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Description of three new species of *Kudoa* Meglitsch, 1947 (Myxozoa: Multivalvulida) in commercial marine fishes from southern China, and new host records

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Abstract: Multivalvulidan myxosporeans (Multivalvulida) of the genera *Unicapsula* Davis, 1924 and *Kudoa* Meglitsch, 1947 are mostly causative agents of latent and imperceptible infection in marine fishes. However, they are sometimes incriminated in causing *post-mortem* myoliquefaction or unsightly cyst formation in commercial fish. Despite the great commercial impacts of multivalvulidan infection, the biodiversity, host range and epidemiology of multivalvulidan species remain to be explored further, including infection of alternative annelid hosts. Therefore, this study aimed to identify multivalvulidan species and their host and/or distribution records in commercial fishes in China. Multivalvulidan infection was detected in ten commercial fish species of seven families from the South and East China Seas (Northwest Pacific Ocean) and the Eastern Central Atlantic Ocean (an imported *Dagetichthys lusitanicus* [de Brito Capello]). Based on morphological and molecular-genetic analyses of their small and large subunit of ribosomal RNA genes, five new host and/or geographical distribution records for five fish species are presented, and three new species in five fish species are described, namely *Kudoa neoscomberomori* sp. n. in *Scomberomorus commerson* (Lacépède); *Kudoa pilosa* sp. n. in *Helicolenus hilgendorfi* (Döderlein) (type host) and *Sebastiscus tertius* (Barsukov et Chen); and *Kudoa tumidisporica* sp. n. in *Photopectoralis bindus* (Valenciennes) (type host) and *Nuchequula nuchalis* (Temminck et Schlegel). This study provides new data on multivalvulidan diversity in the ocean ecosystem.

Keywords: Myxosporea, myxospore, morphology, diversity, molecular-genetic analysis, rDNA, mtDNA, Northwest Pacific Ocean

This article contains supporting files (Table S1, Figs S1–S4) online at http://folia.paru.cas.cz/suppl/2023-71-018.pdf

Multivalvulidan myxosporeans of the genera *Unicapsula* Davis, 1924 and *Kudoa* Meglitsch, 1947 (Cnidaria: Myxozoa: Myxosporea: Multivalvulida) are usually causative agents of latent or imperceptible infection in marine fishes. However, they are sometimes incriminated as vicious pathogens causing *post-mortem* myoliquefaction or unsightly cyst formation in commercial fish (Davis 1924, Matsumoto 1954, Egusa and Nakajima 1980, Lester 1982, Moran et al. 1999, Whipps et al. 2003a,b, Miller and Adlard 2013, Eiras et al. 2014a, Li et al. 2020a).

Moreover, food poisoning by *Kudoa* spp., which causes temporal diarrhoea and vomiting in humans after consumption of raw fish (sushi and sashimi), may be caused by *Kudoa septempunctata* Matsukane, Sato, Tanaka, Kamata et Sugita-Konish, 2010 and additional multivalvulidans (Matsukane et al. 2010, Kawai et al. 2012, Suzuki et al. 2012, 2015, Sugita-Konishi et al. 2014, Ohnishi et al. 2018). Although multivalvulidan infection has negative commercial impacts and presents a potential human health threat, the biological characteristics such as biodiversity, host range and epidemiology of multivalvulidan myxosposporeans have not been extensively explored (Okamura et al. 2015, 2018).

Multiple new species of the genera *Unicapsula* and *Kudoa* as well as new host and/or distribution record have been reported in commercial marine fish in China (Suppl. Table S1; Fujita 1930, Zhou and Zhao 2008, Li et al. 2020a–c, 2022a,b, Inoue et al. 2021). Currently, more than 120 *Kudoa* spp. and 16 *Unicapsula* spp. are known since the first descriptions of *Kudoa quadratum* (Thélohan, 1895) (syn. *Chloromyxum quadratum* Thélohan, 1895), and *Unicapsula muscularis* Davis, 1924, respectively (Miller and Adlard 2013, Eiras et al. 2014a, Sakai et al. 2018, 2019). Myxozoans are endoparasitic radiates of cnidarians that exploit in-

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vertebrate (annelids) and vertebrate (fish) hosts in aquatic environments. However, the life cycle of multivalvulidan myxosporeans is yet to be clarified (Yokoyama et al. 2012, Eszterbauer et al. 2015, Atkinson et al. 2019).

Traditionally, myxozoan classification and taxonomic identification are based on myxospore morphology. For example, members of the genus *Kudoa* (Kudoidae) are characterised as having a myxospore with four or more shell valves (SVs) and a corresponding number of polar capsules (PCs) (Whipps et al. 2003a, 2004, Lom and Dyková 2006, Fiala et al. 2015). Similarly, members of the genus *Unicapsula* (Trilosporidae) are characterised as having a myxospore with three unequal SVs and PCs, one of which is prominent and the other two rudimentary (Lom and Dyková 2006, Alama-Bermejo et al. 2009, Miller and Adlard 2013, Fiala et al. 2015).

In the last two decades, the introduction of molecular techniques such as nucleotide sequencing of the ribosomal RNA gene (rDNA) (Hervio et al. 1997, Anderson et al. 1999, Tops et al. 2005, Atkinson et al. 2015, Fiala et al. 2015) has facilitated reliable identification of myxozoan species of different geographical and/or host origins, including multivalvulidan species (Diamant et al. 2005, Whipps et al. 2003a,b, 2004, Whipps and Kent 2006, Burger et al. 2007, Burger and Adlard 2010a,b, 2011, Kasai et al. 2016a, 2017, Sakai et al. 2019, Li et al. 2013, 2020a–c, 2022a,b).

Furthermore, attempts using mitochondrial DNA (mtDNA) such as the cytochrome *c* oxidase subunit I gene (*cox-1*) and the small subunit and large subunit RNA genes (*rns-rnl*) enables differentiation of cryptic species that show little morphological differences and/or few nucleotide changes in the small subunit (SSU) and large subunit (LSU) rDNA (Takeuchi et al. 2015, 2016, Sakai et al. 2018, 2019, Li et al. 2020b, 2022c). The technical progression of these analytical methods for taxonomical approaches as well as increased interest in myxozoan biodiversity as important fish pathogens in aquaculture and capture fishery have accelerated new species descriptions and new host and/or geographical distribution records worldwide. Hence, this study aimed to use these analytical methods to identify and describe new multivalvulidan myxosporean species from commercial fishes in China and elucidate their host and/or distribution records.

In the present study, we report nine multivalvulidan species, including one *Unicapsula* and eight *Kudoa* spp., from ten commercial fish species collected at fish markets in Zhanjiang City (Guangdong Province) and Taizhou City (Zhejiang Province), China. These cities are located at the South China Sea and East China Sea coasts, respectively. Three new kudoid species and five new host and/or geographical distribution records in five fish species were identified based on myxospore morphology, molecular characterisation of their SSU and LSU rDNA sequences and, if possible, mtDNA. This study further increases our understanding of multivalvulidan diversity in the ocean ecosystem.

Table 1. Fish samples examined and detected myxospoean species in the trunk muscle in a survey in China (2021–2022).

Fish species		Collection date	Collection site	Parasite species detected[a]	Parasite isolate	Recovery[b]
Sciaenidae (Actinopterygii: Eupercaria)						
Protonibea diacanthus (Lacépède)	Blackspotted croaker	28 Dec. 2021	Zhanjiang, Guangdong Province	*Unicapsula andersenae* Miller et Adlard, 2013	Li8-4pU (pseudocyst)	1 / 1 (100%)
Mullidae (Actinopterygii: Mulliformes)						
Upeneus tragula Richardson	Freckled goatfish	13 Oct. 2021	Zhanjiang, Guangdong Province	*Kudoa lutjanus* Wang, Huang, Tsai, Cheng, Tsai, Chen, Chen, Chu, Liaw, Chang et Chen, 2005	Li8-2cK (cyst)	1 / 5 (20%)
Carangidae (Actinopterygii: Carangiformes)						
Decapterus macarellus (Cuvier)	Mackerel scad	16 Jun. 2022	Zhanjiang, Guangdong Province	*Kudoa trachuri* Matsukane, Sato, Tanaka, Kamata et Sugita-Konishi, 2011	Li9-4cK (cyst)	1 / 21 (5%)
Soleidae (Actinopterygii: Pleuronectiformes)						
Dagetichthys lusitanicus (de Brito Capello)	Portuguese sole	13 Oct. 2021	Zhanjiang, Guangdong Province (Imported)	*Kudoa iidae* Li, Inoue, Tanaka, Zhang et Sato, 2020	Li8-1pK (pseudocyst)	1 / 1 (100%)
Scombridae (Actinopterygii: Scombriformes)						
Scomberomorus niphonius (Cuvier)	Japanese Spanish mackerel	12 Jun. 2022	Zhanjiang, Guangdong Province	*Kudoa konishiae* Sakai, Kato, Sakaguchi, Setsuda et Sato, 2018	Li9-2pK (pseudocyst)	2 / 5 (40%)
Scomberomorus commerson (Lacépède)	Narrow-barred Spanish mackerel	15 Jun. 2022	Zhanjiang, Guangdong Province	***Kudoa neoscomberomori* sp. n.**	Li9-3pK (pseudocyst)	3 / 8 (38%)
Sebastidae (Actinopterygii: Perciformes)						
Helicolenus hilgendorfi (Döderlein)	Hilgendorf's saucord	13 Oct. 2021	Taizhou, Zhejiang Province	***Kudoa pilosa* sp. n.**	Li8-3pK (pseudocyst)	5 / 7 (71%)
Sebastiscus tertius (Barsukov et Chen)	Red marbled rockfish	29 Sep. 2021	Taizhou, Zhejiang Province	***Kudoa pilosa* sp. n.**	Li9-1pK (pseudocyst)	1 / 1 (100%)
Leiognathidae (Actinopterygii: Acanthuriformes)						
Photopectoralis bindus (Valenciennes)	Orangefin ponyfish	31 Dec. 2021	Zhanjiang, Guangdong Province	*Kudoa uncinata* Zhou et Zhao, 2008	Li8-5pK1 (pseudocyst)	2 / 4 (50%)
				***Kudoa tumidisporica* sp. n.**	Li8-5pK2 (pseudocyst)	2 / 4 (50%)
Nuchequula nuchalis (Temminck et Schlegel)	Spotnape ponyfish	31 Dec. 2021	Zhanjiang, Guangdong Province	*Kudoa uncinate* Zhou et Zhao, 2008	Li8-6pK1 (pseudocyst)	1 / 1 (100%)
				***Kudoa tumidisporica* sp. n.**	Li8-6pK2 (pseudocyst)	1 / 1 (100%)

[a] All multivalvulid species (*Unicapsula* and *Kudoa* spp.) were found in the trunk muscles.

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

MATERIALS AND METHODS

Fish samples examined

Fifty-four individuals of ten fish species were purchased from fish markets in Zhanjiang City, Guangdong Province, or Taizhou City, Zhejiang Province, China, between September 2021 and June 2022, and parasitological examination was conducted at the Guangdong Ocean University (Table 1). Putative host fish species were identified using DNA barcoding of the mitochondrial *cox-1*, as conducted in our previous studies (Li et al. 2020a–c, 2022a,b). The Portuguese sole, *Dagetichthys lusitanicus* (de Brito Capello), was deep-frozen at purchase. This fish specimen was captured and transported from the Eastern Central Atlantic Ocean, off the West African coast between Mauritania and Angola (based on the species' known distribution), although the exact location of capture was unknown. The remaining fish species were captured in the South China Sea, except for two sebastid fish species, which were captured in the East China Sea.

Parasitological examination

The collected fishes were thawed (if necessary) and dissected, and all organs were carefully examined with the naked eye. To check for the presence of myxosporean plasmodia in cysts or pseudocysts, thin slices of the trunk muscle were placed in physiological saline, pressed between glass plates and examined under a dissection microscope. Muscle tissue pieces containing myxosporean plasmodia were then divided into two groups and fixed in either 10% neutral-buffered formalin solution for morphological examination, or 70% ethanol for molecular analysis.

For morphological examination, the myxospores were released from the formalin-fixed tissues using fine forceps, observed using a light microscope equipped with differential interference contrast imaging (Olympus BX60, Olympus Co., Shinjuku, Tokyo, Japan) and photographed at ×800 magnification. The photographs were processed using Adobe® Photoshop® ver. 11.0 (Adobe Systems, San Jose, California, USA) and printed at a high magnification. Measurements were conducted on multiple printed photographs, following the guidelines of Lom and Arthur (1989).

The myxospore measurements were compared with those from different researchers, paying close attention to the notion that formalin-fixed myxospores may be marginally smaller than fresh myxospores from the same source (Parker and Warner 1970). All measurements are expressed in µm unless otherwise stated and reported as ranges with means, or mean ± standard deviation, in parentheses. The fixed parasite specimens were deposited in the Meguro Parasitological Museum, Tokyo, Japan (collection nos. 25005–25016).

Histological examination of the trunk muscles was performed on a specimen of the narrow-barred Spanish mackerel, *Scomberomorus commerson* (Lacépède). Briefly, a muscle block was immersed in 10% neutral-buffered formalin solution for one week, washed in tap water, dehydrated through a graded ethanol series, cleared in xylene, and embedded in paraffin. Histological sections (5-µm thick) were prepared and stained with hematoxylin and eosin (HE) following a standard protocol.

DNA extraction, PCR amplification and Sanger sequencing

Small pieces of the ethanol-preserved fish muscle tissue containing myxosporean plasmodia were dissected under a stereomicroscope, and myofibres containing the plasmodia were isolated. After washing three times in sterile pure water, DNA was extracted individually from myofibres with a single plasmodium of different origins (at least two plasmodia/isolate, except for the Li8-2cK isolate) using an Illustra™ tissue and cells genomicPrep Mini Spin Kit (GE Healthcare UK, Buckinghamshire, UK) according to the manufacturer's instructions. To determine the fish species of the putative host, amplification of the fish *cox-1* region using polymerase chain reaction (PCR) was performed in a 20-µl reaction solution using primer pairs of FishF1 and FishR1 (Ward et al. 2005), and FishF2_t1 and FR1d_t1 (Zhang and Hanner 2011), as described previously (Li et al. 2020a,c). For molecular characterisation of myxosporean species, overlapping rDNA fragments were amplified by PCR using multiple primer pairs as reported by Li et al. (2013), Tomochi et al. (2014), and Kasai et al. (2015). For further molecular-genetic characterisation of kudoid isolates, PCR amplification and nucleotide sequencing of the mtDNA, i.e. *cox-1* and *rns-rnl* regions, were conducted according to our previous study (Sakai et al. 2018, 2019, Li et al. 2020b).

The resulting PCR products were purified using a FastGene Gel/PCR Extraction Kit (NIPPON Genetics Co., Bunkyo-ku, Tokyo, Japan) and were then directly sequenced by Sanger sequencing through a commercial service (FASMAC, Atugi, Kanagawa, Japan) using PCR amplification and sequencing primers (Li et al. 2013, Tomochi et al. 2014). The resulting nucleotide sequences are available from the GenBank database under the accession nos. LC705434–LC705439 and LC745033–LC745036 (fish *cox-1*), LC777071–LCLC777090 (myxosporean rDNA), and LC777091–LCLC777095 (myxosporean mtDNA).

Phylogenetic analysis

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 SSU and LSU rDNA sequences of 12 multivalvulid isolates in this study and related sequences retrieved from the GenBank database (NCBI) were aligned using ClustalW (Thompson et al. 1994) incorporated into the MEGA7 software (Kumar et al. 2016) with manual adjustments, which involved excluding poorly aligned regions and base sites with gaps or ambiguous nucleotides in any sequences from subsequent analyses. The accession numbers of the analysed sequences are given in the figure of the phylogenetic tree.

Finally, 1,372 characters, of which 402 were variable, and 472 characters, of which 232 were variable, were retained for subsequent analysis of the SSU and LSU rDNA, respectively. Similarly, 437 characters, of which 179 were variable, and 982 characters, of which 474 were variable, were retained for subsequent analysis of the *cox-1* and *rns-rnl* mtDNA, respectively. Maximum likelihood (ML) analysis was performed with the PhyML program (Guindon and Gascuel 2003, Dereeper et al. 2008) provided on the 'phylogeny.fr' website (http://phylogeny.lirmm.fr/phylo_cgi/index.cgi). 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). Seven representative

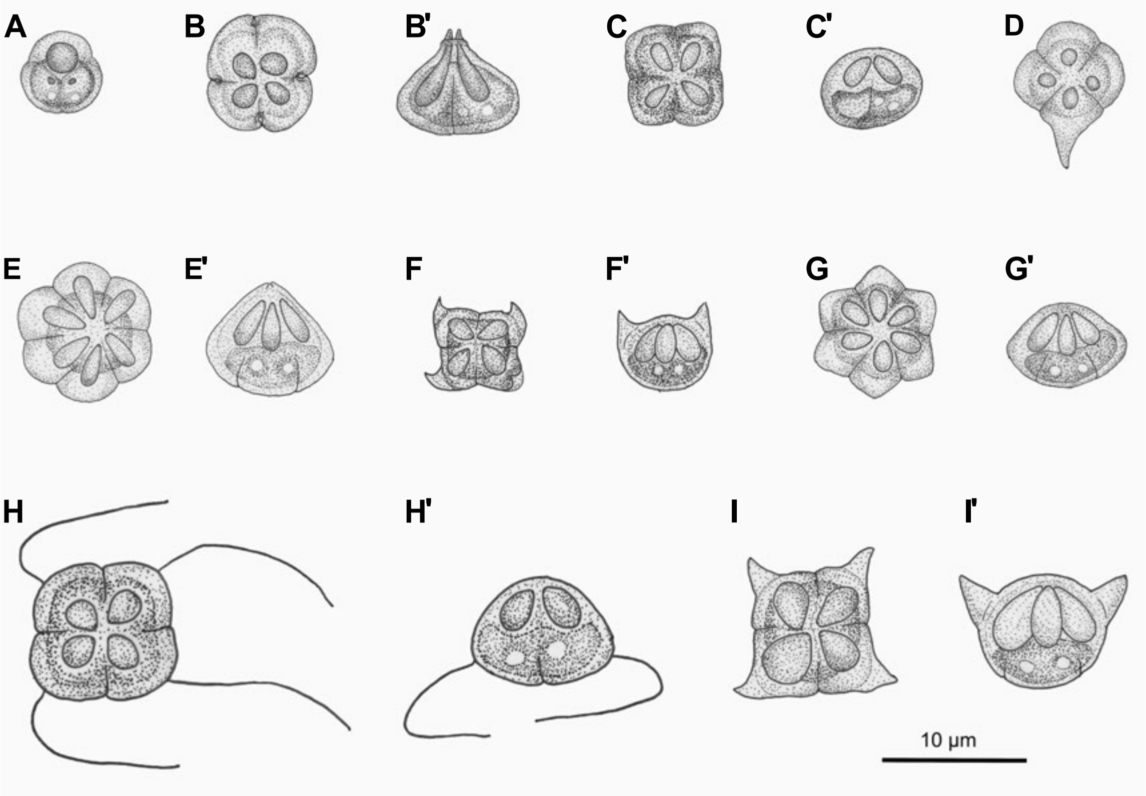

Fig. 1. Schematic diagrams of collected myxospores of multivalvulidan myxozoans. **A** – *Unicapsula andersenae* (Li8-4pU); **B**, **B'** – *Kudoa lutjanus* (Li8-2cK); **C**, **C'** – *Kudoa trachuri* (Li9-4cK); **D** – *Kudoa iidae* (Li8-1pK); **E**, **E'** – *Kudoa konishiae* (Li9-2pK); **F**, **F'** – *Kudoa uncinata* (Li8-5pK1/Li8-6pK1); **G**, **G'** – *Kudoa neoscomberomori* sp. n. (Li9-3pK); **H**, **H'** – *Kudoa pilosa* sp. n. (Li8-3pK/Li9-1pK); and **I**, **I'** – *Kudoa tumidisporica* sp. n. (Li8-5pK2/Li8-6pK2). Except for *U. andersenae* (A, frontal view) and *K. iidae* (D, apical view), myxospores of each species are illustrated as the apical view (left) and lateral view (right). All diagrams at the same magnification (scale bar = 10 µm).

Unicapsula spp. of the order Multivalvulida and a genus closely related to *Kudoa* were used as outgroups to plot ML phylogenetic trees based on the SSU and LSU rDNA nucleotide sequences.

RESULTS

Parasitological examination

Microscopical examination of the organs and/or tissues of ten fish species, which were genetically confirmed based on *cox-1* sequences, detected myxosporean cysts or pseudocysts only in the trunk muscles. Two fish specimens (one *Upeneus tragula* Richardson and one *Decapterus macarellus* [Cuvier]) had myxosporean cysts between myofibres, and 16 fish specimens of eight fish species had myxosporean pseudocysts in the myofibres or the muscular interstitial tissue (Table 1). In total, myxospores of nine morphological types were identified (Fig. 1). One morphological type (Li8-4pU; Fig. 1A) consisted of three SVs and a corresponding number of PCs (one large and two rudimentary PCs), which is congruent with the definition of the genus *Unicapsula*. The remaining morphological types were characterised by four or six SVs and a corresponding number of PCs (Fig. 1B–I), which is congruent with the definition of the genus *Kudoa*. Combined morphological and molecular-genetic characterisations of each isolate are separately detailed below.

As mentioned above, all myxosporean isolates examined in the present study were classified in either the genus *Unicapsula* (one species) or *Kudoa* (eight species).

***Unicapsula andersenae* Miller et Adlard, 2013** (Li8-4pU isolate)

Numerous myxosporean plasmodia were found in an examined specimen of the blackspotted croaker *Protonibea diacanthus* (Lacépède) from the South China Sea. Plasmodia of *U. andersenae* Miller et Adlard, 2013 of the Li8-4pU isolate formed elongated pseudocysts with tapering ends in the myofibres, measuring 2.51–5.86 (3.93 ± 0.96) mm in length and 72–137 (99 ± 18) in width (n = 17).

Bilaterally symmetrical, almost spherical myxospores (Fig. 1A; n = 25) measured 5.2–6.2 (5.6) in width by 4.9–5.9 (5.5) in length. Each myxospore contained one prominent semi-spherical PC, which measured 2.0–2.7 (2.3) by 1.8–2.4 (2.1), and two oval rudimentary PCs, which measured 0.4–0.8 (0.6) by 0.3–0.6 (0.4). Coils of polar tubes in prominent PCs were not discernible.

Two sets of the newly obtained rDNA nucleotide sequences from different plasmodia of the Li8-4pU isolate were identical to each other. They contained partial SSU rDNA (1,742 bp), an internal transcribed spacer (ITS) -1 region (451 bp), 5.8S rDNA (160 bp), ITS-2 region

(261 bp), and LSU rDNA (2,750 bp) (DDBJ/EMBL/GenBank accession no. LC777071).

The rDNA sequences of the Li8-4pU isolate were identical with those of *U. andersenae* isolates from the donkey croaker *Pennahia aneus* (Bloch) (isolate Li4-1; LC474123) and the tigertooth croaker *Otolithes ruber* (Bloch et Schneider) (isolate Li1-1; LC474121) from the South China Sea. An exception was the ITS-1 region where the present Li8-4pU isolate showed 99.1% (446/450) and 95.8% (432/451) similarity with those of the aforementioned past isolates, respectively.

Remarks. Dimensions of myxospores, functional PCs and other morphological features of the present Li8-4pU isolate were highly similar to the known morphology of *U. andersenae* (Miller and Adlard 2013, Li et al. 2020a). Three genetic lineages of *U. andersenae* were reported by us (Li et al. 2020a) in fishes of the families Sciaenidae (*Otolithes ruber*, *Pennahia aneus*, *Pennahia argentata* [Houttuyn]), Cynoglossidae (*Cynoglossus puncticeps* [Richardson]), Gerreidae (*Gerres filamentosus* Cuvier) and Hapalogenyidae (*Hapalogenys analis* Richardson) from the South China Sea.

In addition, two genetic lineages of *U. andersenae* were recorded in fishes of the Sciaenidae (*Argyrosomus japonicus* [Temminck et Schlegel]), Sparidae (*Acanthopagrus australis* [Günther]), Polynemidae (*Eleutheronema tetradactylum* [Shaw]), Lutjanidae (*Lutjanus russellii* [Bleeker]) and Sillaginidae (*Sillago ciliata* Cuvier) from the Australian Coral Sea (Miller and Adlard 2013). The new Li8-4pU isolate reported in this study in *Protonibea diacanthus* (Sciaenidae) belongs to a genetic lineage of *U. andersenae* previously recorded in three sciaenid fishes from the South China Sea and Australian Coral Sea. The present isolation of *U. andersenae* from *Protonibea diacanthus* is a new host record.

***Kudoa lutjanus* Wang, Huang, Tsai, Cheng, Tsai, Chen, Chen, Chu, Liaw, Chang et Chen, 2005** (Li8-2cK isolate)

Three myxosporean plasmodia, forming microscopic oval cysts between the myofibres, were found in the trunk muscles of one of the five fish specimens of the freckled goatfish, *Upeneus tragula*, from the South China Sea. The parasitised mullid specimen measured 11.1 cm in standard body length.

Myxospores of the Li8-2cK isolate were semiquadrate with four equal SVs and an identical number of equal PCs in the apical view (Fig. 1B). Four small suture notches were noticed at the periphery of the spore bottom, and apical protrusions corresponding to the apical ends of PCs were observed. In the lateral view, myxospores were drawstring-pouch in shape, with long pyriform PCs (Fig. 1B'). The myxospores had the following dimensions (n = 20): width 9.5–11.6 (10.8), thickness 8.4–10.7 (9.7), sutural thickness 7.6–10.4 (9.1), length 8.1–10.9 (9.2), length without apical protrusions 6.9–9.6 (8.1), PC length 4.7–6.4 (5.6), and PC width 1.5–1.9 (1.8).

Nucleotide sequences of the SSU and LSU rDNA, 1,694 bp and 759 bp long, respectively, were obtained for this kudoid isolate (DDBJ/EMBL/GenBank accession nos. LC777072 and LC777073, respectively). A BLAST search using the full-length sequence of the newly obtained SSU rDNA showed the highest nucleotide identity with *Kudoa lutjanus* Wang, Huang, Tsai, Cheng, Tsai, Chen, Chen, Chiu, Liaw, Chang et Chen, 2005 (100% [1,690/1,690] (LC493818, LC626064, and LC626066), followed by *Kudoa iwatai* Egusa et Shiomitsu, 1983 (maximum 99.5% [1,685/1,694] with AB553294), *Kudoa bora* (Fujita, 1930) (99.1% [1,679/1,694] with LC483813), and the other *Kudoa* spp.

A BLAST search using the full-length sequences of the newly obtained LSU rDNA showed the highest nucleotide identity with *K. lutjanus* (99.9% [759/760] with one insertion/deletion site (indel) against LC493819, LC626065, LC626067), followed by *K. iwatai* (maximum 97.2% [727/748] with 24 indels against AB693041), *K. bora* (95.7% [704/736] with 23 indels against LC493814), and the other *Kudoa* spp. A BLAST search using the *rns-rnl* region of the mtDNA (1,566 bp) of the present Li8-2cK isolate (accession no. LC777093) showed the highest nucleotide identity with *K. lutjanus* (99.5% [1,557/1,565] identity with one indel against LC494286), followed by *K. iwatai* (87.0% [1,280/1,472] identity with 125 indels against LC494282).

Remarks. *Kudoa lutjanus* has been recorded in four fish species of four families belonging to three different orders from the South China Sea (Wang et al. 2005, Li et al. 2020b): the crimson snapper, *Lutjanus erythropterus* Bloch (Eupercaria: Lutjanidae); the yellowfin seabream, *Acanthopagrus latus* (Houttuyn) (Eupercaria: Sparidae); the bald glassy, *Ambassis gymnocephalus* (Lacépède) (Ovalentaria: Ambassidae); and the saddleback silver-biddy, *Gerres limbatus* Cuvier (Eupercaria: Gerreidae). The present isolation of *K. lutjanus* from the trunk muscle of the freckled goatfish, *U. tragula* (Mulliformes: Mullidae), expands its host spectrum to five fish species of five families belonging to three order-ranks.

***Kudoa trachuri* Matsukane, Sato, Tanaka, Kamata et Sugita-Konishi, 2011** (Li9-4cK isolate)

One of 21 examined specimens of the mackerel scad, *Decapterus macarellus* (Cuvier), was heavily infected with *Kudoa trachuri* (Li9-4cK isolate). This parasitised carangid specimen measured 28.5 cm in standard body length. Numerous ovoid cysts (Suppl. Fig. S1), measuring 0.44–1.69 (0.72 ± 0.27) mm by 0.18–0.48 (0.29 ± 0.08) mm (n = 22), were dispersed in the trunk muscles.

Myxospores were semiquadrate with four equal SVs and PCs in the apical view (Fig. 1C). The lateral view of myxospores was oval, with teardrop-shaped PCs occupying the apical half of myxospores (Fig. 1C'). The myxospores had the following dimensions (n = 25): width 6.8–8.6 (7.6), thickness 5.7–7.3 (6.5), sutural thickness 5.3–6.6 (6.0), length 5.4–6.4 (5.7), PC length 2.3–2.8 (2.5), and PC width 1.3–1.8 (1.5).

Two SSU rDNA nucleotide sequences (1,776 bp) of different plasmodia of the present Li9-4cK isolate were identical with each other (DDBJ/EMBL/GenBank accession no. LC777074), showing almost complete identity with *K. tra-*

Table 2. Morphological comparison of *Kudoa konishiae* from different origins, and closely-related *Kudoa* spp. with *K. scomberomori*-like myxospores[a]

Species	*K. konishiae*	*K. konishiae*	*K. konishiae*	*K. neoscomberomori* sp. n.	*K. scomberomori*	*K. scomberomori*	*K. grammatorcyni*
Locality	South China Sea, off Guangdong, China	Sea of Japan. off Yamaguchi, Japan	Philippine Sea, off Kagoshima, Japan	South China Sea, off Guangdong, China	Great Barrier Reef, Queensland, Australia	South China Sea, off the north central coast of Vietnam	Great Barrier Reef, Queensland, Australia
Number of SV/PC per spore	6	6	6	6	6	6	6
Host	*Scomberomorus niphonius*	*Scomberomorus niphonius*	*Scomberomorus niphonius*	*Scomberomorus commerson*	*Scomberomorus commerson*	*Scomberomorus guttatus*	*Grammatorcynus bicarinatus*
Reference	Present study	Sakai et al. (2018)	Sakai et al. (2018)	Present study	Adlard et al. (2005)	Chinh et al. (2018)	Adlard et al. (2005)
Plasmodium in myofibres	(n = 12)	(n = 8)	(n = 8)	(n = 35)	—	—	—
Length (mm)	0.25–0.52 (0.36)	0.15–0.42 (0.28)	0.22–0.53 (0.34)	0.78–1.94 (1.34)	—	0.4–0.7	2–3
Width	32–69 (45)	70–120 (90)	40–130 (80)	32–59 (44)	—	100–300	—
Myxospore	(n = 4; partially ≥8)	(n = 20)	(n = 20)	(n = 35)	(n = 30)	(n = 30)	(n = 30)
Width	9.3–10.1 (9.7)	8.1–9.7 (9.0)	8.1–9.6 (8.9)	8.2–9.4 (8.7)	6.8–8.2 (7.6)	7.2–7.5 (7.4)	8.0–9.0 (8.6)
Thickness	8.7–9.2 (8.8)	7.2–8.8 (8.2)	7.1–8.8 (8.1)	7.1–8.4 (7.7)	6.2–7.6 (6.8)	6.4–6.6 (6.5)	7.6–8.7 (8.1)
Sutural thickness	7.8–8.7 (8.3)	6.7–7.5 (7.1)	6.0–7.5 (6.7)	6.3–7.5 (6.9)	5.3–6.3 (5.9)	5.7–6.1 (5.8)	7.2–8.2 (7.7)
Length	8.5–9.7 (9.1)	7.1–7.9 (7.5)	7.1–8.8 (8.1)	5.9–7.1 (6.5)	5.0–6.2 (5.4)	6.5–6.7 (6.6)	6.3–6.7 (6.5)
Polar capsule							
Length	3.2–4.3 (3.9)	2.7–4.2 (3.6)	3.2–4.7 (3.8)	2.7–3.8 (3.4)	3.0–3.6 (3.2)	3.3–3.4 (3.4)	3.6–3.8 (3.7)
Width	1.3–1.7 (1.6)	1.2–1.8 (1.5)	1.3–2.5 (1.7)	1.2–1.6 (1.5)	1.3–1.5 (1.4)	1.1–1.4 (1.3)	1.7–1.8 (1.7)

[a] All measurements are in µm, except for plasmodium length, and expressed as range with mean in parentheses. '—' indicates no available data.

churi recorded in various carangid fish from Japanese seawaters and the South China Sea (AB553299, AB693043, LC066367, LC626068, LC626070, LC640000, LC640004, and LC640006). All these sequences including those obtained for the present isolate showed little variations with 99.8% (1,773/1,776)–100% (1,776/1,776) nucleotide identities between different isolates.

Remarks. *Kudoa trachuri* had been understood for more than 10 years as a species forming ovoid macroscopic cysts in the trunk muscle of the Japanese jack mackerel, *Trachurus japonicus* (Temminck et Schlegel), from the sea around western Japan (Matsukane et al. 2011, Abe et al. 2011, Li et al. 2013, Kasai et al. 2015). Recently, we reported its distribution in the South China Sea (Li et al. 2022a), by adding *Decapterus maruadsi* (Temminck et Schlegel) and *Decapterus macrosoma* Bleeker as the hosts of *K. trachuri*. Inoue et al. (2022) added *Pseudocaranx dentex* (Bloch et Schneider), *Decapterus akaadsi* Abe, *Decapterus muroadsi* (Temminck et Schlegel) and *Decapterus tabl* Berry to the list of host fishes for this species. Our present isolation of *K. trachuri* from *Decapterus macarellus* is a new host record. These studies indicate that a variety of carangid fish are susceptible to *K. trachuri* parasitism.

Kudoa iidae Li, Inoue, Tanaka, Zhang et Sato, 2020

(Li8-1pK isolate)

In the trunk muscle of an imported Portuguese sole *Dagetichthys lusitanicus* (35.5 cm in standard body length) from the Eastern Central Atlantic Ocean off the West African coast, ten plasmodia of ovoid or indefinite shape (e.g., 5.56 mm by 2.41 mm, 2.35 mm by 1.08 mm and 1.22 mm by 0.69 mm when three plasmodia were measured) were found; however, their exact location in the tissue was uncertain (Suppl. Fig. S2).

The contents of these plasmodia were kudoid myxospores (Li8-1pK isolate), but almost all of them were deformed (disintegrated). A few myxospores with better morphology showed bilaterally symmetric ray-like form in the apical view with four ovoid PCs (Fig. 1D). One myxospore with better morphology (apical view) showed maximum width (long axis) 11.0, minimum width (short axis) 8.5, thickness 9.3 or 6.9, sutural thickness 7.5 or 6.5, and spherical PC diameter 1.1. Another myxospore (lateral view) showed length 5.5 and spherical PC length 1.1.

Two sets of rDNA nucleotide sequences containing partial SSU rDNA (1,698 bp) and LSU rDNA (2,003 bp) sequences, identical to each other, were obtained for the present Li8-1pK isolate (DDBJ/EMBL/GenBank accession nos. LC777075 and LC777076). All lengths of these sequences were completely identical with the SSU and LSU rDNA nucleotide sequence of *Kudoa iidae* Li, Inoue, Tanaka, Zhang et Sato, 2020 isolated from the Royal threadfin, *Pentanemus quinquarius* (Linnaeus), from the Eastern Central Atlantic Ocean (accession nos. LC493822 and LC493823, respectively).

Remarks. Myxospore deformation (disintegration) was evident in the present Li8-1pK specimen, and made morphological identification of the species difficult. Although the reason(s) why such deformation of myxospores occurred is

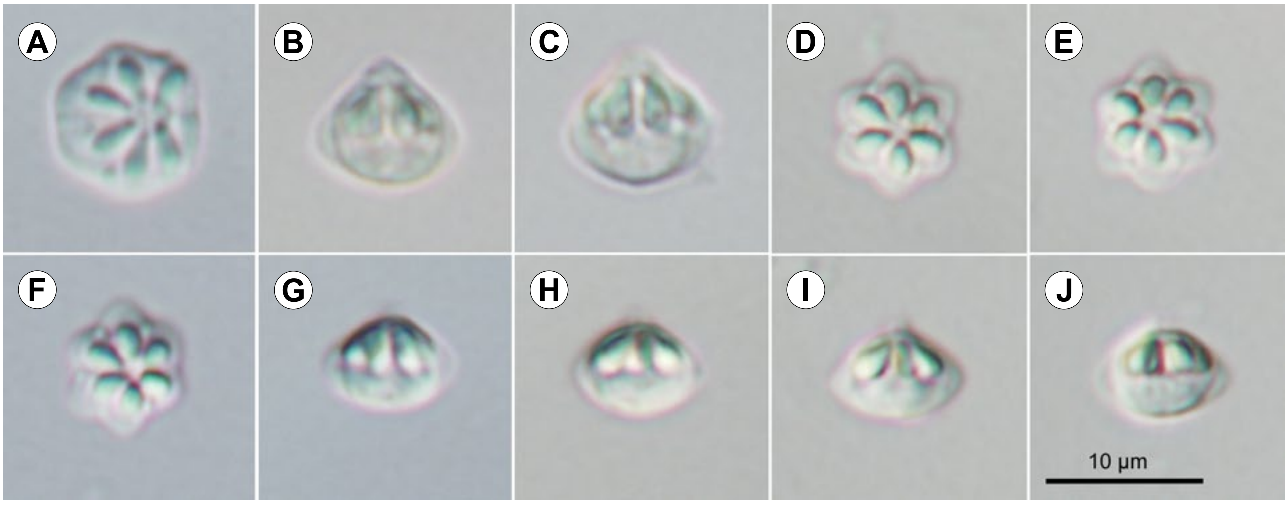

Fig. 2. Microscopic images of the myxospores of *Kudoa konishiae* Sakai, Kato, Sakaguchi, Setsuda et Sato, 2018 from *Scomberomorus niphonius* (Cuvier) (A–C) and *Kudoa neoscomberomori* sp. n. from *Scomberomorus commerson* (Lacépède) (D–J). **A**, **D**–**F** – Spores in apical view; and **B**, **C**, **G**–**J** – Spores in lateral view at the same magnification (scale bar = 10 µm).

uncertain, we hypothesise that it was induced by the processing of the fish between the capture and selling point. Molecular-genetic analyses of the SSU and LSU rDNA sequences of the current isolate greatly helped our reliable species identification. The present record of *K. iidae* in *Dagetichthys lusitanicus* (Actinopteri: Pleuronectiformes: Soleidae) is the second discovery of the species after its original discovery in the royal threadfin (Li et al. 2020c), which is classified in the family Polynemidae (Actinopteri: Carangaria).

Kudoa konishiae **Sakai, Kato, Sakaguchi, Setsuda et Sato, 2018** (Li9-2pK isolate)

Two specimens of the Japanese Spanish mackerel, *Scomberomorus niphonius* (Cuvier), from the South China Sea, were occasionally infected with a kudoid species forming small-sized pseudocysts in the myofibres of trunk muscles. Dimensions of fusiform plasmodia from the present Li9-2pK isolate were 0.25–0.52 (0.36 ± 0.09) mm in length and 32–69 (45 ± 11) in width (n = 12).

Stellate myxospores isolated from the plasmodia had six equal SVs and an identical number of radially arranged PCs in apical view (Figs. 1E, 2A). In lateral view, the myxospores were drawstring-pouch-shaped with pointed apices and convex bottoms, and teardrop-shaped PCs occupied the apical half of the spores (Figs. 1E', 2B,C). The myxospores of the Li9-2pK isolate had dimensions of: width 9.3–10.1 (9.7; n = 4), thickness 8.7–9.2 (8.8; n = 4), sutural thickness 7.8–8.7 (8.3; n = 4), length 8.5–9.7 (9.1; n = 8), PC length 3.2–4.3 (3.9; n = 14), and PC width 1.3–1.7 (1.6; n = 14). Morphometric comparison of different isolates of *Kudoa konishiae* Sakai, Kato, Sakaguchi, Setsuda et Sato 2018 is presented in Table 2.

Two sets of the newly obtained SSU and LSU rDNA nucleotide sequences for different Li9-2pK plasmodia (DDBJ/EMBL/GenBank accession nos. LC777077 and LC777078) were identical with each other. These nucleotide sequences were 1,699 bp and 743 bp in length, respectively, exhibiting 99.9% (1,560/1,561) identity with the SSU rDNA of *K. konishiae* (LC316965; 1,561 bp) and 99.9% (742/743) identity with its LSU rDNA (LC316966; 743 bp). Partial nucleotide sequences of the *cox-1* (711 bp) and *rns-rnl* (1,565 bp) were obtained for the Li9-2pK isolate (accession nos. LC777091 and LC777094), exhibiting 100% (437/437) identity with the *cox-1* of *K. konishiae* (LC316996; 437 bp) and 100% (1,367/1,367) identity with its *rns-rnl* (LC317003; 1,367 bp).

Remarks. *Kudoa konishiae* was originally described in the Japanese Spanish mackerel from the Sea of Japan, off Yamaguchi Prefecture, Japan, and the Philippine Sea, off Kagoshima Prefecture, Japan (ABU and KGS isolates, respectively; Sakai et al. 2018). The new Li9-2pK isolate was detected in the trunk muscle of the same fish species from the South China Sea off Zhanjiang City, Guangdong Province, China. Hence, it is highly possible that *K. konishiae* is widely distributed with its host fish (*S. niphonius*), which are distributed in the subtropical and temperate waters of the Northwest Pacific Ocean, off the eastern parts of the Asian continent. Moreover, this study demonstrates the highest level of genetic conservation (rDNA and mtDNA such as *cox-1* and *rns-rnl*) as well as morphology (particularly, greater relative spore length, i.e., right triangle against flattened isosceles triangle) of *K. konishiae* (see Fig. 2).

Kudoa uncinata **Zhou et Zhao, 2008** (Li8-5pK1 and Li8-6pK1 isolates)

Two types of myxosporean plasmodia, different in width, were frequently found in the trunk muscles of two specimens of the orangefin ponyfish, *Photopectoralis bindus* (Valenciennes), and one specimen of the spotnape ponyfish, *Nuchequula nuchalis* (Temminck et Schlegel), both from the South China Sea. Examined orangefin ponyfish specimens measured 7.3–9.6 (8.4 ± 1.0; n = 4) cm, and an examined spotnape ponyfish specimen measured 9.4 cm in standard body length.

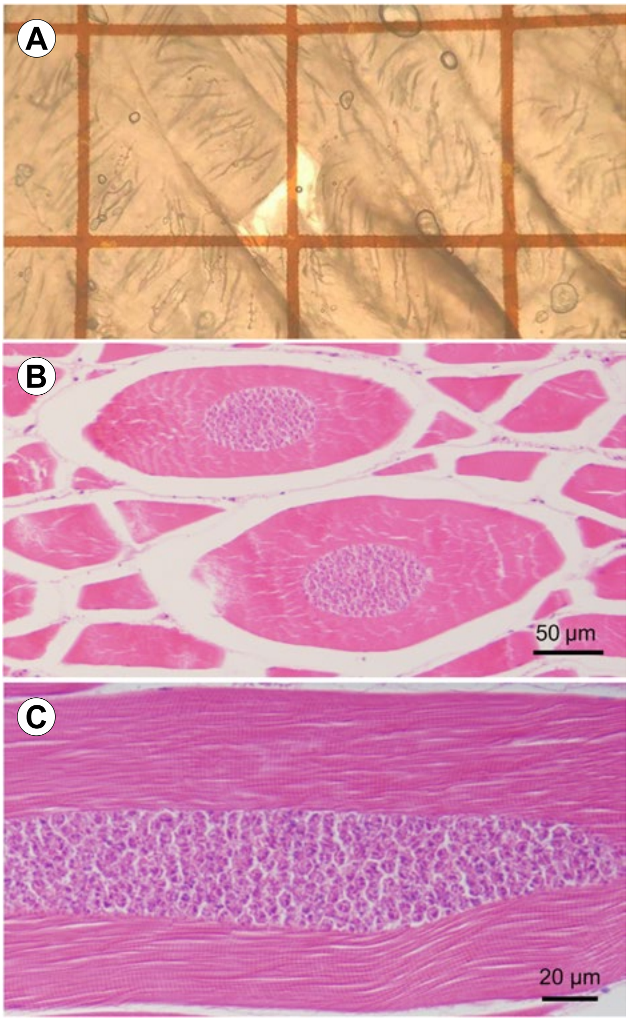

Fig. 3. Muscle tissues of *Scomberomorus commerson* (Lacépède) infected with plasmodia of *Kudoa neoscomberomori* sp. n. **A** – Stereomicroscopic view of plasmodia densely packed in myofibres (blackish brown color). Grid interval = 5 mm. **B** – Transverse histological view of myofibres containing kudoid plasmodia with myxospores (scale bar = 50 µm). **C** – Longitudinal histological view of a myofibre containing a kudoid plasmodium filled with myxospores (scale bar = 20 µm).

Thicker plasmodia (Suppl. Fig. S3), measuring 0.18–2.62 (0.74 ± 0.52) mm in length, and 44–154 (71 ± 20) in width (n = 30), contained kudoid myxospores characterised by semiquadrate shape in the apical view, four almost equal SVs and PCs, and distinct uplifted tips of the SV termini (Figs. 1F), identical to those of *Kudoa uncinata* Zhou et Zhao, 2008. Suture lines were unclear with no apical protrusions. Pyriform PCs occupied at least the apical half of oval myxospores in the lateral view (Fig. 1F'). The myxospores had the following dimensions (n = 30): width 7.2–8.9 (7.9), thickness 5.4–6.6 (6.0), sutural thickness 5.1–6.2 (5.5), length 4.8–6.1 (5.4), PC length 2.6–3.5 (2.9), and PC width 1.1–2.4 (2.0).

The SSU and LSU rDNA nucleotide sequences, 1,697 and 2,341 bp in length, respectively, were obtained for the Li8-5pK1 (DDBJ/EMBL/GenBank accession nos. LC777083 and LC777084, respectively) and Li8-6pK1 isolates (accession nos. LC777087 and LC777088, respectively). A BLAST search for the SSU rDNA exhibited an almost complete nucleotide identity (1,696/1,697 or 1,676/1,676) with the deposited sequence of *K. uncinata* isolates from the spotnape ponyfish (Li1-5pK; accession no. LC493816) and the orangefin ponyfish (Li5-1pK; LC626058) from the South China Sea.

The same search for the LSU rDNA using an 800-bp long sequence of the present isolates exhibited similarly an almost complete identity (800/800 or 739/740) with deposited sequences of *K. uncinata* isolates from the spotnape ponyfish (Li1-5pK; accession no. LC493817) and the orangefin ponyfish (Li5-1pK; LC626059).

Remarks. *Kudoa uncinata* was originally described in the shortnose ponyfish, *Leiognathus brevirostris* (Valenciennes), collected from Xiamen Bay around the border of the South and East China Seas (Zhou and Zhao 2008). Subsequently, we recorded this species from the spotnape ponyfish and orangefin ponyfish in the South China Sea off Guangdong Province (Li et al. 2020b, 2022a). The dimensions of myxospores were variable by reports: for example, the thickness and length of *K. uncinata* myxospores was 5.9–6.6 (6.1) and 5.2–5.6 (5.5) by Zhou and Zhao (2008), and 8.9–10.8 (9.8) and 7.1–8.0 (7.5) by Li et al. (2020b).

The newly collected myxospores from the spotnape ponyfish and orangefin ponyfish specimens exhibited 5.4–6.6 (6.0) in thickness and 4.8–6.1 (5.4) in length, closer to the measurements provided by Zhou and Zhao (2008). Despite such morphometric variation of *K. unicinata* myxospores, different isolates showed identical SSU and LSU rDNA nucleotide sequences. Kudoid myxospores (labelled as '*K. uncinata*') measuring 8.0–10.1 (8.8) in thickness and 6.7–8.5 (7.7) in width, illustrated by Li et al. (2022a), may be those of *Kudoa tumidisporica* sp. n. described later. We hypothesize that Li et al. (2022a) microscopically observed myxospores from a plasmodium of the new species, which are distinct from *K. uncinata*, but molecularly analysed a *K. uncinata* plasmodium due to a limited number of available plasmodia in that orangefin ponyfish specimen examined in that study.

***Kudoa neoscomberomori* sp. n.** (Li9-3pK isolate)

(Table 2; Figs. 1G, G', 2D–J, 3)

ZooBank number for species:
urn:lsid:zoobank.org:act:3E4E82EE-29C3-400A-A709-481D87A666C4

Three specimens of the narrow-barred Spanish mackerel, *Scomberomorus commerson* (Lacépède), measuring around 21.0 cm in standard body length, were severely infected with a kudoid species forming pseudocysts in the myofibres of trunk muscles (Fig. 3). Enlargement of parasitised myofibres with plasmodia filled by myxospores was histologically observed, and no host responses such as inflammation were evident.

Description

Filamentous plasmodia, forming pseudocysts in trunk muscle myofibres, measureing 0.78–1.94 (1.34 ± 0.28) mm by 32–59 (44 ± 7) mm (Li9-3pK; n = 35). Polysporic

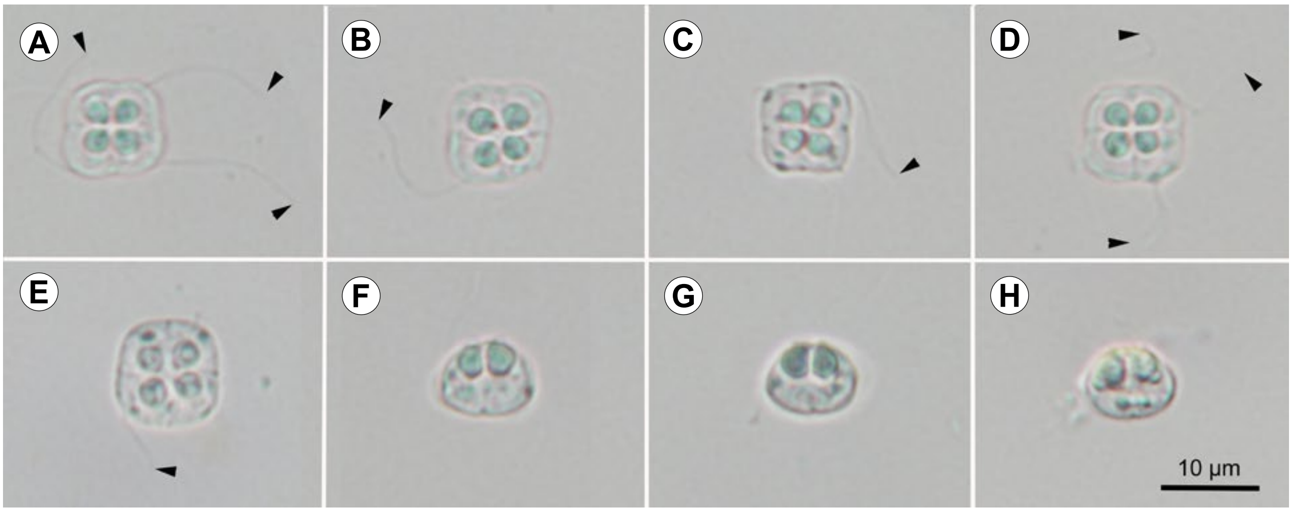

Fig. 4. Microscopic images of the myxospores of *Kudoa pilosa* sp. n. from *Helicolenus hilgendorfi* (Döderlein) (A–D, F, G) and *Sebastiscus tertius* (Barsukov et Chen) (E, H). **A**–**E** – Spores in apical view; and **F**–**H** – Spores in lateral view at the same magnification (scale bar = 10 µm). Myxospores have four filamentous projections (arrowheads) emanating from the corner of each shell valve.

and synchronised myxospore development. Myxospores in apical view stellate, comprised of six equal SVs and teardrop-shaped PCs (Figs. 1G, 2D–F). Suture lines indistinct. Coils of polar tubes not visible in wet preparations. In lateral view, myxospores drawstring-pouch-shaped with rather round apices and convex bottoms, and teardrop-shaped PCs in apical half of myxospore (Figs. 1G', 2G–J). Myxospores having following dimensions (n = 35): width 8.2–9.4 (8.7), thickness 7.1–8.4 (7.7), sutural thickness 6.3–7.5 (6.9), length 5.9–7.1 (6.5), PC length 2.7–3.8 (3.4), and PC width 1.2–1.6 (1.5).

Molecular characterisation

Two sets of the rDNA nucleotide sequences for the Li9-3pK isolate were identical with each other, containing partial SSU rDNA (1,719 bp), ITS-1 region (423 bp), 5.8S rDNA (158 bp), ITS-2 region (594 bp), and LSU rDNA (2,849 bp) (DDBJ/EMBL/GenBank accession no. LC777079).

The BLAST search for the SSU rDNA sequence found the highest nucleotide identity with *Kudoa* spp. with six or more SVs and PCs such as *K. permulticapsula* Whipps, Adlard, Bryant et Kent, 2003 (accession no. AY078429) with 99.6% (1,673/1,679) identity, *K. scomberomori* Adlard, Bryant, Whipps et Kent, 2005 (AY302737) with 99.5% (1,670/1,679) identity with one indel, *K. konishiae* (LC316965) with 99.4% (1,551/1,560) identity, followed by other *Kudoa* spp. The BLAST search for the LSU rDNA using an 800-bp long 5'-terminus sequence of the Li9-3pK isolate found the highest nucleotide identity with *Kudoa* spp. with six or more SVs and PCs such as *K. konishiae* (LC316966) with 94.8% (704/743) identity, *K. hexapunctata* Yokoyama, Suzuki et Shirakashi, 2014 (AB693042) with 94.5% (753/797) identity with three indels, and *Kudoa neothunni* (Arai et Matsumoto, 1953) (AB693049) with 94.4% (752/797) identity with three indels, followed by other *Kudoa* spp.

Partial nucleotide sequences of the *cox-1* (711 bp; accession no. LC777092) and *rns-rnl* (1,561 bp; accession no. LC777095) were obtained for the Li9-3pK isolate. The former sequence exhibited the highest nucleotide identity with the *cox-1* sequence of *K. konishiae* (LC777091) at 88.3% (628/711) or *K. hexapunctata* (LC316992) at 87.1% (619/711), and the latter sequence showed the highest nucleotide identity with the *rns-rnl* of Chinese Li9-2pK isolate of *K. konishiae* (LC777094) at 87.4% (1,362/1,558) with 9 indels, or Japanese ABU isolate of *K. konishiae* (LC317003) at 88.6% (1,206/1,362) with 9 indels.

Remarks. In the trunk muscle of the narrow-barred Spanish mackerel (*S. commerson*) from the Australian Coral Sea, two *Kudoa* spp. have been described (Whipps et al. 2003a, Adlard et al. 2005): *K. permulticapsula*, and *K. scomberomori*. The latter species has been recently reported in the Indo-Pacific king mackerel, *Scomberomorus guttatus* (Bloch et Schneider), in the South China Sea off the north central coast of Vietnam (Quang Binh Province) with a prevalence of 8 out of 15 specimens examined (Chinh et al. 2018).

The present Li9-3pK isolate is characterised by stellate myxospores with six equal SVs and a corresponding number of PCs in apical view, resembling the apical view of *K. scomberomori* myxospores (Figs. 1G, 2D–F). In lateral view, *K. scomberomori* is characterised as smoothly ovoid, but the present new species shows flattened drawstring-pouch-shaped with somewhat dull-pointed lateral SV edges and convex bottoms (Figs. 1G', 2G–J). Myxospores of the present Li9-3pK isolate have larger spore width, thickness and length than those of *K. scomberomori*, although PC dimensions of both species are almost identical (Table 2).

Furthermore, the nucleotide identity of SSU rDNA nucleotide sequences of *K. scomberomori* (AY302737) and the present Li9-3pK isolate is high (99.5% over 1,679-bp length with one indel) but comparable with nucleotide identities between the present Li9-3pK isolate and other kudoid species with myxospores comprised of six or more

Table 3. Morphological comparison of *Kudoa* spp. characterised by myxospores having a filamentous projection emanating from the corner of each SV[a]

Species	***K. pilosa*** **sp. n.**	***K. pilosa*** **sp. n.**	*K. hirsuta*	*K. hirsuta*	*K. caudata*	*K. camarguensis*
	Li8-3pK	Li9-1pK	Li5-7pK	Li5-9pK	–	–
Host	*Helicolenus hilgendorfi*	*Sebastiscus tertius*	*Trachurus japonicus*	*Decapterus macrosoma*	*Scomber japonicus*	*Pomatoschistus minutus*; *Pomatoschistus microps*
Locality	East China Sea, off Zhejiang, China	East China Sea, off Zhejiang, China	South China Sea, off Beihai, China	South China Sea, off Guangdong, China	South Pacific Ocean, off Peru (10°09'S, 82°22'W)	Vaccarès Lagoon, Rhône River Delta, Camargue, France
Reference	Present study	Present study	Li et al. (2022a)	Li et al. (2022a)	Kovaleva and Gaevskaya (1983)	Pampoulie et al. (1999)
Plasmodium in myofibres	(n = 8)	(n = 35)	(n = 15)	(n = 7)	–	–
Length (mm)	1.88–3.98 (2.80)	2.02–5.20 (3.41)	0.55–1.84 (1.06)	0.23–0.54 (0.37)	–	2–5
Width	96–143 (129)	82–231 (160)	24–50 (31)	29–37 (33)	–	1000–2000
Myxospore	(n = 35)	(n = 30)	(n = 20)	(n = 12)	–	(n = 30)
Width	10.0–12.0 (11.1)	10.4–13.6 (12.1)	8.9–10.9 (10.0)	8.7–10.0 (9.2)	8.0–8.6	6.4–8.0
Thickness	8.9–10.6 (9.7)	9.4–11.3 (10.5)	7.8–9.3 (8.6)	7.2–8.0 (7.6)	6.7	4.4–6.0
Sutural thickness	8.3–10.2 (9.1)	8.8–11.1 (10.2)	6.9–8.9 (7.9)	7.2–8.0 (7.6)	–	–
Length	7.0–8.2 (7.6)	7.6–8.9 (8.3)	6.9–8.0 (7.4)	–(6.2)	5.3–6.7	4.4–6.0
Polar capsule						
Length	2.4–2.8 (2.7)	3.0–4.2 (3.5)	1.6–2.2 (2.0)	1.6–2.3 (1.9)	2.0–2.7	2.5
Width	1.9–2.5 (2.1)	2.3–3.0 (2.5)	1.1–1.5 (1.3)	1.1–1.4 (1.2)	1.6–2.0	1.5
Spore vulve filamentous projection						
Length	8.5–13.0 (10.3)	10.2–15.9 (12.8)	4.0–6.2 (4.9)	3.9–4.6 (4.2)	–	ca. 2.0

[a] All measurements are in µm and expressed as range with mean in parentheses. '–' indicates no available data.

SVs and PCs. These include *K. permulticapsula*, *K. konishiae*, *Kudo grammatorcyni* Adlard, Bryant, Whipps et Kent, 2005, *K. neothunni* and *K. hexapunctata* as detailed above. Further genetic comparison is currently impossible due to the lack of deposited LSU rDNA or other gene sequences of *K. scomberomori*.

Based on morphological and molecular-genetic differences of the present Li9-3pK isolate from recorded kudoid species, we propose *Kudoa neoscomberomori* sp. n. for the new isolate (Li9-3pK).

Taxonomic summary

Type host: *Scomberomorus commerson* (Lacépède) (Scombriformes: Scombridae), narrow-barred Spanish mackerel.

Type locality: South China Sea, off Zhanjiang City, Guangdong Province, China.

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

Material deposited: Specimen no. 25010, Meguro Parasitological Museum, Tokyo, Japan.

Deposited rDNA sequences: DDBJ/EMBL/GenBank accession no. LC777079 (rDNA), LC777092 (*cox-1*), and LC777095 (*rns-rnl*).

Prevalence: 38% (3/8).

Etymology: The species name reflects the fact that the myxospore morphology fairly resembles that of *K. scomberomori* recorded in the same host fish from the Australian Coral Sea. However, these two species are differentiated based on morphological and molecular genetic traits.

Kudoa pilosa **sp. n.** (Li8-3pK and Li9-1pK isolates) (Table 3; Figs. 1H,H', 4)

ZooBank number for species:
urn:lsid:zoobank.org:act: 18587222-6A5D-4EF3-A38B-C5602AA5FC1C

Five specimens of the Hilgendorf's saucord, *Helicolenus hilgendorfi* (Döderlein), and one specimen of the red marbled rockfish, *Sebastiscus tertius* (Barsukov et Chen), from the South China Sea, were heavily infected with a kudoid species forming pseudocysts in the myofibres of the trunk muscles. Standard body lengths of specimens were around 17.0 cm (n = 5) for the former fish species and 25.4 cm for the latter fish species. Myxospores from the Hilgendorf's saucord specimens (Li8-3pK isolate) and the red marbled rockfish specimen (Li9-1pK isolate) were identical in morphology, having four filamentous projections emanating from the corner of each SV. This unique myxospore morphology of the present Li8-3pK and Li9-1pK isolates differs from known species as detailed below. A new species, *Kudoa pilosa* sp. n., was designated for the present isolates.

Description

(Based on specimens from the type host *Helicolenus hilgendorfi*): Fusiform or filamentous plasmodia forming pseudocysts in trunk muscle myofibres, measuring 1.88–3.98 (2.80 ± 0.70) mm by 96–143 (129 ± 15) (Li8-3pK; n = 8). Polysporic and synchronised myxospore development. Myxospores in apical view semiquadrate, comprised of four equal SVs and oval PCs (Figs. 1H, 4A–E). Suture lines indistinct. Each SV with filamentous projection protruding from corner, measuring 8.5–13.0 (10.3). Coils of polar tubes not visible in wet preparations. In lateral view, myxospores oval, and oval PCs in apical 2/5 of myxospore (Figs. 1H', 4F–H). No apical protrusions. Myxospores have following dimensions (Li8-3pK; n = 35): width 10.0–12.0 (11.1), thickness 8.9–10.6 (9.7), sutural thickness 8.3–10.2 (9.1), length 7.0–8.2 (7.6), PC length 2.4–2.8 (2.7), PC width 1.9–2.5 (2.1), and 8.5–13.0 (10.3) long filamentous projection from the corner of each SV.

(Based on specimens from the additional host *Sebastiscus tertius*): Fusiform or filamentous plasmodia forming pseu-

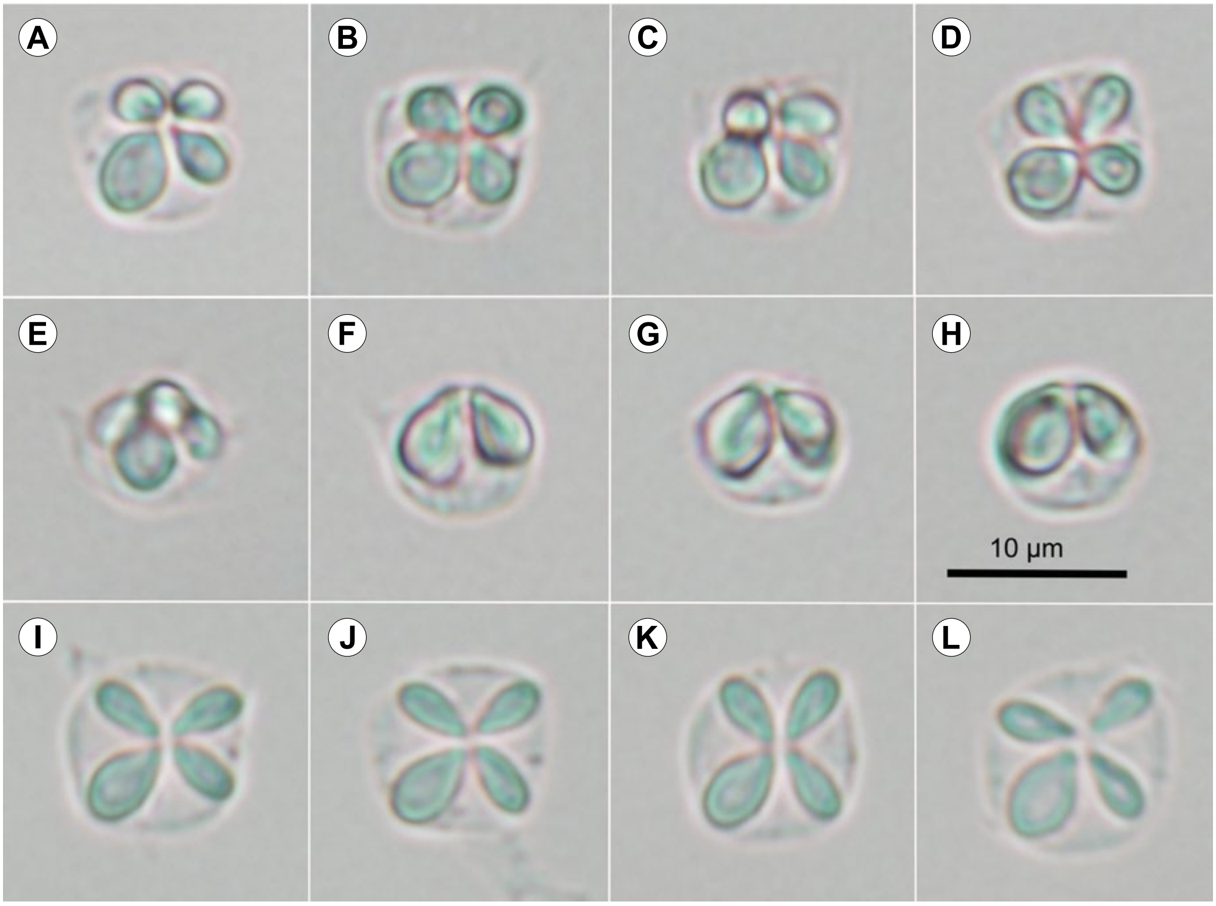

Fig. 5. Microscopic images of the myxospores of *Kudoa tumidisporica* sp. n. from *Photopectoralis bindus* (Valenciennes) and *Nuchequula nuchalis* (Temminck et Schlegel). **A**–**E** – Spores in apical view; **F**–**H** – Spores in lateral view; **I**–**L** – pressed spores in apical view at the same magnification (scale bar = 10 µm).

docysts in trunk muscle myofibres, measuring 2.02–5.20 (3.41 ± 0.76) mm by 82–231 (160 ± 40) (Li9-1pK; n = 35). Myxospores have following dimensions of (Li9-1pK; n = 30): width 10.4–13.6 (12.1), thickness 9.4–11.3 (10.5), sutural thickness 8.8–11.1 (10.2), length 7.60–8.92 (8.3), PC length 3.0–4.2 (3.5), PC width 2.3–3.0 (2.5), and 10.2–15.9 (12.8) long filamentous projection from corner of each SV.

Molecular characterisation

Three sets of the Li8-3pK isolate rDNA nucleotide sequences, each set of which was prepared using a single plasmodium, were identical with each other, containing partial SSU rDNA (1,754 bp), ITS-1 region (607 bp), 5.8S rDNA (158 bp), ITS-2 region (315 bp), and LSU rDNA (2,929 bp) (DDBJ/EMBL/GenBank accession no. LC777080). Similarly, two identical sets of the Li9-1pK isolate SSU rDNA (1,733bp) and LSU rDNA (1,460 bp) were obtained (DDBJ/EMBL/GenBank accession no. LC777081 and LC777082, respectively).

The SSU rDNA nucleotide sequences of the Li8-3pK and Li9-1pK isolates were completely identical over comparable 1,733-bp length. The LSU rDNA nucleotide sequences of the Li8-3pK and Li9-1pK isolates showed 99.7% (1,455/1,460) identity. Almost complete identities of comparable rDNA nucleotide sequences of these two isolates indicate that they belong to a single species.

The BLAST search for the SSU rDNA sequence (LC777080) found less than 90% nucleotide identities with deposited SSU rDNA nucleotide sequences of *Kudoa* spp. For example, the highest nucleotide identity (88.3% over 1,741-bp sequence with 16 indels) was found with *Kudoa acentrogobia* Li, Inoue, Tanaka, Zhang et Sato, 2020 (accession no. LC493824). Similarly, the BLAST search for the LSU rDNA sequence using an 800-bp-long sequence (LC777080) found less than 90% nucleotide identities with any deposited LSU rDNA nucleotide sequences of *Kudoa* spp.

Remarks. *Kudoa* spp. such as *K. caudata* Kovaljova et Gayevskaya, 1983, *K. camarguensis* Pampoulie, Marques, Rosecchi, Crivelli et Bouchereau, 1999 and *K. hirsuta* Li, Inoue, Zhang et Sato, 2022 are characterised by myxospores having four filamentous projections emanating from the corner of each SV (Kovaleva and Gaevskaya 1983, Pampoulie et al. 1999, Li et al. 2022a).

Myxospores of the new species, *Kudoa pilosa* sp. n. (Li8-3pk and Li9-1pK isolates), are larger than those of the other three species with larger PCs and longer SV projections (Table 3). In addition, plasmodium sizes of *K. pilosa* sp. n. and *K. hirsuta* are distinct. For example, plasmodium sizes are 2.02–5.20 (3.41) mm by 82–231 (160) in *K. pilosa* sp. n. Li9-1pK (n = 35; present study) vs. 0.55–1.84 (1.06) mm by 20–60 in *K. hirsuta* Li5-7pK (n = 15; Li et al. 2022a).

Molecular-genetic comparison of rDNA nucleotide sequences confirms the distinction of *K. pilosa* sp. n. from *K. hirsuta,* which was recorded in carangid fishes (*Trichurus japonicus*, *D. macrosoma* and *D. maruadsi*) from the South China Sea (Li et al. 2022a). Due to the lack of deposited nucleotide sequences of *K. caudata* and *K. camarguensis*, phylogenetic relationships of *K. pilosa* sp. n. with these species are uncertain.

Taxonomic summary

Type host: *Helicolenus hilgendorfi* (Döderlein); Hilgendorf's saucord (Perciformes: Sebastidae).

Additional host: *Sebastiscus tertius* (Barsukov et Chen), red marbled rockfish (Perciformes: Sebastidae).

Type locality: South China Sea, off Taizhou, Zhejiang Province, China.

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

Material deposited: Hapantotype no. 25011 (Li8-3pK), and paratype no. 25012 (Li9-1pK), Meguro Parasitological Museum, Tokyo, Japan.

Deposited rDNA sequences: DDBJ/EMBL/GenBank accession no. LC777080–LC777082 (rDNA).

Prevalence: 71% (5/7) in *H. hilgendorfi* and 100% (1/1) in *S. tertius*.

Etymology: The species name reflects the presence of a long hairy projection from each shell valve of a myxospore.

***Kudoa tumidisporica* sp. n.** (Li8-5pK2 and Li8-6pK2 isolates) (Figs. 1I, I', 5)

ZooBank number for species:
urn:lsid:zoobank.org:act:E1E2E8B1-A338-47D2-AC66-FA1D193D3DA8

In the trunk muscle myofibres of two orangefin ponyfish (*P. bindus*) and one spotnape ponyfish (*Nuchequula nuchalis*) specimens, two plasmodium types of different widths were detected as mentioned above (Suppl. Fig. S3). Wider fusiform plasmodia (0.18–2.62 [0.74] mm long by 44–154 [71] wide; n = 30) contained semiquadrate myxospores of *K. uncinata*, which were characterised by four almost equal SVs and PCs, and distinct uplifted tips of the SV termini (Fig. 1F). Narrower fusiform plasmodia (0.19–1.69 [0.85] mm long by 15–51 [32] wide; n = 25) contained spheroid myxospores with four SVs and ballooned teardrop-shaped PCs of unequal sizes (Figs. 1I; 5), which looked like myxospores reported as an aberrant form of *K. uncinata* myxospores in our previous study (Li et al. 2022a). Intense infection of this kudoid species in the present study enabled its satisfactory genetic characterisation, and we describe a new species, *Kudoa tumidisporica* sp. n., for this morphotype.

Description

Fusiform or filamentous plasmodia forming pseudocysts in trunk muscle myofibres, measuring 0.19–1.69 (0.85 ± 0.74) mm by 15–51 (32 ± 9) (Li9-5pK2; n = 25). Polysporic and synchronised myxospore development. Myxospores in apical view rounded rather than semiquadrate, deformed due to presence of one large ballooned pyriform PC and three smaller pyriform PCs (Figs. 1I, 5). Suture lines indistinct. In lateral view, myxospores spheroid with uplifted tips on all four SV termini. Coils of polar tubes in PCs unclear in wet preparation. No apical protrusions. Myxospores have following dimensions (Li8-5pK2 and Li8-6pK2; n = 30): width 9.3–11.0 (10.0), thickness 7.5–9.3 (8.3), sutural thickness 7.2–8.4 (7.9), length 7.7–9.0 (8.2), big PC length 4.5–5.7 (5.1), big PC width 2.9–3.7 (3.2), small PC length 3.2–4.6 (4.1), and small PC width 1.6–2.2 (2.0).

Molecular characterisation

Five identical sets of the SSU (1,696 bp) and LSU rDNA (2,840 bp) nucleotide sequences of the Li8-5pK2 and Li8-6pK2 isolates, each set of which was prepared using a single plasmodium, were newly obtained (DDBJ/EMBL/GenBank accession nos. LC777085, LC777086, LC777089 and LC777090). The BLAST search for the SSU rDNA sequence (LC777085) detected the highest nucleotide identity with *K. uncinata* (accession no. LC626058) exhibiting 99.4% (1,660/1,670) identity and three indels, followed by *Kudoa corniculata* Li, Inoue, Zhang et Sato, 2022 (LC626060) with 98.0% (1,652/1,686) and one indel, *K. longichorda* Inoue, Kasai, Argamjav et Sato, 2022 (LC626077) with 96.8% (1,638/1,692) and three indels, and other *Kudoa* spp. The BLAST search for the LSU rDNA sequence (LC777086) using an 800-bp-long 5'-terminus sequence found the highest nucleotide identity with *K. parvibulbosa* Li, Inoue, Zhang et Sato, 2022 (accession no. LC626079) exhibiting 90.1% (720/799) identity and 11 indels, followed by *K. longichorda* (LC626078) with 89.5% (710/793) and four indel, and other *Kudoa* spp.

Remarks. As mentioned above, a kudoid species characterised by morphologically similar myxospores has been described previously by us as an aberrant form of *Kudoa uncinata* in the same fish host (*P. bindus*) from the same South China Sea (Li et al. 2022a). In that fish, only a few plasmodia (Li5-1pK isolate) were found in the trunk muscle myofibres. Regardless of the aberrant myxospore morphology of the Li5-1pK kudoid isolate, the species was identified as *Kudoa uncinata* based on the highest nucleotide identity of the SSU (99.94% over 1,676-bp) and LSU rDNA (100% over 740-bp) nucleotide sequences with the species previously characterised by us at a different time (Li et al. 2020b).

In the present study, we analysed relatively intense infections with two kudoid morphotypes in the trunk muscles of three ponyfish specimens of the family Leiognathidae, demonstrating that all three ponyfish (*P. bindus* and *N. nuchalis*) specimens were co-infected with two species with different morphotypes (*K. uncinata* and *K. tumidisporica* sp. n.). When the same infection status, i.e., mixed infection, was seen in our previous examination of *P. bindus* and a limited number of small-sized plasmodia were available for morphological and molecular analyses (Li et al. 2022a), mis-

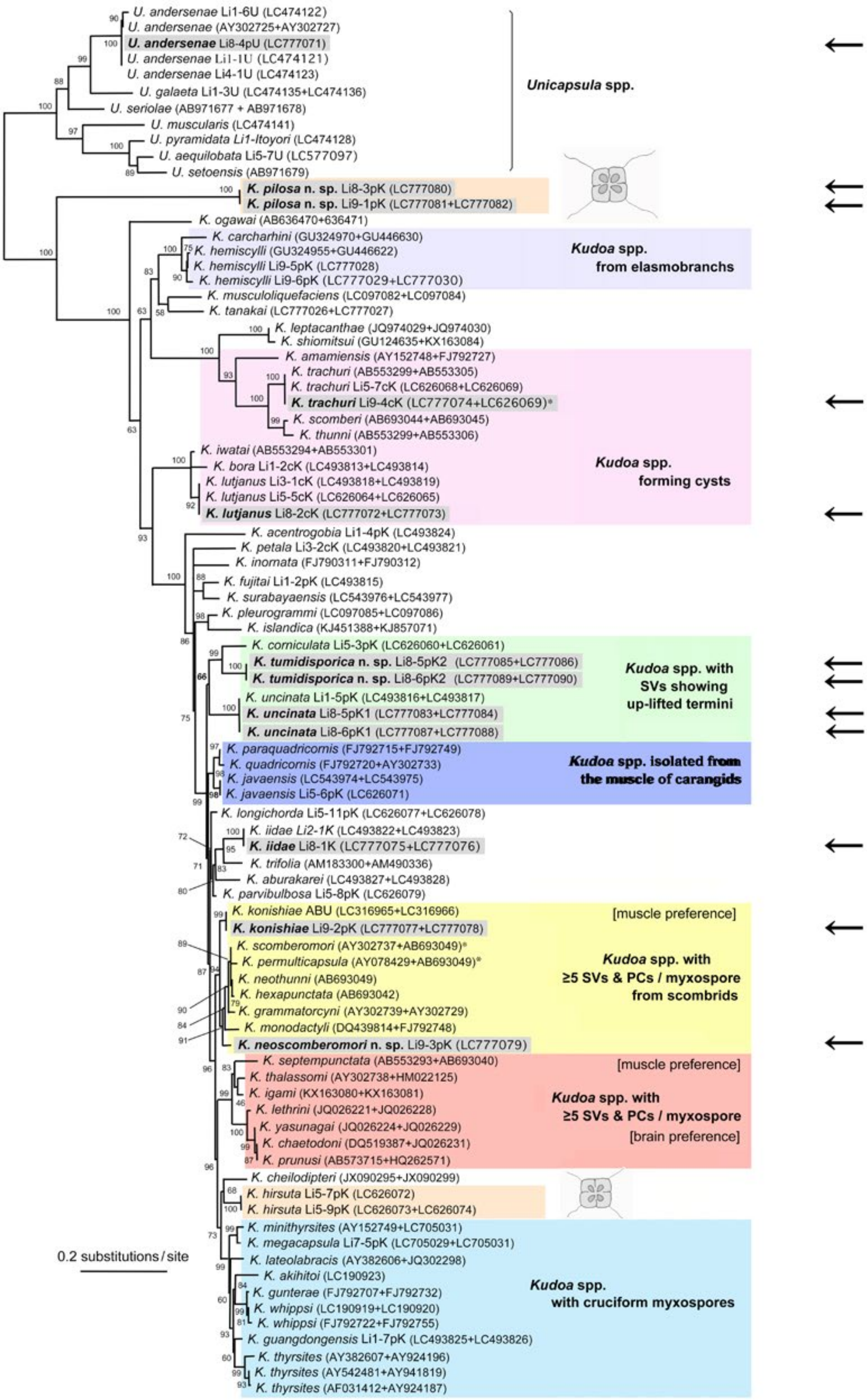

Fig. 6. Maximum likelihood phylogenetic tree based on the concatenated SSU and LSU rDNA nucleotide sequences (1,844 characters, of which 634 are variable) of multivalvulid myxosporeans of the genera *Unicapsula* Davis, 1924 and *Kudoa* Meglitsch, 1947. Each species name (for Chinese isolates along with isolate name) is followed by DDBJ/EMBL/GenBank accession number in parentheses. Sequences newly obtained in this study are marked in bold with grey background and arrows on the right side of the figure. Several groups of *Kudoa* spp. with morphological resemblance are shown by different colour background, and specific characters of grouping are shown on the right side of these colour backgrounds. Location of *Kudoa* spp. with myxospores having filamentous projections on the SV, i.e., *K. pilosa* sp. n. and *K. hirsuta* Li, Inoue, Zhang et Sato, 2022, in this phylogenetic tree is shown by orange background and line-drawings of their myxospores.

identification of a morphotype by molecular-genetic analysis using a co-infected plasmodium may have been made. Although it is uncertain why mixed infection with two species (*K. uncinata* and *K. tumidisporica* sp. n.) could be seen in our ponyfish specimens (Table 1), use of the same alternative annelid host in the same sea area with these two kudoid species could cause such tightly-related mixed infection.

Taxonomic summary

Type host: *Photopectoralis bindus* (Valenciennes), orangefin ponyfish (Acanthuriformes: Leiognathidae).

Additional host: *Nuchequula nuchalis* (Temminck et Schlegel), spotnape ponyfish (Acanthuriformes: Leiognathidae).

Type locality: South China Sea, off Taizhou, Zhejiang Province, China.

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

Material deposited: Hapantotype no. 25014 (Li8-5pK2), and paratype no. 25016 (Li9-6pK2), Meguro Parasitological Museum, Tokyo, Japan.

Deposited rDNA sequences: DDBJ/EMBL/GenBank accession no. LC777085 and LC777089 (SSU rDNA) and LC777086 and LC777090 (LSU rDNA).

Prevalence: 50% (2/4) in *P. bindus* and 100% (1/1) in *N. nuchalis*.

Etymology: The species name reflects the swollen morphology of the myxospores.

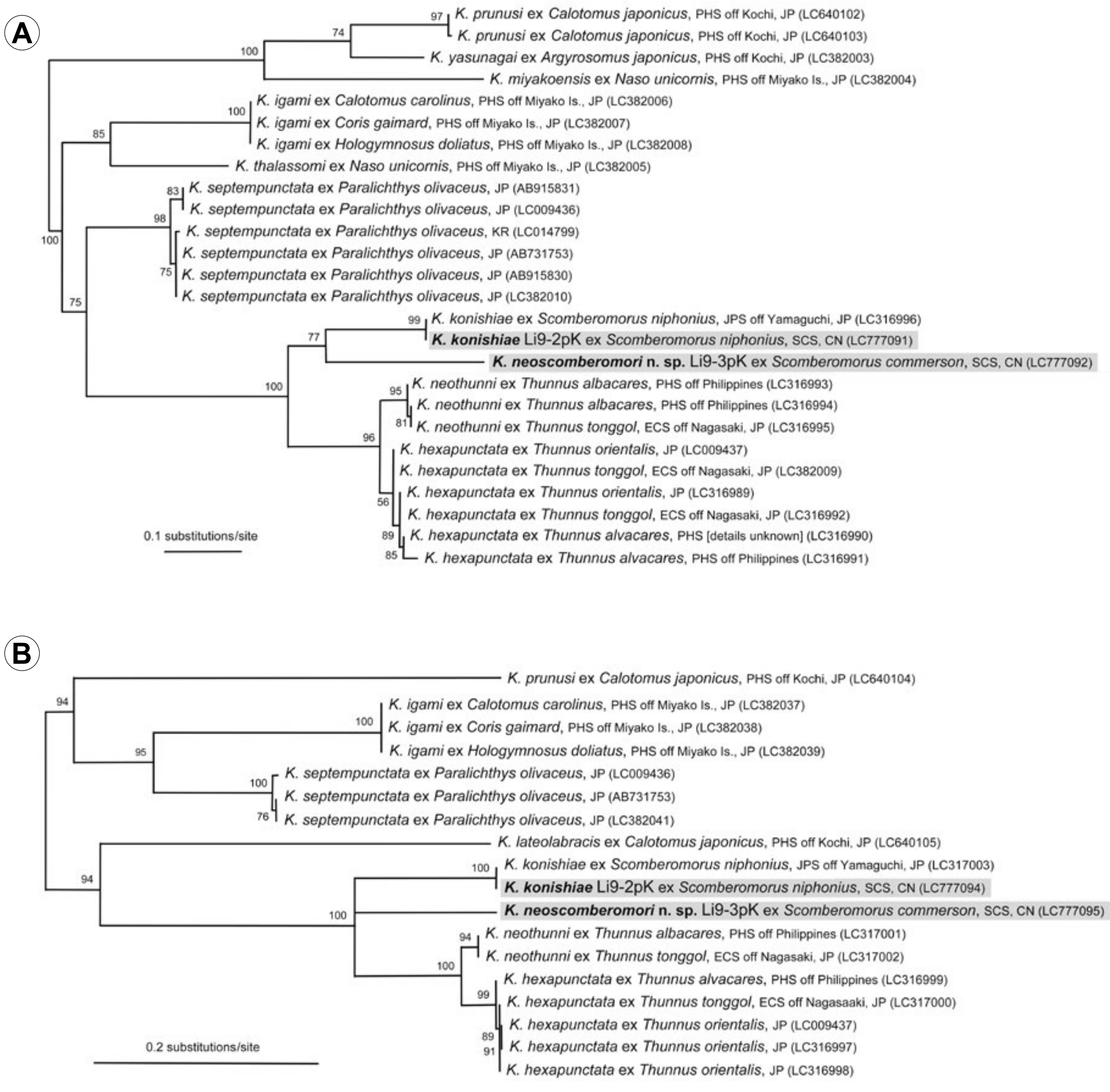

Fig. 7. Unrooted maximum likelihood phylogenetic tree based on partial mitochondrial gene sequences (**A** – *cox-1*; and **B** – *rns-rnl*) of representative *Kudoa* spp. with five or more SV/PC per myxospore. For the *cox-1* and *rns-rnl* mtDNA, 437 characters, of which 179 are variable, and 982 characters, of which 474 are variable, were retained for this analysis, respectively. The species name of the isolates collected in the present study (with grey background) is followed by the name of the fish host, sea area and country name of collection, and DDBJ/EMBL/GenBank accession number in parentheses. Abbreviations of sea area and country names: ECS, East China Sea; JP, Japan; JPS, Japan Sea; KR, Korea; and PHS, Philippine Sea; SCS, South China Sea.

Phylogenetic analyses

A phylogenetic tree was constructed using concatenated SSU and LSU rDNA sequences of various multivalvulidan species including nine species isolated in the present study (Fig. 6). Members of two multivalvulidan genera were clearly segregated into two major clades (*Unicapsula* spp. and *Kudoa* spp.). The unique phylogenetic position of *K. pilosa* sp. n. was evident in this tree: *K. pilosa* sp. n. was placed sister to all other *Kudoa* spp. This was similarly demonstrated in phylogenetic trees based solely on either SSU or LSU rDNA sequences (Suppl. Fig. S4). Morphologically similar *Kudoa* spp. with semiquadrate myxospores and filamentous projections from each corner of four SVs, i.e. *K. pilosa* sp. n. and K. *hirsuta*, were distant in phylogenetic trees based on the rDNA.

As mentioned above, the SSU and LSU rDNA sequences of all new isolates of six known species (*U. andersenae*, *K. iidae*, *K. konishiae*, *K. lutjanus*, *K. trachuri* and *K. uncinata*) showed no or few nucleotide substitutions with those of their previous isolates, reflected in the phylogenetic tree as groupings of different isolates of the same species at an identical site (Fig. 6; Suppl. Fig. S4). As mentioned in our previous study (Li et al. 2020a), *Unicapsula* spp. except for *U. andersenae* showed little or no intraspecific genetic variation in both SSU and LSU rDNA sequences. Apart from such a situation in the phylogenetic relationships of *Unicapsula* spp., several *Kudoa* spp. such as *Kudoa thyrsites* (Gilchrist, 1923), *Kudoa whippsi* Burger et Adlard, 2010, or *Kudoa hemiscylli* Gleeson, Bennett et Adlard, 2010 showed noticeable intraspecific variations in the rDNA nucleotide sequences.

Kudoa spp. with some unique morphological traits of myxospores such as those exhibiting cruciform shape or those with more than four SVs and PCs tended to form independent clades (Fig. 6). *Kudoa* spp. with more than four SVs and PCs were segregated into two clades, of which one consisted of *Kudoa* spp. characterised by stellate myxospores with six equal SVs/PCs, except for *K. permulticapsula* (myxospores with 13 SVs/PCs), isolated exclusively from scombrid fishes (*Scomberomorus* spp., *Grammatorcynus bicarinatus* [Quoy et Gaimard], and *Thunnus* spp.). This clade included *K. konishiae* and *K. neoscomberomori* sp. n. isolated in this study. Exclusively, however, *Kudoa monodactyli* Gunter, Cribb, Whipps et Adlard, 2006 is characterised by myxospores with five SVs/PCs, and recorded in *Monodactylus argenteus* (Linnaeus) classified in the family Monodactylidae Jordan et Evermann. Similar phylogenetic relationships of *Kudoa* spp. with more than four SVs/PCs were seen in phylogenetic trees based on mtDNA (*cox-1* as well as *rns-rnl*) nucleotide sequences (Fig. 7), although analysed species were limited for these genes.

DISCUSSION

At least five *Unicapsula* spp. and 21 *Kudoa* spp. have been recorded from the South and East China Sea, off the eastern coast of China (see Suppl. Table S1; Fujita 1930, Wang et al. 2005, Yokoyama and Itoh 2005, Zhou and Zhao 2008, Li et al. 2020a–c, 2022a,b, Inoue et al. 2021, Li et al. 2022c). Referring to faunas of multivalvulidan species from marine fishes in Australia and Japan (Whipps et al. 2003a,b, Eiras et al. 2014a, Sato and Kasai 2016, Sakai et al. 2018, 2019), it is natural to expect that more variety of multivalvulidan species remain to be recorded in marine fish from the South China Sea. This is despite the fact that fish are limited to those commercially available at local fish markets. For example, *Kudoa amamiensis* Egusa et Nakajima, 1980, *K. musculoliquefaciens* (Matsumoto, 1954), *K. thalassomi* Adlard, Bryant, Whipps et Kent, 2005, *K. thyrsites*, *K. whippsi* or *Kudoa yasunagai* (Hsieh et Chen, 1984) have been recorded from Japan and Australia (Whipps et al. 2003b, Adlard et al. 2005, Sakai et al. 2019, Bolin et al. 2021), but not from an intervening region between these two sea areas, i.e. the subtropical and temperate waters of the Northwest Pacific Ocean, off the Chinese coast.

Shin et al. (2016) indicated that there was a significant bias of kudoid distribution, i.e., numbers of recorded kudoid species in sea areas could be ascribed to researchers' prominent interests or artificial deflection. In addition, possible patchy distribution of kudoids could be an important factor for capricious detection of infected fish (Sugiyama et al. 1999, Okamura et al. 2018). Indeed, Yurakhno et al. (2022) examined fish in the Vietnamese coastal sea (Nha Trang Bay in the Vietnam East Sea), corresponding to the South China Sea, off the Vietnamese coast, and recorded *K. thyrsites*, *K. whippsi*, *K. monodactyli*, two new kudoid species (*Kudoa igori* Yurakhno, Slynko, Chinh, Ha et Whipps, 2022, and *Kudoa borimiri* Yurakhno, Slynko, Chinh, Ha et Whipps, 2022), and two more unspecified multivalvulidan species, all of which have never been encountered in our surveys.

Spanish mackerels of the genus *Scomberomorus* Lacépède (Scombridae) have been reported as host fishes of *Kudoa permulticapsula* (*Scomberomorus commerson* from the Australian Coral Sea), *Kudoa scomberomori* (*S. commerson* from the Australian Coral Sea, and *Scomberomorus guttatus* from the Vietnamese Sea), *Kudoa konishiae* (*S. niphonius* from the Sea of Japan, off western Japan, and Philippine Sea, off western Japan), *Kudoa rayformis* Shin, Shirakashi, Hamano, Kato, Lasso et Yokoyama, 2016 (*Scomberomorus sierra* Jordan et Starks from the Eastern Central Pacific Ocean, off the coast of Panama), and unspecified *Kudoa* sp. (*Scomberomorus brasiliensis* Collette, Russo et Zavala-Camin from the Western Atlantic Ocean, off the coast of Brazil) (Whipps et al. 2003a, Adlard et al. 2005, Eiras et al. 2014b, Shin et al. 2016, Chinh et al. 2018, Sakai et al. 2018).

This study adds *Kudoa neoscomberomori* sp. n. in *S. commerson* from the South China Sea to the list of kudoids from Spanish mackerels of the genus *Scomberomorus*. Three kudoid species from *Scomberomorus* spp., namely *K. scomberomori*, *K. konishiae* and *K. neoscomberomori* sp. n. are characterised by stellate myxospores with six equal SVs/PCs in the apical view, along with *Kudoa grammatorcyni* from *Grammatorcynus bicarinatus* (Scombridae), and *Kudoa neothunni* and *K. hexapunctata* from tunas of the genus *Thunnus* South (Scombridae). As discussed above, these species are differentiated by myxospore morphology, e.g., different SV peripheral margins (rounded vs. pointed), and different myxospore shapes in the lateral view (oval vs. drawstring-pouch in shape or flat bottom vs. convex bottom).

Kudoid species characterised by myxospores with more than four SVs/PCs from scomberid fishes were phylogenetically clustered in a clade as shown in Figs. 6 and 7. *Kudoa rayformis* characterised by ray-form myxospores with four SVs/PCs in *S. sierra* from the Eastern Central Pacific Ocean, off the coast of Panama is phylogenetically distant from the aforementioned clade of kudoids originated from scomberid fishes (not shown in this study; see Shin et al. 2016, Li et al. 2020c). However, it is uncertain whether kudoid species characterised by myxospores with more than four SVs/PCs from the scomberid fish are exclusively distributed in the western Pacific Ocean, and if this phenotypical character of myxospores may have originated in the western Pacific Ocean.

An additional lineage of *Kudoa* spp. characterised by myxospores with more than four SVs/PCs, occurred in a wide spectrum of non-scomberid fish, and this clade includes *Kudo*a spp. with muscle preference (*K. thalassomi*, *Kudoa igami* Shirakashi, Yamane, Ishitani, Yanagida et Yokoyama, 2014; and *K. septempunctata*) and those with brain preference (*K. yasunagai*; *K. neurophila* Grossel, Dyková, Handlinger et Munday, 2003; *K. lethrini* Burger, Cribb et Adlard, 2007; *K. chaetodoni* Burger, Cribb et Adlard, 2007; *K. prunusi* Meng, Yokoyama, Shirakashi, Grabner, Ogawa, Ishimaru, Sawada et Murata, 2011; *K. lemniscati* Miller et Adlard, 2012, and *K. miyakoensis* Sakai, Kawai, Zhang et Sato, 2019).

Differentiation of kudoid species resembling each other in myxospore morphology is well assisted by molecular characterisation of the SSU and LSU rDNA as well as mtDNA *cox-1* and *rns-rnl* sequences. Primer pairs available for amplification of the mtDNA *cox-1* and *rns-rnl* fragments are currently limited to a part of kudoid species due to a suspected great variation of these gene nucleotide sequences (Takeuchi et al. 2015, 2016, Sakai et al. 2018, 2019, Li et al. 2020b; present study).

Furthermore, accumulation of mtDNA *cox-1* and *rns-rnl* nucleotide sequences of kudoids is limited yet, and the usability of these mtDNA markers for intraspecific lineage analyses is currently uncertain. Partial but long nucleotide sequences of the *cox-1* and *rns-rnl* of the Chinese *K. konishiae* isolate (Li9-2pK) are completely identical with those of Japanese isolates of the species (ABU and KGS), suggesting no special biogeographical lineages in *K. konishiae*, although *S. niphonius* is thought to be divided into at least two ecological stocks, East China Sea (China) spawning stock and Seto Inland Sea (Japan) spawning stock (Shui et al. 2009).

Similarly, an exceptional morphotype of *K. neothunni* in the longtail tuna, *Thunnus tonggol* (Bleeker), which was characterised by myxospores with seven SVs/PCs, had an identical *cox-1* and *rns-rnl* nucleotide sequences with the common morphotype with six SVs/PCs of *K. neothunni* from the yellowfin tuna, *Thunnus albacares* (Bonnaterre), as demonstrated by Sakai et al. (2018). These mtDNA markers (*cox-1* and/or *rns-rn*l) are, however, invaluable to differentiate *Kudoa* spp. characterised by myxospores with similar morphology and almost similar SSU and LSU rDNA nucleotide sequences (Sakai et al. 2019).

The current status that approximately two-thirds of the nominal *Kudoa* spp. have only been recorded from a single host (Burger and Adlard 2011) might be ascribed to few incentives to investigate parasites in fish of little commercial value. For approximately 10 years after its first recovery by Matsukane et al. (2011), *Kudoa trachuri* was recorded solely in *Trachurus japonicus*, but recent surveys, including the present study, have added multiple fishes such as *Decapterus* spp. (*D. akaadsi*, *D. muroadsi*, *D. tabl*, *D. maruadsi*, *D. macrosoma,* and *D. macarellus*) and *Pseudocaranx dentex*, all of which are classified in the family Carangidae Rafinesque (Inoue et al. 2022, Li et al. 2022a, present study).

Kudoa septempunctata, one of the *Kudoa* food poisoning causative agents (Kawai et al. 2012, Sugita-Konishi et al. 2014), was originally described from the trunk muscles of the farmed oriental flounder, *Paralichthys olivaceus* (Temminck et Schlegel) (Paralichthyidae) (Matsukane et al. 2010), and later studies have added wild oriental flounder, black scraper, *Thamnaconus modestus* (Günther) (Monacanthidae), grass puffer, *Takifugu alboplumbeus* (Richardson) (Tetraodontidae), and Japanese whiting, *Sillago japonica* Temminck et Schlegel (Sillaginidae), as the host for this species (Takeuchi et al. 2016, Kasai et al. 2016b, Sakai et al. 2019, Shirakashi et al. 2021).

These examples suggest that the known host range of most multivalvulidans is wider, although Burger and Adlard (2011) emphasised that the actual level of threat of multivalvulids to farmed fish is determined by their host specificity, i.e., stenoxenous or euryxenous, or presence of reservoir hosts. As demonstrated in this study, our understanding of multivalvulidan biodiversity with its host spectrum and geographical distribution is under development.

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Authors' contribution. Conceptualisation, YCL, JYZ and HS; methodology, YCL, KI and HS; software, KI and HS; writing—original draft preparation, YCL and KI; writing—review and editing, JYZ and HS; funding acquisition, YCL and HS. All authors have read and agreed to the published version of the manuscript.

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