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Kudoa hexapunctata n. sp. (Myxozoa: Multivalvulida) from the somatic muscle of Pacific bluefin tuna *Thunnus orientalis* and re-description of *K. neothunni* in yellowfin tuna *T. albacares*

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ABSTRACT

Since *Kudoa septempunctata* in olive flounder (*Paralichthys olivaceus*) was indicated to cause food poisoning in humans, other *Kudoa* species are suspected to have pathogenic potential. Recently, a myxosporean possibly associated with food poisoning in humans consuming raw Pacific bluefin tuna, *Thunnus orientalis*, was identified as *Kudoa neothunni*. This is a known causative myxosporean of post-harvest myoliquefaction in yellowfin tuna *Thunnus albacares*. Regardless of the significant differences in the 28S rDNA sequence and the pathological character (with/without myoliquefaction) between the two *T. orientalis* and *T. albacares* isolates, they were considered intraspecific variants of *K. neothunni*. However, the light and low-vacuum electron microscopic observations in the present study revealed that there were two morphotypes; pointed- and round-type spores, which were significantly differentiated by the ratio of suture width to spore width. Furthermore, the two morphotypes were genetically distinguishable by the 28S rDNA sequence analysis. This morphological and molecular evidence validates that the two *Kudoa* types are separate species, and thus the pointed- and round-types are referred to as *K. neothunni* and *Kudoa hexapunctata* n. sp., respectively. *K. neothunni* was detected solely from *T. albacares*, whereas *K. hexapunctata* n. sp. was found not only from *T. orientalis* but also from *T. albacares*.

1. Introduction

Multivalvulid myxosporeans comprise more than 80 species predominantly found in marine fishes [1]. Among them, kudoid myxosporeans contain economically important species. Several muscle-infecting *Kudoa* species either produce macroscopic cysts in the musculature, e.g., *Kudoa amamiensis* in yellowtail *Seriola quinqueradiata*, or are associated with post-mortem myoliquefaction, e.g., *Kudoa neothunni* in the yellowfin tuna (YFT) *Thunnus albacares*. These signs lower the commercial value of the infected fish [2].

In recent years, *Kudoa septempunctata* from the olive flounder, *Paralichthys olivaceus*, has been recognized as a causative food poisoning agent in humans [3]. Subsequently, the potential effects of other *Kudoa* species on human health were suspected to be similar [4]. More recently, a myxosporean isolated from raw Pacific bluefin tuna (PBT) *Thunnus orientalis*, which have been associated with cases of food poisoning, was identified as *K. neothunni* based on its 18S rDNA sequence and spore morphology [5,6]. Regardless of the notable differences in 28S rDNA sequence (22 base pair differences across 2245 bp length) and pathological character (with/without myoliquefaction) between the two isolates from PBT and YFT, Lin et al. [6] considered them to be intraspecific variants of *K. neothunni*. Abe and Maehara [5] also noted 10 substitutions within a 753 bp 28S rDNA overlap between PBT and YFT isolates and proposed that they represented two *K. neothunni* genotypes.

However, because only a few *Kudoa* isolates were analyzed in both studies, the evidence for this species identification seems insufficient. In the present study, therefore, we aimed to solve the question on the classification of *K. neothunni* based on detailed morphological and molecular studies of a larger data set.

2. Materials and methods

2.1. Fish samples

Three specimens of juvenile wild PBT were collected from Nagasaki, Kochi, and Aomori Prefectures, Japan, in December 2013, February 2013, and July 2012, respectively. Four specimens of juvenile cultured PBT (0–1 year old) were obtained from anonymous fish farmers in western Japan between February and July 2012. Seven specimens of sliced raw fish (sashimi) from adult wild YFT were purchased at local markets between June and September 2012. One sample of YFT muscle block (36 kg in body weight) showing typical post-harvest myoliquefaction was provided by Okinawa Prefectural Institute of

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Table 1
Oligonucleotide primers used for PCR assays in the present study.

Primer name		Primer sequence (5′ to 3′)	Position	Accession no.
PBT-F	(Forward)	GGCTAGTGAAAAGCCAACTTATGG	1587–1610	AB693042
PBT-R	(Reverse)	TTCGCGGATTCCAACCTATT	1614–1630	AB693042
PBT-P	(Probe)	CACTTGTGTGGCTAAAT	1633–1652	AB693042
YFT-F	(Forward)	GCCAACTTATGGTCGCACTGT	1599–1619	AB693049
YFT-R	(Reverse)	TTCCCTTTCGCGGATTCC	1621–1637	AB693049
YFT-P	(Probe)	TGTGGCTGGATATAGGT	1639–1656	AB693049
Ku18F1	(Forward)	AGCCATGCAAGTCTAAGTTCA	26–46	AB553293
Ku18R1	(Reverse)	TTTCACTGAGGGCCCAACCTGGATT	1012–1036	AB553293
Ku18F2	(Forward)	ATTAAAAGGGACATTTGAGGG	814–839	AB553293
Ku18R2	(Reverse)	TACGGAAACCTTGTTACGACT	1680–1700	AB553293
Myxo28S1F	(Forward)	AGTAACTGCGAGTGAAGCG	80–98	FJ417058
Ku28R1	(Reverse)	TCACGCATAGTTCACCATCT	946–965	FJ417058
Ku28F2	(Forward)	AGTGAAAGCGAGAGATGAGA	845–864	FJ417058
Myxo28S3R	(Reverse)	GAGCACTGGGCAGAAATC	2506–2523	FJ417058
Ku28F3	(Forward)	ATTAAAACAAAGCATTGCGAT	2459–2479	FJ417058
Ku28R3	(Reverse)	GCATTGCATCACTGGCCTAT	3504–3523	FJ417058

Health and Environment, in November 2011. The latter fish was captured off the Philippines in October 2011 and landed at Naha, Okinawa Prefecture in November 2011. The precise capture locality of YFT bought at markets is not known, and thus the location described in the price tag was used as the origin of each sample. All samples were frozen at −20 °C or −80 °C until spore collection.

After thawing of the samples, several pieces of muscle tissue were taken from different parts of the samples. As for the YFT with myoliquefaction, four pieces of muscle tissue were collected, two from the liquefied and two from the non-liquefied part of the same fish. The tissue was then flattened between two glass plates; pseudocysts were then examined under a dissecting microscope with transmitted light. To avoid cross-contamination of multiple *Kudoa* species, pseudocysts were individually excised from each specimen with fine forceps and squashed on a glass slide with a few drops of phosphate buffered saline (PBS). After the spores had been photographed under a

Table 2
Comparison of spore dimensions (μm, mean ± SD, range in parentheses) of *Kudoa hexapunctata* n. sp. and *K. neothunni* from Pacific bluefin tuna (PBT) and yellowfin tuna (YFT).

Host	Origin	Spore					Polar capsule		Morphotype[a]	Species
		Width	Length	Thickness	Suture width	Suture width/width (%)	Length	Width		
PBT (this study)	Farmed A	8.7 ± 0.4 (8.0–9.2)	5.9 ± 0.4 (5.0–6.4)	7.5 ± 0.3 (7.0–8.2)	6.6 ± 0.4 (5.6–7.4)	76.4 ± 4.2 (67.0–82.6)	2.7 ± 0.4 (2.2–3.1)	1.5 ± 0.2 (1.3–1.8)	Round	*K. hexapunctata* n. sp.
	Farmed B	10.6 ± 0.4 (10.1–11.2)	7.2 ± 0.4 (6.4–7.9)	9.3 ± 0.3 (8.6–10.0)	8.4 ± 0.4 (7.7–9.0)	79.6 ± 4.2 (71.0–87.3)	3.4 ± 0.4 (2.7–4.5)	2.0 ± 0.2 (1.7–2.2)	Round	*K. hexapunctata* n. sp.
	Farmed C	10.1 ± 0.4 (9.2–10.9)	6.8 ± 0.4 (6.2–7.4)	8.6 ± 0.3 (7.9–9.0)	7.4 ± 0.3 (6.9–7.8)	73.7 ± 2.9 (69.0–80.0)	3.4 ± 0.4 (2.5–4.0)	1.8 ± 0.2 (1.4–2.0)	Round	*K. hexapunctata* n. sp.
	Farmed D	8.7 ± 0.4 (8.0–9.2)	5.9 ± 0.3 (5.4–6.3)	7.4 ± 0.2 (7.0–7.8)	6.5 ± 0.2 (6.0–6.8)	75.0 ± 3.3 (67.8–83.3)	2.9 ± 0.4 (2.2–3.4)	1.6 ± 0.1 (1.3–1.9)	Round	*K. hexapunctata* n. sp.
	Nagasaki	10.4 ± 0.5 (9.1–11.4)	7.5 ± 0.5 (6.6–8.2)	9.1 ± 0.5 (8.4–9.9)	7.8 ± 0.5 (6.5–8.5)	75.3 ± 3.4 (67.3–80.7)	3.2 ± 0.5 (2.0–3.9)	1.7 ± 0.2 (1.4–2.0)	Round	*K. hexapunctata* n. sp.
	Kochi	10.3 ± 0.7 (8.9–11.4)	7.3 ± 0.3 (6.7–7.9)	9.0 ± 0.6 (7.9–10.2)	7.7 ± 0.5 (6.9–8.8)	75.3 ± 5.4 (66.5–82.9)	3.3 ± 0.4 (2.7–4.0)	1.8 ± 0.2 (1.5–2.3)	Round	*K. hexapunctata* n. sp.
	Aomori	10.7 ± 0.5 (9.4–11.8)	7.7 ± 0.5 (6.9–8.4)	9.2 ± 0.5 (8.0–10.1)	8.2 ± 0.4 (7.3–8.9)	76.7 ± 4.5 (67.3–85.7)	3.4 ± 0.4 (2.8–4.0)	1.8 ± 0.2 (1.3–2.1)	Round	*K. hexapunctata* n. sp.
YFT (this study)	Pacific Ocean	10.2 ± 0.4 (9.3–11.0)	7.1 ± 0.5 (6.4–8.1)	9.0 ± 0.3 (8.4–9.4)	8.0 ± 0.3 (7.3–8.4)	78.0 ± 3.3 (72.1–84.8)	3.2 ± 0.6 (2.3–4.1)	1.9 ± 0.2 (1.6–2.2)	Round	*K. hexapunctata* n. sp.
	Indian Ocean A	9.7 ± 0.3 (9.3–10.4)	7.0 ± 0.4 (6.0–7.5)	8.5 ± 0.3 (7.9–9.0)	7.3 ± 0.3 (6.7–7.7)	75.5 ± 2.8 (67.9–79.9)	3.6 ± 0.5 (2.3–4.1)	1.9 ± 0.2 (1.5–2.1)	Round	*K. hexapunctata* n. sp.
	Indian Ocean B	9.8 ± 0.4 (9.1–10.7)	7.2 ± 0.3 (6.7–7.4)	8.7 ± 0.6 (7.8–10.3)	7.8 ± 0.6 (7.0–9.3)	79.2 ± 4.0 (72.6–87.6)	3.6 ± 0.4 (2.8–4.1)	1.9 ± 0.2 (1.5–2.3)	Round	*K. hexapunctata* n. sp.
	Vanuatu	11.7 ± 0.7 (10.7–12.9)	7.0 ± 0.4 (6.3–7.5)	9.7 ± 0.5 (8.6–10.5)	7.4 ± 0.3 (6.8–7.9)	63.4 ± 4.0 (57.1–72.7)	3.5 ± 0.7 (2.4–4.5)	2.0 ± 0.1 (1.8–2.2)	Pointed	*K. neothunni*
	South Korea	11.0 ± 0.5 (9.7–11.5)	6.6 ± 0.4 (5.9–7.4)	9.2 ± 0.5 (8.4–10.0)	7.3 ± 0.4 (6.4–7.8)	63.4 ± 4.0 (57.1–72.7)	3.5 ± 0.4 (2.4–4.5)	1.9 ± 0.1 (1.7–2.2)	Pointed	*K. neothunni*
	Philippines	11.8 ± 0.7 (11.1–12.7)	NA	10.4 ± 0.4 (9.7–10.8)	7.9 ± 0.6 (6.9–8.5)	66.9 ± 4.0 (61.4–70.0)	NA	1.7 ± 0.1 (1.6–1.9)	Pointed	*K. neothunni*
	Fiji	12.0 ± 0.5 (11.0–12.7)	6.5 ± 0.4 (5.9–7.4)	10.1 ± 0.5 (9.3–11.0)	7.4 ± 0.4 (6.9–8.5)	62.0 ± 3.5 (57.2–72.3)	3.5 ± 0.4 (2.8–4.1)	2.1 ± 0.1 (1.8–2.3)	Pointed	*K. neothunni*
	Unknown	11.5 ± 0.6 (10.6–12.1)	6.8 ± 0.3 (6.3–7.7)	9.7 ± 0.6 (9.1–10.5)	7.6 ± 0.2 (7.4–7.9)	66.0 ± 2.2 (64.3–69.6)	3.9 ± 0.4 (2.9–4.3)	2.0 ± 0.2 (1.7–2.1)	Pointed	*K. neothunni*
YFT [14]	Banda Sea	11.0 (9.1–13.0)	6.2 (5.3–7.3)	NA	7.1 (5.9–8.7)	64.5	2.5 (2.0–3.1)	1.6 (1.3–2.1)	Pointed	*K. neothunni*
YFT or PBT [5]	Unknown	10.2 or 10.3 (9.0–12.0)	NA	6.8 (6.0–7.5)	NA	NA	NA	NA	?	?
PBT [6]	Unknown	10.6 (9.5–11.4)	7.6 (7.3–7.7)	9.7 (8.9–10.9)	8.1 (7.3–8.6)	76.4	3.8 (3.6–4.1)	2.0 (1.8–2.3)	Round	*K. hexapunctata* n. sp.

[a] Round- and pointed-types were classified above or below 70% in suture width/spore width, respectively.