844) [Oplegnathidae] *Ostorhinchus aureus* (Lacepède, 1802) [Apogonidae] *Ostorhinchus fleurieu* Lacepède, 1802 [Apogonidae] *Platycephalus* sp. [Platycephalidae] *Priacanthus hamrur* (Forsskål 1775) [Priacanthidae] *Rhabdosargus sarba* (Forsskål 1775) [Sparidae] *Scomberomorus niphonius* (Cuvier, 1832) [Scombridae] ***Seriola quinqueradiata*** Temminck et Schlegel, 1845 *Siganus rivulatus* Forsskål et Niebuhr, 1775 [Siganidae] *Sparus aurata* Linnaeus, 1758 [Sparidae]	Egusa and Shiomitsu (1983); Egusa (1986); Diamant et al. (2005); Matsukane et al. (2011); Abe et al. (2011); Kasai et al. (2015)
**Kudoa javaensis* Yunus, Yustinasari, Natalia, Ghosh, Sakuma, Inoue et Sato, 2021	Java Sea (Indonesia)	***Alepes djedaba*** (Forsskål, 1775)	Yunus et al. (2021)
****Kudoa longichorda*** sp. n.	Philippine Sea (Northwest Pacific Ocean, off southwest of Japan)	***Decapterus tabl*** Berry, 1968	Present study
Kudoa megacapsula Yokoyama et Itoh, 2005		*Sphyraena pinguis* Günther 1874 [Sphyraenidae] ***Seriola quinqueradiata*** Temminck et Schlegel, 1845	Yokoyama and Itoh (2005); Yokoyama et al. (2006)
Kudoa neurophila (Grossel, Dyková, Handlinger et Munday, 2003)	Tasmanian Sea; Indian Ocean (Australia)	*Latris lineata* (Forster, 1801) [Latridae] ***Seriola lalandi*** Valenciennes, 1833	Grossel et al. (2003); Burger and Adlard (2010b)
Kudoa nova Naidenova, 1975	Black Sea; Sea of Azov; Mediterranean Sea; Atlantic Ocean (Guinea Gulf, and off the seacoast of northwestern Africa)	*Pomatomus saltatrix* (Linnaeus, 1766) [Pomatomidae] *Dentex macrophthalmus* (Bloch, 1791) [Sparidae] *Euthynnus alletteratus* (Rafinesque, 1810) [Scombridae] *Gobius niger* Linnaeus, 1758 [Gobiidae] *Knipowitschia longecaudata* (Kessler 1877) [Gobiidae] *Neogobius fluviatilis* (Pallas, 1814) [Gobiidae] *Neogobius melanostomus* (Pallas, 1814) [Gobiidae] *Oxyeleotris marmorata* (Bleeker, 1852) [Butidae] *Pagellus acarne* (Risso, 1827) [Sparidae] *Pomatoschistus marmoratus* (Risso, 1810) [Gobiidae] *Ponticola platyrostris* (Pallas, 1814) [Gobiidae] *Ponticola ratan* (Nordmann, 1840) [Gobiidae] *Ponticola syrman* (Nordmann, 1840) [Gobiidae] *Thunnus obesus* (Lowe, 1839) [Scombridae] ***Trachurus mediterraneus*** (Steindachner, 1868) ***Trachurus picturatus*** (Bowdich, 1825) ***Trachurus trachurus*** (Linnaeus, 1758) ***Trachurus trecae*** Cadenat, 1950 *Zosterisessor ophiocephalus* (Pallas, 1814) [Gobiidae]	Burger and Adlard (2011); Yurakhno and Gorchanok (2011); Pascual et al. (2012)
**Kudoa paraquadricornis* Burger et Adlard, 2010	Coral Sea (Australia)	***Caranx ignobilis*** (Forsskål, 1775) ***Carangoides plagiotaenia*** Bleeker, 1857 ***Caranx papuensis*** Alleyne et MacLeay, 1877 ***Caranx sexfasciatus*** Quoy et Gaimard, 1825	Burger and Adlard (2010a)

**Kudoa pericardialis* Nakajima et Egusa, 1978	Philippine Sea (Japan)	***Seriola quinqueradiata*** Temminck et Schlegel, 1845	Nakajima and Egusa (1978)
**Kudoa pyramidalis* Zhou et Zhao, 2008	South China Sea (China)	***Alepes djedaba*** (Forsskål, 1775)	Zhou and Zhao (2008)
Kudoa quadratum (Thélohan, 1895)	Black Sea; Mediterranean Sea; Atlantic Ocean; Indian Ocean (off African coast)	*Myoxocephalus scorpius* (Linnaeus, 1758) [Cottidae] *Callionymus lyra* Linnaeus, 1758 [Callionymidae] *Coris julis* (Linnaeus, 1758) [Labridae] *Entelurus aequoreus* (Linnaeus, 1758) [Syngnathidae] *Parablennius gattorugine* (Linnaeus, 1758) [Blenniidae] *Plicofollis polystaphylodon* (Bleeker, 1846) [Ariidae] *Syngnathus acus* Linnaeus, 1758 [Syngnathidae] *Syngnathus tenuirostris* Rathke 1837 [Syngnathidae] ***Trachurus trachurus*** (Linnaeus, 1758)	Shul'man (1966); Burger and Adlard (2011)
**Kudoa quadricornis* Whipps, Adlard, Bryant et Kent, 2003	Coral Sea (Australia)	***Carangoides fulvoguttatus*** (Forsskål, 1775)	Whipps et al. (2003a)
Kudoa shiomitsui Egusa et Shiomitsu, 1983	Philippine Sea (Japan)	*Takifugu rubripes* (Temminck et Schlegel, 1850) [Tetraodontidae] *Apogon lineatus* Temminck et Schlegel, 1842 [Apogonidae] *Paralichthys olivaceus* (Temminck et Schlegel, 1846) [Paralichthyidae] ***Seriola dumerili*** (Risso, 1810) *Stephanolepis cirrhifer* (Temminck et Schlegel, 1850) [Monacanthidae] *Thunnus orientalis* (Temminck et Schlegel 1844) [Scombridae]	Egusa and Shiomitsu (1983); Ogawa and Inoue (1997); Zhang et al. (2010); Kasai et al. (2016)
**Kudoa trachuri* Matsukane, Sato, Tanaka, Kamata et Sugita-Konishi, 2011	Sea of Japan and Philippine Sea (Japan); South China Sea (China)	***Trachurus japonicus*** (Temminck et Schlegel, 1844) ***Decapterus akaadsi*** Abe, 1958 ***Decapterus macrosoma*** Bleeker, 1851 ***Decapterus maruadsi*** (Temminck and Schlegel,1844) ***Decapterus muroadsi*** (Temminck et Schlegel, 1844) ***Decapterus tabl*** Berry, 1968 ***Pseudocaranx dentex*** (Bloch et Schneider, 1801)	Matsukane et al. (2011); Abe et al. (2011); Li et al. (2013); Kasai et al. (2015); Present study; Li et al. (2022)
Kudoa yasunagai (Hsieh et Chen, 1984)	Coral Sea (Australia); Philippine Sea and Sea of Japan (Japan); East and South China Sea (China)	*Lateolabrax japonicus* (Cuvier, 1828) [Lateolabracidae] *Argyrosomus japonicus* (Temminck & Schlegel, 1843) [Sciaenidae] *Calotomus japonicus* (Valenciennes, 1840) [Scaridae] *Oplegnathus fasciatus* (Temminck et Schlegel, 1844) [Oplegnathidae] *Pagrus major* (Temminck et Schlegel, 1843) [Sparidae] *Paralichthys olivaceus* (Temminck & Schlegel, 1846) [Paralichthyidae] *Plotosus japonicus* Yoshino et Kishimoto, 2008 [Plotosidae] *Plotosus lineatus* (Thunberg, 1787) [Plotosidae] ***Seriola aureovittata*** Temminck and Schlegel, 1845 (syn. ***Seriola lalandi*** in Shin et al. 2016a) ***Seriola quinqueradiata*** Temminck et Schlegel, 1845 *Sillago ciliata* Cuvier, 1829 [Sillaginidae] *Takifugu rubripes* (Temminck & Schlegel 1850) [Tetraodontidae] *Thunnus orientalis* (Temminck et Schlegel, 1844) [Scombridae]	Yasunaga et al. (1981); Hsieh and Chen (1984); Egusa (1986); Ogawa and Inoue (1997); Burger et al. (2007); Zhang et al. (2010); Burger and Adlard (2010b, 2011); Yokoyama (2016); Shin et al. (2016a); Sakai et al. (2019)
**Unicapsula seriolae* Lester, 1982	Coral Sea (Australia); Philippine Sea and Sea of Japan (Japan); South China Sea (China)	***Seriola lalandi*** Valenciennes, 1833 *Epinephelus malabaricus* (Bloch et Schneider, 1801) [Serranidae] ***Seriola dumerili*** (Risso, 1810) ***Selaroides leptolepis*** (Cuvier, 1833)	Lester (1982); Abe et al. (2011); Miller and Adlard (2013); Tomochi et al. (2014); Yokoyama et al. (2014); Inoue et al. (2021)
**Unicapsula aequilobata* Inoue, Li, Ghosh, Yunus, Zhang et Sato, 2021	South China Sea (China)	***Decapterus maruadsi*** (Temminck et Schlegel, 1843)	Inoue et al. (2021)

Species names marked with an asterisk indicate that the type host of the species is a carangid fish. Underlined fish species names are type host species, and carangid fish species are shown in bold without family names.

lae was originally described from the Australian yellowtail amberjack *Seriola lalandi* Valenciennes, and Lester (1982) suggested that this infection might be a cause of *postmortem* myoliquefaction during cooking or smoking. Although this problem has not been reported in Japan, after the consumption of raw greater amberjack *Seriola dumerili* (Risso) as sashimi or sushi, sporadic outbreaks of food-borne disease, manifested clinically by diarrhoea and vomiting like 'Kudoa food poisoning' (Kawai et al. 2012, Suzuki et al. 2012, 2015, Sugita-Konishi et al. 2014), have been reported and ascribed to parasitisation by *U. seriolae* in the musculature (Tomochi et al. 2014, Ohnishi et al. 2018).

Recently, in addition to a new host record of *Selaroides leptolepis* (Cuvier) for *U. seriolae*, two new species (*Kudoa javaensis* Yunus, Yustinasari, Natalia, Ghosh, Sakuma, Inoue et Sato, 2021, and *Unicapsula aequilobata* Inoue, Li, Ghosh, Yunus, Zhang et Sato, 2021) have been reported from the carangid fishes *Alepes djedaba* (Forsskål) and *Decapterus maruadsi* (Temminck et Schlegel), respectively (Inoue et al. 2021, Yunus et al. 2021). In the present study, to extend our knowledge of multivalvulid infection in carangid fish, we have examined four commercial carangid species fished in the Philippine Sea, off southwestern Japan, and characterised two multivalvulid species by morphology and genetic analyses of the ribosomal RNA gene (rDNA) for their taxonomic identification.

MATERIALS AND METHODS

Fish samples and parasitological examination

Whole fish bodies of six individuals each of four carangid species were purchased from local fish markets in Kagoshima or Kochi Prefectures, western Japan (Table 2). *Pseudocaranx dentex* (Bloch et Schneider) was fished in the Philippine Sea (North West Pacific Ocean), off Kagoshima, Japan, on 25 December 2015; their standard lengths (SL) ranged from 23 to 26 (mean 24.3) cm and body weights (BW) were 315–403 (mean 363) g. Three scad species of the genus *Decapterus* Bleeker were fished in the Philippine Sea, off Kochi, Japan, on 15 June 2016: *Decapterus akaadsi* Abe with SL 23–24 (23.3) cm and BW 206–236

Table 2. Fish samples examined and *Kudoa* spp. detected in the trunk muscle of carangid fishes in this study.

Fish species	Collection date	Locality	Parasite species detected	Type of plasmodia	Prevalence[a]	Intensity[b]
Pseudocaranx dentex (Bloch et Schneider 1801) White trevally	Dec. 25, 2015	Pacific Ocean, off Kagoshima, Japan	*Kudoa trachuri* Matsukane, Sato, Tanaka, Kamata et Sugita-Konishi, 2011	Cyst (trunk muscle)	2/6 (33%)	9, 35
Decapterus akaadsi Abe, 1958 —	Jun. 15, 2016	Pacific Ocean, off Kochi, Japan	*Kudoa trachuri*	Cyst (trunk muscle)	2/6 (33%)	>18, >100
Decapterus muroadsi (Temminck et Schlegel, 1844) Amberstripe scad	Jun. 15, 2016	Pacific Ocean, off Kochi, Japan	*Kudoa trachuri*	Cyst (trunk muscle)	3/6 (50%)	2, 26, 46
Decapterus tabl Berry, 1968 Roughear scad	Jun. 15, 2016	Pacific Ocean, off Kochi, Japan	*Kudoa trachuri*	Cyst (trunk muscle)	2/6 (33%)	4, 23
			Kudoa longichorda sp. n.	Pseudocyst (trunk muscle)	4/6 (67%)	4–78 plasmodia / g muscle

[a] Number of positive fish / number of examined fish (percentage).

[b] Total number of myxosporean cysts detected in the trunk muscles of a fish. For plasmodia forming pseudocysts in myofibers, number of positive myofibres per gram trunk muscle

(218) g; *Decapterus muroadsi* (Temminck et Schlegel) with SL 24 (24) cm and BW 222–241 (230) g; and *Decapterus tabl* Berry with SL 30–31 (30.5) cm and BW 336–397 (368) g. These fish samples were transported on ice to the laboratory in Yamaguchi University within one day after purchase.

Parasitological examinations of the fish samples were performed as described previously (Li et al. 2020a,c). Briefly, to check for the presence of myxosporean cysts or pseudocysts, fishes were cut open and their gills, viscera, and brain removed and examined under a dissection microscope. Thin slices of all available trunk muscle were placed in physiological saline, pressed between glass plates, and examined under a dissection microscope. Myxosporean plasmodia from a fish sample were divided into two groups for fixation in either 10% neutral-buffered formalin solution or 70% ethanol. Fresh myxospores were measured following the guidelines of Lom and Arthur (1989). All measurements are expressed in µm unless otherwise stated and are presented as ranges with the means in parentheses. The specimens collected for this study, fixed in 10% neutral-buffered formalin solution or 70% ethanol, were deposited in the Meguro Parasitological Museum, Tokyo, Japan, under collection nos. 21258–21261, and 21776.

Scanning electron microscopy (SEM)

For SEM, a portion of the formalin-fixed myxospores was washed three times in 0.2 M Na_2HPO_4–NaH_2PO_4 solution (PB) at pH 7.8, immersed in a NanoSuit® solution Type III (Nisshin EM, Tokyo, Japan) diluted three times in 25% glycerine-70% ethanol, and dropped on a dry paper for wrapping powdered medicine. After trimming, the paper with myxospores was mounted on a stub, and observed using a scanning electron microscope (model S-3400; Hitachi, Tokyo, Japan) at an accelerating voltage of 5 kV (Takaku et al. 2017). In addition, standard SEM of myxospores fixed in 2.5% glutaraldehyde and post-fixed in 1% (w/v) osmium tetroxide in PB was conducted as described previously (Sekiya et al. 2019). In the latter observation, the specimens were observed using a scanning electron microscope (model JSM-6100; JEOL, Tokyo, Japan) at an accelerating voltage of 15 kV.

DNA extraction, amplification and sequencing

Parasite DNA was extracted from the myxosporean plasmodia fixed in 70% ethanol; At least one plasmodium (cyst) from one host individual of different host species for *Kudoa trachuri*, and a single or a few plasmodia (pseudocysts) from each *D. tabl* individual (four individuals in total) for *Kudoa* sp. DNA extraction, amplification of certain lengths of rDNA fragments by polymerase chain reaction (PCR), and the purification of the PCR products were performed as described previously (Li et al. 2013, 2020c, Kasai et al. 2015, Kato et al. 2017). Amplification of 18S rDNA fragments proved difficult using the primers reported previously. Therefore, we used different primers described by Hallett and Diamant (2001) and Fiala (2006); a primer combination of 18e (5'-CTG GTT GAT TCT GCC AGT-3') and MYX4r (5'-CTG GTT GAT TCT GCC AGT-3'), and another combination of MyxospecF (5'-TTC TGC CCT ATC AAC TWG TTG-3') and S.r.18S-SSU18R (5'-TGA TCC TTC YGC AGG TTC AC-3'). For sequencing of these two rDNA fragments, Myxo18S_575F (5'-CGC GGT AAT TCC AGC TCC AG-3'), NSF1179/18 (5'-AAT TTG ACT CAA CAC GGG-3'), 1430_fwd (5'-TTA GTT CGT GGA GTG ATC TG-3'), Mbseq2r (5'-CCT TGG TGA GCC ACT ACC TC-3'), and NSR1438/20 (5'-GGG CAT CAC AGA CCT GTT AT-3') were used with reference to previous studies (Freeman et al. 2008, Matsukane et al. 2010, Li et al. 2013, Kato et al. 2017).

When direct sequencing results were not satisfactory, purified PCR products were cloned into the pTA2 plasmid vector (TArget Clone™; TOYOBO, Dojima Hama, Osaka, Japan) according to manufacturer's instructions. Following propagation, the plasmid DNA was extracted using a FastGene Plasmid Mini Kit (NIPPON Genetics Co., Tokyo, Japan) and inserts from multiple independent clones, at least three, were sequenced using universal M13 forward and reverse primers. The nucleotide sequences obtained in this study are available from the GenBank databases under the accession nos. LC640000–LC640011.

Phylogenetic analyses

Fragments of the newly obtained rDNA sequences were analysed to identify highly similar nucleotide sequences using the Basic Local Alignment Search Tool (BLAST) of the National Center for Biotechnology Information website (NCBI; https://www.ncbi.nlm.nih. gov/). For phylogenetic analysis, the newly obtained 18S and 28S rDNA sequences of *Kudoa* spp. from this study and related sequences retrieved from the GenBank database (NCBI) were aligned using the MEGA7 software (Kumar et al.

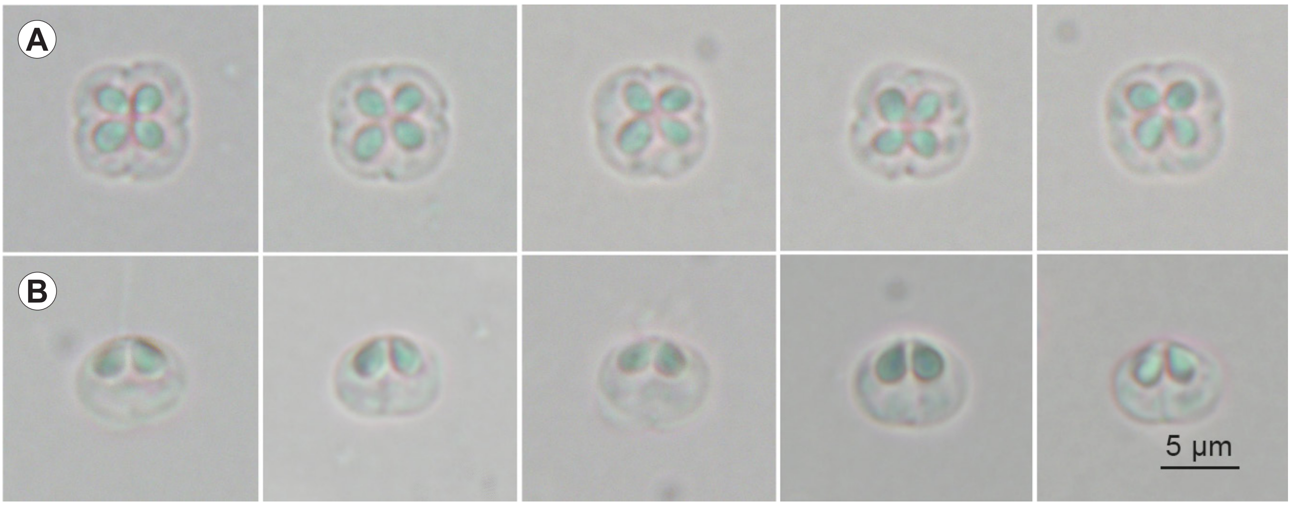

Fig. 1. Microscopic images of the myxospores of *Kudoa trachuri* Matsukane, Sato, Tanaka, Kamata et Sugita-Konishi, 2011 from *Pseudocaranx dentex* (Bloch et Schneider). **A** – spores in apical view; **B** – spores in lateral view at the same magnification.

2016), with subsequent manual adjustments. Regions judged to be poorly aligned were excluded from subsequent analyses; 1,484 characters, of which 385 were variable, remained for subsequent analysis of the 18S rDNA, and 505 characters, of which 230 were variable, remained for subsequent analysis of the 28S rDNA.

Furthermore, concatenated sequences of the 18S and 28S rDNA were prepared for the phylogenetic analysis; 1,989 characters, of which 602 were variable, remained for subsequent analysis of the 18S + 28S rDNA. For an isolate of *K. trachuri* in *Decapterus macrosoma* Bleeker from the South China Sea and five isolates of *K. trachuri*-like species recently recorded in the muscle of sparid and serranid species in the Eastern Central Atlantic Ocean around the Canary Island (Rodríguez-Ponce et al. 2019), the 28S rDNA nucleotide sequence of *K. trachuri* (AB553305) was supplementarily used to prepare their 18S + 28S concatenated sequences, since these isolates lack deposition of the sequences at the GenBank database. Maximum likelihood (ML) analysis was performed with the program, PhyML (Guindon and Gascuel 2003, Dereeper et al. 2008) provided on the ‘phylogeny.fr’ website (http://www.phylogeny.fr/). This program is a built-in package of the substitution model ‘HKY85’. The probability of inferred branching was assessed by the approximate likelihood-ratio test (aLRT), an alternative to the non-parametric bootstrap estimation of branch support (Anisimova and Gascuel 2006). Representative sequences of *Unicapsula* spp. of the same order Multivalvulida and a genus closely related to *Kudoa* were used as an outgroup for the construction of the ML phylogenetic trees. The accession numbers of the analysed sequences are given in a figure for phylogenetic tree.

RESULTS

Parasitological examination

Trunk muscle slices of 24 individuals of carangid fish were examined microscopically. Myxosporean infection was observed in 11 fish individuals of four species. A kudoid species forming macroscopic cysts lined by thin fibrous tissues between myofibres was detected in two *Pseudocaranx dentex*, two *Decapterus akaadsi*, three *D. muroadsi*, and two *D. tabl* individuals (Table 2). A second kudoid species forming pseudocysts was found in the myofibres of four *D. tabl* individuals. No site preference of these two *Kudoa* spp. in the trunk muscles was noted. Two *D. tabl* individuals were coinfected with both *Kudoa* spp. No multivalvulid infection was found in other organs.

Kudoa trachuri **Matsukane, Sato, Tanaka, Kamata et Sugita-Konishi, 2011**

This species formed oval milky white cysts in the trunk muscle of all four carangid species examined. The number of cysts per individual fish ranged from two to more than 100. Cysts measured 0.35–2.16 mm by 0.25–0.74 mm (Table 3). Myxospores were semiquadrate with four equal shell valves (SVs) and polar capsules (PCs) in the apical view (Fig. 1). The lateral view of myxospores was oval, with teardrop-shaped PCs occupying the apical half of myxospores. The dimensions of the myxospores isolated from these four carangid species (Table 3) are comparable with measurements provided in previous records of *Kudoa trachuri*, although different isolates show some variations in morphometry.

The 18S rDNA nucleotide sequences of four isolates from carangid fishes collected in the present study (GenBank accession nos. LC640000, LC640002, LC640004, LC640006 and LC640008) and four isolates from *Trachurus japonicus* (Temminck et Schlegel) retrieved from the DNA databases (accession nos. AB553299, AB626095, AB693043 and LC066367) and two other carangid fish in the South China Sea (LC626068 and LC626070) showed few nucleotide substitutions with high nucleotide similarities of 99.94%–100% over the 1,776 bp length. The 5’-terminus 28S rDNA sequences, 758-bp long, of the four aforementioned isolates in the present study (nos. LC640001, LC640003, LC640005, LC640007 and LC640009) were identical to those of *K. trachuri* from *Trachurus japonicus* fished from the sea around western Japan (AB553305, AB638616 and LC066367) except for two variable base positions.

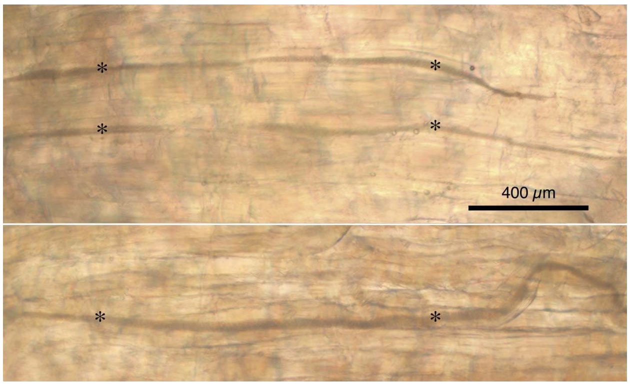

Fig. 2. Microscopic image of the filamentous plasmodia (darker stripes marked by asterisks) of *Kudoa longichorda* sp. n. in myofibres of the trunk muscle of *Decapterus tabl* Berry. Upper and lower photographs were taken at the same magnification.

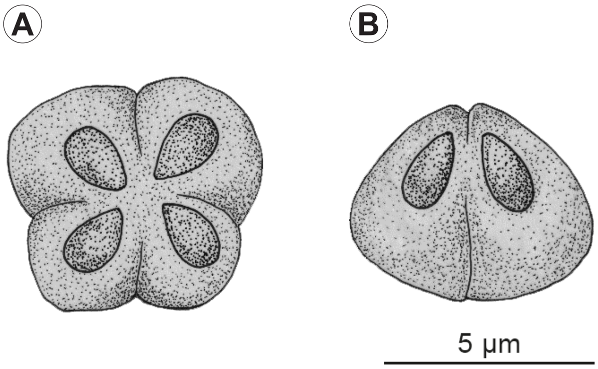

Fig. 4. Illustrations of *Kudoa longichorda* sp. n. from *Decapterus tabl* Berry. **A** – apical view; **B** – lateral view.

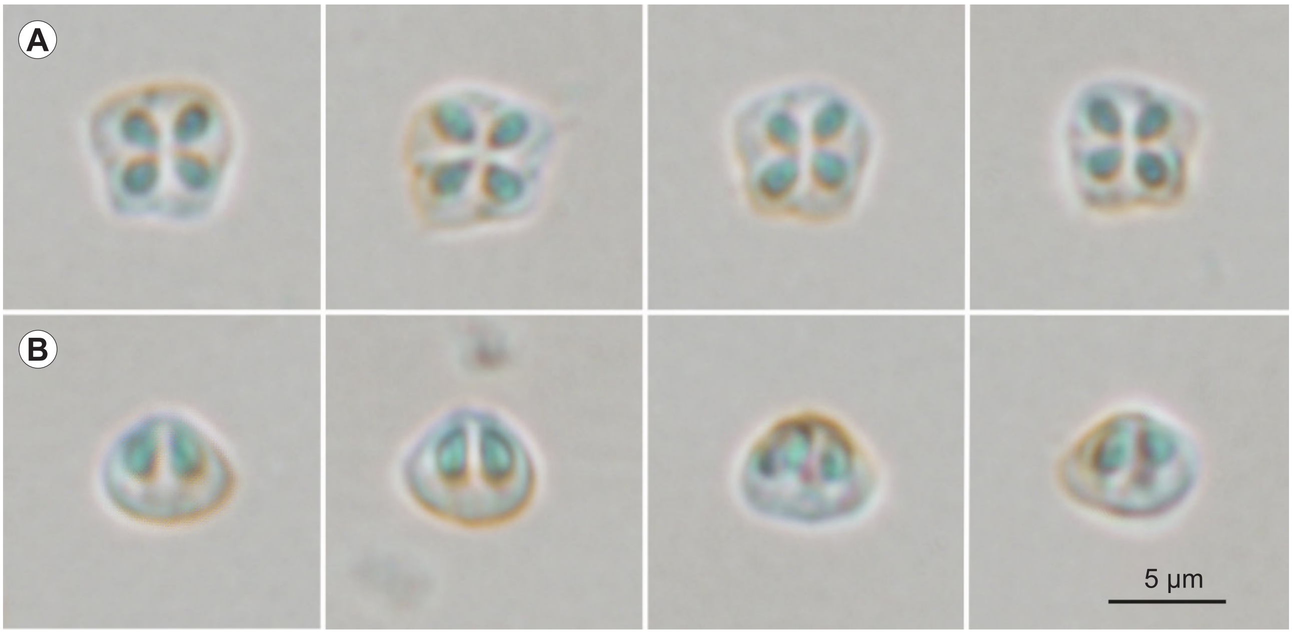

Fig. 3. Microscopic images of the myxospores of *Kudoa longichorda* sp. n. from *Decapterus tabl* Berry. **A** – spores in apical view; **B** – spores in lateral view at the same magnification.

Remarks. *Kudoa trachuri* has been recorded as a species forming oval macroscopic cysts in the trunk muscle of *Trachurus japonicus* fished in the sea around western Japan (Abe et al. 2011, Matsukane et al. 2011, Li et al. 2013, Kasai et al. 2015). The present discovery of *K. trachuri* in *P. dentex* and three species of scads of the genus *Decapterus* (*D. akaadsi*, *D. muroadsi* and *D. tabl*) establishes new host records for *K. trachuri.* Our separate study (Li et al. 2022) also found *K. trachuri* in additional hosts, i.e., *Decapterus macrosoma* Bleeker and *Decapterus maruadsi* fished in the South China Sea (see Table 1).

Taxonomic summary

Type host: *Trachurus japonicus* (Temminck et Schlegel) (Carangiformes: Carangidae).

Additional hosts: *Pseudocaranx dentex* (Bloch et Schneider), *Decapterus akaadsi* Abe, *Decapterus muroadsi* (Temminck et Schlegel), *Decapterus tabl* Berry (Carangiformes: Carangidae).

Type locality: Sea of Japan, off Nagasaki, Japan.

Additional locality: Philippine Sea (North West Pacific Ocean), off Kagoshima and Kochi, western Japan.

Site of infection: Cysts between the myofibres of trunk muscles.

Material deposited: Specimen nos. 21258–21260 and 21776, Meguro Parasitological Museum, Tokyo, Japan.

Deposited rDNA sequences: GenBank accession nos. LC640000–LC640009 (rDNA).

Prevalence: 33% (2/6) or 50% (3/6) as shown in Table 2.

Kudoa longichorda sp. n. Figs. 2–5

ZooBank number for species:
urn:lsid:zoobank.org:act:92FC9AFC-BFB6-4B57-8491-EC73108ABD88

This species was found in the trunk muscle of *Decapterus tabl*. During microscopic examination, long filamentous plasmodia forming pseudocyst in the myofibres were evident and intensely dispersed (Fig. 2). The slightly asymmet-

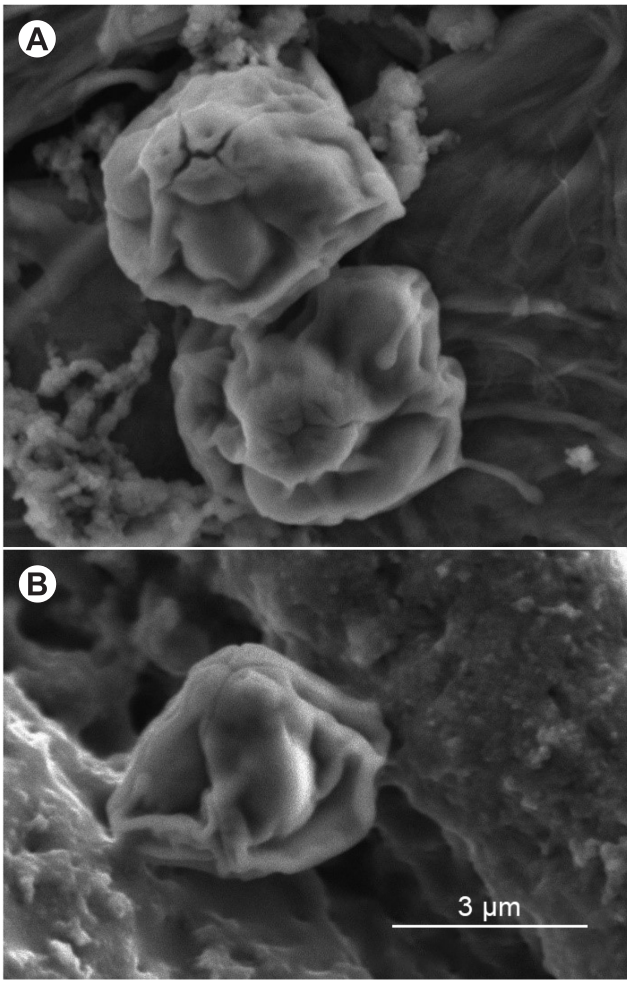

Fig. 5. Scanning electron microscopy of NanoScuit®-treated myxospores of *Kudoa longichorda* sp. n. from *Decapterus tabl* Berry. **A** – apical view; **B** – lateral view at the same magnification. Wrinkles at shell valves are artifacts during their processing. By standard SEM, similar figures of myxospores were observed.

ric myxospores were irregularly subquadrate with slightly different-sized SVs (Figs. 3–5). We erect a new species, *Kudoa longichorda* sp. n., for this isolate based on its morphological and molecular-genetic distinctness from known species.

Description

Polysporic and synchronised spore development in plasmodium, forming pseudocysts in trunk muscle myofibre, measuring 0.7–2.4 (1.6) mm in length by 0.03–0.10 (0.05) mm in width (n = 20). In apical view, myxospores irregularly semiquadrate, composed of four slightly unequal SVs and equal pyriform PCs. Coils of polar filament not visible in wet preparation. In lateral view, myxospores pyriform, and teardrop-shaped PCs in apical half of myxospore length. No apical protrusions. Dimensions of myxospores (n = 20): length, 4.3–5.5 (4.9), width 6.0–6.8 (6.4), thickness, 4.8–6.3 (5.5), sutural thickness 4.4–5.6 (4.9), polar capsule length 2.3–3.1 (2.7), polar capsule width 1.1–1.7 (1.4).

The 18S rDNA nucleotide sequence obtained from the isolate 1,720 bp long (GenBank accession no. LC640010) showed the highest nucleotide BLAST identity with sequences from *Kudoa javaensis* (98.2% [1,685/1,716] against LC543974) and *Kudoa hexapunctata* Yokoyama, Suzuki et Shirakashi, 2014 (98.0% [1,685/1,720] with two sites of nucleotide insertion/deletion (indels) against LC381991), followed by other species of *Kudoa*. A BLAST search using the initial 800 bp of the obtained 28S rDNA nucleotide sequence (total length 2,411 bp; LC640011) showed the highest nucleotide identity with sequences from *Kudoa neothunni* (Arai et Matsumoto, 1953) (94.6% [752/795] with six indels against LC200499) and *K. hexapunctata* (94.6% [752/795] with six indels against LC381991), followed by *Kudoa konishiae* Sakai, Kato, Sakaguchi, Setsuda et Sato, 2018 (94.2% [700/743] with one indel against LC316966) and other species of *Kudoa*.

Taxonomic summary

Type host: *Decapterus tabl* Berry, 1968 (Actinopterygii: Perciformes: Carangidae: Caranginae).

Type locality: Philippine Sea (North West Pacific Ocean), off Kochi, western Japan.

Site of infection: Pseudocysts in the myofibres of trunk muscles.

Type material deposited: Specimen no. 21261, Meguro Parasitological Museum, Tokyo, Japan.

Deposited rDNA sequences: GenBank accession nos. LC640010 and LC640011 (rDNA).

Prevalence: 67% (4/6) as shown in Table 2.

Etymology: The species name comes from long filamentous plasmodia.

Differential diagnosis

It is fairly difficult to differentiate myxospores of *K. longichorda* sp. n. from those of multiple *Kudoa* spp. with myxospores with four SVs and PCs and spore dimensions (width, thickness and length) less than 8 µm (see Tables 3 and 4 of Yunus et al. 2021) based solely on morphological criteria. Fourteen *Kudoa* spp. listed in tables 3 and 4 in Yunus et al. (2021) include four kudoid species from marine fishes from the Indo-Pacific region such as South China Sea and Java Sea, namely *Kudoa fujitai* Li, Tamemasa, Zhang et Sato, 2020, *Kudoa acentrogobia* Li, Inoue, Tanaka, Zhang et Sato, 2020, *Kudoa javaensis* and *Kudoa surabayaensis* Yunus, Yustinasari, Natalia, Ghosh, Sakuma, Inoue et Sato, 2021. From these four species of *Kudoa* and additional six species from fishes in the Atlantic Ocean, Mediterranean Sea, and South-East Pacific Ocean, *K. longichorda* sp. n. is molecularly distinct when their 18S and/or 28S rDNA sequences are compared.

Four other *Kudoa* spp. remain to be characterised molecularly in the future, including *Kudoa branchiata* Joy, 1972 from *Leiostomus xanthurus* Lacepède in the West Atlantic Ocean, *Kudoa camarguensis* Pampoulie, Marques, Rosecchi, Crivelli et Bouchereau, 1999 from *Pomatoschistus microps* (Krøyer) in the Malagroy Lagoon, facing Mediterranean Sea, *Kudoa peruvianus* Mateo-Salas, 1972 from *Merluccius gayi* (Guichenot) in the South-East Pacific Ocean, off the coast of Peru, and *Kudoa shkae* Dyková,

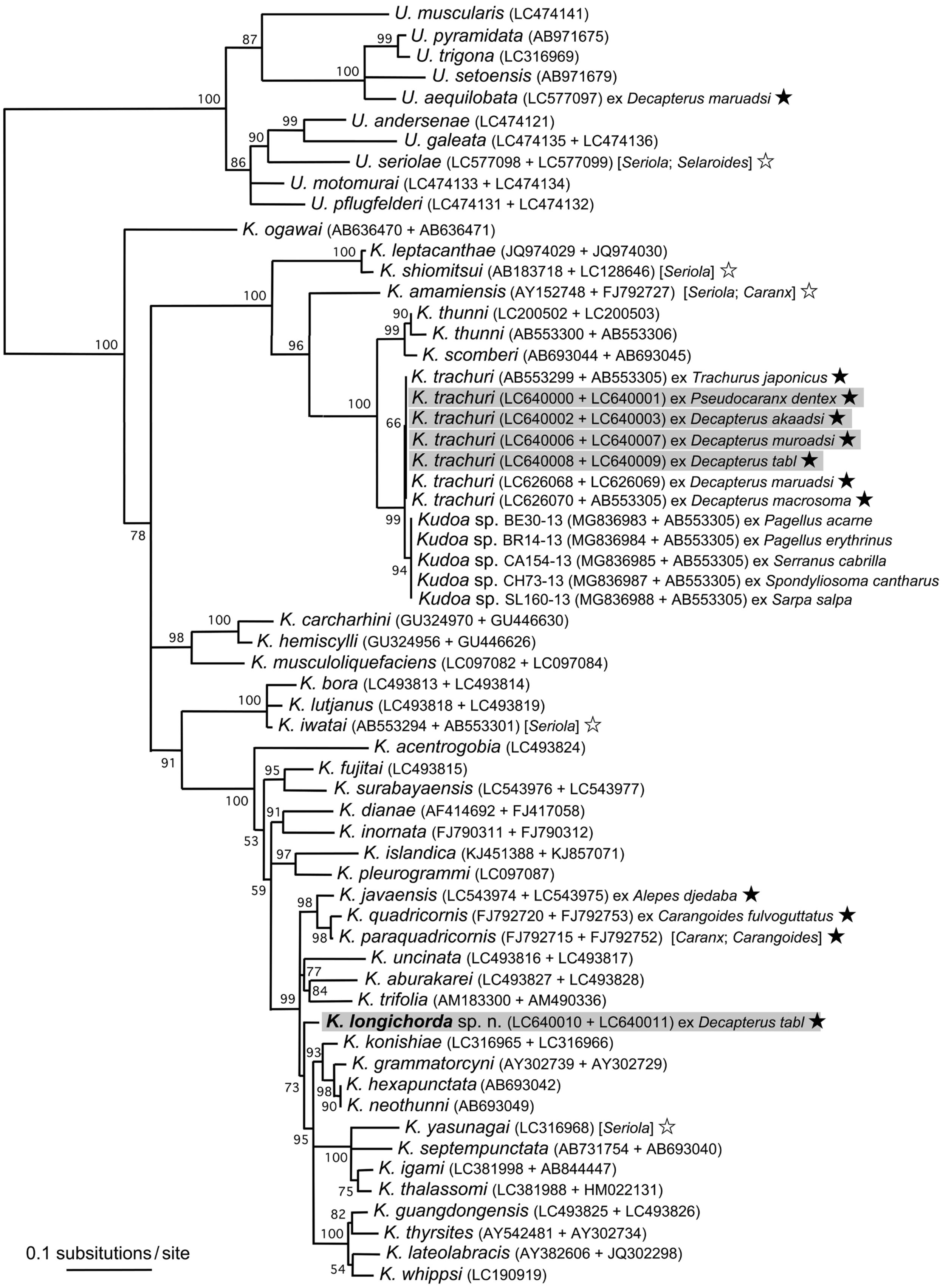

Fig. 6. Maximum likelihood phylogenetic tree based on concatenated 18S and 28S rDNA sequences (1,989 characters) of species of *Kudoa* Meglitsch, 1947. Species recorded only from carangid fish are shown with the host species or genus, and marked with a closed star, whereas species recorded from multiple fish families, including the Carangidae, are shown with the genus of fish origin, and marked with an open star. Sequences newly obtained in this study are marked with grey background.

Table 3. Morphometric comparison of *Kudoa trachuri* and *Kudoa* sp. of Rodríguez-Ponce et al. (2019) from different sources[a]

Isolate name	KG_shimaAji	KC_AkaAji	KC_MuroAji	KC_Oakamuro	Aji/080911	Aji/081204	Aji/140630	*Kudoa* sp. of Rodríguez-Ponce et al. 2019		
Host fish species	*Pseudocaranx dentex*	*Decapterus akaadsi*	*Decapterus muroadsi*	*Decapterus tabl*	*Trachurus japonicus*	*Trachurus japonicus*	*Trachurus japonicus*	*Pagellus acarne*	*Serranus cabrilla*	*Spondiliosoma cantharus*
Lacality	Pacific Ocean, off Kagoshima, Japan	Pacific Ocean, off Kochi, Japan	Pacific Ocean, off Kochi, Japan	Pacific Ocean, off Kochi, Japan	Sea of Japan, off Nagasaki, Japan	Sea of Japan, off Nagasaki, Japan	Sea of Japan, off Yamaguchi, Japan	Eastern Central Atlantic Ocean around the Canary Islands	Eastern Central Atlantic Ocean around the Canary Islands	Eastern Central Atlantic Ocean around the Canary Islands
Date of collection	Dec. 25, 2015	June 15, 2016	June 15, 2016	June 15, 2016	Sep. 11, 2008	Dec. 4, 2008	Jun. 30, 2014	—	—	—
Reference	Present study	Present study	Present study	Present study	Matsukane et al. (2011)	Matsukane et al. (2011)	Kasai et al. (2015)	Rodríguez-Ponce et al. (2019)	Rodríguez-Ponce et al. (2019)	Rodríguez-Ponce et al. (2019)
Cyst surrounding a plasmodium	(n = 13)	(n = 20)	(n = 20)	(n = 20)	(n = 3)	(n = 8)	—	(n>50)	(n>50)	(n>50)
Length (mm)	0.43–1.28 (0.87)	0.35–1.49 (0.82)	0.75–2.16 (1.27)	0.75–2.16 (1.27)	1.5–1.6 (1.6)	0.5–1.1 (0.7)	—	1.3–3.0	0.5–1.6	0.3–1.7
Width (mm)	0.25–0.56 (0.46)	0.30–0.54 (0.41)	0.31–0.74 (0.53)	0.31–0.74 (0.53)	0.6–1.1 (0.9)	0.3–0.6 (0.4)	—	1.1–1.4	0.3–1.1	0.2–0.9
Myxospore	(n = 20)	(n = 20)	(n = 20)	(n = 20)	(n = 20)	(n = 20)	(n = 20)	(n = 36)	(n = 50)	(n = 50)
Width	7.4–9.2 (8.2)	6.8–8.0 (7.5)	7.9–8.9 (8.5)	7.9–8.9 (8.5)	7.0–8.5 (7.9)	5.9–6.8 (6.3)	7.1–8.8 (8.1)	6.0–6.6 (7.8)	5.4–8.9 (7.1)	6.4–9.2 (7.8)
Thickness	6.7–8.1 (7.3)	6.2–7.3 (6.7)	6.9–8.2 (7.6)	6.9–8.2 (7.6)	5.3–6.2 (5.8)	4.5–5.6 (5.1)	5.9–7.2 (6.7)	4.0–6.6 (5.3)	3.9–7.0 (5.4)	4.9–6.2 (5.6)
Sutural thickness	5.2–6.9 (6.2)	5.2–6.3 (5.9)	6.1–7.0 (6.6)	6.1–7.0 (6.6)	—	—	—	—	—	—
Length	4.9–6.0 (5.5)	5.0–6.1 (5.6)	5.8–6.6 (6.1)	5.8–6.6 (6.1)	5.5–6.9 (6.1)	6.7–7.5 (7.0)	5.5–7.0 (6.1)	—	—	—
Polar capsule										
Length	2.4–3.2 (2.8)	2.5–3.5 (3.0)	2.4–3.5 (2.9)	2.5–3.7 (3.1)	2.6–3.5 (2.9)	2.2–2.9 (2.7)	2.1–3.3 (2.8)	2.6–4.4 (3.5)	2.7–4.5 (3.6)	2.3–3.6 (2.9)
Width	1.6–2.0 (1.8)	1.5–2.0 (1.8)	1.6–2.3 (1.9)	1.5–2.2 (1.8)	1.6–2.2 (2.0)	1.7–2.2 (2.0)	1.8–2.4 (2.0)	1.8–3.1 (2.4)	1.7–3.1 (2.4)	1.3–2.4 (1.8)

[a] Unless otherwise indicated, all measurements are in µm and expressed as ranges with means in parentheses. '—' indicates no available data.

Lom et Overstreet, 1994 from *Ariopsis felis* (Linnaeus) in the Northern Gulf of Mexico (Joy 1972, Mateo-Salas 1972, Dyková et al. 1994, Pampoulie et al. 1999).

In addition to marginally overlapped myxospore dimensions of *K. longichorda* sp. n. with *K. branchiata* and *K. shkae* (4.8–6.3 µm vs. 4.4–4.9 µm or 7.0–8.1 µm in thickness, respectively), there are additional differences, such as distinct localisation and form of plasmodia (cysts between gill filaments in *K. branchiata*, and fusiform pseudocysts in the trunk muscle in *K. shkae*), and different lateral view of myxospores (pyriform vs. oval) (Joy 1972, Pampoulie et al. 1999). Apical protrusions and a tubular extension from each of the SVs in the *K. camarguensis* are distinct morphological features from the new species, in addition to its oval lateral view of myxospores, and formation of fusiform pseudocysts in myofibres in the trunk muscle (Dyková et al. 1994). Although myxospore morphometrics of *K. peruvianus* and *K. longichorda* sp. n. show apparent overlaps in width, thickness and length, lateral views of myxospores of these two species are different in the apical end (pointed *vs*. dull) and proportion of myxospore length against width or thickness (relatively small *vs*. high) (Mateo-Salas 1972). Therefore, *K. longichorda* sp. n. is both morphologically or molecular-genetically distinct from previously recorded *Kudoa* spp.

Phylogenetic analyses

The ML phylogenetic tree based on concatenated sequences of the 18S and 28S rDNA (Fig. 6) showed that *K. trachuri* in the trunk muscle of five carangid fish species was phylogenetically distant from *K. longichorda* sp. n. from *D. tabl*. Kudoids that form cysts in the trunk muscle, such as *K. trachuri*, *Kudoa scomberi* Li, Sato, Tanaka, Ohnishi, Kamata et Sugita-Konishi, 2013 from *Scomber japonicus* Houttuyn, and *Kudoa thunni* Matsukane, Sato, Tanaka, Kamata et Sugita-Konishi, 2011 from tunas (*Thunnus* South) had sister phylogenetic relationships with each other, forming a clade closer to *Kudoa amamiensis*, which forms cysts in the trunk muscle of various pomacentrid and carangid fishes. Although the BLAST search of 18S and 28S rDNA sequences of *K. longichorda* sp. n. suggested high similarity with those of *K. javaensis* or kudoid species with five or more SVs/PCs, sister relationships of *K. longichorda* sp. n. with the above-mentioned kudoid clades were not evident phylogenetically (Fig. 6).

A BLAST search of the 18S rDNA sequence of *K. trachuri* detected highly similar rDNA sequences of *Kudoa* sp. from four sparid fish species, namely *Pagellus acarne* (Risso), *Pagellus erythrinus* (Linnaeus), *Spondyliosoma cantharus* (Linnaeus), and *Sarpa salpa* (Linnaeus), as well as *Serranus cabrilla* (Linnaeus), a serranid fish species living around the Canary Islands, Spain (Rodríguez-Ponce et al. 2019). In the ML phylogenetic tree, *K. trachuri* and *Kudoa* sp. from Canary sparid and serranid fishes exhibited the phylogenetically closest relationship, but the nucleotide similarities were between 99.0% (1,710/1,728) and 99.1% (1,712/1,728) with 6 indels, suggesting the distinctness of these two species. The 28S rDNA sequences of cyst-forming *Kudoa* sp. from Canary sparid and serranid fish species are not available for comparison and there are limited morphological observations, e.g., there is no record of lateral views of the myxospores.

DISCUSSION

For a decade, *Kudoa trachuri* has been recorded solely from *Trachurus japonicus* in the sea around western Japan (Abe et al. 2011, Matsukane et al. 2011, Li et al. 2013, Kasai et al. 2015). Thus, our observations of *K. trachuri* cysts in the trunk muscles of four additional carangid species (white trevally *Pseudocaranx dentex*, akaaji scad *Decapterus akaadsi*, amberstripe scad *D. muroadsi*, and roughear scad *D. tabl*) along with our recent detection of the species in two other carangid species (*D. macrosoma*, and *D. maruadsi*; Li et al. 2022) suggests its broad host specificity as seen for *Kudoa amamiensis* and *Unicapsula seriolae* (Table 1). However, records of *K. trachuri* remain limited to fishes of the family Carangidae to date. According to Burger and Adlard (2011), approximately two thirds (57/87) of *Kudoa* spp. were recorded from a single host at that time. This is ascribed partially to the exceptional number of extensive and intensive surveys of multivalvulid myxosporeans in a variety of marine fish (Whipps et al. 2003b, Diamant et al. 2005, Heiniger et al. 2013, Sakai et al. 2019).

Rodríguez-Ponce et al. (2019) reported a *K. trachuri*-like species, with similar myxospore morphology in the apical view and the closest molecular phylogenetic relationship to *K. trachuri* based on 18S rDNA sequences. High prevalence of this species was observed in four sparid fish species and a serranid fish species around the Canary Islands, ranging from 19% (6/31) to 87% (39/45). Although these two species both form cysts in the musculature, there was molecular differentiation, i.e., 16–18 substitutions over 1,728 bp, and six indels in the 18S rDNA sequences. While the intra-populational variations of 18S rDNA nucleotide sequences of *K. trachuri* and *Kudoa* sp. of Rodríguez-Ponce et al. (2019) was zero or few substitutions across almost their entire length regardless of host origins (five fish species each), inter-populational differences in the nucleotide sequences were evident and confirmed in this study. In addition, these two species have been recorded from distant sea areas (North-Central Atlantic Ocean around the Canary Islands vs. seawaters around the western Japan), and from different categories of fish (Sparidae and Serranidae vs. Carangidae), suggesting speciation of *K. trachuri* and *Kudoa* sp. reported by Rodríguez-Ponce et al. (2019) from a common ancestor followed by dispersal to different host fish populations.

Kudoa nova has been recorded to form macroscopic cysts in the trunk muscle of four European carangid fish of the genus *Trachurus* Rafinesque (as well as 15 fish species from the families Butidae, Gobiidae, Pomatomidae, Scombridae and Sparidae (Burger and Adlard 2011; see Table 1). These cysts are characterised by myxospores that morphologically resemble those of *K. trachuri* and *Kudoa* sp. of Rodríguez-Ponce et al. (2019), at least in the apical view. Based on the 18S rDNA, *K. nova* is phylogenetically distinct from both species, although the deposited rDNA of *K. nova* is currently limited to a single sequence of an isolate from the round goby *Neogobius melanostomus* (Pallas) from the Sea of Azov, Ukraine (EF644198; Pascual et al. 2012). It remains to be determined whether specimens of *K. nova* reported from a variety of fish species really belong to a single species. If *Kudoa* spp. phylogenetically related to *K. trachuri* and/or *Kudoa* sp. reported by Rodríguez-Ponce et al. (2019) are included in the currently recognised '*K. nova*', links between phylogenetically close species distributed in Asia (Pacific Ocean) and Europe (Atlantic Ocean) could be revealed.

Two multivalvulid species forming pseudocysts in the muscle of carangid fish have recently been recorded: *Kudoa javaensis* from *Alepes djedaba* in the Java Sea and *Unicapsula aequilobata* from *D. maruadsi* in the South China Sea (Inoue et al. 2021, Yunus et al. 2021). The former species, *K. javaensis*, is a sister species to *K. quadricornis* Whipps, Adlard, Bryant et Kent, 2003 and *K. paraquadricornis* Burger et Adlard, 2010 from carangid fishes of the genera *Caranx* and *Carangoides* in the Coral Sea of Australia (Whipps et al. 2003a, Burger and Adlard 2010a). Based on 18S and 28S rDNA sequences, these three species formed an independent clade of kudoids that form pseudocysts in the musculature of carangid fishes (see Fig. 6), although they can be morphologically differentiated from each other. This clade was phylogenetically distant from *K. longichorda* sp. n. In contrast, *K. fujitai* and *K. surabayaensis*, which form pseudocysts in the musculature and have myxospore morphology closely resembling that of *K. longichorda* sp. n., formed a clade phylogenetically distant from this new species (see Fig. 6). Phenotypical resemblance of myxospores is not related to phylogenetic closeness as discussed previously (Shin et al. 2016b, Li et al. 2020c).

Recently, host-independent exploration of environmental DNA has highlighted our limited understanding of the true myxozoan diversity in global waters (Hartikainen et al. 2016). Taxonomic identification and differentiation of multivalvulid species have progressed explosively in the last two decades (Whipps et al. 2004, Lom and Dyková 2006, Eiras et al. 2014, Atkinson et al. 2015). However, the evolutionary relationships of described species remain to be elucidated since phenotypical characteristics as well as geographical and host origins of the currently known species could only partially explain their evolutionary trends

(Shin et al. 2016b). Further investigation of undescribed and insufficiently recorded species is necessary to reveal the actual biodiversity and biogeography of multivalvulid species in the global waters.

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