Kudoa amamiensis n. sp. (Myxosporea: Multivalvulida) Found in Cultured Yellowtails and Wild Damselfishes from Amami-Ohshima and Okinawa, Japan

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A description is given of a new species of myxosporian parasite located in the musculature of the damselfishes Abudefduf sexfasciatus, A. vagiensis, Chromis isharai, C. notatus, Chrysiptera assimilis from Amami-Ohshima and Okinawa and the yellowtail Seriola quinqueradiata from the Marine Ranch of the International Ocean Exhibition at Motobu, Okinawa. The name Kudoa amamiensis n. sp. is suggested for this parasite. The cysts are spherical to ellipsoidal, measure up to 5 mm in diameter, have a thick wall of connective tissue and contain quadrangular spores which measure 4.5-5 μm in length, 5-6 μm in breadth and thickness. Observed in the scanning electron microscope the spores are characterized by the presence of short finger-shaped projections at the top and papillae on the inflated corners.

From February through June, 1975 about 25,000 yellowtails, Seriola quinqueradiata, of one- or two-year-old were transferred from yellowtail farms in Shikoku to the Marine Ranch of the Okinawa International Ocean Exhibition at Motobu, Okinawa Island. The fish were stocked in a wide net enclosure to give public exhibitions and fed mainly frozen mackerels which were shipped from the main land of Japan. Although the fish received foods at relatively low feeding rates, they grew well without showing any mortality thanks to high water temperatures and good environmental conditions. In the net enclosure various kinds of wild fishes including damselfishes were found in fairly large numbers.

In September, 1975 several yellowtails of about 50 cm in fork length were examined for parasites and myxosporian cysts were observed to exist in large numbers in the flesh of all the fish examined. Subsequent examinations from October, 1975 through August, 1976 revealed that the incidence of the myxosporian infection was always 100%.

Once the same myxosporian infection occurred in 1970 in yellowtails cultured in net pens of a commercial farm in Amami-Ohshima Island which lies 250 km north of Okinawa Island. In this farm too, young yellowtails were transferred from farms in Shikoku and fed frozen trash fishes shipped from the main land. The incidence of the infection was about 10 to 30%. Infected fish had no commercial value and the farm was closed. No scientific report was published about this accident.

Okinawa and Amami-Ohshima Islands lie close to the tropics and have many coral reefs around them and the fish fauna on these coasts is different from Kyushu, Shikoku and the south of the main land of Japan. The yellowtail is not found on the coasts of Amami-Ohshima and Okinawa Islands.

From the above-mentioned facts it was assumed that there might be some native host fishes of the myxosporian on these coasts and the yellowtail might be an accidental host for it. About 2500 wild fish of 87 species of 48 genera collected from the waters from October, 1975 through March, 1978 were examined for myxosporian infections. As a result the same myxosporian as found in the yellowtail was detected in damselfishes, Abudefduf sexfasciatus, A. vagiensis, Chromis isharai, C. notatus and Chrysiptera assimilis. The myxosporian was identified as a new species of the genus Kudoa and named Kudoa amamiensis n. sp. The present paper deals with the description and identification of this species.

Materials and Methods

The fish studied were yellowtails obtained from

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the Okinawa Marine Ranch and the aforementioned damselfishes caught in Amami-Ohshima and Okinawa coasts. The trunk muscles of each fish were exposed in order to observe myxosporidian cysts.

Spore suspensions were obtained by crushing cysts in a physiological saline. Smears were prepared from the suspensions. Some smears were stained with Giemsa solution or treated with 5% KOH solution to make polar filaments extrude. The spore size and the polar filament length were measured under the light microscope. For scanning electron microscopic observation spores were fixed in 2% glutaraldehyde and 2% sodium tetraoxide, dehydrated with ethanol series, dried with a critical point dryer, coated with golden ion, and observed with the Hitachi MSM-6 at an accelerating voltage of 25 kV. For histological observation cysts and the surrounding muscular tissues were fixed in 10% formalin. Paraffin sections were prepared from them and stained with hematoxylin and eosin.

The material is deposited in the National Science Museum, Tokyo. Cat. No. NSMT-Pr 102.

Results

Cyst

The cysts were opaque, whitish to creamy, spherical to ellipsoidal and had a thick, elastic host-produced wall of fibrous connective tissue, which measured up to 50 μm in thickness. All the cysts examined were packed with mature spores. They localized in the skeletal musculature. The severity of infection differed characteristically between the yellowtail and the damselfishes.

In the yellowtail cysts were present in innumerable numbers in almost all parts of the skeletal muscles. In heavily infected fish cysts were found in the heart muscle, serosa, skin and fins, also (Figs. 1–4). The cysts varied in size with the size of host or the season. For example, the cysts obtained from fish 46.0–53.5 cm in folk length in October, 1975 were about 1 mm and those from fish 57.4–70.8 cm in January, 1976 were 3.2–4.6 × 2.2–2.6mm. The largest cyst found in fish 55.5–72.0 cm in June, 1976 was 5.0 mm in length.

Whereas in the damselfishes usually only one and rarely two to three small ellipsoidal cysts measuring up to 2 mm were found in the skeletal musculature near the median plane of the trunk or pleural ribs (Figs. 5 and 6).

Spore

Spores obtained from the yellowtail and those from the damselfishes were identical in the following observations. Observed with the light microscope the spore of the present species was quadrangular in polar view with round corners between which the margin was concaved. Seen in side view it was teardrop shaped with an attenuated anterior end. The polar capsules were pyriform with a slightly pointed inner end (Fig. 7). Extruded polar filaments were short, being equal to or slightly longer than the spore length (Fig. 8).

Observed with the scanning electron microscope, the spores were characterized by the presence of a short finger-shaped projection at the anterior end of each shell valve and several papillae about the middle of the valve surface, which was not rugose. The sutral lines were visible as fine grooves (Figs. 9 and 10).

Dimensions of spores preserved in a physiological saline on 20 measurements were as follows: length 4.5–5.0 μm; breadth and thickness 5–6 μm; polar capsule length 1.5–2 μm; polar capsule breadth 1–1.2 μm; extruded polar filament length 4.8–5 μm. Observed with the scanning electron microscope spores were 4.0–4.4 (average 4.1) μm in length and 4.6–5.5 (4.9) μm in breadth and thickness.

Discussion

In a previous paper2) the present authors described Kudoa pericardialis which was often found in the pericardial cavity of the yellowtail cultured in farms located in Shikoku, Kyushu, and the mainland of Japan. The present species is distinctly different from K. pericardialis in spore dimensions (Table 1) and infection site. It was not found in the pericardial cavity of any of the fish examined. In addition, the surface architecture of the spore of the present species, as seen in the scanning electron microscope, is clearly different from that of K. pericardialis. The spore of the latter has a rugose surface with 4 deep depressions, polar plates and polar lids, all of which are not observed in the present species and has neither finger-shaped projections nor papillae which are characteristics of the present species. Besides there is a difference between the two in the shape of the polar capsules as stained with Giemsa solution. The polar capsules of the present species are globular (Fig. 7), while those of K. pericardialis are slender.

Five species of the genus Kudoa which form cysts
Fig. 1. Opened trunk of *Seriola quinqueradiata* heavily infected with *Kudoa amamiensis*. Numerous spherical to ellipsoidal cysts are seen in the skeletal musculature and serosa. × 1/2.5

Fig. 2. Pectoral fin with cysts in the fin membrane of *S. quinqueradiata* heavily infected with *K. amamiensis*. × 1

Fig. 3. Section of trunk muscle containing 2 cysts from an infected *S. quinqueradiata*. × 17

Fig. 4. Thick wall (C) of fibrous connective tissue of a cyst of *K. amamiensis* in the trunk muscle of an infected *S. quinqueradiata*. M: muscle; S: spores. × 280
Fig. 5. *Chromis notatus* harbouring a cyst (arrow) of *K. amamiensis*. ×1.3

Fig. 6. Cyst (arrow) of *K. amamiensis* in the lateral muscle near the vertebrae of *Abudefduf vagi-ensis*. ×2.5

Fig. 7. Spores of *K. amamiensis* stained with Giemsa solution. Polar view. ×2,000

Fig. 8. Spores of *K. amamiensis* with polar filaments extruded, treated with 5% KOH solution. ×1,800

Fig. 9 and 10. *K. amamiensis* spores as seen in the scanning electron microscope, with somewhat shrunken appearance of the shells. 9. A spore in an upper anterior view. Notice the characteristic projections at the top of the spore and the papillae on the inflated corners. Sutural lines are visible as fine grooves. 10. A spore in side view. ×10,000.
Table 1. Dimensions of the spores of species of *Kudoa* which form cysts in the skeletal musculature of brackish water and marine fishes and those of *K. pericardialis* (μm)

<table>
<thead>
<tr>
<th>Species</th>
<th>Host and location</th>
<th>Spore</th>
<th>Polar capsules</th>
<th>Polar filaments</th>
<th>Source</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Length range (μ)</td>
<td>Breadth range (μ)</td>
<td>Thickness range (μ)</td>
<td>Length range (μ)</td>
</tr>
<tr>
<td><em>K. amamiensis</em></td>
<td><em>Abudelfesh sexfasciatus</em></td>
<td>4.5–5</td>
<td>5–6</td>
<td>5–6</td>
<td>1.5–2</td>
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<td></td>
<td><em>A. vagiensis</em></td>
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<td></td>
<td><em>Chromis isharai</em></td>
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<td></td>
<td><em>C. notatus</em></td>
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<td></td>
<td><em>Chrysiptera assimilis</em></td>
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<td></td>
<td><em>Seriola quinqueradiata</em> (Amami-Ohshima, Okinawa, Japan)</td>
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<tr>
<td><em>K. bora</em></td>
<td><em>Mugil cephalus</em> (Taiwan)</td>
<td>8.0–8.5</td>
<td>11–12</td>
<td>11–12</td>
<td>5.5</td>
</tr>
<tr>
<td><em>K. chipeidae</em></td>
<td><em>Clupea harangus</em></td>
<td>4.8–5</td>
<td>5–6</td>
<td>5–6</td>
<td>1.5–2</td>
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<td></td>
<td><em>Allosa pseudoharengus</em></td>
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<td></td>
<td><em>A. aestivalis</em> (Wood Hole, U.S.A.)</td>
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<td></td>
<td><em>Brevoortia tyrannus</em> (Beaufort, U.S.A.)</td>
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<tr>
<td><em>K. crunena</em></td>
<td><em>Scombreromus maculatus</em> (South Florida, U.S.A.)</td>
<td>6.8–8.2</td>
<td>9.3–10.4</td>
<td>8.2–9.7</td>
<td>3.2–4.6</td>
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<tr>
<td><em>K. fundulit</em></td>
<td><em>Fundulus heteroclitus</em> (Barneget Bay, U.S.A.)</td>
<td>6.7</td>
<td>6.7</td>
<td>8.4</td>
<td>1.5</td>
</tr>
<tr>
<td>A. Kudoa*¹</td>
<td><em>Tenmodon saltator</em> (Morocco)</td>
<td>5–6</td>
<td>7–8</td>
<td>7–8</td>
<td>2–2.5</td>
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<tr>
<td>A. Kudoa*²</td>
<td><em>Thunnus thynnus</em></td>
<td>4.4–4.2</td>
<td>4.5–5</td>
<td>4.5–5</td>
<td>2.4–3</td>
</tr>
<tr>
<td><em>K. pericardialis</em></td>
<td><em>Seriola quinqueradiata</em> (Japan)</td>
<td>4.4–4.2</td>
<td>4.5–5</td>
<td>4.5–5</td>
<td>2.4–3</td>
</tr>
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*¹ and *² They were tentatively identified as *K. chipeidae* by the authors.
in the skeletal musculature have been described from several brackish water and marine fishes (Table 1). Recently Rhode\(^3\) reported that the king fish, *Seriola grandis* from Great Barrier Reef of Australia were heavily infected with a *Kudoa*, which made their flesh tasteless and inedible. But he did not give any description of its cyst and spore.

The present species distinctly differs in the shape and structure of cyst from *K. clupeidae*\(^4,5\) from clupeoid fishes of the Atlantic coast of North America, though both species closely resemble each other in the shape of spore. The cysts of *K. clupeidae* are usually slender in shape, being 1-3 × 1-0.4 mm in size according to Meglitsch\(^5\). They were first discovered and named 'pseudocysts' by Linton\(^6\). Hahn\(^4\) observed that the pseudocysts composed almost entirely of mature spores and stated that when pressed with a tip of a scalpel, mashed up just like a bit of soft cheese. This suggests that the cyst of *K. clupeidae* has no thick wall. In fact neither Hahn\(^4\) nor Meglitsch\(^5\) recognized the existence of any membranous structure encasing a mass of spores. Further Sindermann's photomicrograph of a cyst of *K. clupeidae* represented in his book\(^7\) clearly shows that the cyst is a mass of spores and has no thick wall.

As far as the shape and structure of cyst are concerned the present species closely resemble *K. bora*\(^8\) from *Mugil cephalus*, *K. crumenata*\(^9\) from *Scomberomorus maculatus* and a *Kudoa*\(^10,11\) from *Temnodon saltator* and *Thunnus thynnus*. The cysts of these species are all spherical to ellipsoidal, a few millimeters in diameter and have a thick wall of connective tissue. The spores of these three species, however, and that of *K. funduli*\(^12\) from *Fundulus heteroclitus*, are larger in all dimensions than that of the present species (Table 1).

The differences mentioned above between the *Kudoa* we found in both the damselfishes and the yellowtail and the other reported species seem sufficient to give the *Kudoa* specific status. The specific name *Kudoa amamiensis* n. sp. is proposed.

It has become a decisive and inevitable procedure for the taxonomy of Myxosporea to bring out the surface patterns of spores by means of the scanning electron microscope. Thus far, no data exist on scanning electron microscope observations of all the reported species of the Genus *Kudoa*, except *K. pericardialis*. Accordingly the possibility that *K. amamiensis* may be reidentified as any of the reported species in the future is not excluded.

**References**