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ORIGINAL PAPER

Light and electron microscopic studies on *Kudoa pagrusi* sp. n. (Myxosporea: Multivalvulida) infecting the heart of sea bream *Pagrus pagrus* (L.) from the Red Sea

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Abstract A new multivalvulid species, *Kudoa pagrusi* sp. n., was described from the sea bream *Pagrus pagrus*. The cysts were oval to ellipsoidal and restricted to the cardiac muscles. The mean spore measurements were 7.0 μm in length and 6.4 μm in width as well as in thickness, while the mean polar capsule measurements were 3.7 μm in length and 1.5 μm in width. The ultrastructural features of the present species proved that the spore have four polar capsules with four shell valves that are the main criteria for genus *Kudoa*.

Introduction

Myxozoans are economically important fish parasites with over 2,180 described species (Lom and Dykova 2006). Genus *Kudoa* is a myxosporean parasite of marine fishes. It has worldwide distribution and infects wide range of host species. This parasite is responsible for causing economic losses to the fisheries sector by causing postmortem "myoliquefaction," softening of the flesh to such an extent that the fish becomes unmarketable (Moran et al. 1999; Whipps et al. 2003).

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Regarding the type of fish infected, muscle-infecting species is predominant. Other parasite species have been found in extramuscular localizations as brain, kidney, gills, gallbladder, and ovaries (Swearer and Robertson 1999; Dykova et al. 2002).

During a recent survey on the myxosporean parasites from selected Red Sea fish, *Kudoa pagrusi* sp. n. is described as new species from the heart muscle of the common sea bream *Pagrus pagrus* (L.).

Materials and methods

Fresh caught fishes were purchased from fishermen at Gulf of Suez during visits between March 2006 and March 2007. A total of 100 *P. pagrus* fish were examined. Gross examination of all organs and body fluids was carried out for myxosporean infection. The highly infected heart muscles were fixed in 10% neutral buffered formalin for histological preparations. For the electron microscopy, small parts of the highly infected heart muscles were isolated and fixed in 3% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4), washed in the same buffer and post-fixed with 2% OsO_4 in the same buffer. The tissue pieces were dehydrated in graded ethanol and embedded in araldite. Ultrasections were stained with uranyl acetate and lead citrate and examined with Philips (208) electron microscope at 80–100 kV. Fresh spores were measured and photographed using Zeiss Axiovert 100 microscope with C80 Camera. Descriptions and measurements of spores followed the guidelines of Lom and Arthur (1989). Measurements were based on 30 fresh spores, and data were presented as mean±SD (range). Drawings were made with the aid of a camera lucida. For permanent preparations, air-dried smears were stained with Giemsa after fixation in acetone-free absolute methanol.

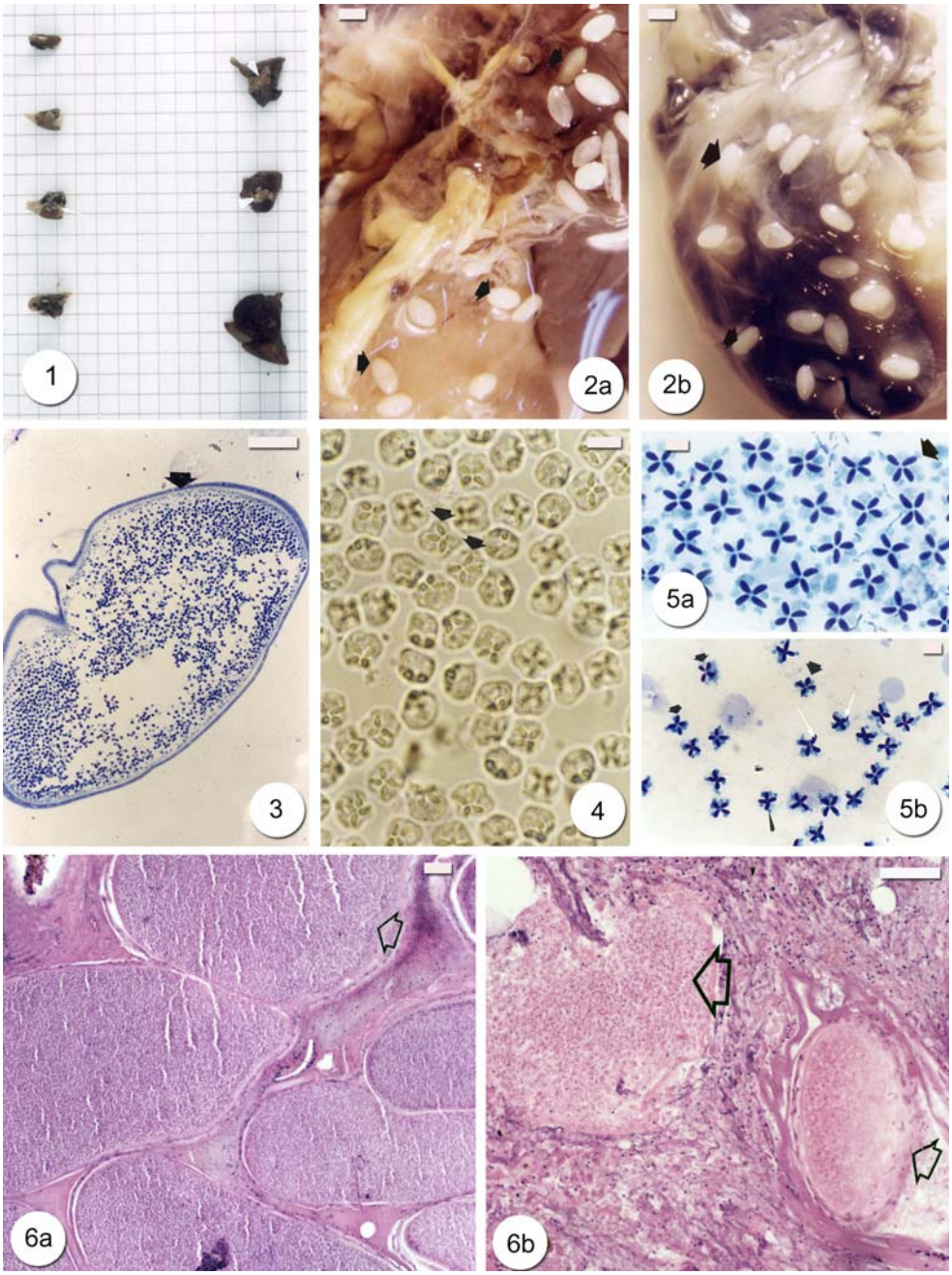

Fig. 1 Photograph of heavily infected hearts of sea bream *Pagrus pagrus* with *Kudoa pagrusi* sp. n. cysts (arrow), (square=1 mm)
Fig. 2 **a**, **b** Photomicrographs of *Kudoa pagrusi* sp. n. cysts in the heart of sea bream *Pagrus pagrus* (*arrow*; *scale bar*=1 mm)
Fig. 3 Photomicrograph of the semithin section of *Kudoa pagrusi* sp. n. cyst surrounded with connective tissue (*arrow*) and stained with toluidine blue (*scale bar*=0.2 mm)
Fig. 4 Photomicrograph of fresh spores of *Kudoa pagrusi* sp. n. (*arrow*; *scale bar*=5 μm)
Fig. 5 **a**, **b** Giemsa-stained spores with polar capsules (*arrow*) of *Kudoa pagrusi* sp. n. (*scale bar*=5 μm)
Fig. 6 **a**, **b** Photomicrographs of histological section through infected heart with cysts of *Kudoa pagrusi* sp. n (*arrow*). The cysts were surrounded with connective tissue (*scale bar*= 0.1 mm, 0.5 mm, respectively)

Results

Light microscopy

Vegetative stage

The plasmodia of the present parasite species were detected in the heart muscles. Up to 12 cysts were counted per infected fish. Cysts were whitish in color, elliptical to oval in shape, and measured 1.64±0.4 (1.27–2.2) mm in length and 0.75± 0.6 (0.70–0.86) mm in width (Figs. 1 and 2a,b).

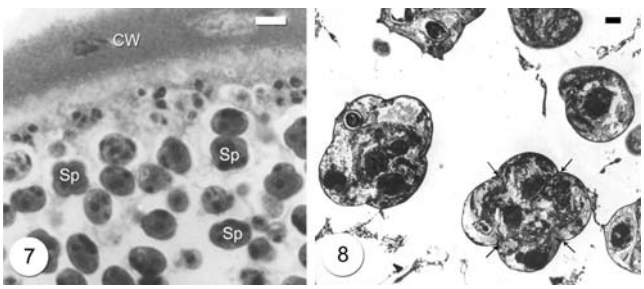

Fig. 7 Photomicrographs of the semithin section through the cyst of *Kudoa pagrusi* sp. n. surrounded with cyst wall (*CW*) and containing spores (*Sp*) and stained with toluidine blue (*scale bar*=5 μm)
Fig. 8 Electron micrograph of *Kudoa pagrusi* immature spore with distinct sutures at the periphery (*arrow*; *scale bar*=0.5 μm)

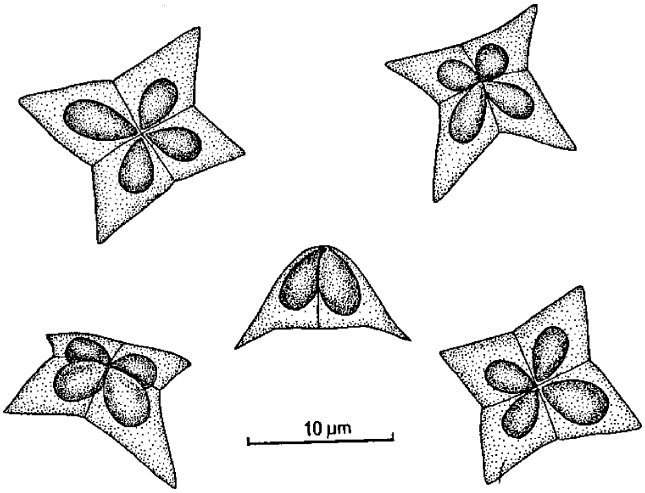

Fig. 9 Line diagram of *Kudoa pagrusi* spores infecting the heart of *Pagrus pagrus*

Spores

Spores were typically of genus *Kudoa*. Mature spores were quadrate in the apical view with nearly rounded edges. Spore membrane was delicate, while the suture was distinct (Figs. 4, 5a,b, and 9). The spore length was 7.0±0.8 (6.5–8.6) µm in the lateral view, while the width and thickness were 6.4±0.4 (5.8–7.2) µm. Four polar capsules were pyrifom in shape and were equal in size. They measured 3.7±0.3 (2.6–4.2) µm in length and 1.5±0.2 (1.0–1.8) µm in width. Polar filament turns were not clearly seen, but when straight released, they reached to 10–12 µm long nearly four times longer than the polar capsule length.

Histology

The infection site of the present species was the heart muscles, where fully developed plasmodia were detected containing only mature spores (Figs. 3, 4, 5a,b, and 6a,b). No inflammatory reactions were observed. The only host reaction was manifested by the encapsulation of the plasmodia with a thick layer of connective tissue (Figs. 6a,b and 7).

Electron microscopy

Plasmodia of the present species were surrounded with a single unit membrane with few and small pinocytotic channels. Late

Table 1 Comparative descriptive measurements (µm) of *Kudoa pagrusi* sp. n. with morphologically similar species

Species	Spore		Polar capsule		Site	Locality	References
	Length	Width	Length	Width			
Kudoa thyrsitis	12.7 (10.0–14.0)	7.1 (6.0–8.0)	5.5 (4.5–6.2)	–	Muscle	Canada	Kabata and Whitaker 1981
Kudoa clupeidae	6.4 (6.3–7.5)	5.1(4.0–5.3)	(1.5–2.6)	–	Muscle	Atlantic	Meglitsch 1948
K. branchiate	4.7 (4.4–4.9)	4.2 (3.9–4.9)	1.5	–	Gills	Texas	Joy 1972
Kudoa cerebralis	6.2 (5.6–7.0)	5.2 (4.5–6.5)	3.4 (2.7–4.8)	–	Nervous system	Virginia	Paperna and Zwerner 1974
Kudoa kabatai	(5.0–7.7)	(4.0–5.0)	(1.5–2.0)	–	Muscle	North sea	Kovaleva et al. 1979
Kudoa amamiensis	(5.0–6.0)	(4.5–5.0)	(1.5–2.0)	–	Muscle	Japan	Egusa and Nakajima 1980
Kudoa paniformis	5.9 (5.0–6.5)	5.3 (4.5–6.0)	2.6 (1.9–3.3)	–	Muscle	Canada	Kabata and Whitaker 1981
Kudoa lunatas	10.0 (9.0–11.4)	5.3 (4.5–6.2)	2.5 (2.0–3.0)	–	Muscle	Czechoslovakia	Lom et al. 1983
Kudoa cynoglossi	14.1 (13.8–14.4)	6.2 (5.8–6.5)	(2.9–3.2)	–	Muscle	West Africa	Obiekezie and Lick 1994
Kudoa miniauriculata	7.9 (7.0–8.5)	5.4 (4.9–5.9)	2.2 (1.8–2.3)	–	Muscle	California	Whitaker et al. 1996
Kudoa cascasi	8.0 (6.6–8.2)	6.6 (5.8–6.9)	3.1 (2.4–3.4)	–	Intestine	India	Sarker and Chaudhury 1996
Kudoa ciliatae	6.4 (6.3–7.3)	5.7 (4.5–5.9)	3.1 (2.4–3.4)	–	Intestine	Australia	Hallett et al. 1997
Kudoa aegyptia	10.1 (9.1–11.4)	8.1 (7.1–8.5)	4.3 (3.2–5.9)	–	Heart	Egypt	Koura 2000
Kudoa dianae.	5.0 (4.5–5.5)	6.0 (5.5–6.5)	2.0	1.5	Esophagus, Mesenteries	Czech	Dykova et al. 2002
Kudoa monodactyli	8.0 (7.3–9.2)	8.5 (7.7–9.1)	3.8 (3.2–4.6)	2.4 (1.9–2.7)	Muscle	Australia	Gunter et al. 2006
Kudoa pagrusi sp. n.	7.0 (6.5–8.6)	6.4 (5.8–7.2)	3.7 (2.6–4.2)	1.5 (1.0–1.8)	Heart	Egypt	Present study

stages of spore development, including immature and mature spores, were only recognized in the studied plasmodia (Fig. 7).

Early sporogonic stages were not observed. The earliest recognizable stage was the immature spore (Fig. 8). As the spore proceeded toward maturation, there was a structural progress in the capsulogenesis, sporoplasm maturation, and valvogenesis (Fig. 8).

The spore was limited from the outline by two pairs of peripheral valvogenic cells separated from each other by the spore sutures (Figs. 8 and 9).

Discussion

Class Myxosporea comprises two orders, the Multivalvulida and Bivalvulida (Lom and Dykova 1992). Genus *Kudoa* have been assigned to the Multivalvulida species that have four shell valves and with four polar capsules (Whipps et al. 2003).

Most members of the genus *Kudoa* form macroscopic cysts in the muscles and cause tissue degradation in a broad range of hosts (Egusa 1986; Moran et al. 1999). The infection of *Kudoa pagrusi* sp. n. described in the present study was restricted to cardiac muscles. Spore measurements of the present species were more or less different from the majority of the previously described species (Table 1). Therefore, the comparison was restricted to those species of the same site of infection, and species of other sites of infection were excluded from the comparison. Accordingly, only *Kudoa aegyptia* (Koura 2000) could be compared to the current myxosporean. *K. aegyptia* can be distinguished from the present species in having quite longer and wider spores. The detection of *Kudoa pagrusi* spores in the cardiac muscle of sea bream *P. pagrus* was of interest because no other reports appeared on the cardiac myxosporidiosis in fresh or marine fish in Egypt except *K. aegyptia*. Therefore, we justify of the new species, *K. pagrusi* sp. n.

The histological studies showed that the cysts were embedded partly or completely in the heart tissues. The growing cysts caused distortion at their site of infection and also in the adjacent layers. These observations are in agreement with those of Kabata and Whitaker (1981).

Dykova et al. (2002) stated that using light and electron microscopy is the best way to identify and classify genus *Kudoa*. To the best of our knowledge, ultrastructural characteristics were studied only for few described species. However, the ultrastructural features of the present species were similar to most studied myxosporean species (Dykova et al. 2002; Gunter et al. 2006). The presence of four sutures and four polar capsules in the spore of the present species supported the view that there were four shell valves, and this is in agreement with Meglitsch (1948), Dykova et al. (2002), and Whipps et al. (2003).

Taxonomic summary

Host: *Pagrus pagrus* (Linnaeus, 1758)

Locality: Gulf of Suez (lat. 30°N and long. 32.5°E)

Location in the host: Heart

Prevalence: 70/100 (70%)

Type material: Syntypes on slide no. Myx.-19 is deposited at the museum of Zoology department, Faculty of Science, Beni-Suef University, Beni-Suef, Egypt.

Etymology: The parasite is named after the fish host.

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