Mycoscience 38: 433–436, 1997

Short Communication

Mycotic granulomatosis found in two species of ornamental fishes imported from Singapore

Chutima Hanjavanit[1], Hiroki Suda[2] and Kishio Hatai[2]

[1] Department of Biology, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand
[2] Division of Fish Diseases, Nippon Veterinary and Animal Science University, 1–7–1, Kyonan-cho, Musashino, Tokyo 180, Japan

Accepted for publication 1 September 1997

Golden gourami, *Trichogaster trichopterus*, and snakehead, *Channa pleurophthalmus*, were newly discovered to be hosts of mycotic granulomatosis caused by *Aphanomyces* sp., the first such cases to be reported in Japan.

Key Words——*Aphanomyces*; fungal disease; golden gourami; mycotic granulomatosis; snakehead.

The fungal disease caused by *Aphanomyces* has been described as mycotic granulomatosis (MG) and was originally found in ayu, *Plecoglossus altivelis* Temminck & Schlegel (Egusa and Masuda, 1971; Miyazaki and Egusa, 1973a). It has also been reported in goldfish, *Carassius auratus* Linnaeus (Miyazaki and Egusa, 1972), bluegill, *Lepomis macrochirus* Rafinesque (Miyazaki and Egusa, 1973b), and some wild fishes (Kumamaru, 1973; Miyazaki and Egusa, 1973c). The fungus isolated from affected ayu was identified as *Aphanomyces piscicida* Hatai (Hatai and Egusa, 1977; Hatai, 1980). Recently, similar inflammatory responses found in many species of freshwater and estuarine fishes from Southeast Asia were described as epizootic ulcerative syndrome (EUS) (Lilley et al., 1992; Roberts et al., 1993). Fungi in the genus *Aphanomyces* were also consistently isolated from fishes with EUS (Roberts et al., 1993). The aim of the present study is to report the histopathological features of systemic aphanomycosis in such ornamental fishes as golden gourami, *Trichogaster trichopterus*, and snakehead, *Channa pleurophthalmus*.

The fishes were purchased from a petshop in Tokyo and underwent gross observation and histopathological examination. We found fungal hyphae in a lesion of the mid-trunk of the golden gourami, which was haemorrhagic with dermal swelling and scale loss (Fig. 1). The snake head showed a massive, cottony, whitish area of fungal hyphae in the muscle trunk adjacent to the left operculum (Fig. 2). No sporangia or spores could be found in the lesions. A fungus was isolated from both fishes. Small pieces of the trunk muscles and internal organs were fixed in 10% phosphate-buffered formalin solution, embedded in paraffin, and sectioned at 4 to 5 μm. Sections were stained with methenamine silver-nitrate-Grocott's variation and counter staining with haematoxylin and eosin (Grocott-H&E). Serial sections were stained by Giemsa and periodic acid Schiff (PAS) reagent. Some sections were also stained with the Ziehl-Nielsen (ZN) method for acid-fast bacteria.

During microscopic examination of the skin lesion of the golden gourami, numerous fungal hyphae invading the myotome musculature were revealed by staining with Grocott-H&E or PAS. Hyphae were aseptate, 8 to 20 μm in width and sparsely branching. Most of the hyphae were surrounded with granulomatous tissue, which consisted of many layers of epithelioid cells (Fig. 3). In some areas, there were many granulomas without hyphal growth, but necrotic cells with pyknotic nuclei and eosinophilic matrix appeared in the central part of granulomas. Severe inflammatory responses, and degenerative and necrotic changes in the muscle were observed at the invaded site. In this case, mycotic granulomas were also observed in the kidney, which showed histological findings similar to those found in the muscular lesions (Fig. 4).

Histopathological examination of the skin lesion of the snakehead is shown in Fig. 5. A large number of fungal hyphae had infiltrated the underlying musculature. Most of the hyphae were surrounded by many layers of epithelioid cells, which formed a large number of mycotic granulomas (Fig. 6). Extensive degenerative changes and necrosis had occurred in muscle fibers. A large number of inflammatory cells infiltrating between the degenerative changes of the myotic fibers were observed. In some areas, many hyphae were present within the myotome without any cellular responses. The fungal hyphae grew in the muscular tissues adjacent to the gill filaments, kidney, pancreas, liver and muscular layer of intestinal wall, and the kidney in particular showed granulomatous responses to the fungal elements (Fig. 7). Sections stained by the ZN method did not reveal the presence of any acid-fast bacterial pathogens.

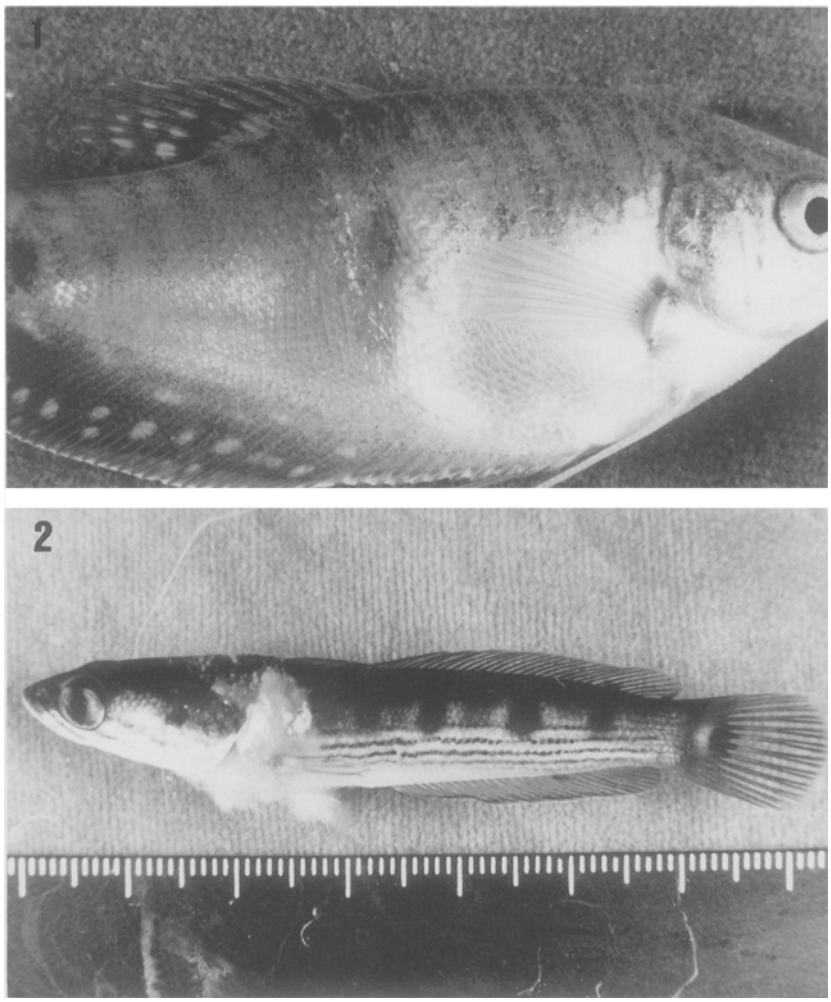

Fig. 1. Gross appearance of a diseased golden gourami. Note swelling and haemorrhage of the abdomen.
Fig. 2. Gross appearance of an affected snakehead. The fungal growth appears adjacent to the base of the left pectoral fin.

Hyphae of the fungi cultured from the skin lesions of the golden gourami and the snakehead by using GY agar morphologically resembled those seen in hisotopathological sections of the diseased fish as shown in Fig. 8. All of the cultures were identified as *Aphanomyces* sp. from morphological observation, especially the mode of zoospore formation.

The histopathological findings in the present cases were very similar to those of mycotic granulomatosis in dwarf gourami, *Colisa lalia* (Hatai et al., 1994) and to other reports of EUS affecting wild and cultured freshwater fishes (Lilley et al., 1992; Roberts et al., 1993). In the present case, the isolates were identified as the fungus isolated from mycotic granulomatous lesions of dwarf gourami (Hatai et al., 1994) and in fishes with EUS (Roberts et al., 1993). It seems likely that *Aphanomyces* infection occurs not only in cultured fishes but also in ornamental fishes. Recently, many species of fishes including ornamental fishes are being imported from Southeast Asia to Japan. Some imported fishes such as the dwarf gourami from Singapore showed severe fungal infection caused by *Aphanomyces* sp. (Hatai et al., 1994). This implies that the disease may in future be introduced into Japan through such ornamental fishes. In addition to the granulomatous responses observed in the trunk muscular tissues, hyphae were also found to have penetrated internal organs such as the kidney in the present case, indicating that the disease was an invasive fungal infection.

In the present study, golden gourami and snakehead were newly discovered to be hosts of mycotic granulomatosis caused by *Aphanomyces* infection, the first such cases to be reported in Japan.

Literature cited

Egusa, S. and Masuda, N. 1971. A new fungal disease of *Plecoglossus altivelis*. Fish Pathol. **6**: 41–46. (In Japanese.)

Hatai, K. 1980. Studies on pathogenic agents of saprolegniasis in fresh water fishes. Spec. Rep. Nagasaki Pref. Inst. Fish. **8**: 1–95. (In Japanese.)

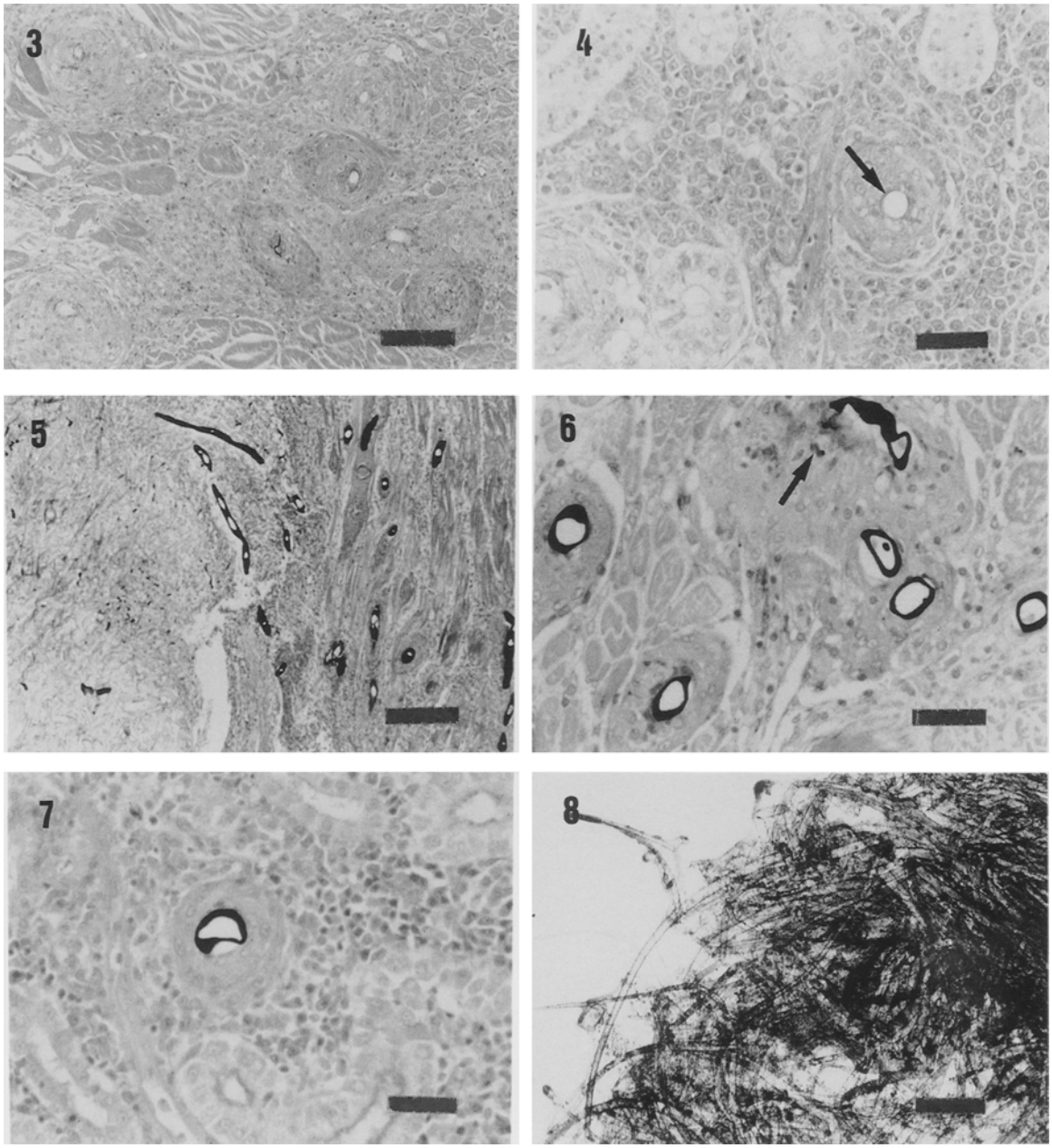

Fig. 3. Mycotic lesion in the trunk muscle of the golden gourami. Note granulomatous tissues surrounding fungal hyphae and severe inflammatory response around degenerative muscle fibers. Stained with Grocott-H&E. Bar=100 μm.

Fig. 4. Mycotic lesion in the kidney of the golden gourami. Note the hyphae surrounded by granulomatous tissues (arrow). Stained with Grocott-H&E. Bar=50 μm.

Fig. 5. Mycotic lesion in the skin lesion of the snakehead. Numerous fungal hyphae have invaded the muscular tissues. Note severe degeneration and necrosis of muscle fibers and inflammatory responses. Stained with Grocott-H&E. Bar=250 μm.

Fig. 6. Mycotic granulomas in the trunk muscle lesion of the snakehead. Note fungal hyphae encapsulated by many layers of epithelioid cells and some necrotic cells appearing adjacent to the hyphae (arrow). Stained with Grocott-H&E. Bar=50 μm.

Fig. 7. Fungal hyphae invading the kidney of the snakehead display granulomatous formation. Stained with Grocott-H&E. Bar=50 μm.

Fig. 8. Hyphal mass found on the trunk of the snakehead. Unfixed and unstained, bar=250 μm.

Hatai, K. and Egusa, S. 1977. Study on the pathogenicity fungus of mycotic granulomatosis—I. Isolation and pathogenicity of the fungus from cultured ayu infected with the disease. Fish Pathol. **12**: 129–133. (In Japanese.)

Hatai, K., Nakamura, K., Yuasa, K. and Wada, S. 1994. *Aphanomyces* infection in dwarf gourami (*Colisa lalia*). Fish Pathol. **29**: 95–99.

Kumamaru, A. 1973. A fungal disease of fishes in lake

Kasumigaura and lake Kitaura. Rep. Stan. Ibaraki Pref. **11**: 129–142.

Lilley, J. H., Phillips, M. J. and Tonguthai, K. 1992. A review of epizootic ulcerative syndrome (EUS) in Asia. Aquatic Animal Health Research Institute and Network of Aquaculture Centres in Asia-Pacific, Bangkok.

Miyazaki, T. and Egusa, S. 1972. Studies on mycotic granulomatosis in freshwater fish I. Mycotic granulomatosis in goldfish. Fish Pathol. **7**: 15–25. (In Japanese.)

Miyazaki, T. and Egusa, S. 1973a. Studies on mycotic granulomatosis in freshwater fish II. Mycotic granulomatosis prevailed in goldfish. Fish Pathol. **7**: 125–133. (In Japanese.)

Miyazaki, T. and Egusa, S. 1973b. Studies on mycotic granulomatosis in freshwater fish III. Mycotic granulomatosis in bluegill. Fish Pathol. **8**: 41–43. (In Japanese.)

Miyazaki, T. and Egusa, S. 1973c. Studies on mycotic granulomatosis in freshwater fish IV. Mycotic granulomatosis in some wild fishes. Fish Pathol. **8**: 44–47. (In Japanese.)

Roberts, R. J., Willoughby, L. G. and Chinabut, S. 1993. Mycotic aspect of epizootic ulcerative syndrome (EUS) of Asian fishes. J. Fish Dis. **16**: 169–183.