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FISH PARASITOLOGY - ORIGINAL PAPER

Occurrence of *Kudoa prunusi* and *K. lateolabracis* (Myxozoa: Myxosporea: Multivalvulida) in Philippine-Sea Japanese parrotfish (*Calotomus japonicus*)

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Abstract

Multiple *Kudoa* spp. (Myxozoa: Myxosporea: Multivalvulida) have been recorded in Japanese parrotfish (*Calotomus japonicus*) from the Philippine Sea (Northwest Pacific Ocean), off southwestern Japan; *Kudoa yasunagai* in the brain, and *K. igami*, *K. lateolabracis*, and *K. thalassomi* in the muscles. This study examined eight Philippine Sea Japanese parrotfish samples collected in January and February 2019 and found *K. prunusi* in the brain (3–57 plasmodia/fish; average 17.9) and *K. lateolabracis* plasmodia in the trunk muscle of all fish individuals examined. The *K. prunusi* in this study was characterized by myxospores predominatetly with six shell valves (SVs) and a corresponding number of polar capsules (PCs), contrasting with the original description of the species from farmed Pacific bluefin tuna (*Thunnus orientalis*) brain that characterized the species as having predominately five SVs/PCs. Molecular-genetic characterization of 18S and 28S ribosomal RNA genes and mitochondrial DNA genes (cytochrome *c* oxidase subunit 1 and small and large ribosomal RNA subunits) clearly differentiated the *K. prunusi* isolate from *K. yasunagai*, commonly characterized by six or seven, but rarely five, SVs/PCs myxospores. The Japanese parrotfish is a new host record for *K. prunusi* and speculated to be an important reservoir host in its natural waters. *Kudoa lateolabracis* myxospores isolated from pseudocysts in the myofiber were morphologically and phylogenetically close to a clade of the *Kudoa* spp. that exhibit cruciform myxospores similar to *K. thyrsites*. This study is the first to sequence a mitochondrial DNA of small and large subunit ribosomal RNA of *K. lateolabracis*.

Keywords Myxozoa · Multivalvulida · *Kudoa* · Morphology · rDNA · mtDNA · New host record · Pacific Ocean · Japan

Introduction

Multiple *Kudoa* spp. (Cnidaria: Myxozoa: Myxosporea: Multivalvulida) have been recorded in the Japanese parrotfish *Calotomus japonicus* (Valenciennes, 1840) from the Philippine Sea (Northwest Pacific Ocean), off southwestern Japan. *Kudoa yasunagai* (Hsieh et Chen, 1984) was recorded in the brain, and *K. igami* Shirakashi, Yamane, Ishitani, Yanagida et Yokoyama, 2014, *K. lateolabracis* Yokoyama, Whipps, Kent, Mizuno et Kawakami, 2004, and *K. thalassomi* Adlard, Bryant, Whipps et Kent, 2005 were recorded in the trunk muscle (Shirakashi et al. 2014; Sakai et al. 2019).

Kudoa igami was originally characterized by stellate myxospores with five or six shell valves (SVs) and a corresponding number of polar capsules (PCs), forming pseudocysts in the myofiber of the Japanese parrotfish (Shirakashi et al. 2014). Later, Shin et al. (2016) found the species in the trunk muscle of the olive flounder *Paralichthys olivaceus* (Temminck et Schlegel, 1846) farmed in the Philippine Sea, where the *K. igami* infections in Japanese parrotfish were recorded. More recently, Sakai et al. (2019) recorded the species in the trunk muscle of the Carolines parrotfish *Calotomus carolinus* (Valenciennes, 1840), African coris *Coris gaimard* (Quoy et Gaimard, 1824), and the pastel ring wrasse *Hologymnosus doliatus* (Lacepède, 1801) from the border of the Philippine and East China Seas, off Miyako Island, Okinawa, Japan. Sakai et al. (2019) observed, however,

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seven to nine SVs/PCs in myxospores of their *K. igami* isolates, in contrast to five or six SVs/PCs previously reported by Shirakashi et al. (2014) and Shin et al. (2016). Morphological variations in the SV and PC numbers in myxospores from the same or different plasmodia are also established among other *Kudoa* spp. characterized by myxospores with more than four SVs/PCs: e.g., *K. yasunagai* (Egusa 1986; Burger and Adlard 2010b; Miller and Adlard 2012; Sakai et al. 2019); *K. septempunctata* Matsukane, Sato, Tanaka, Kamata et Sugita-Konishi, 2010 (Matsukane et al. 2010; Kasai et al. 2016b; Yokoyama et al. 2017); *K. thalassomi* (Burger and Adlard 2011; Shirakashi et al. 2014; Sakai et al. 2019); *K. neothunni* (Arai et Matsumoto, 1953) (Kasai et al. 2017b); *K. chaetodoni* Burger, Cribb et Adlard, 2007 (Burger et al. 2007; Miller and Adlard 2012); *K. lemniscati* (Miller and Adlard 2012); and *K. miyakoensis* Sakai, Kawai, Zhang et Sato, 2019 (Sakai et al. 2019).

Postharvest myoliquefaction caused by multivalvulidan infection in commercially important fish has a significant economic impact on natural water marine fisheries and aquaculture (Egusa 1986; Moran et al. 1999). The known causative species of this phenomenon include *Unicapsula seriolae* Lester 1982, *K. thyrsites* (Gilchrist, 1924), *K. lateolabracis*, *K. megacapsula* Yokoyama et Itoh, 2005, *K. musculoliquefaciens* (Matsumoto 1954), *K. neothunni*, *K. paniformis* Kabata et Whitaker, 1981, *K. pleurogrammi* Kasai, Li, Mafie et Sato, 2016, *K. rosenbuschi* (Gelormini, 1944), and *K. aburakarei* Li, Inoue, Tanaka, Zhang et Sato, 2020 (Matsumoto 1954; Kabata and Whitaker 1981; Lester 1982; Moran et al. 1999; Yokoyama et al. 2004, 2006; Yokoyama and Itoh 2005; Whipps and Kent 2006; Li et al. 2013, 2020b; Kasai et al. 2016a). The parasitism of these species is not directly related to postharvest myoliquefaction, as enzymatic digestion of host muscle can be affected by multiple factors (Konagaya 1984; Dawson-Coates et al. 2003; Funk et al. 2007, 2008; Zhou and Li-Chan 2009). Originally, *K. lateolabracis* was described as a cause of postharvest myoliquefaction in Chinese sea bass *Lateolabrax* sp. cultured in western Japan (Yokoyama et al. 2004). However, all Japanese parrotfish parasitized with *K. lateolabracis* did not exhibit this phenomenon when Shirakashi et al. (2014) examined 17 infected fish samples.

In this study, we examined eight Japanese parrotfish specimens fished in the Philippine Sea, distant from the previous study on Japanese parrotfish kudoid infection (Shirakashi et al. 2014), and found two *Kudoa* spp. in the brain and trunk muscles. We attempted to identify the isolated kudoid species based on morphological criteria and molecular-genetic characterization.

Materials and methods

Fish samples and parasitological examination

Four whole Japanese parrotfish each were purchased on January 10 and February 4, 2019, from a local fish market in Kochi, southwestern Japan. They were fished in the Philippine Sea (Northwest Pacific Ocean), off Kochi, Japan. The samples were transported on ice to the laboratory in Yamaguchi University within 1 day of purchase. Parasitological examinations of the fish samples were performed as previously described (Inoue et al. 2021). Briefly, the gills, viscera, and brain were removed and examined under a dissection microscope. Thin slices of the trunk muscle were placed in physiological saline, pressed between glass plates, and examined under a dissection microscope.

Fresh myxospores were measured according to Lom and Arthur (1989). All measurements are expressed in μm unless otherwise stated, and the ranges are presented with the means in parentheses. The tissue-embedded myxosporean plasmodia were divided into two groups and fixed in either 10% neutral-buffered formalin solution or 70% ethanol for further analyses. The specimens in this study were deposited in the Meguro Parasitological Museum, Tokyo, Japan, under collection numbers 21774 and 21775.

DNA extraction, amplification, and sequencing

Parasite DNA was extracted from the myxosporean plasmodia frozen after isolation at − 20 °C. The methods for DNA extraction, amplification of rDNA fragments by polymerase chain reaction (PCR), and purification of the PCR products were performed as previously described (Li et al. 2013; Kasai et al. 2015). Further molecular-genetic characterization of kudoid isolates was conducted on the mitochondrial DNA (mtDNA), i.e., cytochrome *c* oxidase subunit 1 (*cox-1*) and small and large ribosomal RNA subunits (*rns-rnl*), according to our previous study (Sakai et al. 2018). When direct sequencing results were not satisfactory, purified PCR products were cloned into the pTA2 plasmid vector (TArget Clone™; TOYOBO, Dojima Hama, Osaka, Japan) according to the manufacturer's instructions. Following propagation, the plasmid DNA was extracted using a FastGene Plasmid Mini Kit (NIPPON Genetics Co., Tokyo, Japan), and inserts from multiple independent clones, at least three, were sequenced using universal M13 forward and reverse primers. The nucleotide sequences obtained in this study are available from the DDBJ/EMBL/GenBank databases under accession numbers LC640102–LC640108.

Phylogenetic analyses

Fragments of the newly obtained rDNA sequences were analyzed to identify highly similar nucleotide sequences using the Basic Local Alignment Search Tool (BLAST) of the National Center for Biotechnology Information website (NCBI; https://www.ncbi.nlm.nih.gov/). For phylogenetic analysis, the newly obtained 18S and 28S rDNA sequences of *Kudoa* spp. from this study and related sequences retrieved from the GenBank database (NCBI) were aligned using the MEGA7 software (Kumar et al. 2016), with subsequent manual adjustments. The accession numbers of the analyzed sequences are provided in the figures of the phylogenetic trees. Regions judged to be poorly aligned and characters with a gap in any sequences were excluded from subsequent analyses; 1415 characters, of which 194 were variable, remained for subsequent analysis of the 18S rDNA, and 591 characters, of which 216 were variable, remained for subsequent analysis of the 28S rDNA. Similarly, 437 characters, of which 172 were variable, remained for subsequent analysis of the *cox-1* mtDNA, and 961 characters, of which 471 were variable, remained for subsequent analysis of the *rns-rnl* mtDNA. Maximum likelihood (ML) analysis was performed with the program, PhyML (Guindon and Gascuel 2003; Dereeper et al. 2008) provided on the "phylogeny.fr" website (http://www.phylogeny.fr/). 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). Outlier *Kudoa* spp. forming cysts, such as *K. bora* (Fujita, 1930), *K. iwatai* Egusa et Schiomitsu, 1983, and *K. lutjanus* Wang, Huang, Tsai, Cheng, Tsai, Chen, Chen, Chu, Liaw, Chang et Chen, 2005, to a majority of *Kudoa* spp. were used as an outgroup for construction of the ML phylogenetic trees.

Results

Parasitological examination

Two batches of the Japanese parrotfish samples, with four samples in each batch, were purchased in January and February 2019, and kudoid plasmodia were found in the cranial cavity and trunk muscles of all eight samples. Myxospores isolated from plasmodia located in different organs exhibited distinct morphology.

Kudoa prunusi Meng, Yokoyama, Shirakashi, Grabner, Ogawa, Ishimaru, Sawada et Murata, 2011

Kudoa prunusi was identified as round to oval plasmodia floating in the subarachnoid space (Fig. 1), measuring

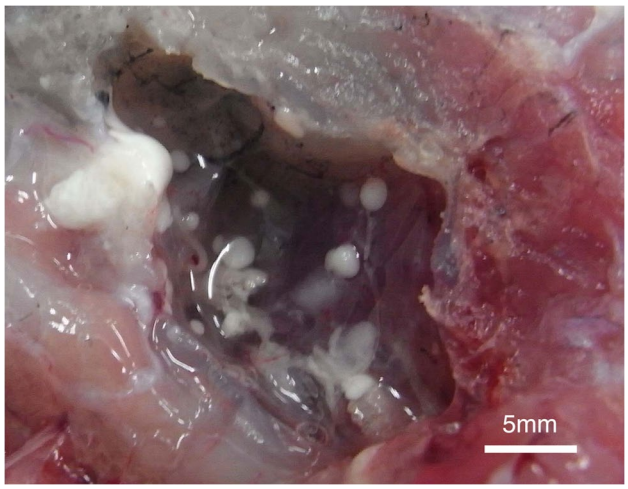

Fig. 1 *Kudoa prunusi* plasmodia in cranial cavity after removing the brain

0.22–1.93 (0.73) mm by 0.21–1.65 (0.64) mm ($n = 80$). There were 3–57 (17.9) plasmodia of varying dimensions per fish. The myxospores were stellate in apical view, with six or five SVs and a corresponding number of PCs (Fig. 2). The ratio of myxospores with six SVs/PCs to those with five SVs/PCs was 75%:25% ($n = 40$). The lateral view of the myxospores was rounded-pyramidal. The dimensions of the *K. prunusi* myxospores isolated in this study are shown in Table 1.

BLAST searches using the 18S and partial 28S rDNA nucleotide sequences, 1719-bp and 800-bp in length, respectively, of the *K. prunusi* isolate (DDBJ/EMBL/GenBank accession no. LC640106) showed the highest nucleotide identity with an isolate of a *K. prunusi* isolate from the brain of cultured *Thunnus orientalis* (Temminck et Schlegel, 1844) in Wakayama, western Japan, followed by various *Kudoa* spp. isolated from brain tissue (Table 2). Two partial fragments of *K. prunusi* mitochondrial genes, *cox-1* and *rns-rnl*, were sequenced for the first time (LC640102–LC640104). The *cox-1* sequences of the two *K. prunusi* isolates exhibited a high affinity with *K. yasunagai* (LC382003), exhibiting 91.08% (398/437) and 91.30% (399/437) identity, followed by *K. miyakoensis* (LC3820004) with 82.84% (362/437) and 83.07% (363/437) identity.

Remarks

Kudoa prunusi was originally described from the brain of juvenile Pacific bluefin tunas cultured in Wakayama Prefecture, Japan, at the edge of the Philippine Sea (Meng et al. 2011). The myxospores of the species were characterized as penta-radiate in apical view, with five (rarely six) SVs/PCs per myxospore. The ratio of these two morphotypes in a plasmodium was 80:20. As in the original description (Meng et al. 2011), we also found two myxospore morphotypes in the same plasmodium in the present

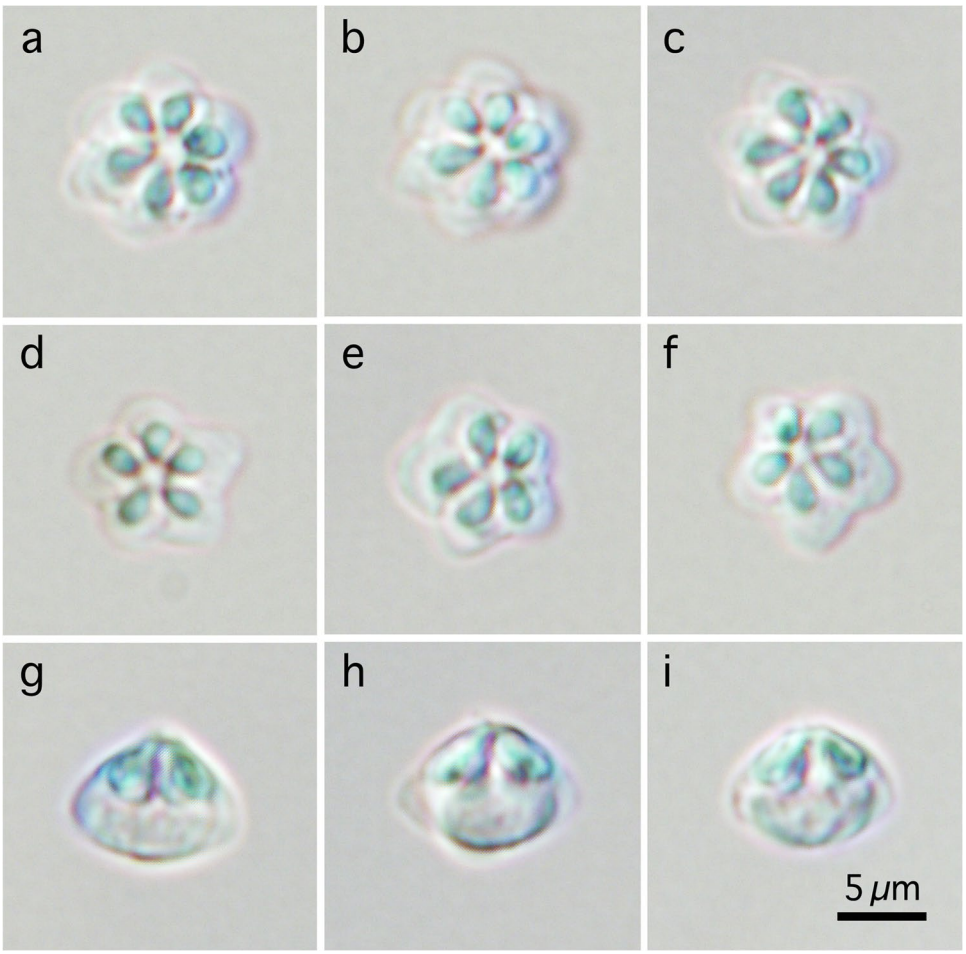

Fig. 2 *Kudoa prunusi* myxospores. Apical view showing six SVs and PCs (**a**–**c**), apical view showing five SVs and PCs (**d**–**f**), and lateral view (**g**–**i**)

isolate; however, the ratio was approximately opposite, 25:75 for five and six SVs/PCs, respectively. The morphometric values of the present isolate were comparable with those of *K. prunusi*, *K. yasunagai*, and *K. neurophila* (Table 1). The nucleotide sequences of the 18S and 28S rDNA of the present isolate identified it as *K. prunusi*. The *K. prunusi* parasitism in Japanese parrotfish brain tissue established a new host record and expanded its geographical distribution to the open sea area of the Philippine Sea.

Taxonomic summary

Host: *Calotomus japonicus* (Valenciennes, 1840): Japanese parrotfish (Actinopteryi: Eupercaria/misc: Scaridae-dae: Sparisomatinae).

Locality: Philippine Sea (Northwest Pacific Ocean), off Kochi, western Japan.

Site of infection: Subarachnoid space.

Materials deposited: Specimen no. 21775, Meguro Parasitological Museum, Tokyo, Japan.

Deposited rDNA sequences: DDBJ/EMBL/GenBank accession nos. LC640106 (rDNA) and LC640102–LC640104 (mtDNA).

Prevalence: 100% (8/8).

Kudoa lateolabracis Yokoyama, Whipps, Kent, Mizuno et Kawakami, 2004

Kudoa lateolabracis was frequently found in the trunk muscle of all eight Japanese parrotfish examined in this study. The plasmodia, developed in pseudocysts in the myofibers, measured 0.98–3.79 (2.01) mm by 0.09–0.33 (0.16) mm. The myxospores were cruciform in apical view, with four SVs and a corresponding number of PCs. The sizes of the SVs and pyriform PCs were unequal, with one large PC/SV opposite one small PC/SV and two intermediate PCs/SVs between the former two (Fig. 3). The lateral view of the myxospores was asymmetric-pyramidal. The myxospore dimensions of *K. lateolabracis* isolated in this study are shown in Table 3.

BLAST searches using 18S and partial 28S rDNA nucleotide sequences, 1721-bp and 800-bp in length, respectively, of the present *K. lateolabracis* isolate (LC640107 and LC640108), showed an absolute or close to absolute similarity to *K. lateolabracis* from *Lateolabrax* sp. (AY382606) and *Calotomus japonicus* (AB844442). A

Table 1 Morphometric comparison of *Kudoa prunusi* of different origin and two other *Kudoa* spp. with brain tropism[a]

Species	*K. prunusi*		*K. prunusi*	*K. yasunagai*		*K. yasunagai*	*K. neurophila*
Host fish species	*Calotomus japonicus*		*Thunnus orientalis* (farmed)	*Sillago ciliata*		*Calotomus japonicus*	*Seriola lalandi*
Locality	Philippine Sea (Northwest Pacific Ocean), off Kochi, Japan		Aquacultured in Wakayama, Japan (Philippine Sea)	Great Barrier Reef, Queensland, Australia		Philippine Sea, off Kochi, Japan	Great Barrier Reef, Queensland, Australia
Date of collection	Jan. 10 and Feb. 4, 2019		—	—		Oct. 12, 2016	—
Reference	Present study		Meng et al. (2011)	Burger et al. (2007)		Sakai et al. (2019)	Burger and Adlard (2010b)
Cyst surrounding a plasmodium	$n=80$		Numerous			$n=4$	
Length (mm)	0.22–1.93 (0.73)		≤0.5 in diameter (round)	—		0.35–0.97 (0.54)	—
Width (mm)	0.21–1.65 (0.64)			—		0.31–0.83 (0.45)	—
Myxospore	**5 SVs/PCs ($n=10$)**	**6 SVs/PCs ($n=25$)**	**5 SVs/PCs ($n=20$)**	**5 SVs/PCs ($n=53$)**	**6 SVs/PCs ($n=31$)**	**6 SVs/PCs ($n=20$)**	**4–5 SVs/PCs**
Width	8.5–10.6 (9.6)	8.8–11.4 (10.5)	8.5–10.3 (9.6)	10.2–11.9 (10.8)	10.5–12.9 (11.6)	10.6–12.8 (11.6)	8.6–10.3 (9.2)
Thickness	7.7–9.6 (8.8)	8.5–10.2 (9.5)	—	9.2–10.9 (10.0)	9.8–12.2 (10.7)	8.7–11.6 (10.4)	6.8–8.0 (7.3)
Sutural thickness	—	7.3–8.6 (8.0)	—	—	7.6–10.2 (8.4)	7.2–9.0 (8.4)	7.2–8.6 (7.8)
Length	7.0–8.3 (7.5)	7.0–8.3 (7.5)	6.7–8.6 (7.5)	6.8–8.1 (7.3)	6.8–8.1 (7.3)	7.0–8.5 (7.7)	—
Polar capsule							
Length	2.3–3.2 (2.9)	2.3–3.6 (3.0)	2.8–4.1 (3.7)	2.5–4.1 (3.5)		3.9–5.0 (4.3)	2.0–2.6 (2.3)
Width	1.5–1.8 (1.6)	1.5–1.9 (1.6)	1.7–2.2 (2.0)	1.5–2.4 (1.9)		1.8–2.7 (2.2)	1.4–1.8 (1.6)
Variation of SV/PC number[b]	25% (5):75% (6) [$n=40$]		80% (5):20% (6) [$n=?$]	86% (5):14% (6) [$n=?$]		72% (6):26% (7):2% (8) [$n=50$]	4–5 (ratio not indicated)

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

[b]Ratio of spores with different numbers of SV/PC indicated in parentheses is shown

Table 2 Nucleotide similarity of rDNA sequence of the present *Kudoa prunusi* isolate to *Kudoa* spp. retrieved from the DNA databases[a]

Species	Accession no	Host fish	Locality	Difference of nucleotide[b]	Indels	Percentage of similarity
18S rDNA						
K. prunusi	AB573715	*Thunnus orientalis*	Japan: Wakayama	1/1676	0	99.94
K. yasunagai	JQ026224	*Lutjanus ehrenbergii*	Australia: Coral Sea	4/1599	0	99.75
	JQ026226	*Liza vaigiensis*	Australia: Coral Sea	4/1589	0	99.75
	LC216968	*Calotomus japonicus*	Japan: Kochi	6/1719	0	99.65
	LC216967	*Argyrosomus japonicus*	Japan: Kochi	6/1719	0	99.65
	AY302741	*Paralichthys olivaceus*	Japan	6/1679	0	99.64
K. chaetodoni	JQ026223	*Lutjanus carponotatus*	Australia: Coral Sea	6/1608	2	99.63
	JQ026218	*Caesio cuning*	Australia: Coral Sea	6/1599	0	99.62
K. lemniscati	JQ026222	*Lutjanus lemniscatus*	Australia: Coral Sea	6/1588	0	99.62
K. miyakoensis	LC381986	*Naso unicornis*	Japan: Okinawa	7/1719	0	99.59
K. lethrini	JQ026221	*Lutjanus fulviflamma*	Australia: Coral Sea	14/1575	0	99.11
	JQ026220	*Lutjanus fulviflamma*	Australia: Coral Sea	14/1518	0	99.08
	JQ026219	*Lutjanus ehrenbergii*	Australia: Coral Sea	15/1563	0	99.04
K. neurophila	AY172511	*Latris lineata*	Australia: Coral Sea	23/1551	1	98.52
28S rDNA						
K. prunusi	HQ262571	*Thunnus orientalis*	Japan: Wakayama	0/664	0	100.00
K. yasunagai	GU808777	*Scolopsis monogramma*	Australia: Coral Sea	10/669	0	98.51
	LC216967	*Argyrosomus japonicus*	Japan: Kochi	12/800	0	98.50
	KX163085	*Seriola lalandi*	Japan: Wakayama	11/732	0	98.50
	GU808775	*Sillago ciliata*	Australia: Coral Sea	11/669	0	98.36
	LC216968	*Calotomus japonicus*	Japan: Kochi	14/800	0	98.25
	AY302736	*Paralichthys olivaceus*	Japan	12/671	0	98.21
	JQ026229	*Lutjanus ehrenbergii*	Australia: Coral Sea	13/637	0	97.96
	JQ026230	*Liza vaigiensis*	Australia: Coral Sea	14/637	0	97.80
K. chaetodoni	GU808771	*Chaetodon unimaculatus*	Australia: Coral Sea	10/669	0	98.51
	JQ026231	*Caesio cuning*	Australia: Coral Sea	10/637	0	98.43
K. lemniscati	JQ026232	*Lutjanus lemniscatus*	Australia: Coral Sea	8/637	8	98.74
K. miyakoensis	LC381986	*Naso unicornis*	Japan: Okinawa	13/800	0	98.38
K. lethrini	GU808772	*Gymnocranius audleyi*	Australia: Coral Sea	23/669	14	96.56
	JQ026227	*Lutjanus ehrenbergii*	Australia: Coral Sea	23/637	14	96.39
K. neurophila	AY302735	*Latris lineata*	Australia: Coral Sea	31/667	6	95.35
	GU808774	*Seriola lalandi*	Australia: Coral Sea	31/665	6	95.34

[a]*Kudoa prunusi* in this study from the Japanese parrotfish *Calotomus japonicus* (DDBJ/EMBL/GenBank accession no. LC640106) compared with deposited nucleotide sequences of *Kudoa* spp

[b]The number of nucleotide differences over the number of overlapping nucleotides

partial fragment of *K. lateolabracis* mitochondrial *rns-rnl* was sequenced for the first time (LC640105).

Remarks

Kudoa lateolabracis was originally described from the liquefied muscle of Chinese sea bass farmed in the Inland Sea of Japan, off Ehime Prefecture (Yokoyama et al. 2004), from fish seeds imported from China. Additional records of the species came from the muscle of the Japanese parrotfish at the edge of the Philippine Sea, off Wakayama, Japan (Shirakashi et al. 2014), and the olive flounder farmed in Wakayama (Shin et al. 2016). The two previous reports suggested that *K. lateolabracis* might be endemic in the seawater around western Japan, and farmed Chinese sea bass might be infected in the farming sea, the Inland Sea of Japan. The current record of *K. lateolabracis* in Japanese parrotfish from the Philippine Sea, off Kochi, further supports the hypothesis that *K. lateolabracis* is an endemic kudoid species in the sea around southwestern Japan.

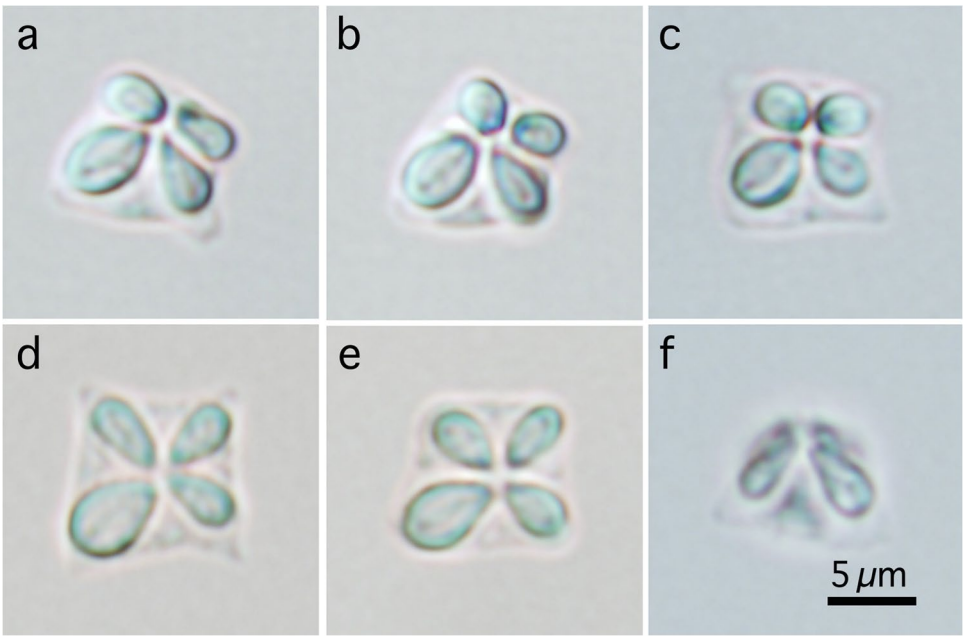

Fig. 3 Apical to oblique view of *Kudoa lateolabracis* myxospores (**a**–**f**)

Table 3 Morphometric comparison of *Kudoa lateolabracis* from different sources[a]

Species	*K. lateolabracis*	*K. lateolabracis*	*K. lateolabracis*	*K. thyrsites*
Host fish species	*Calotomus japonicus*	*Calotomus japonicus*	*Lateolabrax* sp. (farmed)	*Paralichthys olivaceus* (farmed)
Locality	Philippine Sea (Northwest Pacific Ocean), off Kochi, Japan	Philippine Sea (Northwest Pacific Ocean), off Wakayama, Japan	Inland Sea of Japan (Northwest Pacific Ocean), off Ehime, Japan	Inland Sea of Japan (Northwest Pacific Ocean), off Ehime, Japan
Date of collection	Jan. 10 and Feb. 4, 2019	2012–2013	Mar. 6 and Jun. 30, 2003	Jan. 10 and May 7, 2003
Reference	Present study	Shirakashi et al. (2014)	Yokoyama et al. (2004)	Yokoyama et al. (2004)
Plasmodium	$n=50$			
Length (mm)	0.98–3.79 (2.01)	—	—	—
Width (mm)	0.09–0.33 (0.16)	—	—	—
Myxospore	$n=25$	$n=20$	$n=20$	$n=20$
Width	11.4–15.0 (12.9)	13.0–16.0 (14.0)	9.9–12.9 (11.5)	12.9–17.8 (14.7)
Thickness	9.3–11.8 (10.6)	8.6–11.4 (10.2)	8.4–9.9 (9.3)	7.9–11.9 (10.0)
Sutural thickness	7.1–9.8 (8.7)	—	—	—
Length	5.8–7.0 (6.4)	6.4–8.9 (7.7)	5.4–6.9 (6.4)	6.9–8.9 (7.8)
Large polar capsule				
Length	4.6–6.4 (5.7)	3.9–5.3 (4.4)	4.0–5.9 (5.2)	4.0–5.9 (5.0)
Width	2.9–4.4 (3.6)	3.3–4.3 (3.7)	2.5–3.5 (2.8)	1.5–3.0 (2.5)
Intermediate polar capsule				
Length	4.3–5.3 (4.8)	—	—	—
Width	2.3–3.1 (2.7)	—	—	—
Small polar capsule				
Length	3.9–5.1 (4.3)	2.5–4.1 (3.2)	3.0–4.0 (3.6)	2.0–4.0 (2.8)
Width	1.9–2.9 (2.5)	2.4–3.3 (2.7)	1.5–2.0 (1.9)	1.5–2.5 (1.6)

[a]All measurements are in μm and expressed as ranges with means in parentheses unless otherwise stated. "—" indicates no available data

Taxonomic summary

Host: *Calotomus japonicus* (Valenciennes, 1840): Japanese parrotfish (Actinopteryi: Eupercaria/misc: Scaridaedae: Sparisomatinae).

Locality: Philippine Sea (Northwest Pacific Ocean), off Kochi, western Japan.

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

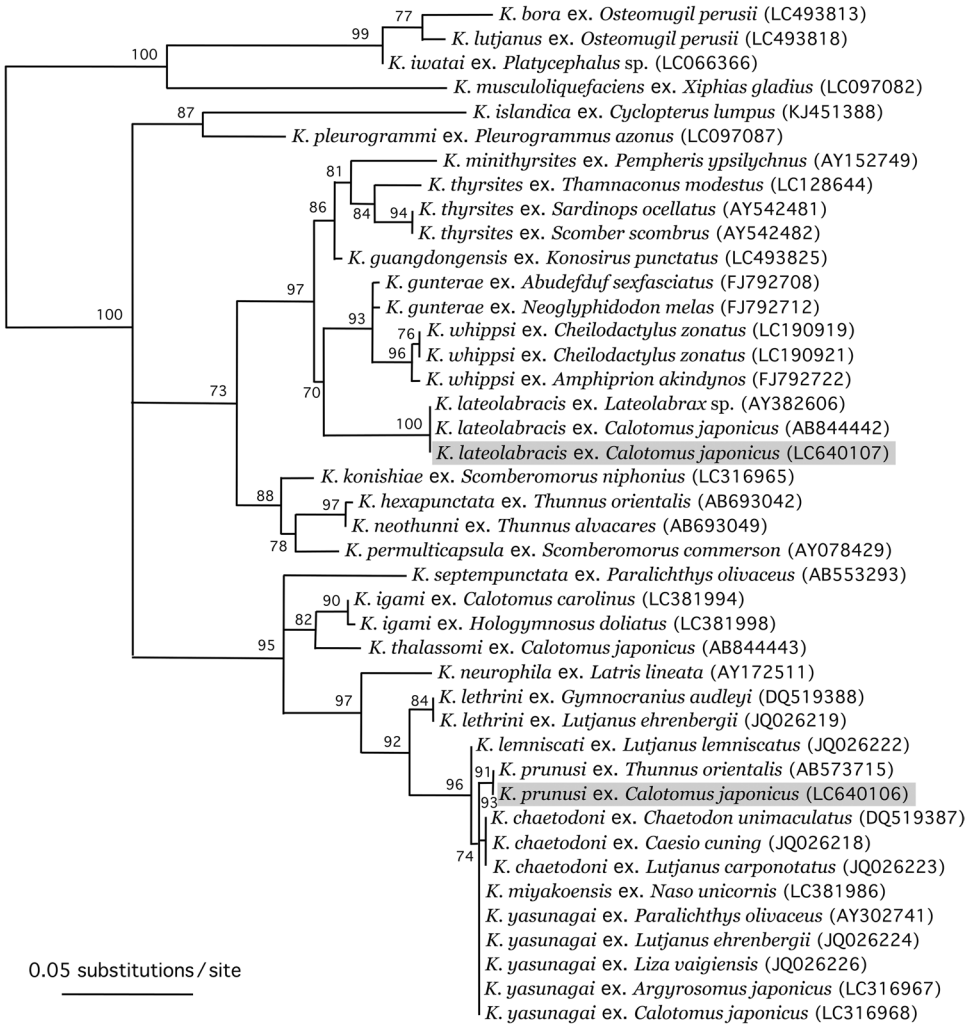

Fig. 4 Maximum likelihood phylogenetic tree of *Kudoa* spp. based on 18S rDNA sequences (1415 characters). Each species name is followed by its host fish name and DDBJ/EMBL/GenBank accession number in parentheses. New sequences from this study are marked with gray backgrounds

Materials deposited: Specimen no. 21774, Meguro Parasitological Museum, Tokyo, Japan.

Deposited rDNA sequences: DDBJ/EMBL/GenBank accession nos. LC640107 (18S rDNA), LC640108 (28S rDNA), and LC640105 (mtDNA).

Prevalence: 100% (8/8).

Phylogenetic analyses

Phylogenetic trees based on the 18S and 28S rDNA of *Kudoa* spp. with cruciform myxospores (including *K. lateolabracis*) and *Kudoa* spp. with brain tropism (including *K. prunusi*) were constructed using the cyst-forming kudoid species (*K. iwatai*, *K. lutjanus*, and *K. bora*) as an outgroup. The two aforementioned kudoid groups formed separate robust clades (Figs. 4 and 5). Phylogenetically, *K. prunusi*, *K. yasunagai*, *K. lemniscati*, *K. chaetodoni*, and *K. miyakoensis* were closely related to each other, and *K. neurophila* (Grossel, Dyková, Handlinger et Munday, 2003) and *K. lethrini* Burger, Cribb et Adlard, 2007 were positioned relatively distantly at the root of the five *Kudoa* spp. Similarly, *Kudoa* spp. with cruciform myxospores, including *K. lateolabracis*, formed a robust clade in the 18S or 28S rDNA phylogenetic trees (Figs. 4 and 5). The topological position of *K. lateolabracis* in the tree differed using different rDNA regions.

Phylogenetic trees based on the mitochondrial DNA genes *cox-1* and *rns-rnl* were constructed using the newly obtained sequences of *K. prunusi* and *K. lateolabracis* (Fig. 6). The available kudoid species were limited to eight *Kudoa* spp. for *cox-1* and seven for *rns-rnl*. The phylogenetic relationships between different species were approximately similar to phylogenetic relationships based on the rDNA regions.

Discussion

This study detected *K. prunusi* plasmodia in the brain and *K. lateolabracis* plasmodia in the trunk muscle of all eight Japanese parrotfish samples examined, which originated from

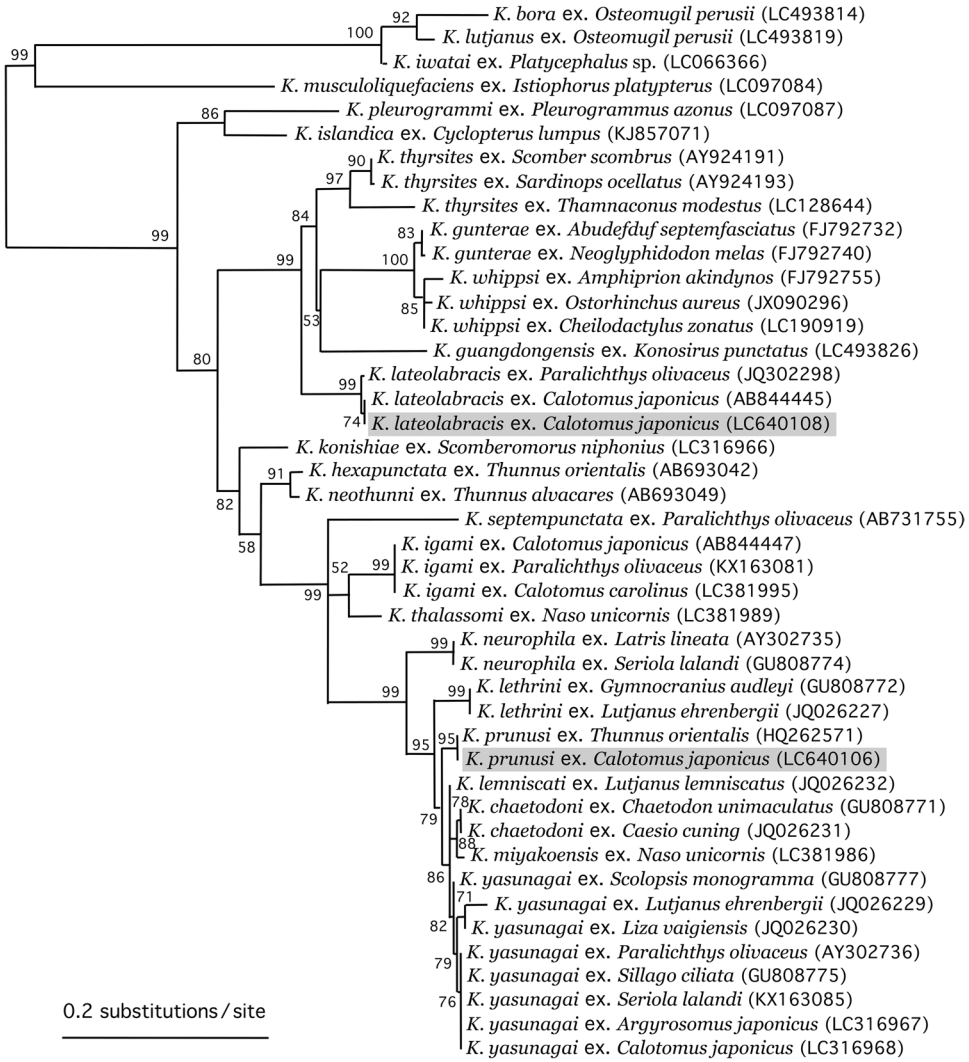

Fig. 5 Maximum likelihood phylogenetic tree of *Kudoa* spp. based on 28S rDNA sequences (591 characters). Labeling of each isolate is similar to Fig. 4 legend. New sequences from this study are marked with gray backgrounds

the northwestern Philippine Sea, off southwestern Japan (Kochi). *Kudoa prunusi* was characterized by myxospores with predominately six SVs/PCs, in contrast to its original description from the brain of cultured Pacific bluefin tuna, in which the species was characterized as having predominately five SVs/PCs (Meng et al. 2011). Molecular-genetic characterization of the 18S and 28S rDNA and mitochondrial DNA genes (*cox-1*) clearly differentiated this *K. prunusi* isolate from *K. yasunagai*, characterized by a myxospore with six or seven, rarely five, SVs/PCs. Accordingly, the Japanese parrotfish has set a new host record for *K. prunusi* and is speculated as an important reservoir host of the species in its natural waters. Shirakashi et al. (2014) recorded *K. yasunagai* prevalence of 94.1% (16/17) in the brain of Japanese parrotfish fished in the Philippine Sea, off Wakayama. Furthermore, our previous study (Sakai et al. 2019) detected *K. yasunagai* in the brain of one of three Japanese parrotfish collected from the same area as this study.

As discussed by Meng et al. (2011), reliable species differentiation of *K. prunusi* from related species with brain tropism, such as *K. yasunagai*, *K. miyakoensis*, and *K. chaetodoni*, is not feasible due to the high morphological variations of myxospores, e.g., the number of SVs/PCs, overlapping measurements, and low molecular-genetic variations (few nucleotide substitutions) in the 18S and 28S rDNA (Shin et al. 2016; Sakai et al. 2019; Inoue et al. 2021). This study (Fig. 6) suggests the possibility of assessing *cox-1* mtDNA sequencing as an alternative for specific identification. However, more isolates need to be sequenced to clarify the intra- and inter-specific variations in *Kudoa* spp. with brain tropism.

Shirakashi et al. (2014) reported *K. lateolabracis* plasmodia, which formed pseudocyst in the myofibers, in the trunk muscle of the Japanese parrotfish at a prevalence of 41.5% (17/41). Their report was the second host record for the species after its original description in

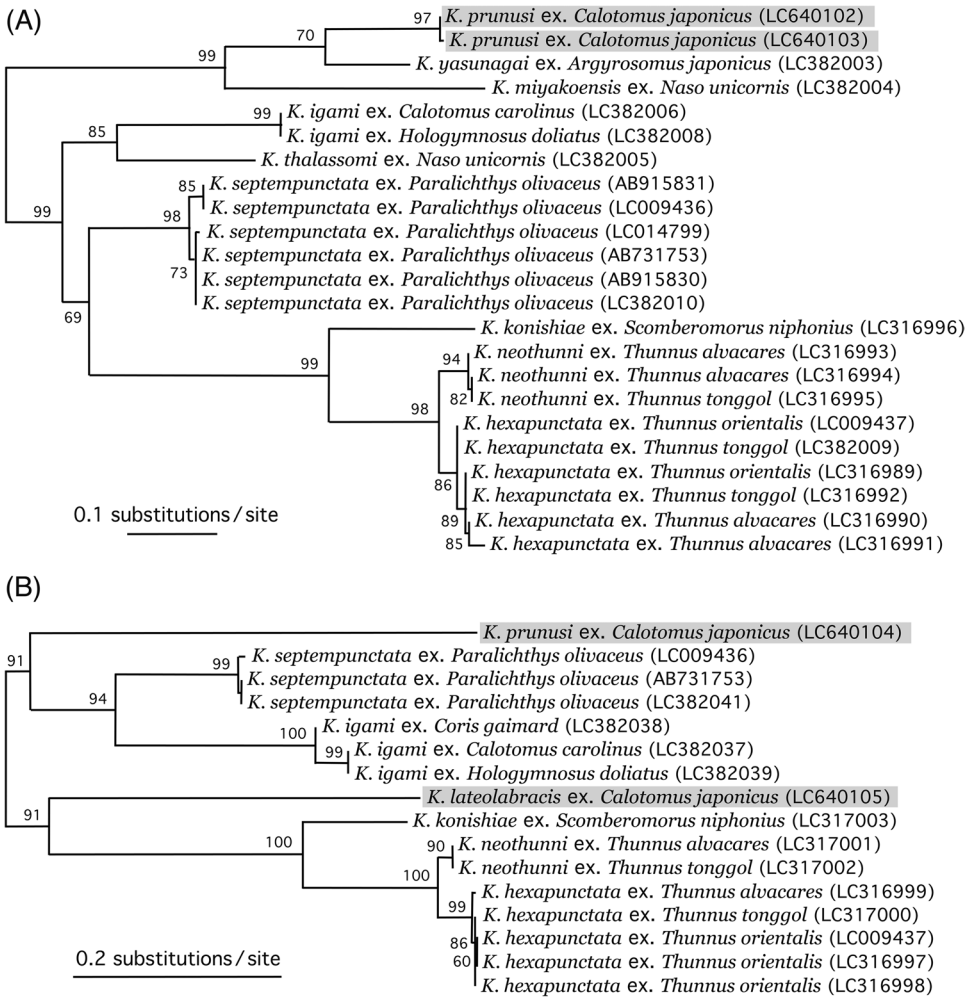

Fig. 6 Unrooted maximum likelihood phylogenetic trees based on partial mitochondrial gene sequences (**A**, *cox-1* and **B**, *rns-rnl*) of representative *Kudoa* spp. with five or more SVs/PCs per myxospore, and *K. lateolabracis* with cruciform myxospores comprised four SVs and PCs. Labeling of each isolate is similar to Fig. 4. New sequences from this study are marked with gray backgrounds

the liquefied muscles of Chinese sea bass farmed in the Inland Sea of Japan, off Ehime (Yokoyama et al. 2004). As mentioned above, the common occurrence of *K. lateolabracis* in Japanese parrotfish in its natural waters, in the Philippine Sea, off southwestern Japan, indicates the endemicity of the species in the waters around Japan. In this study, a partial *rns-rnl* mtDNA sequence of *K. lateolabracis* characterized by cruciform myxospores with four SVs/PCs was obtained for the first time (Takeuchi et al. 2016; Sakai et al. 2018, 2019; Li et al. 2020a). It might be possible to use mtDNA genes to identify the geographical origin of an isolate, as postulated for *K. septempunctata* (Takeuchi et al. 2016; Yokoyama et al. 2017).

Several *Kudoa* spp. with cruciform myxospores have been differentiated from the well-known *K. thyrsites* (Gilchrist, 1924) in the last two decades using rDNA molecular-genetic characterization: e.g., *K. mirabilis* Naidenova et Gaevskaya, 1991; *K. minithyrsites* Whipps, Adlard, Bryant, Lester, Findlay et Kent, 2003; *K. lateolabracis*; *K. whippsi* Burger et Adlard, 2010; *K. gunterae* Burger et Adlard, 2010; *K. cheilodipteri* Heiniger, Cribb et Adlard, 2013; *K. parathyrsites* Kasai, Li, Mafie et Sato, 2016; *K. akihitoi* Kasai, Setsuda et Sato, 2017; and *K. aburakarei* Li, Inoue, Tanaka, Zhang et Sato, 2020 (Whipps et al. 2003; Yokoyama et al. 2004; Burger and Adlard 2010a; Heiniger et al. 2013; Kasai et al. 2016b, 2017a; Li et al. 2020b; Giulietti et al. 2020). For any kudoid species, reliable species identification and disclosure of substantial biodiversity in multivalvulidan myxosporeans could be achieved by integrated taxonomic approaches with morphological observation, intense molecular-genetic characterization, and ecological investigation (Atkinson et al. 2015). This study contributes to the understanding of the biogeography and epidemiological status of multivalvulidans, which is a particularly important issue for commercial edible fish farming and wild fishes living in aquaculture areas (Egusa and Nakajima 1980; Sugiyama et al. 1999; Burger et al. 2008).

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Declarations

Conflict of interest The authors declare no competing interests.

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