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A new strain of *Cryptocaryon irritans* from the cultured olive flounder *Paralichthys olivaceus*

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ABSTRACT: An obligate parasite, *Cryptocaryon irritans*, which is responsible for the white spot disease of marine fish is known to develop in the temperature regime over 19°C. Recently, however, we found white spot disease of olive flounder *Paralichthys olivaceus* during winter at water temperatures ranging between 12 and 16°C in Korea. In the present study we isolated a *C. irritans*-like ciliate from the affected fish and investigated its reproductive characters to compare the newly found ciliate with typical *C. irritans*. The newly found ciliate had an additional process in the reproductive stage, characterized by a budding before palintomic division, and it showed a higher ability to carry out tomitogenesis at a low temperature (16°C) than at a high temperature (24°C). Nevertheless, the present ciliates still had much in common with typical *C. irritans* with respect to clinical, histopathological, and morphological characters, suggesting that it is a new strain of *C. irritans*, adapted to lower water temperature.

KEY WORDS: *Cryptocaryon irritans*-like ciliate · White spot disease · Olive flounder

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INTRODUCTION

White spot disease of marine fish caused by *Cryptocaryon irritans* Brown 1951 has been a major problem in the development of mariculture (Nigrelli & Ruggieri 1966, Cheung et al. 1980, 1981), accounting for high mortalities in cultured food fishes (Huff & Burns 1981, Colorni 1985, Kaige & Miyazaki 1985, Dimant et al. 1991). *C. irritans* is an obligate parasite of warm-water marine fish from areas where minimum sea temperatures exceed 19°C (Wilkie & Gordin 1969). The life-cycle of the parasite is direct: growth and development takes place within the fish epidermis, and a free-living phase follows in which the parasite forms a cyst, then undergoes several divisions and produces theronts (Brown 1963, Colorni 1985, Lom & Dykova 1992, Burgess & Matthews 1994). In Korea, marine fish such as olive flounder *Paralichthys olivaceus*, red seabream *Pagrus major* and puffer *Takifugu obscrus* cultured at several fish farms were frequently affected by the parasites during high water-temperature periods. Recently, however, we found white spot disease of olive flounder during the winter season at water temperatures ranging between 12 and 16°C. In the present study, we investigated the reproductive characteristics of the ciliates found and compared them with typical *C. irritans*.

MATERIALS AND METHODS

Fish. From December 1997 to February 1998, olive flounder *Paralichthys olivaceus* weighing 150 to 300 g and showing white spots on the skin were obtained from an embankment farm on the East Coast of the Korean peninsula.

Histopathology. Tissue of infected fish was fixed in 10% neutral formalin. Following serial dehydration with ethanol, tissue was embedded in paraffin wax. Sections 4 to 5 µm in thickness were stained with hematoxylin-eosin or Giemsa for light microscopic observation.

Morphology. The morphological characteristics of trophonts, tomonts and theronts were observed with a

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light or phase-contrast microscope, and the sizes of each stage were measured with a micrometer.

Reproduction. The detached mature trophonts were transferred to a 96-well plate filled with filtered seawater, and incubated at 16°C. The morphological changes and reproductive patterns were observed daily with a phase-contrast microscope. To determine whether water temperature influences tomite development and excystment, 2 groups of ciliates (28 and 32 individuals) were incubated at 2 different temperatures (16 and 24°C, respectively). The experiment was carried out 4 times with a total number of 240 populations. The time to beginning of excystment and success rate of excystment was recorded daily. For experimental infections under laboratory conditions, 10 healthy, juvenile flounders in a 30 l seawater aquarium (16°C, 32 ± 2‰) were cohabited with 2 infected fish.

RESULTS

Clinical signs

The infected fish showed multiple pin-point sized white spots on the body surface, fins and gills (Fig. 1). An excessive production of mucus on the body surface and the gills was seen in affected fish. No clinical signs of disease were seen in the internal organs of the fish.

Histopathology

Many ciliates were found above the basal membrane in the epithelium of gill filaments and skin, and caused hyperplasia of epithelial and mucus cells in the parasitic areas. In the gills, a large ciliate was observed in the blood vessel of primary gill filaments. Sometimes, a small ciliate was found in the interlamellar space in the early stage of infection and evoked slight epithelial proliferation. In the skin, ciliates of variable sizes were seen in the subepithelia, and caused marked hyperplasia of mucus cells. Empty spaces were present along with vacuolated and necrotic cells (Fig. 2).

Morphology

The general morphology of the present ciliates was similar to that of typical *Cryptocaryon irritans*, but they were larger than typical *C. irritans* (Table 1).

Reproduction pattern

The detached, mature trophonts formed a cyst wall, then the encysted cell divided into 3 to 6 equal size cells by budding within a few hours. During the division process, each budding cell went through the cyst wall and formed a new cyst wall (Fig. 3). Each divided

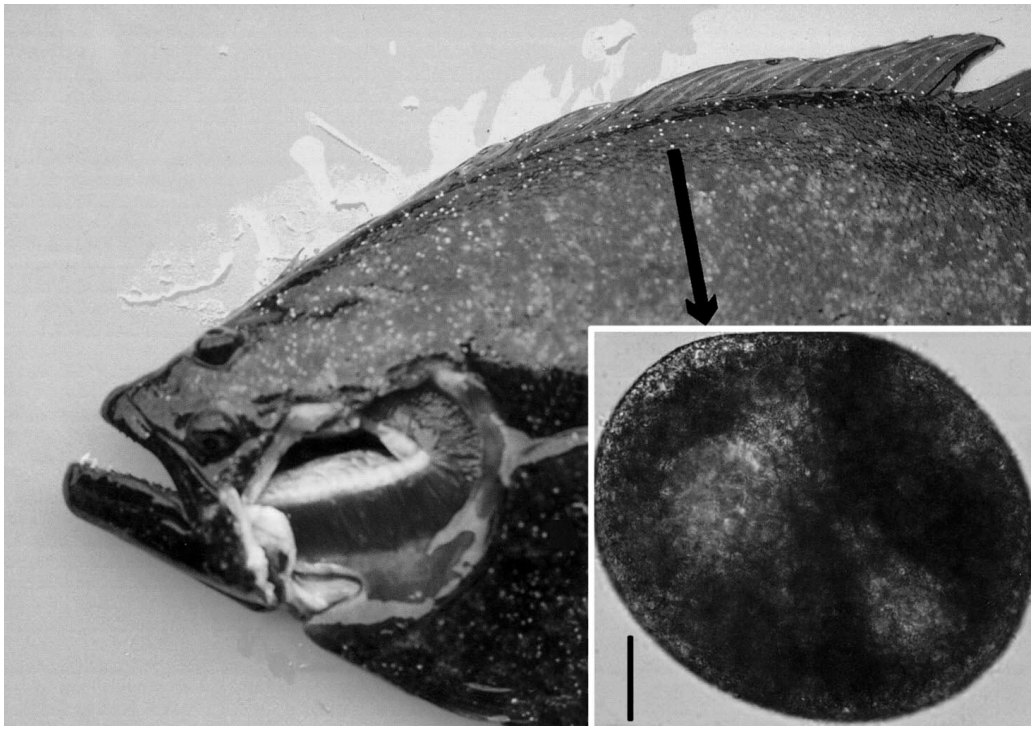

Fig. 1. White spots on the gill and skin of the olive flounder *Paralichthys olivaceus*. Note spherical shape of trophont under light microscopy (arrow). Scale bar = 100 µm

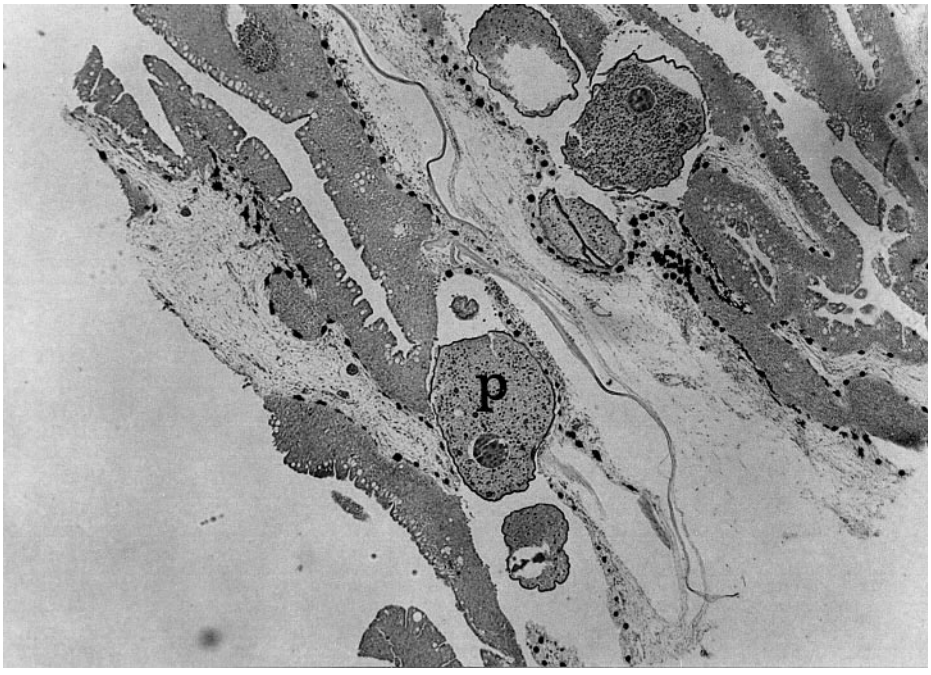

Fig. 2. Necrotic area of the skin from olive flounder showing parasite (P). H&E

tomont underwent a series of divisions and produced tomites. The number of tomites produced per daughter tomont was no more than 100. The time to complete tomitogenesis was 13 to 15 d at 16°C and 7 to 13 d at 24°C. The success rate of tomite production was 76.6% at 16°C and 31.6% at 24°C (Table 2). In the infection experiment, 3 fish among 10 juveniles showed white spots on the body surface on Day 4 after exposure. The trophonts released from the infected juveniles showed reproductive patterns identical to those mentioned above.

DISCUSSION

Diggles & Lester (1996b) reported that the heaviest infection of *Cryptocaryon irritans* on *Acanthopagrus australis* occurred at 17°C and infections were still recorded at 15°C, a temperature at which *C. irritans* infections were found not to occur in previous studies (Wilkie & Gordin 1969). This suggested the possible existence of different strains of *C. irritanse* which can be differentiated by their temperature tolerance. The present study demonstrates that white spot disease also occurs at low water temperature in cultured olive flounder. The considerably higher success rate of tomites production at 16 than at 24°C clearly indicates that these ciliates are well adapted to low water temperature.

The clinical and histopathological signs of the present infected fish showed more excessive mucus production and hyperplasia of epithelial cells in the gill lamellae than in the previous reports (Nigrelli & Ruggieri 1966, Kaige & Miyazaki 1985).

Diggles & Lester (1996a) reported that *Cryptocaryon irritans* exhibited morphometric variability on different

Table 1. Comparison of *Cryptocaryon irritans* from the literature and the ciliates recovered from olive flounder *Paralichthys olivaceus*

	Brown (1951)	Nigrelli & Ruggieri (1966)	Colorni (1985)	Burgess & Matthews (1994)	Present study
Hosts	Various	Various	*Sparus aurata*	*Chelon labrosus*	*Paralichthys olivaceus*
Temperature (°C)	20–25	22–25	23–25	24–26	12–16
Mean trophont diameter (µm)	180–450	350–450	–	203–452	350–700
Mean tomont diameter (µm)	200–450	170–441	160–370	160–406	300–700
Mean theront diameter (µm)	40–75	57	50–70	40–69	60–100
Reproduction pattern	Palintomy	Palintomy	Palintomy	Palintomy	Budding and palintomy
No. of theront/tomont	–	Up to 200 or more	No more than 200	119–292	No more than 100

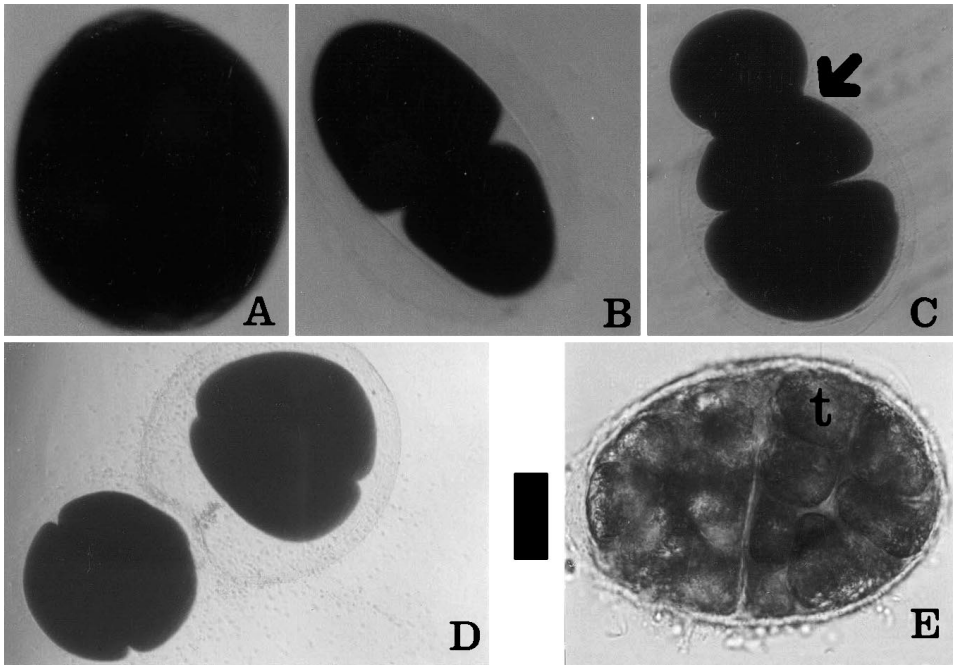

Fig. 3. Reproduction of the parasite recovered from olive flounder. Live specimen. Scale bar = 100 µm. (A) Free-swimming ciliate. (B) Ciliate reproducing within cyst. (C) Daughter cell budding out from a cyst (arrow). (D) Daughter cell forming a new cyst wall. (E) Daughter cell showing palintomy division within the cyst

Table 2. Effects of temperatures on tomitogenesis at constant salinity (35‰). Each value represents the mean of 4 replicate experiments

Temperature (°C)	No. collected	No. encysted	No. excysted	Encysted rate (%)	Encysted time (day)
16	30	24	23	76.6	13–15
24	32	13	10	31.6	7–13

hosts and under different temperature conditions. Diggles & Lester (1996b) also found that the size of *C. irritans* varied inversely with water temperature. A similar relationship between water temperature and parasite size is well known for *Ichthyophthirius multifiliis* (MacLennan 1942) and *Amylloodinium ocellatum* (Paperna 1984). Although the size of the present ciliates in all life stages was larger than that reported for *C. irritans* (Brown 1963, Nigrelli & Ruggieri 1966, Cheung et al. 1981, Colorni 1987, Colorni & Diamant 1993), there were no differences in morphological features. It seems that the fact that present ciliates were larger than the typical *C. irritans* resulted from lower ambient temperatures, which was also pointed out by Diggles & Lester (1996b).

There are numerous reports on the reproduction pattern of *Cryptocaryon irritans* (Brown 1963, Nigrelli & Ruggieri 1966, Cheung et al. 1980, Colorni 1987, Colorni & Diamant 1993). According to these reports, reproduction starts when a mature trophont drops off the host and forms a cyst wall without a host. The encysted ciliate (tomont) undergoes a series of divisions producing numerous tomites. Compared to the reproduction pattern of typical *C. irritans* described in other reports, the present ciliates showed a different sequence of divisions, i.e., production of several daughter tomonts by budding before palintomic division. This peculiar reproduction pattern of *C. irritans* has not been reported previously. Therefore, we tentatively define the present ciliate population as a new strain of *C. irritans* which has adapted to a new environment, mainly to lower water temperature.

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