Mycoscience 36: 365–368, 1995

Short Communication

Morphological aspects of *Saprolegnia diclina* Type 1 isolated from pejerrey, *Odonthetes bonariensis*

Nilubol Kitancharoen, Kei Yuasa and Kishio Hatai

Nippon Veterinary and Animal Science University, 1-7-1, Kyonan-cho, Musashino-shi, Tokyo 180, Japan

Accepted for publication 22 June 1995

***Saprolegnia diclina* Type 1 (syn. *S. parasitica*) discovered in pejerrey, *Odonthetes bonariensis*, is described and illustrated herein.**

Key Words——*Odonthetes bonariensis*; pejerrey; *Saprolegnia diclina* Type 1; *Saprolegnia parasitica*; saprolegniasis.

Pejerrey, *Odonthetes bonariensis* Cuvier & Valenciennes, imported from Argentina for culture in Japan, has been frequently affected by problems of disease. One massive infestation was presumed to have been caused by fungal diseases. Recently, in Tochigi Prefecture Fisheries Experimental Station, a severe disease in pejerrey occurred. The disease affected 50-97% of the cultured fish. The fish revealed fungal hyphae at the mouth and caudal fin, which were eroded and haemorrhagic (Fig. 1). The external signs were similar to saprolegniasis, a prominent fungal disease in freshwater fish. The fungus was isolated by inoculating a piece of lesion with fungus from a diseased fish onto glucose-yeast extract agar (GY; 10 g glucose, 2.5 g yeast extract, 12 g agar in 1 L distilled water) and maintained as a stock culture, with the collection number of NJM 9302. In GY broth, the fungus exhibited non-septate, slender, moderately branched and round-tip hyphae, 9-44 μm in diam. The fungal hyphae were transferred into sterilized tap water to observe zoospore formation. After incubation for 24 h at 20°C, abundant zoosporangia were formed. Zoosporangia were predominantly clavate or filiform, frequently irregular, straight or bent, 120-184 × 16-52 μm in size. Zoosporangia renewed by internal proliferation or basipetalous succession (Figs. 2, 6A,B). Zoospores discharged in saprolegnoid fashion through the exit pore. Encysted spores were 12-13 μm in diam and exhibited both direct and indirect germination (Figs. 3, 6G). Gemmae were not observed. From the results described above, the isolate was classified into the genus *Saprolegnia*. An attempt to induce oogonial production was performed in hemp seed culture at 10°C. The

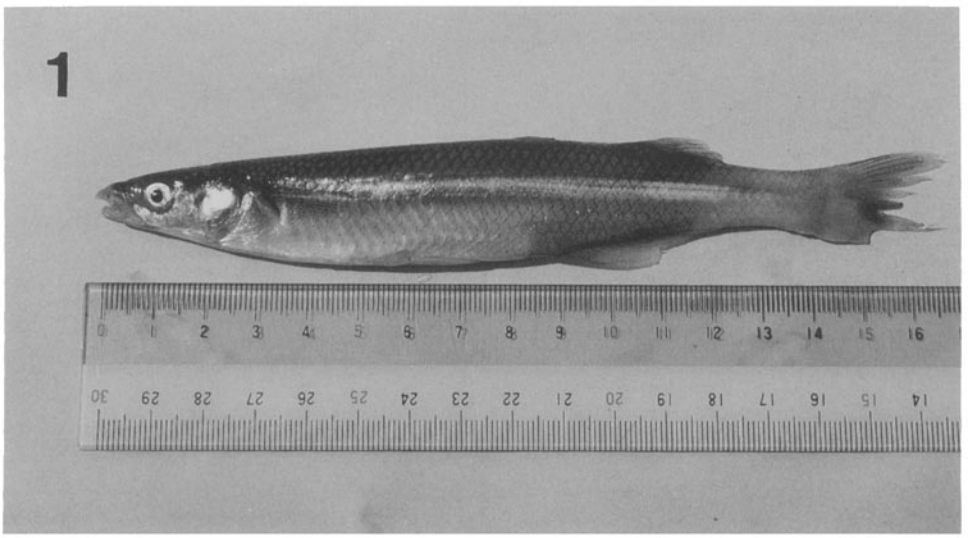


Fig. 1. External signs of pejerrey, *Odonthetes bonariensis*. Haemorrhages, fungal hyphae entanglement and erosion of the caudal fin and mouth were noted.

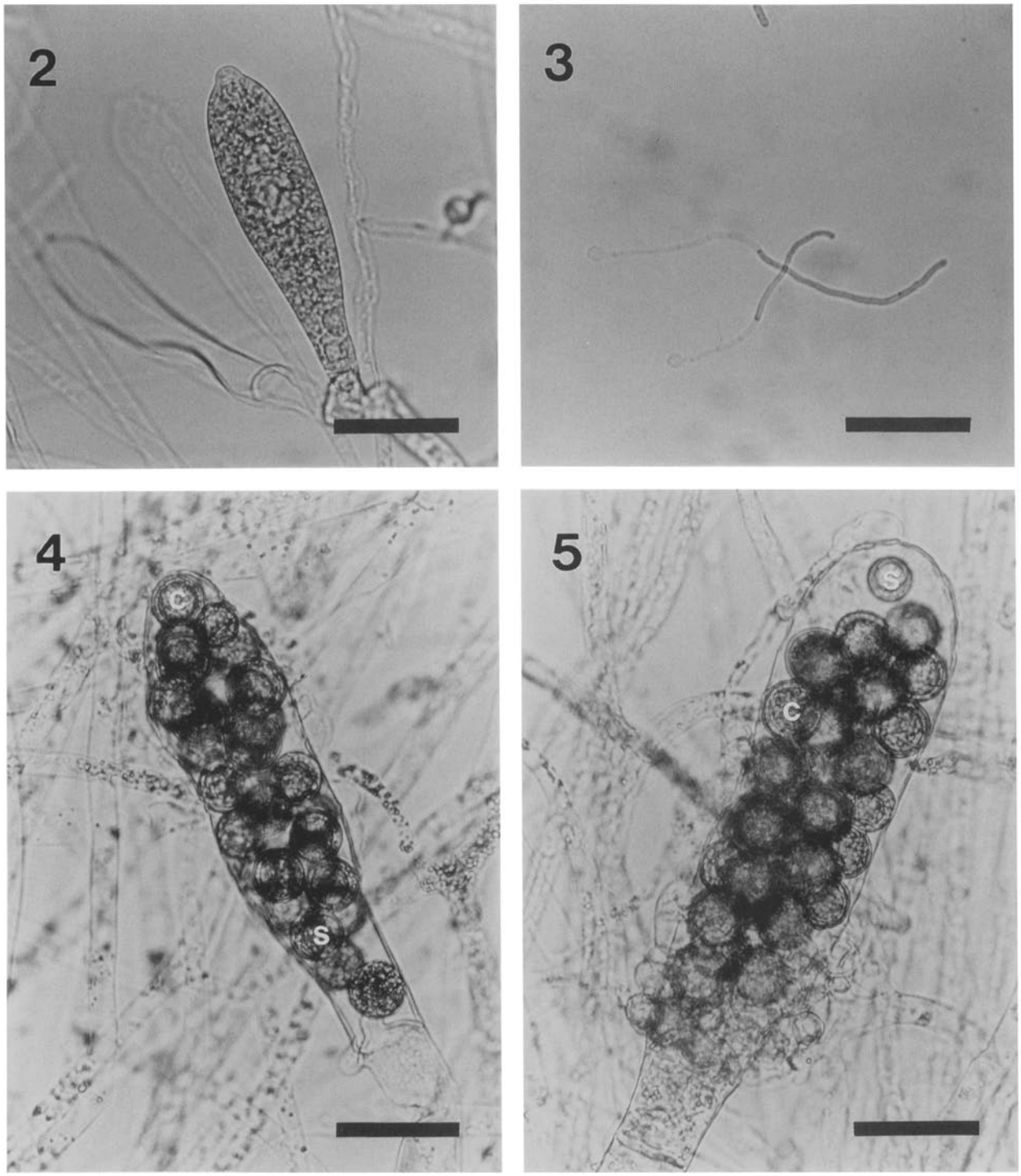
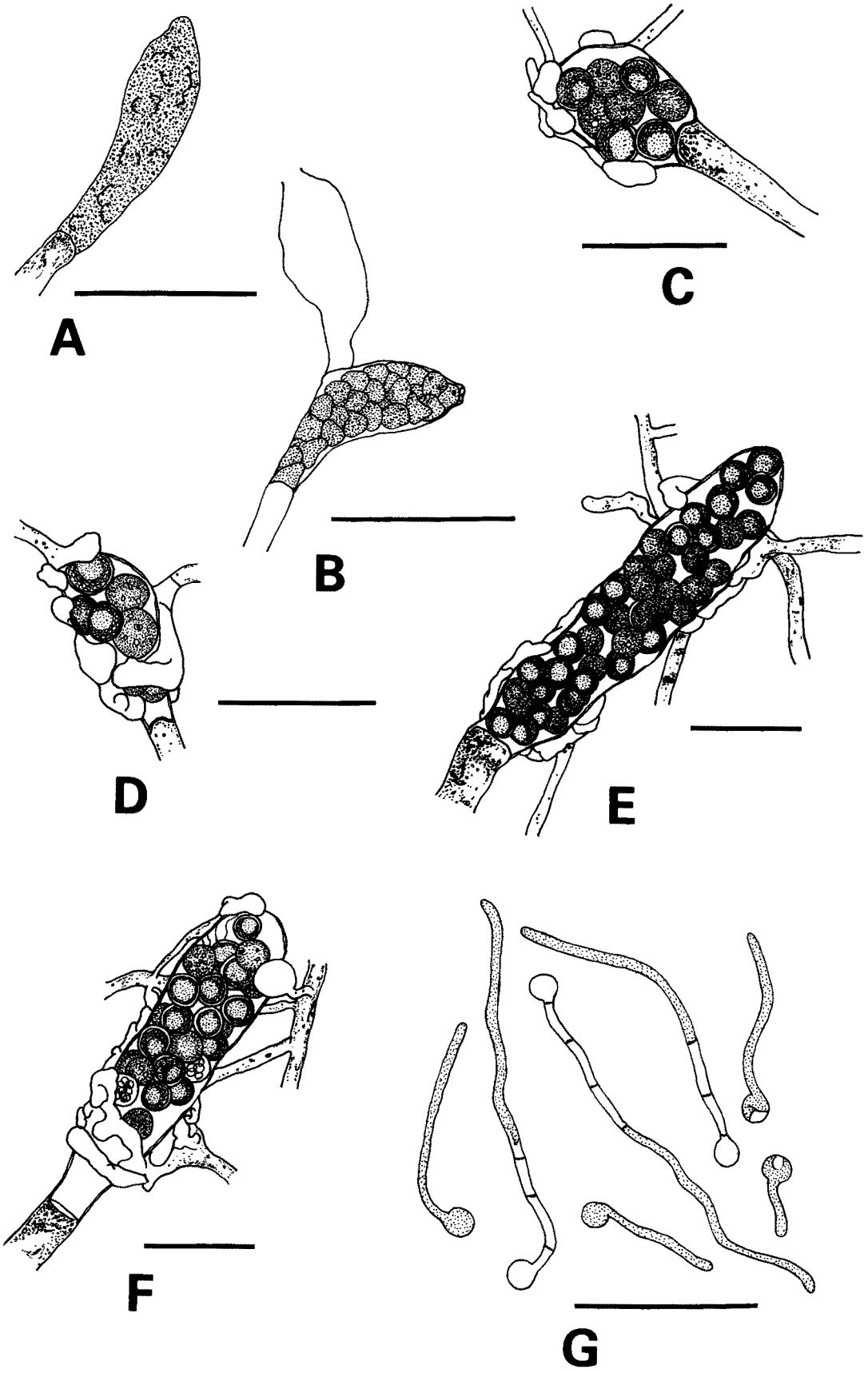


Fig. 2. Zoosporangia renewed in basipetalous succession. (Scale bar = 60 μm.)
Fig. 3. Encysted spores showed in indirect germination. (Scale bar = 120 μm.)
Figs. 4, 5. Pitted elongated oogonia with centric (C) and subcentric (S) oospores attached with diclinous antheridial branches. Antheridial cells were clavate or irregular. (Scale bars = 60 μm.)

Fig. 6. *Saprolegnia diclina* Type 1, NJM 9302. A. Young zoosporangium; B. Zoosporangium renewed in basipetalous succession; C. Obovate oogonium with subcentric oospores and immature oospores surrounded with antheridia; D. Pyriform oogonium wrapped with antheridia, antheridial cells were irregularly branched; E, F. Elongated oogonia with centric, subcentric and immature oospores attached with antheridia; G. Direct and indirect germination. (Scale bars = 100 μm.)


A
B
C
D
E
F
G

production of oogonia was sparse even after a prolonged period. Oogonia were elongate, clavate or pyriform, 158-320(225)×47-88(63) μm for elongated oogonia and 48-93 (58) μm for pyriform oogonia, and oogonial walls were pitted. Elongated oogonia with a length /breadth (L/B) ratio$\geqq$2 as described by Willoughby (1978), were prevalent (53.7%) in this isolate. The isolate revealed a dominance of the diclinous antheridial branches abundantly surrounding the oogonium. Antheridial cells were tubular, clavate, or irregular, infrequently branched, and laterally or apically appressed on the oogonia. The structure of the oospore was not only subcentric, but also centric (Figs. 4, 5, 6C-F). Oospores were 20-23 μm in size, usually filling the oogonium. The zoospore germinating pattern was also examined in 1/4 (v/v) GY broth and sterilized tap water. The rate of occurrence of indirect germination for this isolate was 82.8%.

Water molds of the genus *Saprolegnia* have long been known as external parasites of freshwater fishes, amphibians and reptiles, which notably cause saprolegniasis (Tiffney, 1939; Scott, 1964; Willoughby, 1970, 1978; Willoughby and Copeland, 1984; Hatai and Hoshiai, 1992; Bly et al., 1992; Blaustein et al., 1994). However, in the taxonomic system of the genus *Saprolegnia*, it has proved very difficult to discriminate between some species, with the distinction between *S. parasitica* Coker and *S. diclina* Humphrey being especially doubtful. Coker (1923) originally proposed the name *S. parasitica* for the isolates from parasitized fish and fish eggs which produced only an asexual stage and neither sexual stage. Kanouse (1932) reported the sexual stage of *S. parasitica* isolated from diseased fish in hatcheries in Michigan. According to Seymour (1970), *S. parasitica* was previously distinguished from *S. diclina* by the present of subcentric oospores and lack of centric oospores. Willoughby (1978) disproved the disparity of oospore characteristics between the two species by finding that *Saprolegnia* isolates which are pathogens in salmonids exhibit both subcentric and centric oospores, irrespective of the oogonium of origin, and thus could not be identified as *S. parasitica*. However, Willoughby proposed that the isolates of *S. diclina* could be classified into 3 types as *S. diclina* Types 1, 2 and 3 based on L/B ratios of oogonia. Ensuing discussions among mycologists have sustained the idea that *S. diclina* Humphrey Type 1 and *S. parasitica* Coker are synonymous.

The isolate studied herein showed similarities to *S. diclina* Type 1 formerly described by Willoughby with both subcentric and centric types of oospore, pitted oogonia and also a 53.7% L/B ratio of elongated oogonia, whereas the range of 13.3-55.3 was reported in *S. diclina* Type 1 (Willoughby, 1978). Furthermore, this isolate also revealed indirect germination, identical to the pathogenic strain obtained from diseased char, *Salvelinus alpinus* L. described by Willoughby et al. (1983). The disparities between this isolate and *S. parasitica* described by Kanouse (1932) were that the strain of Kanouse revealed unpitted oogonia and subcentric oospores. From the results and discussion above, the isolate NJM 9302 was identified as *S. diclina* Humphrey Type 1 (syn. *S. parasitica* Coker). This is the first time for *S. diclina* Type 1 to be described in Japan.

Literature cited

Blaustein, A. R., Hokit, D. G., O'Hara, R. K. and Holt, R. A. 1994. Pathogenic fungus contributes to amphibian losses in the Pacific Northwest. Biol. Conserv. **67**: 251-254.

Bly, J. E., Lawson, L. A., Dale, D. J., Szalai, A. J., Durborow, R. M. and Clem, L. W. 1992. Winter saprolegniosis in channel catfish. Dis. Aqua. Organisms **13**: 155-164.

Coker, A. C. 1923. "The Saprolegniaceae, with notes on other water molds," Univ. North Carolina Press, North Carolina. 201 p.

Hatai, K. and Hoshiai, G. 1992. Mass mortality in cultured coho salmon (*Oncorhynchus kisutch*) due to *Saprolegnia parasitica* Coker. J. Wildlife Dis. **28**: 532-536.

Kanouse, B. 1932. A physiological and morphological study of *Saprolegnia parasitica*. Mycologia **24**:431-452.

Scott, W. W. 1964. Fungi associated with fish diseases. In: "Developments in industrial microbiology, 5," pp. 109-123. American Institute of Biological Sciences, Washington, DC.

Seymour, R. L. 1970. The genus *Saprolegnia*. Nova Hedwigia. **19**: 1-124.

Tiffney, W. N. 1939. The host range of *Saprolegnia parasitica*. Mycologia **31**: 310-320.

Willoughby, L. G. 1970. Mycological aspects of a disease of young perch in Windermere. J. Fish Biol. **2**: 113-116.

Willoughby, L. G. 1978. Saprolegnias of salmonid fish in Windermere: A critical analysis. J. Fish Dis. **1**: 51-57.

Willoughby, L. G. and Copeland, J. W. 1984. Temperature-growth relationships of *Saprolegnia* pathogenic to fish, especially eels cultivated in warm water. Nova Hedwigia **34**: 35-55.

Willoughby, L. G., McGrory, C. B. and Pickering, A. D. 1983. Zoospore germination of *Saprolegnia* pathogenic to fish. Trans. Br. Mycol. Soc. **80**: 421-435.