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Amoebic gill infection of turbot, *Scophthalmus maximus*

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Abstract. Amoebae were found to cause severe gill tissue damage in turbot, *Scophthalmus maximus* L. from a grow-out facility in northwestern Galicia (Spain). The nature and extent of lesions along with negative results of bacteriological and virological examination made this agent responsible for mortalities in four turbot stocks supplied with water from a single source. We present our findings, although we failed to isolate amoebae, since there was a clear evidence of their primary role in the development of disease condition and occurrence of mortalities. In addition, this is a record both of a new host endangered by amoebae in intensive cultures and pathogenesis of the gill lesions.

According to the data available thus far, there are very few species of amoebae living as endocommensals in fish. All other amoebae infecting fish are free-living forms which can, under certain conditions, colonize fish (Lom and Dyková 1992). The presence of free-living amoebae in fish tissues is not always associated with a disease condition (Taylor 1977, Franke and Mackiewicz 1982). This applies to internal organs and probably the more so to the gills, which are in direct contact with the aquatic environment. Free-living amoebae use to be trapped on the surface of gill filaments and incorporated into the resident microflora, which is in a balanced relation with the host. Thus far only several species of free-living amoebae were recorded as agents of gill diseases.

The most important outbreaks of amoebic gill diseases resulting in mortalities were recorded in salmonids in both freshwater and marine cultures. *Thecamoeba hoffmani*, discovered on the gills of *Oncorhynchus mykiss*, *O. kisutch* and *O. tschawytscha* and designated by Sawyer et al. (1974) as a free-living species, caused or contributed to mortalities of salmonid fingerlings (Sawyer et al. 1975). Etiology of the proliferative gill disease of rainbow trout, *O. mykiss*, was tentatively attributed to amoebae of the family Cochliopodidae (Daoust and Ferguson 1985). *Paramoeba pemaquidensis* infections in the gills of coho salmon, *O. kisutch*, reared in sea water (in net pens and land-based tanks), continued the sequence of important findings of amoebic gill diseases. Mortalities related to this agent occurred in three subsequent years (Kent et al. 1988). *Paramoeba* gill infections and associated lesions caused in Atlantic salmon, *Salmo salar*, were also described in Tasmania (Roubal et al. 1989). Recently, a recurrent amoebic gill infection was recorded in rainbow trout, *O. mykiss*, cultured in semiclosed recirculating system. The agent, which was introduced to the system with the juveniles, was tentatively assigned to the genus *Cochliopodium* (Noble et al. 1994).

Less numerous were records of amoebic gill diseases in other fish families. Proliferative gill disease associated with the presence of amoebae was described in *Tilapia aurea* by Rogers and Gaines (1975). The same type of gill lesions was also present in systemic amoebiasis in cultured European catfish, *Silurus glanis*. The agent of the latter case was tentatively assigned to the genus *Acanthamoeba* (Nash et al. 1988).

All the above mentioned amoebic gill infections were related to intensive forms of aquaculture. They had in common the course of the initial phase of infection: necrosis of surface epithelial cells due to the attachment of multiplying amoebae, followed by hypertrophy and hyperplasia of cells adjacent to individual amoebae. Also fusion of secondary lamellae was mentioned in most descriptions.

MATERIALS AND METHODS

Clinically diseased specimens of turbot, *Scophthalmus maximus*, reared in a grow-out facility in northwestern Galicia (Spain) were sampled for diagnostic purposes. In addition to bacteriological and virological examinations, tissue samples fixed in Davidson's seawater fixative, embedded in Paraplast and stained with hematoxylin and eosin were examined histologically. In total 11 moribund specimens of turbot from four different stocks were examined in April 1992 to September 1994. The survivors of the last outbreak (nine big specimens

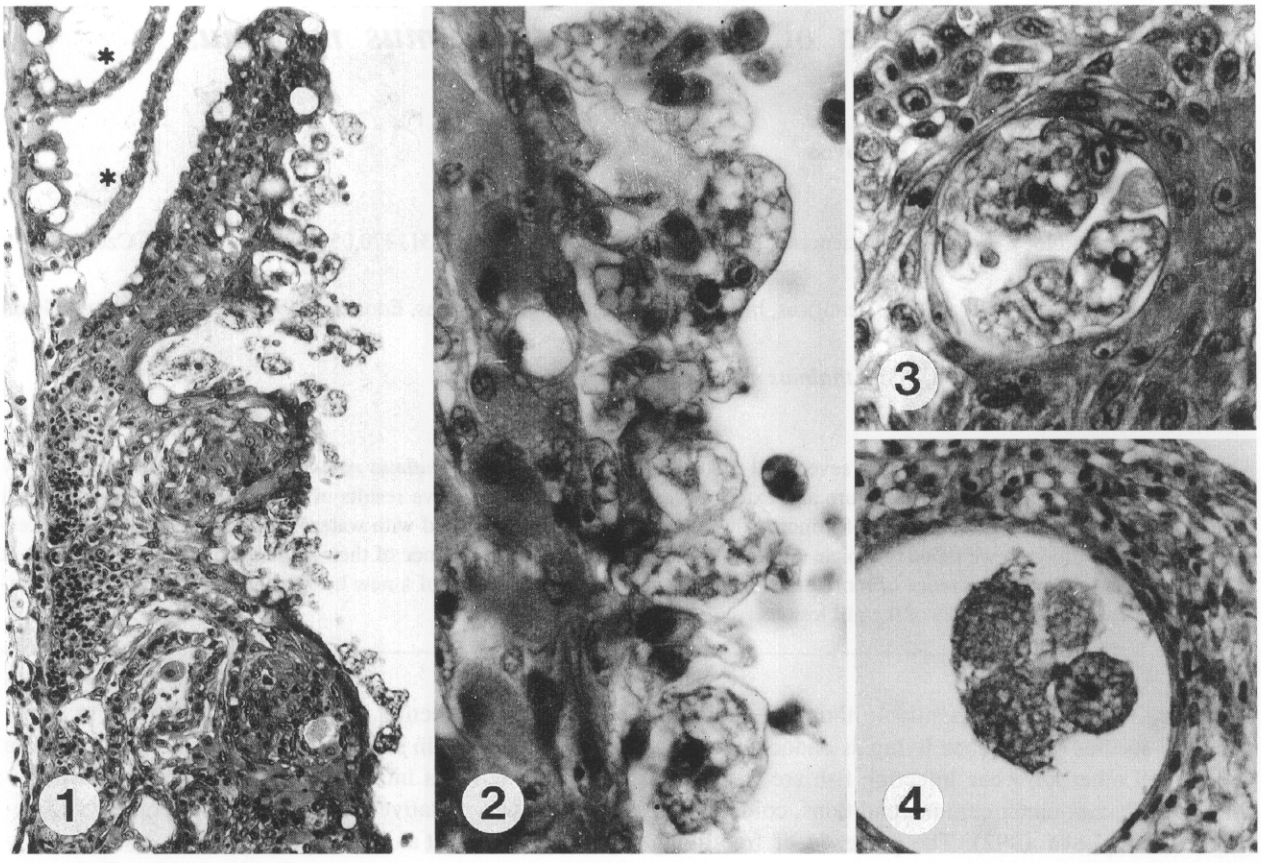

Fig. 1. Part of the turbot gill filament with secondary lamellae altered due to massive infection with amoebae. Two normal, uninfected secondary lamellae marked with asterisks are at the top. HE, × 268. **Fig. 2.** Conspicuously vacuolized trophozoites of amoebae attached to the surface of secondary lamellae. HE, × 947. **Fig. 3.** Two trophozoites in a small cavity between two adjacent secondary lamellae. HE, × 867. **Fig. 4.** Spherical, cyst-forming or "resting" stages of amoebae with equal-sized vacuoles. HE, × 600.

of turbot) were sampled in order to isolate amoebae from the gills. An attempt at isolation of amoebae on petri plates with Bacto marine agar 2216 was made seven months after the last outbreak, when the intensity of infection obviously decreased; only a few amoebae were seen in fresh mounts from gills.

RESULTS

Histological examination of diseased and moribund specimens of turbot revealed the presence of large numbers of amoebae on the surface of the gill tissue, while other organs were free of amoebic infection (Figs. 1–2).

The size of fixed amoebae could be estimated only approximately from sectioned specimens. The maximum length of trophozoites (Figs. 2–3) was 29 μm. Spherical cyst-forming or "resting" stages (Fig. 4) were 18.5 (18.3–19.4) μm in diameter. Numerous vacuoles of a variable size were present in all trophozoites and gave them a foamy appearance.

A comprehensive material available for histological investigation covered different stages of infection and allowed to speculate on the sequence of tissue changes, starting with the most delicate ones up to the loss of branchial structure in large segments of gill filaments.

The gill tissue changes initiated and developed mostly in the secondary lamellae. Their thickening (Figs. 5–6) due to hypertrophy and hyperplasia of epithelium associated with the attachment of amoeba trophozoites and accumulation of amoebae in the interlamellar spaces was followed by the lamellar fusion (Fig. 7). Neighbouring secondary lamellae first adhered to one another and then fused completely, or partly, or focally. Along with the fusion of hypertrophic secondary lamellae, additional alterations occurred. The most important of those were regressive changes manifested as desquamation of the epithelium, local disturbances of blood circulation, and progressive changes represented by inflammation. In the initial stage of lamellar thickening, numerous chloride cells were found also in the distal parts of secondary lamellae (Fig. 6). The major

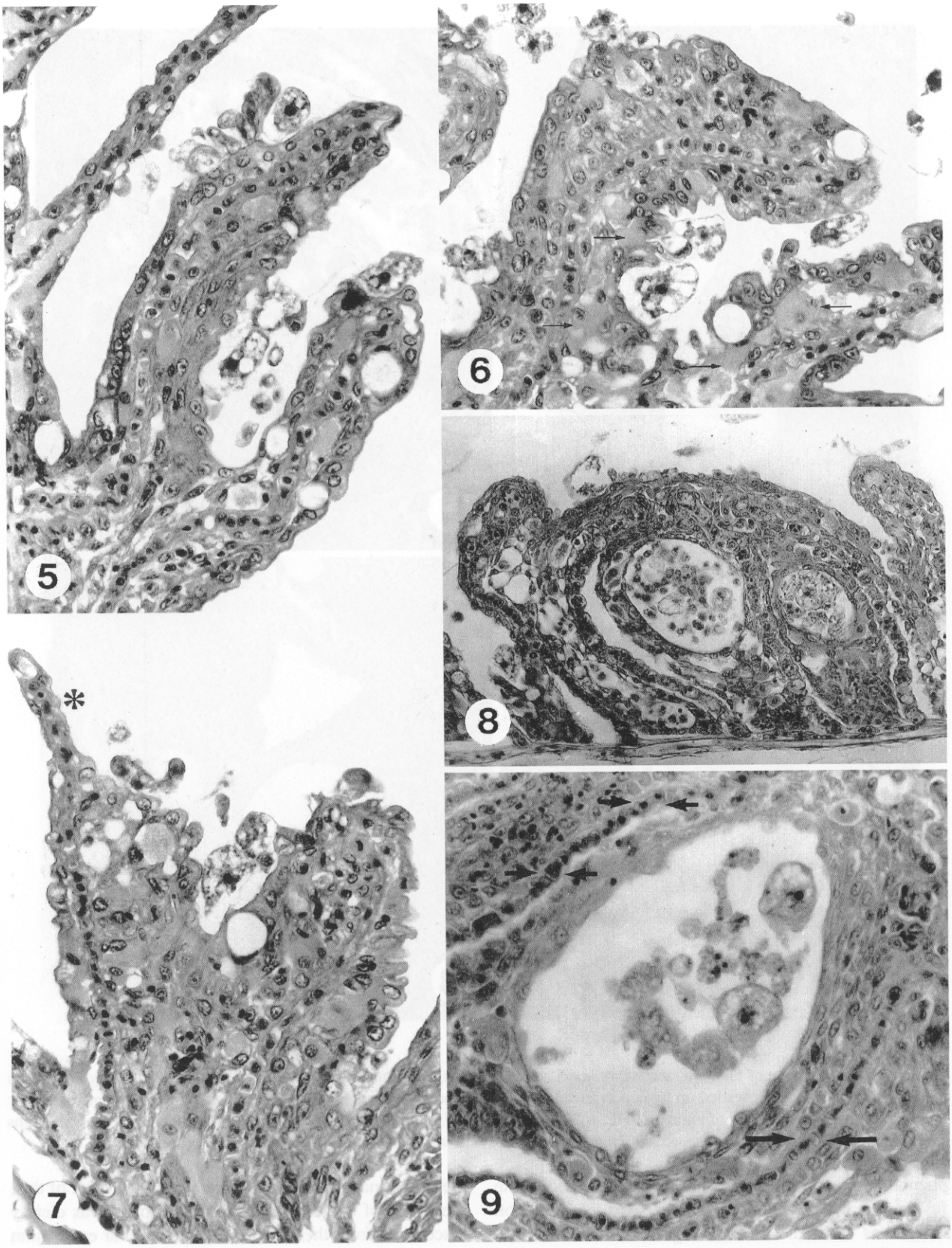

Fig. 5. Thickening of the gill secondary lamellae of turbot with trophozoites of amoebae attached to the surface. HE, × 538. **Fig. 6.** Hypertrophy and hyperplasia of the lamellar epithelium inclusive of specialized chloride cells (arrows) preceding adhesion and fusion of secondary lamellae. HE, × 538. **Fig. 7.** Fusion of two adjacent secondary lamellae. Note their original thickness on the tip of one of the fused lamellae (asterisk). HE, × 538. **Fig. 8.** Part of the gill filament with incompletely fused secondary lamellae and cavities formed among them. HE, × 290. **Fig. 9.** Cavity between two adjacent secondary lamellae; arrows point at the capillaries. HE, × 530.

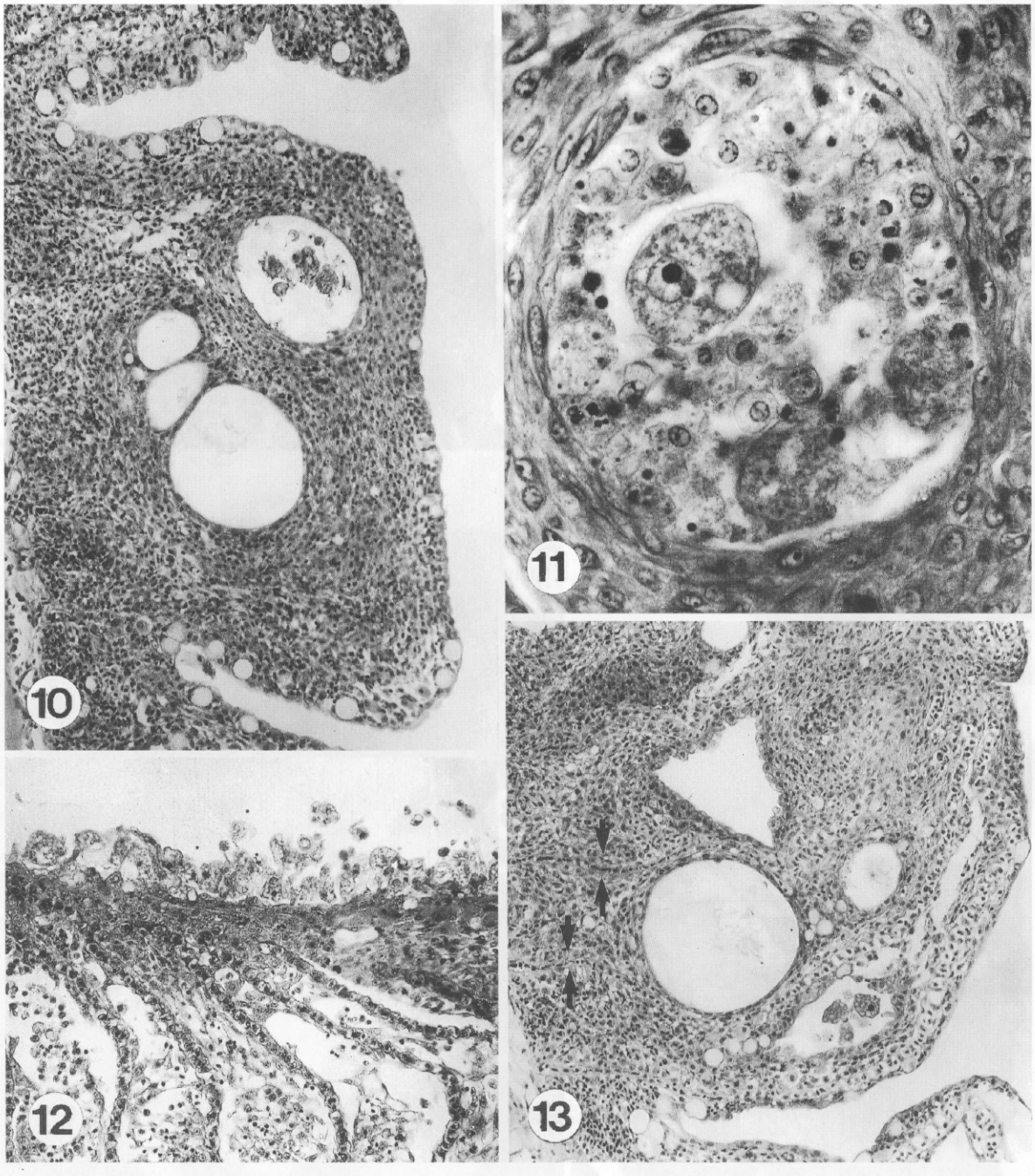

Fig. 10. Advanced stage of fusion of secondary lamellae, revealing signs of inflammation. HE, × 220. **Fig. 11.** Cavity filled with amoebae and tissue debris walled up by concentrically arranged hyperplastic cells. HE, × 875. **Fig. 12.** Fused tips of secondary lamellae covered with a layer of amoebae. HE, × 225. **Fig. 13.** Final stage of changes provoked by the infection; the only signs of the original gill structure are remnants of lamellar capillaries (arrows). HE, × 225.

phenomenon observed in connection with incomplete fusion of leaf-like secondary lamellae was the formation of cavities between lamellae (Figs. 8–11). The cavities contained proliferating amoebae and were the only places where cyst formation was observed. In the advanced stage of infection, secondary lamellae of long segments of gill filaments were fused and disfigured by the inflammatory changes (Figs. 10, 13). In spite of this, the origin of cavities in the confluent parts was clearly discernible from the presence of the lamellar epithelium or capillaries (Fig. 9). The fusion of the tips of secondary lamellae, associated with the oedematous separation of the epithelium in the distal part of the gill lamellar layer, was rather exceptional and difficult to distinguish

from initial postmortal changes. In such cases an almost continuous layer of amoebae was observed on the surface of gill filaments (Fig. 12). While in the initial stage of the lamellar fusion the multiplying trophozoites localized in newly formed cavities were still surrounded by hypertrophic epithelium, later on in the stage of proliferative inflammation, the thin layer of connective tissue formed a discrete wall around the agent (Fig. 11). The number of amoebae localized on the modified gill surface notably diminished in the late stage of infection with the most pronounced inflammatory changes, while small accumulations of trophozoites and stages resembling cysts were still present in the cavities formed originally between two adjacent secondary lamellae. In this stage, the loss of gill respiratory surface area was most pronounced.

The attempt to isolate amoebae from the gills of turbot failed. This was probably because of the late time of sampling (seven months after the last outbreak) when single amoebe only were found in fresh gill scrapings, while bacterial microflora which included *Vibrio* spp. was dominant.

DISCUSSION

Observations and hypotheses on the influence of various predisposing factors on the development of amoebic gill disease (Sawyer et al. 1974, Bullock et al. 1994, Nowak and Munday 1994) were taken into account in the evaluation of pathogenesis of the amoebic gill infection in turbot. No evidence of tissue changes associated with other agents was recognized in the samples of gills from the specimens of various stocks and age groups. Although lamellar hypertrophy is a frequently reported reaction to a wide range of exogenous toxicants and pathogens, individual uninfected secondary lamellae, present in all stages of amoebic infection, did not display any structural changes attributable to the negative influence of water quality. We cannot exclude pre-existing long-lasting or short-term conditions of stress related to intensive form of culture as well as other factors the monitoring of which is very difficult. Pathogenesis of the branchial lesions and the lack of tissue changes prior to the appearance of epithelial hyperplasia associated with the attachment of amoebae convinced us that amoebae were the primary agent.

In addition to the cellular proliferation adjacent to individual amoebae we found other similarities with previously described amoebic gill diseases. Among gill structural changes, formation of cavities was mentioned also in *Paramoeba pemaquidensis* infection. Kent et al. (1988) called them vesicles or interlamellar vesicles. Nowak and Munday (1994) described formation of cribriform three-dimensional lamellar complex after the fusion of secondary lamellae.

Although stress-related compensatory mechanisms of gas exchange ability have already been mentioned (Goldes et al. 1988), the drastic reduction of surface respiratory area of gills caused by the adherence and fusion of secondary lamellae and the consequent disappearance of interlamellar spaces in the course of amoebic infection, seems to be the critical factor for respiratory efficiency and general health condition.

There were no doubts of the appurtenance of the agent to the subphylum Amoebozoa, but the diagnostic features available in histological sections did not permit a more accurate diagnosis. Our plans for future isolation and determination of the amoebae have to take into account that there might be other free-living species of amoebae with the same pathogenic potential.

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