

*Piscinoodinium pillulare* (SCHÄPERCLAUS, 1954) LOM, 1981  
(DINOFLAGELLIDA) INFECTION IN CULTIVATED  
FRESHWATER FISH FROM THE NORTHEAST REGION OF  
SÃO PAULO STATE, BRAZIL. PARASITOLOGICAL AND  
PATHOLOGICAL ASPECTS

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**ABSTRACT**

The Aquaculture Center of Unesp, Jaboticabal, SP, Brazil, received fishes for diagnosis from fish farmers reporting fish crowding at pond edges and in water inlets. Fifty-three out of 194 cases showed round to oval, immobile whitish structures, measuring up to 162 µm diameter, identified as the dinoflagellate *Piscinoodinium pillulare*. In 34 diagnosed cases the parasites were present in the gills, in 2 on body surface, and 9 in both. Thirty-one out of 53 were tambacu hybrids hosts; 7, *Piaractus mesopotamicus*; 6, *Colossoma macropomum*; 5, *Leporinus macrocephalus*; 3, *Oreochromis niloticus*; and 1, *Prochilodus lineatus*. Fish showed increased mucous production on body surface and gills, while ecchymosis in the caudal peduncle and operculum was present. The gills also showed paleness, congestion, and petechiae. Histopathology presented a great number of trophonts situated between secondary lamellae, fixed to or detached from the epithelium. Primary lamellae presented interstitial hemorrhages, severe hyperplasia of the epithelium, goblet cells, and mononuclear infiltrates. The present work is the first report of *P. pillulare* in Brazil and emphasizes the importance of this dinoflagellate which caused significant economic losses from 1995 through 1997.

*Key words:* *Piscinoodinium pillulare*, morphology, pathology, cultivated fish, Brazil.

**RESUMO**

**Infecção por *Piscinoodinium pillulare* (Schäperclaus, 1954) Lom, 1981 (Dinoflagellida) em peixes de água doce cultivados na região Nordeste do Estado de São Paulo, Brasil. Aspectos parasitológicos e patológicos**

O Centro de Aqüicultura, Unesp, Jaboticabal, SP, Brasil, recebeu peixes para diagnose, os quais apresentavam aglomeração nas bordas dos viveiros e na entrada da água. Dos 194 casos diagnosticados, 53 apresentavam estruturas brancas circulares ou ovais, imóveis, medindo 162 µm de diâmetro, identificadas como o dinoflagelado *Piscinoodinium pillulare*. Em 34 casos, os parasitos estavam presentes nas brânquias, em 2 casos, no corpo e em 9 casos, em ambos. Dos 53 casos observados, 31 eram o híbrido tambacu; 7, o *Piaractus mesopotamicus*; 6, o *Colossoma macropomum*; 5, o *Leporinus macrocephalus*; 3, o *Oreochromis niloticus*; e 1, o *Prochilodus lineatus*. Os peixes apresentaram aumento da produção de muco no corpo e nas brânquias e equimoses no pedúnculo caudal e nos opérculos. As brânquias também apresentaram palidez, congestão e petéquias. A histopatologia revelou a presença de grande número de trofontes situados entre as lamelas secundárias, fixados ou não ao epitélio. As lamelas primárias

mostraram hemorragias intersticiais, severa hiperplasia do epitélio e das células calciformes e infiltrado inflamatório. O presente trabalho é o primeiro relato de *P. pillulare* no Brasil e enfatiza a importância dos dinoflagelados, que causaram significativas perdas econômicas entre 1995 e 1997.

*Palavras-chave:* *Piscinoodinium pillulare*, morfologia, patologia, peixe cultivado, Brasil.

## INTRODUCTION

Dinoflagellates may be responsible for lethal diseases in marine fishes (Brown, 1934; Brown & Hovasse, 1946; Noga *et al.*, 1991; Smith *et al.*, 1994; Quod *et al.*, 1994). The first dinoflagellate parasite recorded in a fish host was *Oodinium ocellatum* (Brown, 1931). Nigrelli (1936) described morphology, cytology, and life cycle of *O. ocellatum*. Jacobs (1946) and Lucký (1970) reported the occurrence of *O. limneticum* and *O. pillularis* in aquarium fishes, while Williams Jr. (1972) observed *O. cyprinodontum* in the gills of *Fundulus similis*. Later, on, Lom (1981) proposed a new genus *Piscinoodinium* and chose *Oodinium* Chatton, 1912 genus as a synonym. Since then, *Amyloodinium* genus has been reported to have infected marine fishes, and *Piscinoodinium* genus to have infected freshwater fishes. The parasite was observed in the gills of *Oreochromis niloticus*, *Sarotherodon galilaeus*, and *Clarias* sp., from Kainji Lake, Nigeria (Okaeme & Okojie, 1989). Moreover, the presence of *P. pillulare* in Malaysia caused mortality by inducing hyperplastic gills and obstruction of the respiratory surface (Shaharom-Harrison *et al.*, 1990).

Until now, observations related to the occurrence of and infection with this parasite were unknown in Brazil. In the present work, the authors describe histopathological and morphological aspects of *P. pillulare* infection in cultivated fishes from the Northeast region of São Paulo State.

## MATERIAL AND METHODS

From 1995 to 1997, 265 fishes were examined after necropsy at the Laboratory Aquatic Organisms Pathology at the Aquaculture Center, Unesp, Jaboticabal, São Paulo State, Brazil. Water quality was measured with a pH-meter, a conductivity meter (Corning), dissolved oxygen with portable oxymeter (Hach), and water temperature with a mercury bulb thermometer. To search for parasites, the body surface was scraped from tail

to head and pieces of gill 2-3 mm in diameter were mounted between glass microscope slide coverslips for microscopical observation. Bacteriological and fungal examinations were carried out according to Austin & Austin (1987) and Martins & Machado (1994). Parasites found were stained with lugol solution for detecting amyloid granules. A camera lucida was used to draw 39 parasite specimens. All measurements taken were transformed into mean values and are shown with their respective change intervals. For scanning electron microscopy, the infected gill filaments were excised and fixed at room temperature in a 3% glutaraldehyde solution in a 0.1 M phosphate buffer (pH 7.4). Afterwards, they were dehydrated with serial concentrations of acetone, dried with CO<sub>2</sub>, assembled, and coated with 20 nm gold paladium. Coated filaments were examined with a JEOL JSM-25-S2 microscope. Parasitized tissues were fixed in 10% buffered formalin and embedded in a paraffin block. Tissue sections 6 µm thick were cut and stained with haematoxylin-eosin for histopathological analysis.

## RESULTS

The ponds where the fishes originated were situated in Barretos, Barrinha, Cravinhos, Franca, Guariba, Jaboticabal, Sertãozinho, Taiacu, Taiúva, and Taquaral with 240 to 22,000 m<sup>2</sup> of area with 1 to 8 fish/m<sup>2</sup> stocking density. Water quality showed pH 7.8 ± 0.5 (6.9 to 8.3), electric conductivity 136.0 ± 48.1 µS/cm (29 to 205), dissolved oxygen 4.2 ± 1.3 (2.7 to 7.0), and water temperature 17 to 24°C.

It had been reported that fish generally gathered near the pond edges or near the water inlets. Feeding activity decreased because of appetite loss; fish became lethargic, and swam erratically with loss of equilibrium. Mortality progressively increased. According to the farmers, fish ponds were rarely cleaned. In three cases, chicken bowels were fed to the fish.

From January 1995 to August 1997, fish mortality caused by dinoflagellates resulted in 23,000

dead fish, 3,000 of which died in the period of May 1 to 15, 1996. In the same month, 4,000 fishes died in 24 hours at just one fish farm. One hundred ninety-four cases of a total of 265 occurred during the cold season, from May to August, when the water temperature ranged between 17 and 24°C.

Fifty-three cases of a total of 194, showed round to oval, immobile, whitish structures on gills and body surface, measuring up to 162 µm in diameter. Of these, 34 were on the gills, 2 on the body surface, and 9 on both. Thirty-one out of 53 diagnosed cases occurred in a tambacu hybrid (*Colossoma macropomum* female x *Piaractus mesopotamicus* male) of 5.0 to 36.0 cm length; 7 (15.5%) in pacu, *Piaractus mesopotamicus* Holmberg, 1887 of 5.0 to 28.5 cm; six (13.3%) in tambaqui, *Colossoma macropomum* Cuvier, 1818 of 5.0 to 18.0 cm; 5 (11.1%) in piauçu, *Leporinus macrocephalus* Garavello and Britski, 1988 of 26.0 to 31.0 cm; three (6.6%) in tilápia, *Oreochromis niloticus* Trewavas, 1983 of 15.0 to 26.0 cm and 1 (2.2%) in curimbatá, *Prochilodus lineatus* Steindachner, 1881 of 42.0 cm.

Of all examined fish, tambacu hybrid showed 61.3% mortality, with 4,000 fish dying in just one day, and 11,000 in one fish farm. The *P. mesopotamicus* showed 42.8% mortality, *C. macropomum* 40%, and *O. niloticus* and *L. macrocephalus* 33.3%. Secondary infections with myxobacteria and *Saprolegnia* sp. on the body surface were also recorded.

### Parasitology

Immobile, round to oval whitish structures on the gills and body surface of infected fish were observed. Amyloid granules in the parasites were stained by Lugol's solution. Parasite measurements

were as follows: 102.7 µm (62 to 162) in diameter (Fig. 1); in the basal region of the parasites an attachment disc was seen measuring 38.6 µm (24 to 52) diameter; the cell of the attached trophont was pyriform or sac-like (Lom & Schubert, 1983) measuring 47.7 µm (36 to 70) in length and 33.5 µm (22 to 48) in width; the basal end was narrow, measuring 15.1 µm (10 to 20). Pyriform cells were observed in large number on the gills of parasitized fish. A short structure extending from the basal end, measuring 21.4 µm in length, was observed. The parasite was identified as the dinoflagellate *Piscinoodinium pillulare*.

Scanning electron microscopy showed trophonts attached to the gill epithelium in different developmental stages (Figs. 2 and 3).

### Structural changes

On the body surface, increase in mucous production, scalelessness, suffusion, ecchymosis, petechiae, and small ulcers were observed. The gills also presented increase of mucous production besides epithelial hyperplasia, suffusion, petechiae, congestion, edema, and brownish areas (Table 1). Histopathology showed a great number of trophonts present in the secondary lamellae, sometimes with rhizocysts embedded in the epithelium which penetrated firmly into the epithelial cells. Degeneration and focal epithelial necrosis were observed. The primary lamellae showed congestion, subepithelial edema, focal interstitial hemorrhages, severe hyperplasia of epithelial and goblet cells, and inflammatory cells. The secondary lamellae were fused because of a hyperplastic process that formed cavities in which the parasites were present (Fig. 4).

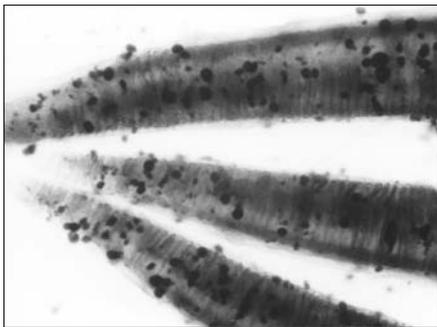


Fig. 1 — Wet mount of gill filament infected by *P. pillulare*. Tambacu hybrid (40 x).

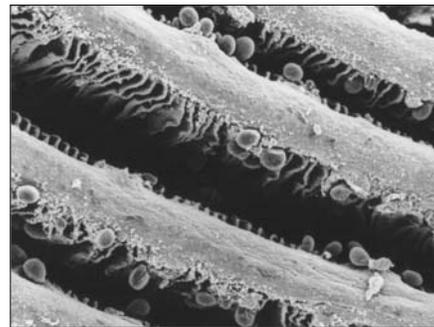
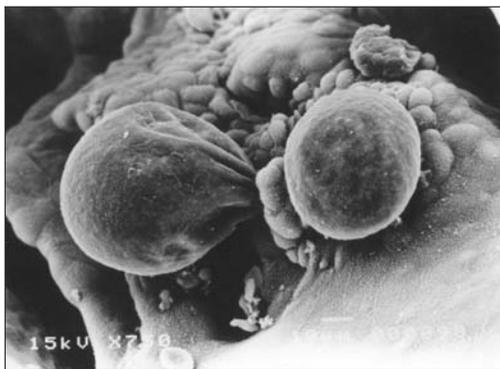
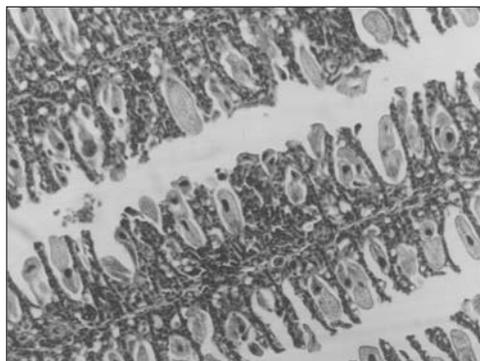


Fig. 2 — Scanning electron microscopy of gill filament infected by *P. pillulare*. Severe infection in tambacu hybrid (SEM 100 x).



**Fig. 3** — Scanning electron microscopy of trophont attached to the gill filament of tambacu hybrid (SEM 750 x).



**Fig. 4** — Parasitic trophonts of *P. pillulare* attached to the gill filament of tambacu hybrid (HE 150 x).

**TABLE 1**  
Macroscopical lesions of Brazilian fish infected with *Piscinoodinium pillulare*.

Fish species	Body surface	Gills
Tambacu hybrid	increase of mucous production, scalelessness of epithelium, suffusion on the operculum and caudal peduncle	paleness, increase of mucous production, congestion, petechiae, hyperplasia
<i>P. mesopotamicus</i>	suffusion, small ulcers	increase of mucous production, edema, petechiae
<i>C. macropomum</i>	scalelessness of epithelium	
<i>L. macrocephalus</i>		tissue with brownish color
<i>O. niloticus</i>	scaleness, suffusion	suffusion
<i>P. lineatus</i>	petechiae, ecchymosis	

In addition, the extension of the hyperplastic process of the epithelial cells filled all interlamellar the space. These epithelium masses obstructed the respiratory surface of the gills (Fig. 5).

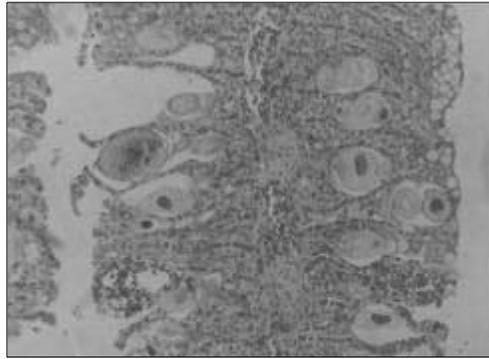
## DISCUSSION

The present work showed the nonspecificity of *P. pillulare* that infects a great number of fish species in different regions of São Paulo State. High mortality caused by severe infection related to lack of cleaning of fish ponds; much organic matter as well as overcrowding were observed. These facts contributed to significant economic losses. Similar parasitosis associated with high levels of organic matter was described by Shaharom-Harrison *et al.* (1990) in *Puntius gonionotus*. The addition of chicken bowels to the fish diet, over population,

and great quantity of food were the main causes of decreasing water quality, favoring parasitic reproduction (Martins, 1998) as observed in the present work.

Of the cases diagnosed, 73.3% occurred from May to August, demonstrating high fish susceptibility to parasites in the cold season when water temperature ranged from 17 to 24°C. Temperatures below 24°C or above 31.5°C have contributed to mortality, according to Shaharom-Harrison *et al.* (1990).

This fact is probably related to better conditions for parasite reproduction and fish resistance decrease caused by stress. Temperatures below 10°C inhibit inflammatory response in ectothermic animals and cause high susceptibility to infections (Bisset, 1948 cited by Finn & Nielsen, 1971; Dias & Sinhorini, 1991; Matushima & Mariano, 1996).



**Fig. 5** — Infection with *P. pillulare*. Note severe hyperplasia with complete obstruction of the respiratory surfaces and haemorrhages (arrow). Tambaçu hybrid (HE 300 x).

Mortality rate was higher in the hybrid tambacu than *P. mesopotamicus* and *C. macropomum*. However, tambacu and *P. mesopotamicus* were the most cultivated fishes in the fish farms.

Severe infections with *P. pillulare* in *P. gonionotus* cultivated in Malaysia from January 1986 through December 1987 were reported by Shaharom-Harrison *et al.* (1990). Some authors observed loss of appetite, lethargy, and agglomeration below the water surface (Siti & Rokiah, 1988; Shaharom-Harrison *et al.*, 1990), corroborating the present observations. Histopathological changes described in Brazilian cultivated fish were the same as those found by Brown (1934), Jacobs (1946), Lucký (1970), Cheung *et al.* (1981a), and Shaharom-Harrison *et al.* (1990) although the geographic region and breed of fish were different.

These lesions initiate a hyperplastic and edematous process in the gills causing changes in the gas and ion interchange surface with consequences for the fish metabolism (Martins & Romero, 1996).

From January 1995 to July 1997, 20% of the cases showed the dinoflagellate *P. pillulare* as the etiological agent. An attachment disc and rhizocysts inserted in the epithelial cells of the host (Lom, 1981) and the presence of amyloid granules (Jacobs, 1946) were used for identification. A flagellate structure situated in the basal extremity of the parasites was observed in just one case in this work, as was also reported by Lom & Schubert (1983). According to Lom (1977, 1981), rhizocyst characteristics, their penetration into the host cells, and presence of chloroplasts constitute the main characteristics of the genus *Piscinoodinium* in freshwater fishes.

Nigrelli (1936) described various stages of the life cycle of *O. ocellatum*. When parasites are

collected from the gills, rhizocysts and flagella retract. For this reason good observation of such structures was not possible in wet mounts. With the aid of scanning electronic microscopy, Cheung *et al.* (1981b) observed a severe epithelial irritation with hyperplastic reaction caused by *O. ocellatum* in the gills of *Zebrassoma flavescens*. These changes provoked increased mucous production and fusion of the lamellae. High organic matter content, low oxygen level, and overcrowding favoured parasite proliferation, causing stress and increasing susceptibility of fish to parasitism.

In conclusion, the present work is the first in Brazil that emphasizes the significance of dinoflagellates in cultivated fishes. The authors add that prophylaxis and water quality are essential factors in management of fish culture.

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