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Ceratomyxa seriolae n. sp. and *C. buri* n. sp. (Myxozoa: Myxosporea) from the gall-bladder of cultured yellowtail *Seriola quinqueradiata*

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Abstract

Ceratomyxa seriolae n. sp. and *C. buri* n. sp. (Myxozoa: Myxosporea) were found in the gall-bladder of cultured yellowtail *Seriola quinqueradiata* Temminck & Schlegel (Carangidae) in Japan. Mature spores of *C. seriolae* n. sp. were elongate and 6.5 (6.0-7.5) μm long and 33.7 (28.0-41.5) μm thick. Disporous plasmodia of *C. seriolae* n. sp., 40-100 μm in size, were amoeboid to spherical. *C. buri* n. sp. were elliptical with a flattened posterior end, 6.5 (5.5-7.5) μm long and 14.3 (11.0-16.5) μm thick. Spherical plasmodia of *C. buri* n. sp., 15-20 μm in diameter, were disporous. In periodical sampling of yellowtail bile from August, 1999 to February, 2000, the two new species of *Ceratomyxa*, as well as *Myxobolus spirosulcatus* Maeno, Sorimachi, Ogawa & Kearn 1995, first appeared in October, and the prevalences were very variable (20-100%) during the study period.

Introduction

Yellowtail *Seriola quinqueradiata* (‘buri’ in Japanese) is the most successfully cultured marine fish in Japan. Neverthless, an increasing number of disease problems has been reported in recent years (Nakajima et al., 1998; Kusuda & Kawai, 1998). About 20 species of parasites have been found in cultured yellowtail (Ogawa & Yokoyama, 1998). Of these, *Myxobolus spirosulcatus* Maeno, Sorimachi, Ogawa & Kearn, 1995 is the only described myxosporean species from the gall-bladder (Maeno et al., 1995). Recently, Maita et al. (1997) reported that a myxosporean infection in the gall-bladder probably caused the liver to turn green in the infected yellowtail, which were fed non-fish meal diet. However, they did not identify the species, and the pathogenic potential of the parasites remained unclarified. During our parasitological investigations of cultured yellowtail, we found two new myxosporeans in the gall-bladder. In this study, we describe them in detail and investigate their seasonal occurrence through the period of sampling.

Materials and methods

A total of 98 cultured 0-year old yellowtail (9-10 fish in each sampling) were periodically sampled (once or twice a month) from a net-cage at Oita Institute of Marine and Fisheries Science, Oita Prefecture from August, 1999 to February, 2000. Average body weights in each sampling ranged from 173 to 395 g. Bile was withdrawn by sterile syringes and transported to the laboratory at the University of Tokyo. Wet mounts were observed by light microscopy, and fresh spores were embedded in 1.5% melted agar and photographed with an oil immersion objective. Measurements were based on 20 fresh spores, unless otherwise stated. KOH (10%) was applied to fresh spores to induce extrusion of the polar filaments, and the lengths of polar filaments were then measured. Smear preparations were made and stained with Diff-Quik. The parasites are described according to the guidelines of Lom & Arthur (1989). Prevalences of infection were determined by the presence or absence of mature spores in the bile.

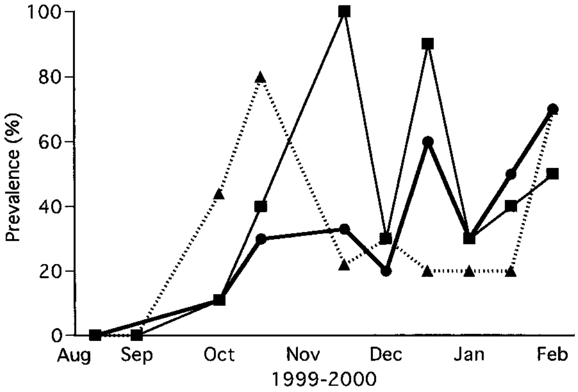


Figure 1. Prevalences of infections with *Ceratomyxa seriolae* n. sp. (circles), *C. buri* n. sp. (squares) and *Myxobolus spirosulcatus* (triangles) in the gall-bladder of cultured yellowtail *Seriola quinqueradiata* from August, 1999 to February, 2000.

Results

In the periodical survey, three species of myxosporeans were found in the bile of cultured yellowtail: *Myxobolus spirosulcatus*, *Ceratomyxa seriolae* n. sp. and *C. buri* n. sp. No myxosporeans were observed in the bile in August and September 1999. The three myxosporeans first appeared in October and were still present in February, 2000. Prevalences of infection increased gradually, but there were marked fluctuations during the study period (Figure 1). Infections of *M. spirosulcatus* peaked in October (at 80% prevalence), *C. buri* n. sp. was next with an infection peaking in November (at 100% prevalence), and *C. seriolae* n. sp. was last, peaking in February (at 70% prevalence). No clear correlation between the infection and host weight was found.

Ceratomyxa seriolae n. sp. (Figures 2A, 3-7)

Type-host: Yellowtail, *Seriola quinqueradiata* Temminck & Schlegel (Perciformes: Carangidae).
Type-locality: Oita Prefecture, Japan.
Site of infection: Gall-bladder.
Prevalence of infection: Up to 70%.

Description

Vegetative stages: Plasmodia variable in shape, mostly amoeboid to spherical, containing numerous refractile bodies and granules (Figure 3). Disporous plasmodia 40-100 μm long (Figure 4).
Spores: Mature spores elongate with slightly convex anterior end and flattened posterior end (Figures 2A, 5-7). Valves tapering to round or often truncated tips. Two spherical to ovoid polar capsules situated medially in anterior part of spores. Sutural line visible but not conspicuous. Spores 6.5 (6.0-7.5) μm long, 33.7 (28.0-41.5) μm thick. Diameter of 2 equal polar capsules 1.9 (1.5-2.0) μm. Number of turns of polar filament coil appears to be 3-4. Polar filaments (n = 17) 9.4 (5-20) μm long. Sporoplasm with 2 nuclei, occupying about half of the spore cavity. Aberrant spores with 3 polar capsules occasionally observed.
Type-material: Syntype specimens deposited in the collections of the National Science Museum, Tokyo, Japan, accession no. NSMT-Pr-171 & 172.

Ceratomyxa buri n. sp. (Figures 2B, 8-11)

Type-host: Yellowtail, *Seriola quinqueradiata* Temminck & Schlegel (Perciformes: Carangidae).
Type-locality: Oita Prefecture, Japan.
Site of infection: Gall-bladder.
Prevalence of infection: Up to 100%.
Etymology: The specific name refers to the Japanese name of the type-host.

Description

Vegetative stages: Plasmodia mostly globular, containing numerous refractile granules and inner generative cells (Figure 8). Disporous plasmodia 15-20 μm in diameter (Figure 9).
Spores: Mature spores elliptical to crescent-shaped, with flattened or slightly convex anterior end and flattened posterior one (Figures 2B, 10-11). Two spherical to ovoid polar capsules situated medially in anterior part of spores. Spores 6.5 (5.5-7.5) μm long and 14.3 (11.0-16.5) μm thick. Diameter of 2 equal polar capsules 2.4 (2.0-3.0) μm. Polar filaments 10.1 (5-25) μm long. Numbers of turns of polar filament coil appears to be 3-4. Sporoplasm with 2 nuclei, occupying most of spore cavity.

Type-material: Syntype specimens deposited in the collections of the National Science Museum, Tokyo, Japan, accession no. NSMT-Pr-171 & 172.

Discussion

In the present study, two new species of *Ceratomyxa* Thelohan, 1892 are described from the gall-bladder of

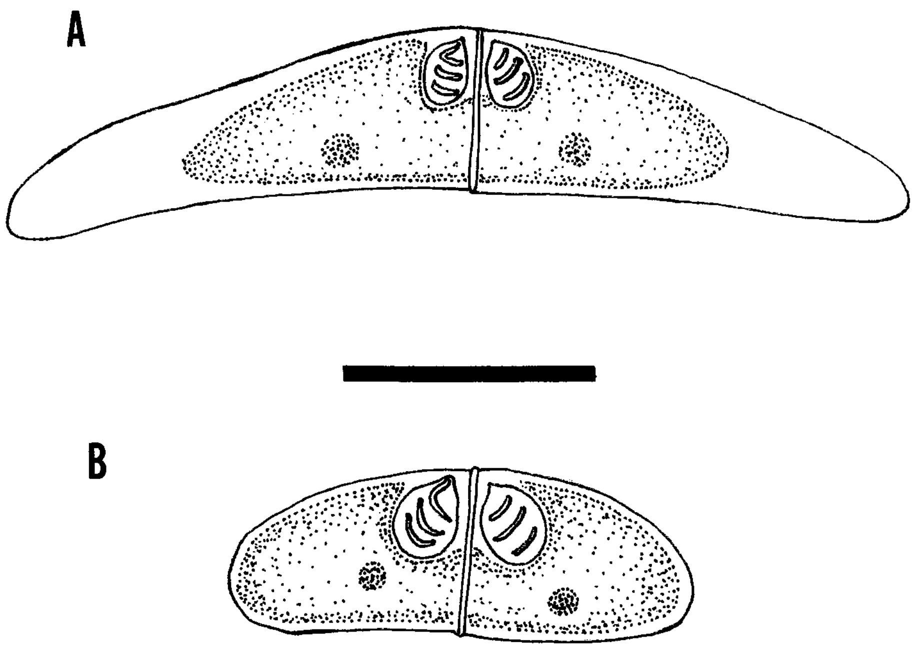


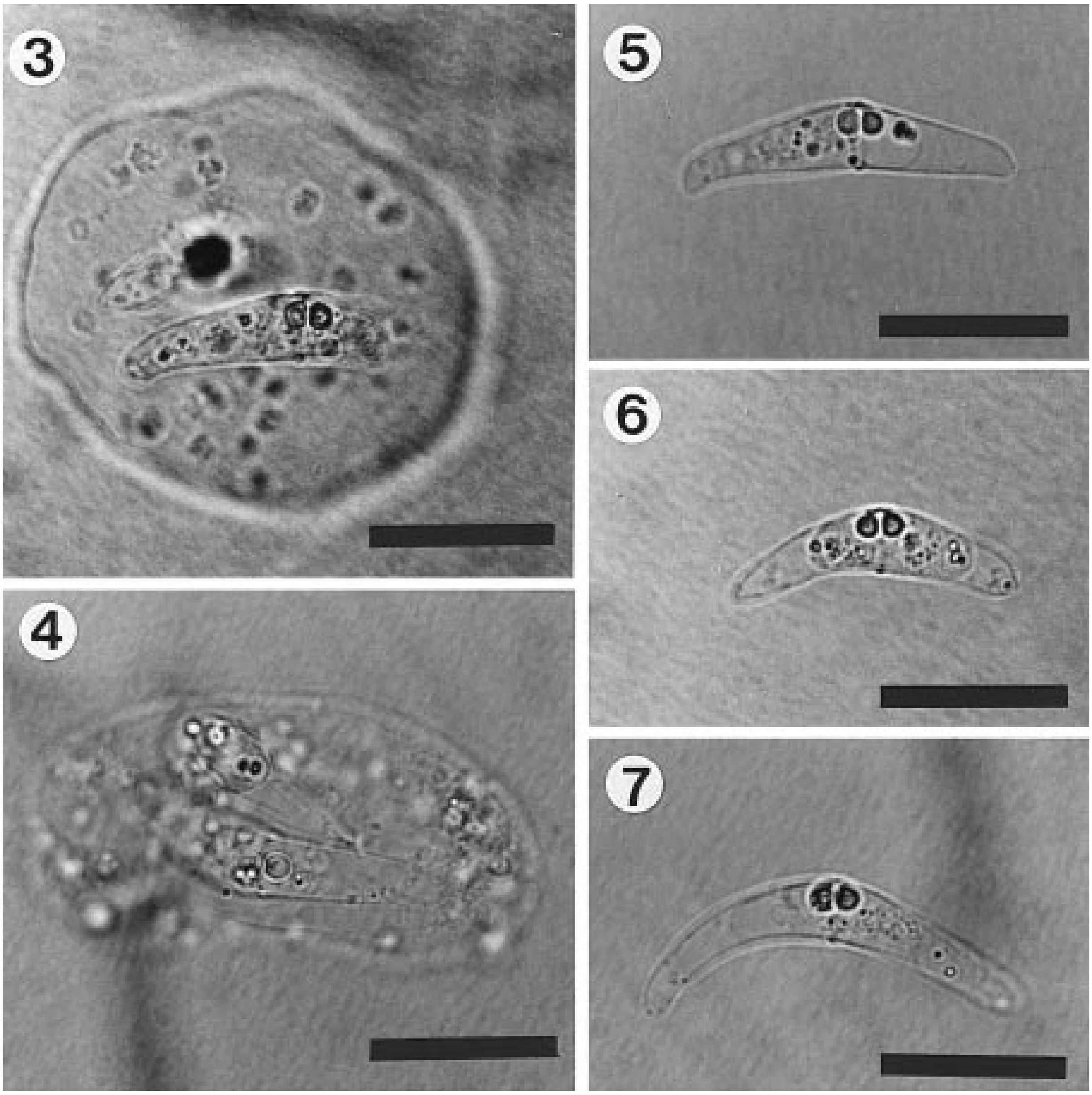
Figure 2. Line drawings of *Ceratomyxa seriolae* n. sp. (A) and *C. buri* n. sp. (B). *Scale-bar*: 10 μm.

Table 1. Comparison of *Ceratomyxa seriolae* n. sp. with related species in the genus. Mean and range (in parentheses) of the measurements are expressed in micrometres.

Species	Spore		Polar capsule	Host	Literature
	Length	Thickness	Length		
C. seriolae n. sp.	6.5 (6.0-7.5)	33.7 (28.0-41.5)	1.9 (1.5-2.0)	*Seriola quinqueradiata*	Present study
C. appendiculata	7.75 (6-8.5)	32.5(27-40)	2.75	*Lophius budegassa*	Lubat et al. (1989)
C. moenei	5.7 (4.5-7.1)	30.2(25.3-35.5)	2.7 (2.3-3.4)	*Polyprionum moene*	Meglitsh (1960)

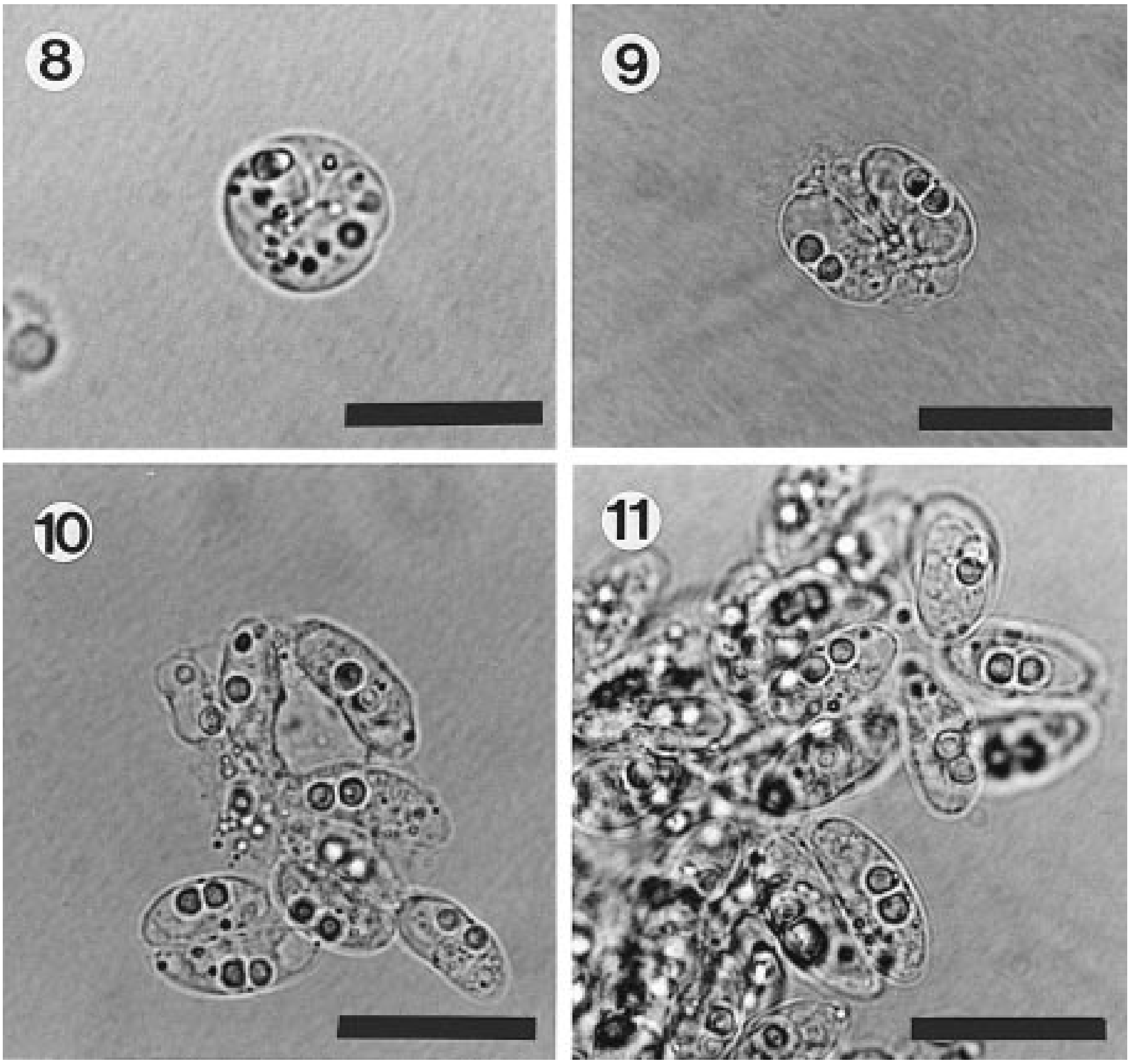
Table 2. Comparison of *Ceratomyxa buri* n. sp. with related species in the genus. Mean and range (in parentheses) of the measurements are expressed in micrometres.

Species	Spore		Polar capsule	Host	Literature
	Length	Thickness	Length		
C. buri n. sp.	6.5 (5.5-7.5)	14.3 (11.0-16.5)	2.4 (2.0-3.0)	*Seriola quinqueradiata*	Present study
C. beloneae	7.25 (6.75-7.5)	16.05 (14-18)	2.75	*Belone belone*	Lubat et al.(1989)
C. castigatoides	5.9 (5.1-7.3)	14.7 (9.8-17.8)	2.0 (1.8-2.6)	*Pseudolabrus coccineus*	Meglitsh (1960)
C. declivis	5.9 (5.1-6.8)	14.4 (13.5-15.2)	2.4 (1.7-2.8)	*Cyttus novaezelandiae*	Meglitsh (1960)
C. faba	6.2 (5.6-6.7)	12.7 (10.7-14.1)	2.4 (2.0-3.1)	*Caulopsetta scapha*	Meglitsh (1960)
C. navicularia	6 (5-7.5)	16 (14-22)	2	*Paralichthys dendatus*	Davis (1917)
C. opisthocentri	5-7	14-17	1-1.5	*Opisthocentrus ocellatus*	Shulman (1966)
C. sparusaurati	5.65 (4.5-7.5)	15.76 (14-17.5)	2.79 (2.2-3.4)	*Sparus aurata*	Sitja-Bobadilla et al.(1995)
C. sprenti	5.7 (4-8)	16.3 (14.0-23.0)	2.4 (2-3)	*Chaetodon aureofasciatus*	Moser et al. (1989)



Figures 3–7. Fresh mounts of *Ceratomyxa seriolae* n. sp. 3, 4. Disporous plasmodia; 5-7. Variable forms of mature spores. *Scale-bar*: 20 μm.

yellowtail. So far more than 140 species of *Ceratomyxa* have been described, mainly from marine fishes (Lom & Dyková, 1992). *C. seriolae* n. sp. closely resembles *C. appendiculata* Lubat, Radujkovic, Marques & Bouix, 1989 and *C. moenei* Meglitsh, 1960 in spore dimension (Table 1). However, *C. appendiculata* tapers to sharply pointed end and is greatly curved in side view (Lubat et al., 1989). *C. moenei* is relatively more convex at the anterior margin (Meglitsh, 1960). Also, the polar capsules of *C. seriolae* are much smaller than those of the other two species. *C. buri* n. sp. resembles several species (Table 2), but it can be differentiated from them by morphological characteristics. Spores of *C. beloneae* Lubat, Radujkovic, Marques & Bouix, 1989 are greatly curved and the polar capsules are pyriform (Lubat et al., 1989); those of *C. castigatoides* Meglitsh, 1960 and *C. declivis* Meglitsh, 1960 are convex at the anterior margin (Meglitsh, 1960); those of *C. faba* Meglitsh, 1960 are stubby (Meglitsh, 1960); those of *C. navicularia*



Figures 8–11. Fresh mounts of *Ceratomyxa buri* n. sp. 8, 9. Disporous plasmodia, 10, 11. Mature spores. *Scale-bar*: 20 μm.

Davis, 1917 are boat-shaped and slightly compressed parallel to the longitudinal plane (Davis, 1917); those of *C. opisthocentri* Dogiel, 1948 have smaller polar capsules (Shulman, 1966); those of *C. sparusaurati* Sitja-Bobadilla, Palenzuela & Alvarez-Pellitero, 1995 are commonly curved and crescent-shaped in side view (Sitja-Bobadilla et al., 1995); and those of *C. sprenti* Moser, Kent & Dennis, 1989 are relatively elongate, and the trophozoites are long and spindle-shape (Moser et al., 1989). Based on these differences in morphological characteristics, geographical distribution and host species, the two myxosporeans are considered as new species.

The seasonal investigation of the yellowtail bile suggested that infections with *C. seriolae*, *C. buri* and *M. spirosulcatus* commenced in October. Presumably the three myxosporeans invade the host fish from October and are continuously released in association with the secretion of bile. Several other myxosporeans have shown seasonal and annual patterns of infection caused by endogenous cycles of the parasites, the availability of susceptible hosts or the effects of environmental factors (Foott & Hedrick, 1987). In the present study, observed variation in prevalences from October to February may be explained by the variable condition of bile secretion at each sampling period. Endogenous developmental cycles remain to be studied, and *in vitro* sporulation techniques will solve this question in the future (Yokoyama & Fukuda, 1999). No pathological changes were observed in the host fish in this study, but the pathogenic potentials of the parasites, in particular their possible relationship with green liver, should be further studied.

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