Mixed Infection of an Acid-fast Bacterium and an Imperfect Fungus in a Napoleon Fish (Cheilinus undulatus)

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Mixed Infection of an Acid-fast Bacterium and an Imperfect Fungus in a Napoleon Fish (*Cheilinus undulatus*)

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ABSTRACT: A Napoleon fish (*Cheilinus undulatus*) was infected with both an acid-fast bacterium and an imperfect fungus. This is the first report of an acid-fast bacterial infection in *Cheilinus undulatus*, and the first observation of an imperfect fungus in the swim bladder of a tropical marine fish.

Key words: Napoleon fish, acid-fast bacterium, imperfect fungus, histopathology, *Cheilinus undulatus*.

Napoleon fish (*Cheilinus undulatus*) are frequently exhibited in commercial Japanese aquariums. There is no information, however, on the diseases of this species. This is the first known report of a simultaneous infection with *Mycobacterium* sp. and an imperfect fungus in Napoleon fish (*Cheilinus undulatus*).

The Napoleon fish was a male weighing 14.6 kg, with a total body length of 934 mm, captured at Padang Bay, Indonesia (8°15′S, 115°29′E) in May 1990. The fish was transported to a dealer in Shizuoka Prefecture, Japan, in June 1990, and reared in a concrete aquarium. In April 1991 the fish was introduced to the Yomiuri Land Marine Aquarium, Tokyo, Japan.

The fish died three days after arriving at the aquarium and was necropsied immediately. Heart, liver, kidney, and swim bladder were fixed in 10% phosphate-buffered formalin, embedded in paraffin, and sectioned at 4 to 5 μm. Sections were stained with hematoxylin and eosin. Some sections also were stained by the Ziehl-Neelsen

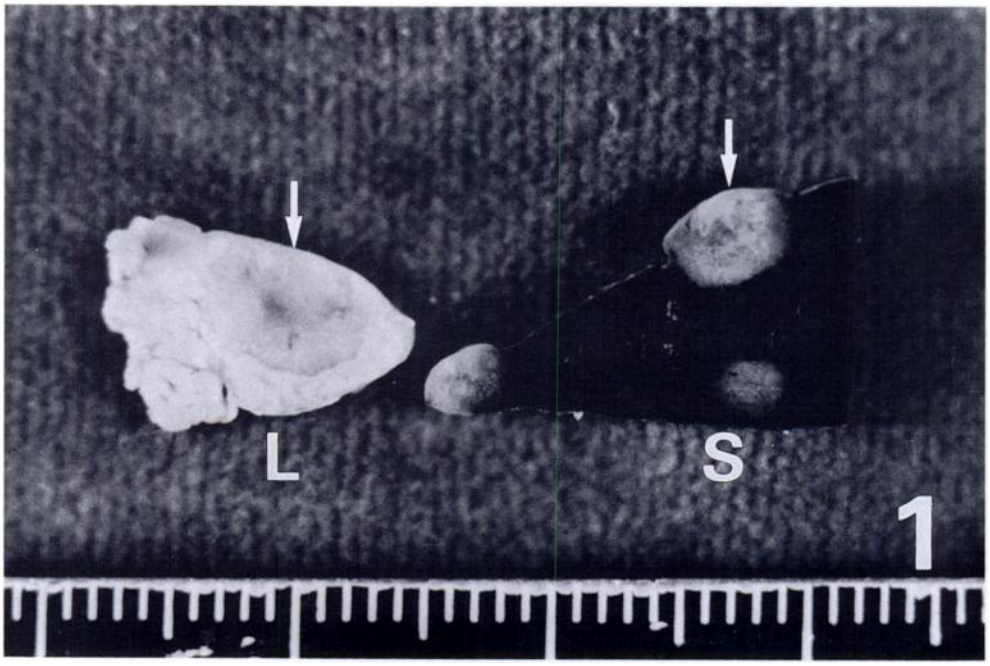


FIGURE 1. Cut surfaces of the liver (L) and the spleen (S) with white nodules (arrows). Small units on bar = 1 mm.

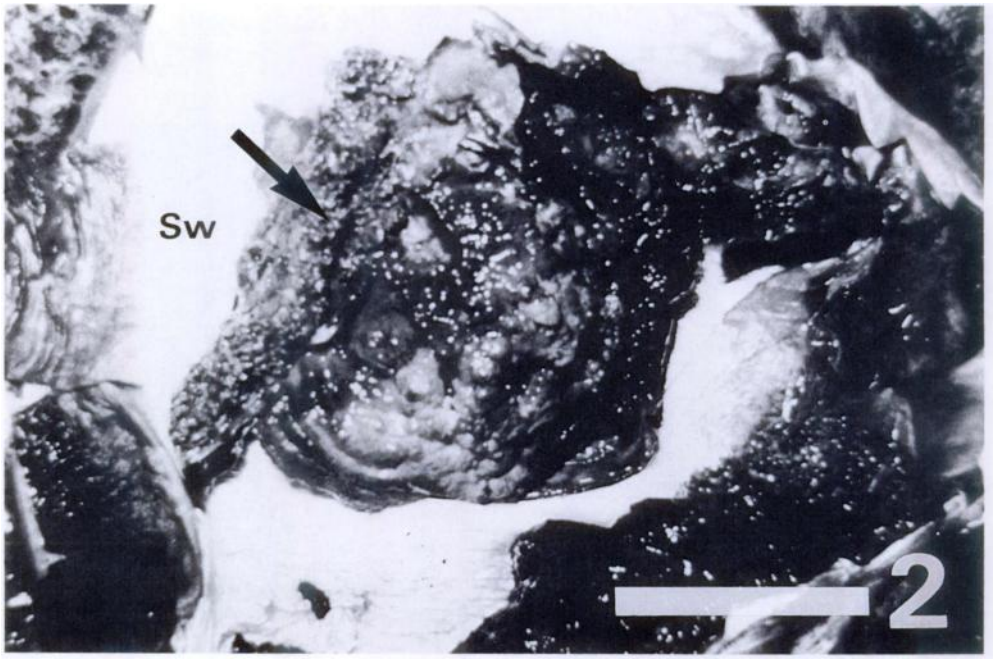


FIGURE 2. Swim bladder with large brown flat mass with rough surface (arrow). Sw, the wall of the swim bladder. Bar = 5 cm.

method for acid-fast bacteria and decolorized with 3% hydrochloric acid in 70% alcohol (Kageyama and Watanabe, 1978); we also used the Hucker-Conn method for bacteria (Kageyama and Watanabe, 1978), the periodic acid Schiff (PAS) reaction (Okudaira, 1985), the methenamine silver-nitrate-Grocott's variation (Grocott, 1955), and the Schmorl method (Naoe and Kuroiwa, 1977) on some sections.

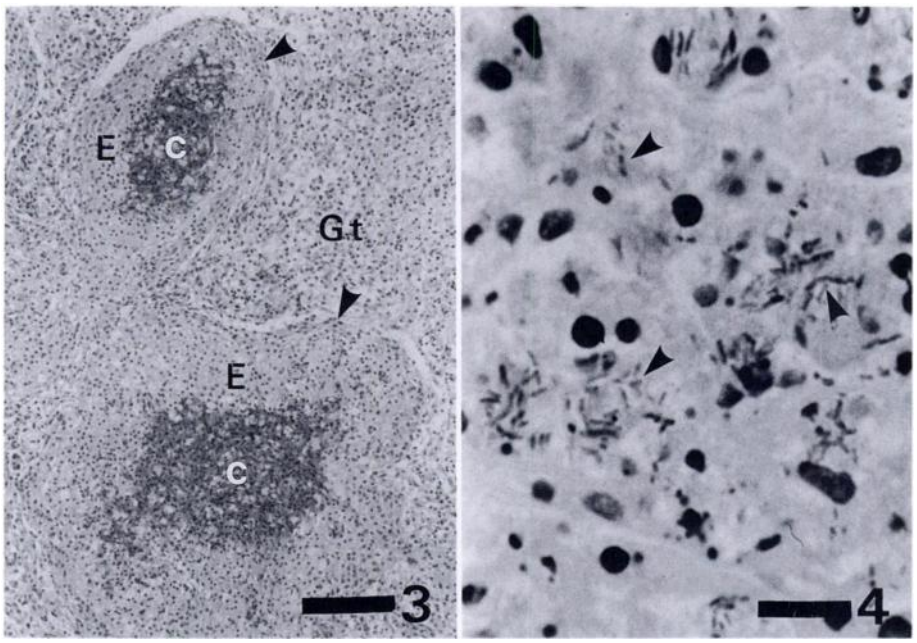
There were a number of abrasions on the body surface. No other clinical signs were observed.

The liver had a yellow discoloration and white nodules, 4 to 5 mm in diameter, on its capsule. Nodules similar in color, and 0.5 to 1.0 cm in diameter, also were observed in the spleen. These nodules extended into the parenchyma of both organs (Fig. 1).

In the swim bladder, a large brown flat mass with a rough surface, approximately 15 cm in diameter, was attached to the inner wall of the bladder (Fig. 2). Fungal hyphae were observed in small pieces of the mass, using light microscopy.

Isolation of the bacterium and the fungus from these lesions was not attempted. No gross lesions were observed in the other organs.

The white nodules in the liver and the spleen were chronic proliferative lesions composed of multiple caseous granulomata with surrounding granulation tissue (Fig. 3). Histologically, similar lesions also were found in the parenchyma of the liver and the spleen. The granuloma was composed of a central caseous area and a surrounding layer of ovoid to spindle-shaped epithelioid cells, but no giant cells were observed in these tissues. The granulation tissue surrounding the granulomas was composed of macrophages, eosinophilic granulocytes (EGC's), and fibroblasts. The EGC's contained weakly eosinophilic, small multiple granules and an eccentric nonlobed nucleus. Loose connective tissue existed in the boundary region between the granulomas and the granulation tissue, and the marginal region of the granulation tissue. In the central caseous area, a number of colonies of slender, long, Gram + rods



FIGURES 3 and 4. Figure 3. Liver. Caseous granulomas (arrow heads) with surrounding granulation tissue (Gt). E, epithelioid cell layer. C, central caseous area. H&E. Bar = 100 μm. Figure 4. Acid-fast bacteria (arrow heads) in the central caseous area of the granulomas. Ziehl-Neelsen. Bar = 10 μm.

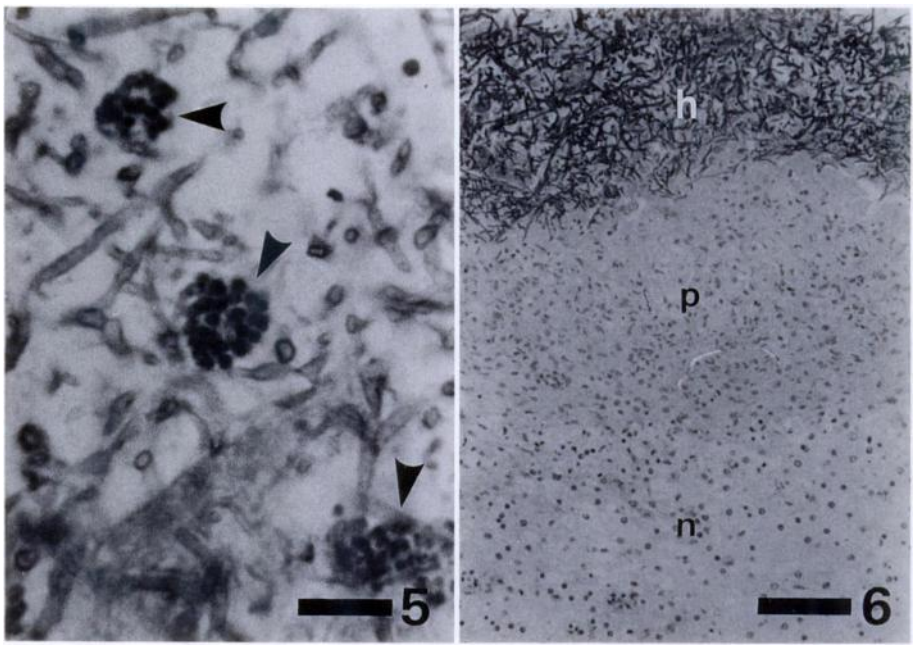
were found to be acid-fast by the Ziehl-Neelsen method (Fig. 4). These acid-fast bacteria also were observed in the epithelioid cells and in the macrophages of the granulation tissue. Similar chronic proliferative lesions associated with acid-fast bacteria were not detected in other organs.

The central region of the swim bladder mass was occupied almost completely by mats of fungal hyphae with many clusters of round-shaped conidia, 1.5 to 2.1 μm in diameter (Fig. 5). The hyphae were branched, septate, and of irregular width; in paraffin sections the hyphae were 1.0 to 2.0 μm in width. Although the hyphae were clearly stained with PAS and Grocott's preparation, the hyphae were not stained positively by the Schmorl method. The marginal region of the mass was composed of elongated hyphae and chronic proliferative lesions. The lesion contained epithelioid granulomata around the elongated hyphae and a wide area of granulation tissue, but had no giant cells (Fig. 6).

The granulation tissue around the epithelioid granulomata was composed of macrophages, EGC's, lymphocytes, fibroblasts, and newly generated capillaries; it was encapsulated by loose connective tissue. The hyphae did not reach either the epithelioid cell layer or the granulation tissue (Fig. 6). The lesion associated with the fungus was not observed in other organs.

In fishes, acid-fast bacteria cause various lesions and many infections have typical epithelioid granulomas without giant cells (Mori et al., 1986; Chinabut et al., 1990). Granulomas associated with the acid-fast bacteria described in this paper were similar to those reported by previous workers, but differed by having a wide area of granulation tissue surrounding them. From their morphological characteristics of the acid-fast bacteria, it is highly probable that the bacteria are *Mycobacterium* sp.

From its morphological characteristics, the fungus in the swim bladder was con-



FIGURES 5 and 6. Figure 5. Note clusters of round shaped conidia (arrow heads) in central region of the mass in the swim bladder. PAS reaction. Bar = 10 μm. Figure 6. Epithelioid cell granuloma found in the marginal region of the swim bladder mass. Note three layers of the granuloma; mats of fungal hyphae (h), pyknotic epithelioid cell layer (p), and non-pyknotic epithelioid cell layer (n). PAS reaction. Bar = 50 μm.

sidered to be an imperfect fungus. Imperfect fungi have been reported as a cause of local and systemic infection in marine fish (Hatai, 1989).

Of these fungal agents, *Exophiala pisciphila* (Gaskins and Cheung, 1986), *Aureobasidium* sp. (Otte, 1964) and *Sarcinomyces crustaceus* (Todaro et al., 1983) have been classified as dematiaceous fungi (Hawksworth et al., 1983). Naoe and Kuroiwa (1977) observed that dematiaceous fungi are stained positively by the Schmorl method. However, the fungus reported in this paper was Schmorl negative. Further, we could not detect the canoe-shaped macroconidia characteristic of the genus *Fusarium* (Nelson et al., 1983). Therefore, we believe that the fungus in the lesion was a new fungus from fish; we could not determine its identity nor its mode of invasion into the swim bladder. Fungal infections of the swim bladder previously have been found in hatcheries, but not in marine fish (Bruno, 1989). Thus our natural case was the first report of such an infection in a tropical marine fish.

We do not know when the pathogens infected the fish; however, from the clinical history, we speculate that the fish became infected while being reared in the commercial fish dealer's concrete aquarium.

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