

**THE FIRST RECORD OF *Kudoa yasunagai* (Hsieh & Chen, 1984)
(Myxosporea: Multivalvulida) PARASITIZING THE BRAIN OF BARHEAD
SPINEFOOT *Siganus virgatus* (Valenciennes) FROM VIETNAM**

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ABSTRACT

A brain-infecting myxozoan, *Kudoa yasunagai* (Hsieh & Chen, 1984), was identified using both morphological and molecular methods in a marine fish from Van Phong Bay, Khanh Hoa province, Vietnam. Cysts containing spores were observed in the brain of one out of 10 *Siganus virgatus* (Valenciennes) individuals (10%). The myxospores were radially symmetrical, containing a majority of 7 equal shell valves and polar capsules. The spores measured 6.6 ± 0.1 (6.4–6.7) μm in length, 7.6 ± 0.5 (7.0–8.4) μm in width, and 7.0 ± 0.4 (6.5–7.7) μm in thickness. Polar capsules measured 2.8 ± 0.2 (2.6–3.0) μm in length and 1.4 ± 0.1 (1.2–1.5) μm in width, containing a filament inside. The SSU rDNA sequence from the Vietnamese specimen showed > 99.94 % identity with *K. yasunagai* from Japan and Australia. This report marks a new geographical and host record for *K. yasunagai*.

Keywords: Kudoidae, brain, marine fish, SSU rDNA, Khanh Hoa province.

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INTRODUCTION

The Barhead spinefoot *Siganus virgatus* (Valenciennes) (Perciformes: Siganidae) is a common marine fish in Southeast Asia. In Vietnam, this species inhabits coral reefs in the coastal water and is a target for local fisheries. It is also farmed in small-scale fish farms, making it a commercially important fish in Vietnam's food market.

Myxosporeans (Myxozoa) are a diverse group of metazoan parasites belonging to the phylum Cnidaria Hatscheck, 1888. Almost all myxosporeans parasitize fishes, though some have been recorded in amphibians, reptiles, birds, and mammals (Lom & Dyková, 2006). To date, more than 2,600 myxosporean species have been described worldwide and they are classified into two orders: Bivalvulida Shulman, 1959 and Multivalvulida Shulman, 1959, each with 57 and 5 genera, respectively (Lom & Dyková, 2006; Fiala et al., 2015; Okamura et al., 2018; Eiras et al., 2021; Wang et al., 2022). In the order Multivalvulida, the genus *Kudoa* Meglitsch, 1947 is the largest genus with more than 120 nominal species described to date, predominantly from marine fish (Lom & Dyková, 2006; Eiras et al., 2014; Li et al., 2020a, b). *Kudoa* is characterized by myxospores possessing four or more shell valves and polar capsules (Whipps et al., 2004; Lom & Dyková, 2006). To date, only seven *Kudoa* species have been recorded in the somatic muscle of marine fishes in Vietnam, including *Kudoa borimiri*, *Kudoa igori*, *Kudoa monodactyli*, *Kudoa scomberomori*, *Kudoa thyrsites*, *Kudoa thunni*, and *Kudoa whippsi* (Hoai et al., 2022; see Chinh et al., 2023).

During the survey of myxosporean parasites on South-Central coast of Vietnam from May to July 2023, we detected myxosporean cysts in the brain of Barhead spinefoot *Siganus virgatus* (Valenciennes). This study aims to identify this brain-infecting myxosporean using both morphological and molecular methods.

MATERIALS AND METHODS

Fish sampling and parasite collection

Ten individuals of fresh *S. virgatus* (25–37 cm in total length, 0.28–0.37 kg in body weight) were purchased from a fish farm in Van Phong Bay, Khanh Hoa province, Vietnam. The samples were randomly selected, and there is no information on the occurrence of disease at the fish farm. The fish were temporally stored in a cooler box with ice during transportation to the laboratory at the Department of Parasitology, Institute of Ecology and Biological Resources in Hanoi. The presence of the myxosporean parasite in various organs of each fish, such as skin, fins, gills, brain, muscle, gall bladder, stomach, intestine, and kidney, was examined under the dissecting microscope (SZ61, Olympus, Tokyo, Japan). When myxosporean cysts were detected, some were squished between a glass slide and a cover slip with a drop of saline solution and subjected to microscopy for spore morphology and measurements. The remaining cysts were preserved in an 80% ethanol solution for subsequent molecular analysis.

Morphological analyses

The fresh myxospores were photographed at 1000× magnification under a light microscope (Eclipse Ni-U, Nikon Corporation, Tokyo, Japan) and connected to a digital camera (DS-Ri2, Nikon Corporation, Tokyo, Japan). Measurements were conducted on 30 mature myxospores based on the digital photographs obtained, using CorelDraw X6® software (Corel Corp., Ottawa, Canada) following the guidelines of Lom & Arthur (1989). All measurements are presented as mean ± standard deviation (range) in micrometers (µm). Representative illustrations of mature spores were created using Adobe Illustrator CS2 software (Adobe Systems Inc., San Jose, California).

Molecular analyses

The total DNA was extracted from preserved cysts using the Qiagen DNeasy Blood and Tissue Kit (Qiagen Inc., Hilden,

Germany) following the manufacturer's instructions. A partial small subunit ribosomal DNA (SSU rDNA) sequence was amplified using the primer pairs: 18E (5'-CTGGTTG ATTCTGCCAGT-3') (Hillis & Dixon, 1991) - MyxospecR (5'-CTACGGAAACCTTGTT ACG-3') (Whipps et al., 2003), and MyxospecF (5'-TTCTGCCCTATCAACTW GTTG-3') (Fiala, 2006)-18R (5'-CTACGG AAACCTTGTTACG-3') (Whipps et al., 2003). PCR was performed in a 25 µL volume containing 12.5 µL of KOD One™ PCR Master Mix (2X) (Toyobo Co. Ltd., Osaka, Japan), 10 pmol of each primer, and 1 µL of DNA template. PCR was run on a PCR Eppendorf Mastercycler Nexus Thermal Cyclers (Eppendorf, Hamburg, Germany) with 45 cycles including denaturation at 98 °C for 10 s, annealing at 56 °C for 5 s, and extension at 68 °C for 5 s. A portion of PCR products were evaluated by 1.0% agarose gel electrophoresis, and stained with Gel Red™ (Biotium Inc., Hayward, CA, USA). The remaining products were purified using QIAquick® PCR Purification Kit (250) (Qiagen Inc., Hilden, Germany) following the manufacturer's instructions before being sent to the limited liability company ATGC (Thanh Xuan, Ha Noi, Vietnam) for sequencing using both forward and reverse primers used for the PCR. The obtained DNA sequence chromatograms were reviewed and edited using ChromasPro 2.1.8 software (Technelysium Pty. Ltd., Queensland, Australia).

Phylogenetic analyses

The obtained sequence of SSU rDNA was compared with 37 sequences of *Kudoa* spp. from the GenBank database with similarity greater than 95% and query cover over 88%. Sequence of *Unicapsula* sp. CMW-2003 (AY302724) was used as an outgroup. All the selected sequences were aligned and trimmed to 1458 bp. A phylogenetic analysis was conducted using the maximum likelihood (ML) method in MEGA 6.0 software (Tamura et al., 2013). The best-fit substitution model (CTR + G + I) was selected on the basis of the lowest Bayesian information criterion (BIC)

score. Bootstrap confidence values were calculated with 1000 replicates.

RESULTS AND DISCUSSION

Three myxosporean cysts were observed on the optic lobes of the brain of one individual of *S. virgatus* (Fig. 1). No cysts were detected in the other 9 individuals, resulting in a 10% infection prevalence. Myxospores within the cysts were radially symmetrical and contained 7 polar capsules (90%), some spores contained 6 polar capsules (10%), and possessed the general characteristics of the genus *Kudoa*. Based on the morphological and molecular characteristics listed below, the myxosporean species was identified as *K. yasunagai* (Hsieh & Chen, 1984).

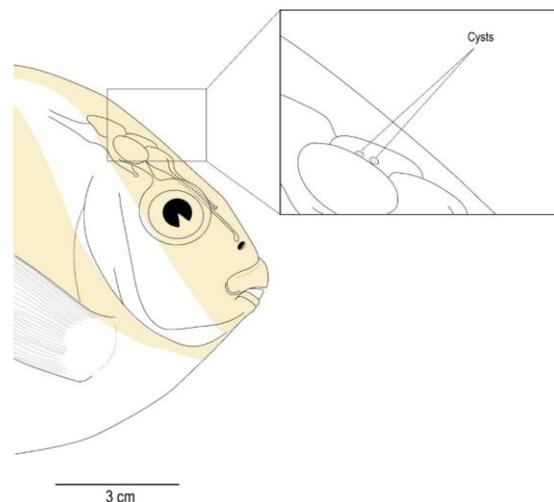


Figure 1. Location of *Kudoa yasunagai* cysts found in the brain of Barhead spinefoot *Siganus virgatus* (Valenciennes)

Kudoa yasunagai (Hsieh & Chen, 1984)

Phylum: Cnidaria Hatschek, 1888
 Class: Myxosporia Bütschli, 1881;
 Order: Multivalvulida Shulman, 1959
 Family: Kudoidae Meglitsch, 1960
 Genus *Kudoa* Meglitsch, 1947

Description of myxospores

White rounded cysts measuring approximately 0.13–0.22 mm were located on

the optic lobes of the brain of *S. virgatus*. Each cyst contained numerous mature myxospores. In the apical view, mature spores were radially symmetrical from the center with 7 (6) equal shell valves and polar capsules. The polar capsules open in the center of the anterior pole of the spores at the same distance from each other. In the longitudinal view, spores were pyramidal in shape with a slightly pointed

anterior pole and a rounded posterior pole. Length of spores 6.6 ± 0.1 (6.4–6.7), $n = 5$; width 7.6 ± 0.5 (7.0–8.4), $n = 25$; thickness 7.0 ± 0.4 (6.5–7.7), $n = 25$. Polar capsules were pear-shaped with measurements 2.8 ± 0.2 (2.6–3.0) in length, and 1.4 ± 0.1 (1.2–1.5) in width. The polar capsules contain a coiled filament inside. The turn of the filament was not clear. (Figs. 2 & 3).

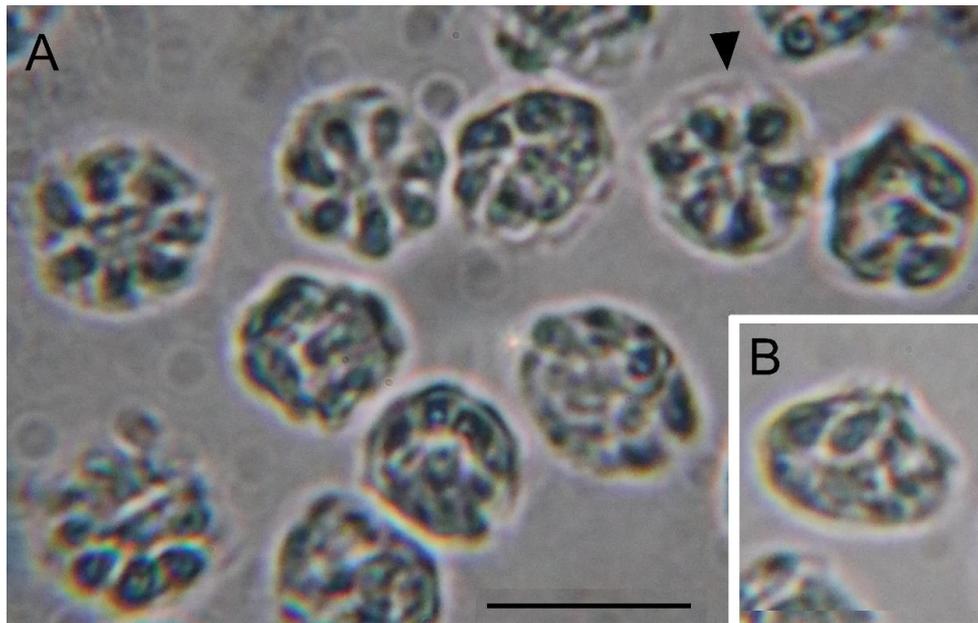


Figure 2. Light photomicrographs of a *Kudoa yasunagai* myxospore from *Siganus virgatus* in apical view (A) and sutural view (B). Scale bar = 10 μ m

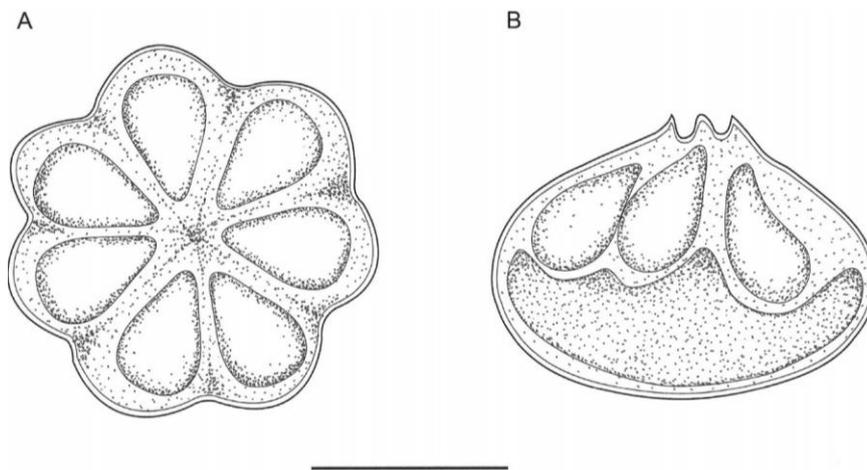


Figure 3. Representative drawing of fresh mature myxospores of *Kudoa yasunagai* in apical view (A) and sutural view (B). Scale bar = 5 μ m

Taxonomic summary

Host: Barhead spinefoot *Siganus virgatus* (Valenciennes, 1835) (Acanthuriformes: Siganidae)

Site of infection: Brain

Locality: Ninh Hoa district, Khanh Hoa province (12°22'50.8"N 109°18'55.6"E) (Van Phong Bay).

Prevalence of infection: 1/10 (10%).

Material: glass slides containing myxosporean spores were deposited in the collection of the Department of Parasitology, Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology, Ha Noi, Vietnam (code 2023-02-KH22).

Molecular data: A partial SSU rDNA sequence of *Kudoa yasunagai* of 1603 base pairs was deposited in GenBank under the accession number: OR781805.

Genus *Kudoa* is an important group of myxosporean parasites in marine fish and some freshwater fish in the Amazon region (Brazil) (Eiras et al., 2014; Velasco et al., 2019). These species generally infect the muscle of their hosts, and some are seen in the heart, gills, kidney, brain, gall bladder, ovary and intestines (Abdel-Ghaffar et al., 2012, 2016; Eiras et al., 2014). To date, more than 120 species of *Kudoa* have been described and some are considered significant threats to fisheries industries. For example, *Kudoa amamiensis* and *Kudoa iwatai* form unsightly macroscopic cysts in the somatic muscle and diminish the market value of the fish (Yokoyama et al., 2004). Some other muscle infecting species, such as *Kudoa musculoliquefaciens*, *Kudoa paniformis*, *Kudoa clupeidae*, *Kudoa miniauriculata*, *K. neothunni*, *K. thyrsites*, *Kudoa lateolabracis*, etc, produce proteolytic enzymes (Patashnik et al., 1982; Tsuyuki et al., 1982; Yokoyama et al., 2004; Matsukane et al., 2010). These enzymes can destroy the myofibers of host fish, causing postmortem myoliquefaction of flesh, leading to significant economic losses (Levsen et al., 2008; Buron et al., 2017). In

addition, the genus *Kudoa* has recently been receiving more attention due to public health issues, as some species such as *Kudoa septempunctata*, *Kudoa hexapunctata*, and *K. iwatai* have been recognized as causative food poisoning agents in humans (Kawai et al., 2012; Suzuki et al., 2012, 2015; Iwashita et al., 2013; Yokoyama et al., 2014; Tachibana & Watari, 2021).

The species *K. yasunagai* (Hsieh & Chen, 1984) was first recorded in the brain of *Lateolabrax japonicus* (Cuvier) from Japan (Hsieh & Chen, 1984). To date, this species has been recorded from 16 fish species belonging to 14 families and 10 orders of fish from Japan, Philippines, and Australia. The reported hosts of *K. yasunagai* include *Argyrosomus japonicus* (Temminck & Schlegel), *Calotomus japonicus* (Valenciennes), *Ellochelon vaigiensis* (Quoy & Gaimard), *Lateolabrax japonicus* (Cuvier), *Lutjanus ehrenbergii* (Peters, 1869), *Oplegnathus fasciatus* (Temminck & Schlegel), *Pagrus major* (Temminck & Schlegel), *Paralichthys olivaceus* (Temminck & Schlegel), *Plotosus lineatus* (Thunberg), *Scolopsis monogramma* (Cuvier), *Seriola lalandi* Valenciennes, *Seriola quinqueradiata* Temminck & Schlegel, *Sillago ciliata* (Cuvier), *Sillago* spp., *Takifugu rubripes* (Temminck & Schlegel), *Thunnus orientalis* (Temminck & Schlegel) (Egusa, 1986a, b; Cheung & Nigrelli, 1990; Whipps et al., 2004; Burger et al., 2007; Burger & Adlard, 2010; Zhang et al., 2010; Shirakashi et al., 2012; Ishimaru et al., 2014; Shin & Shirakashi, 2017; Sakai et al., 2018). *Kudoa yasunagai* forms relatively large cysts, some can be as large as over 0.5 mm in diameter, in the host brain, and this causes abnormal behavior and skeletal deformation, leading to mortality in severe cases (Yasunaga et al., 1981; Egusa, 1986a; Yamamoto, 2007; Ishimaru et al., 2014). Infection prevalence in farmed fish can be as high as 80%, and problems associated with *K. yasunagai* infection have been reported in *Lateolabrax japonicus* (Cuvier), *Seriola*

quinqueradiata Temminck & Schlegel, and several other farmed fishes (Shirakashi et al., 2012). With its wide geographical

distribution and host range, *K. yasunagai* is considered a potentially dangerous pathogen for aquaculture.

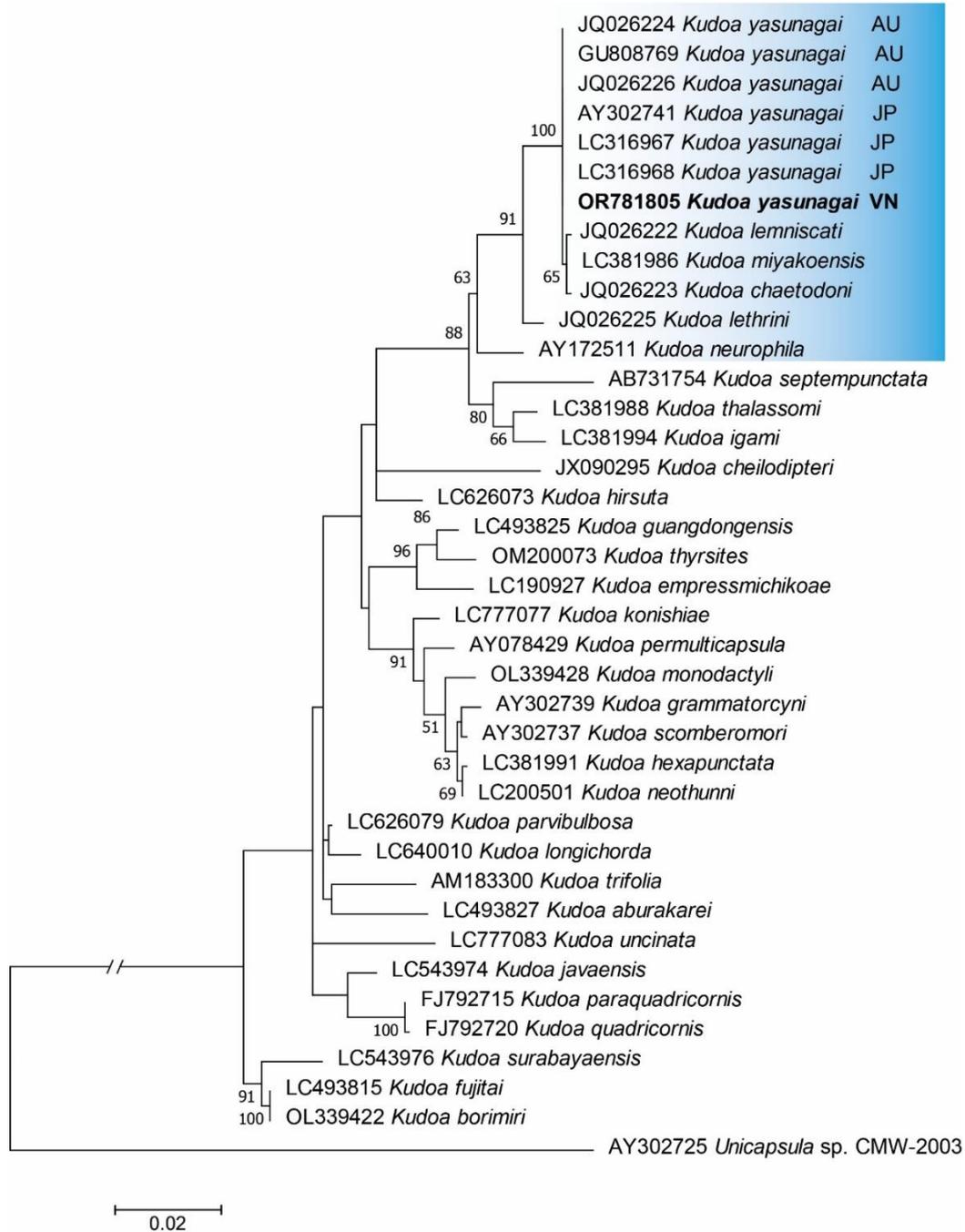


Figure 4. Maximum likelihood phylogenetic tree based on the SSU rDNA sequence of selected *Kudoa* species (the bootstrap values less than 50 were not showed). Blue: Species infecting the brain of their host; VN: Vietnam; JP: Japan; AU: Australia

Table 1. Comparison of spore dimensions of *Kudoa yasunagai* from previous reports and the present study. (All measurements are in μm)
(NA: Data not available)

Family of host	Siganidae	Lateolabracidae	Nemipteridae	Mugilidae	Lutjanidae	Sciaenidae	Scaridae
Host	<i>Siganus virgatus</i> (Valenciennes)	<i>Lateolabrax japonicus</i> (Cuvier)	<i>Scolopsis monogramma</i> (Cuvier)	<i>Ellochelon vaigiensis</i> (Quoy & Gaimard)	<i>Lutjanus ehrenbergii</i> (Peters)	<i>Argyrosomus japonicus</i> (Temminck & Schlegel)	<i>Calotomus japonicus</i> (Valenciennes)
Locality	Vietnam	Japan	Australia	Australia	Australia	Japan	Japan
Spore width	7.0–8.4 (7.6)	9.4–13.9 (11.7)	10.7–12.3 (11.5)	11.5–14.6 (13.2)	11.1–14.5 (12.9)	10.5–12.3 (11.5)	10.6–12.8 (11.6)
Spore thickness	6.5–7.7 (7.0)	7.1–10.2 (8.3)	9.5–11.1 (10.3)	11.0–14.0 (12.7)	11.6–13.8 (12.8)	8.6–11.2 (10.3)	8.7–11.6 (10.4)
Spore length	6.4–6.7 (6.6)	4.3–7.3 (6.2)	NA	NA	NA	6.6–8.4 (7.5)	7.0–8.5 (7.7)
Polar capsule length	2.6–3.0 (2.8)	3.4–4.3 (3.6)	2.3–2.7 (2.5)	2.8–3.9 (3.3)	2.7–3.5 (3.1)	3.6–4.8 (4.2)	3.9–5.0 (4.3)
Polar capsule width	1.2–1.5 (1.4)	2.2–2.9 (2.5)	1.4–1.8 (1.6)	1.7–2.3 (1.9)	1.6–2.2 (1.9)	1.6–2.6 (2.1)	1.8–2.7 (2.2)
Number of SV/PC	7 (6)	7 (6 or 8)	6–7	7–8	6–7	6 (5)	5-6-7
Reference	This study	Hsieh & Chen, 1984	Burger & Adlard, 2010	Miller & Adlard, 2012	Miller & Adlard, 2012	Sakai et al., 2018	Sakai et al., 2018

In this study, myxosporean spores collected from the brain of *S. virgatus* (Valenciennes) were identified as *K. yasunagai* by molecular and morphological methods. The SSU rDNA sequence of this myxosporean sample (OR781805) was 100% identical to the sequences of *K. yasunagai* isolated from *Calotomus japonicus* (Valenciennes) (LC316968), *Argyrosomus japonicus* (Temminck & Schlegel) (LC316967), *Paralichthys olivaceus* (Temminck & Schlegel) (AY302741), *Ellochelon vaigiensis* (Quoy & Gaimard) (JQ026226), *Lutjanus ehrenbergii* (Peters) (JQ026224), and *Sillago ciliata* Cuvier (GU808769) (1603/1603, 1603/1603, 1603/1603, 1589/1589, 1589/1589, 1432/1432, respectively). In the phylogeny tree, all these sequences of *K. yasunagai* were clustered in the same subclade with a high bootstrap value (100%) (Fig. 4). In addition, *Kudoa* spp. infecting the brain of their host were grouped in a separate clade (blue box) in the phylogeny tree (Fig. 4). This result indicated that *Kudoa* spp. infecting fish brain have a close genetic relatedness. This result is consistent with the opinion of Burger et al. (2007) and Shin et al. (2016) who indicated an association between the tissue tropism of *Kudoa* spp. and their genetic relationships. Morphologically, the measurements of *K. yasunagai* myxospore in this study were smaller than those from Australia and Japan (Table 1). These variations may be attributed to differences in host and geographical location. More precise molecular analysis with DNA regions of higher resolution, such as the large subunit ribosomal DNA and mitochondrial COI gene, may reveal possible host/geographical distinction in this cosmopolitan *Kudoa* species.

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