

Neoparamoeba sp. and other protozoans on the gills of Atlantic salmon *Salmo salar* smolts in seawater

Mairéad L. Bermingham*, Máire F. Mulcahy

Environmental Research Institute, Aquaculture and Fisheries Development Centre, Department of Zoology, Ecology and Plant Science, National University of Ireland, Cork, Ireland

ABSTRACT: Protozoan isolates from the gills of marine-reared Atlantic salmon *Salmo salar* smolts were cultured, cloned and 8 dominant isolates were studied in detail. The light and electron-microscopical characters of these isolates were examined, and 7 were identified to the generic level. Structure, ultrastructure, a species-specific immunofluorescent antibody test (IFAT), and PCR verified the identity of the *Neoparamoeba* sp. isolate. Five other genera of amoebae, comprising *Platyamoeba*, *Mayorella*, *Vexillifera*, *Flabellula*, and *Nolandella*, a scuticociliate of the genus *Paranophrys*, and a trypanosomatid (triosomatid-bodonid incertae sedis) accompanied *Neoparamoeba* sp. in the gills. The pathogenic potential of the isolated organisms, occurring in conjunction with *Neoparamoeba* sp. in the gills of cultured Atlantic salmon smolts in Ireland, remains to be investigated

KEY WORDS: Amoebic gill disease · *Neoparamoeba* sp. · Amoebae · *Platyamoeba* sp. · Scuticociliates · Trypanosomatids

—Resale or republication not permitted without written consent of the publisher—

INTRODUCTION

Various protozoans have been associated with gill disease in fish. Those causing the most serious mortalities in fish are generally free-living species of amoebae (Dyková et al. 2000). The first free-living amoeba to be associated with gill disease was *Vahlkamfia mucicola* in the peacock wrasse *Symphodus tinca* and corkwing wrasse *S. melops* (Chatton 1909). Subsequently, other amoebae species have been implicated in gill disease including *Thecamoeba hoffmani* in rainbow trout *Oncorhynchus mykiss*, *Neoparamoeba pemaquidensis* in Atlantic salmon *Salmo salar*, *Platyamoeba* sp. in turbot *Scophthalmus maximus*, and other unidentified amoebae in Atlantic salmon and rainbow trout (Leiro et al. 1998, Bermingham & Mulcahy 2004, Dyková & Lom 2004, Buchmann et al. 2004).

In Ireland, as in Tasmania, *Neoparamoeba* sp. has been implicated as the primary agent of amoebic gill disease (AGD) in Atlantic salmon (Munday et al.

1990, Palmer et al. 1997). However, simultaneous isolation of amoebae other than *Neoparamoeba* sp. from the gills of clinically diseased fish has raised the question of the possible involvement of such amoebae in the disease (Dyková et al. 2000, Bermingham & Mulcahy 2004). Six genera in addition to *Neoparamoeba* have been isolated from the gills of Atlantic salmon with, and at risk of, AGD in Tasmania, comprising: *Acanthamoeba*, *Flabellula*, *Heteroamoeba*, *Platyamoeba*, *Vannella* and *Vexillifera* (Howard 2001). Species belonging to the genera *Platyamoeba*, *Vannella* and *Flabellula* were found to accompany *Neoparamoeba* sp. in clinically diseased turbot in Spain (Dyková et al. 1999). In addition, protozoans other than amoebae have been isolated along with *Neoparamoeba* sp. from AGD-affected gills, such as scuticociliates from turbot, European sea bass, *Dicentrarchus labrax* and sharp snout seabream *Puntazzo puntazzo* (Dyková & Novoa 2001), and flagellates and scuticociliates from Atlantic salmon smolts (Bermingham & Mulcahy 2004). Fur-

*Email: mbermingham@hotmail.com

thermore, in the Irish situation, *Neoparamoeba* sp. has been observed in the gills of Atlantic salmon smolts throughout the year, but with no statistical association with gill lesions, wherein it appears to be outcompeted by other protozoans colonising the gills (Bermingham 2004, Bermingham & Mulcahy 2004).

This research examined the diversity of protozoans colonising the gills of marine cultured Atlantic salmon smolts with, or at risk of, AGD in Ireland.

MATERIALS AND METHODS

An enclosed sheltered site 'Marine Harvest Ireland' (Marine Harvest), the Cranford site, based in Kindrum, Fanad, County Donegal, was selected for study. AGD problems have been experienced annually at this site since a 1995 outbreak, when mortalities reached 60% (C. McManus pers. comm.). Atlantic salmon smolts (S1, 66 to 72 g) at this site were transferred to the sea at the Cranford site in October 2000 and 2001. A monthly gill sample (n = 10) was taken from April to September 2001 during Year 1, and a sample was taken twice monthly from May to September 2002 in Year 2.

Protozoans were isolated on non-seeded 0.6%/75% seawater/agar and subsequently cultured on *Pseudomonas* sp.-seeded 1.5%/75% seawater/agar, according to the methods described by Dyková et al. (1998) and Howard (2001). The 1.5% agar medium proved too dry for the highly motile flagellate and scuticociliate isolates, so a medium of 0.6%/75% seawater/agar was prepared. Isolates were cloned (Taylor 1977) and subcultured every 4 to 6 d. Each isolate was subcultured at least 5 times before being stored in liquid nitrogen (−196°C).

The isolates were observed employing the hanging drop method (Dyková et al. 2000). The petroleum jelly cover slip method (Foissner 1991) was employed to retard the highly motile scuticociliate and flagellate isolates, in order that the living cells could be measured and structural details photographed. The isolates were measured under a light microscope and photographed (400 ASA black and white Kodak film) employing a microscope equipped with Normarski differential interference optics. For examination of ultrastructure/surface membranes, the isolates were fixed in 3% glutaraldehyde fixative in 1.0 M cacodylate buffer, postfixed in 1% osmium tetroxide solution, and prestained with 5% uranyl acetate, embedded in Araldite-resin, cut into ultrathin sections, stained with uranyl acetate and lead citrate, and examined using a Jeol JEM 100CX transmission electron microscope operating at 50 kV.

The *Neoparamoeba* sp. isolates were verified using *Neoparamoeba* sp.-specific immunofluorescent anti-

body test (IFAT) (Howard 2001) and polymerase chain reaction (PCR) of *N. pemaquidensis* 18S ribosomal RNA gene (rRNA), using specific forward *N. pemaquidensis* primer fPA-Hxe23bl and reverse primer rPA-Hx49 (Elliott et al. 2001).

The Protargol technique was used to reveal the infraciliature and silverline system of the scuticociliate isolate (Foissner 1991). The slides were examined under a light microscope, and drawings revealing the position of macro and micronucleus; oral and infraciliatures were made. The scuticociliates were prepared for scanning electron microscopy (SEM) by Dr. I. Dyková, M. Vererková and Dr. I. Fiala at the Institute of Parasitology, Academy of Sciences of the Czech Republic, according to the methods described by Foissner (1991). Observations were made using a Jeol 6300 scanning electron microscope with TESCAN 1101 system image analysis.

RESULTS

Of 92 isolates cultured from the gills of Atlantic salmon smolts over the study period, only 8 of the most dominant isolates were examined in detail due to time constraints. Species-specific IFAT and PCR demonstrated that 16 prepared isolates (34%) belonged to the genus *Neoparamoeba*.

Isolate 1, composed of flabellate trophozoites with a flattened anterior hyaloplasm, occupying approximately $\frac{1}{3}$ to $\frac{1}{2}$ of the cell, extending around the sides of a thicker granuloplasmic mass (Fig. 1A), was assigned to the family Vannellidae. Transmission electron microscopy (TEM) revealed a thin amorphous glycocalyx, composed of tightly packed filamentous elements (Fig. 1B), which lacked the distinct pentagonal glycostyles of the genus *Vannella*, assigning this isolate to the genus *Platyamoeba* (Page 1988). Isolate 1 closely resembled *P. bursella*, with trophozoites ranging between 15 and 36 μm (\bar{x} 23 μm), L:B (length/breadth ratio) 1.0 to 1.8 (\bar{x} 1.3) and possessed a thin amorphous glycocalyx extending 20 nm above the plasma membrane (Page 1983).

Isolate 2, composed of compressed trophozoites, which were longer than broad that produced digitiform hyaline subpseudopodia from an anterior hyaloplasm (Fig. 1C), was assigned to the family Paramoebidae (Page 1988). The thick cuticle, composed of a fibrillar matrix (Fig. 1D), assigned this isolate to the genus *Mayorella* (Page 1988). Isolate 2 closely resembled *M. pussardi*, with trophozoites ranging between 13 and 26 μm (\bar{x} 16 μm), L:B 1.0 to 2.4 (\bar{x} 1.2), and possessing a thick cuticle 274 nm in diameter (Page 1983).

Isolate 3, composed of trophozoites with an irregular shape that produced slender subpseudopodia from an

anterior hyaloplasm (Fig. 1E), a thin cuticle (14 nm), and a glycocalyx differentiated into glycostyles (Fig. 1F), was assigned to the family Vexilliferidae (Page 1988). The subpseudopodia, which were carried back along to the posterior end, giving a spiny appearance to the isolate, and the fine structure of glycostyles, which extended approximately 47 nm beyond the plasma membrane, assigned this isolate to the genus *Vexillifera* (Page 1988). Isolate 3 closely resembled *V. armata* with trophozoites ranging between 13 and 23 μm (\bar{x} 19 μm), L:B 1.1 to 2.2 (\bar{x} 1.4) (Page 1983).

Isolate 4, composed of flattened flabellate trophozoites with a prominent anterior hyaloplasm, and an irregular and rapidly changing form (Fig. 1G), was assigned to the family Flabellulidae (Page 1988). The absence of subpseudopodia and the formation of hyaline clefts during forward motion assigned this isolate to the genus *Flabellula* (Page 1983). Isolate 4 most closely resembled *F. calkinsi* with trophozoites ranging between 13 and 23 μm (\bar{x} 19 μm), L:B 1.1 to 2.2 (\bar{x} 1.4) with a thin amorphous glycocalyx 13 nm above the plasma membrane (Fig. 1I). Though a limax-like form was observed (Fig. 1H), the uriodal filaments described in *F. calkinsi* and *F. citata* were not observed (Page 1983, Howard 2001).

Isolate 5, a limax amoeba with tubular mitochondrial cristae (Fig. 1K), was assigned to the family Hartmannellidae (Page 1988). The eruptive activity, production of hyaline bulges (Fig. 1J), and thick surface coat composed of a fibrillar matrix (Fig. 1L) assigned this isolate to the genus *Nolandella* (Page 1983). Isolate 5 resembled *N. hibernica*, with limax trophozoites ranging between 10 and 23 μm (\bar{x} 16 μm), L:B 1 to 1.5 (\bar{x} 1.2). However, the trophozoites possessed a thick glycocalyx extending 209 nm above the plasma membrane, which is much thicker than that reported for *N. hibernica* (Page 1983).

Isolate 6 comprised irregularly shaped trophozoites with a raised posterior granular region and more flattened anterior hyaline zone from which digitiform subpseudopodia were produced (Fig. 1M), and a thick glycocalyx (274 nm) differentiated into glycostyles (Fig. 1N), and was assigned to the family Vexilliferidae (Page 1988). An oval parasome (3 μm) (Fig. 1M) that moved with the nucleus (3 μm) was present in the living amoebae. The parasome was not observed in TEM. The presence of or more parasomes adjacent to the nucleus, and absence of scales, assigned this isolate to the genus *Neoparamoeba* (Dyková et al. 2000). Species-specific IFAT and PCR confirmed the identification of this isolate (Fig. 1O to P). Isolate 6 closely resembled *N. pemaquidensis* with trophozoites ranging between 13 and 26 μm (\bar{x} 21 μm), L:B 1.0 to 2.0 (\bar{x} 1.2), with conspicuous hair-like glycostyles extending 134 nm above the plasma membrane (Page 1983, Kent

et al. 1988). However, the secondary fibrillar matrix (Fig. 1N) that enveloped the glycostyles may either represent a processing related artefact or indicate that this isolate represents a separate species. Because the primer set employed in this study has shown cross-reactivity with the recently identified *N. branchiphila* (Dyková et al. 2005), it is possible it could cross-react with other *Neoparamoeba* spp.

Isolate 7 was a scuticociliate, which lacked a ribbed wall between the paroral dikinetid toward the cytostome and paroral velum (Fig. 1S), and was placed in the order Philasterida. The midventral postoral alignment of the paroral segment (scuticoves-tige) (Figs. 1S,U & 2C) assigned this isolate to the family Orchitophryidae (available at: www.uoguelph.ca/~ciliates). The orchitophryids of Isolate 8 were composed of slender scuticociliates with a cylinder body shape that tapered at the apical end (Figs. 1Q,S–U & 2A–C), an oral field half the cell length, a buccal apparatus consisting of 3 closely distributed membranes (Figs. 1T,U & 2C) to the left-hand side of a distinctly bipartite paroral membrane (Figs. 1S & 2C), and a prolonged caudal cilium (13 μm) (Figs. 1Q & 2A), and were assigned to the genus *Paranophrys*. A large macronucleus (10 to 15 μm , \bar{x} 12 μm ; L:B 1.1 to 2.2 μm , \bar{x} 1.4 μm) was located centrally and a micronucleus (1 to 3 μm , \bar{x} 2 μm ; L:B 1.0 to 2.0 μm , \bar{x} 1.1 μm) positioned anteriorly to the macronucleus (Fig. 2A,B). A single contractile vacuole was terminally located (Figs. 1Q & 2A) and opened at lower end of somatic kinet 2 (Figs. 1S & 2C,D). Isolate 8 most closely resembled *P. marina* in size (15 to 36 μm , \bar{x} 22 μm ; L:B 1.6 to 4.5 μm , \bar{x} 2.6 μm) and form. However the *Paranophrys* sp. isolate possessed only 9 somatic monokinetids (Figs. 1V & 2B–D), as opposed to the 10 dikinetids of *P. marina* (Song et al. 2002). A resting cyst stage was also observed, with its greatest dimensions ranging from 10 to 21 μm (\bar{x} 15 μm), L:B 1.0 to 2.0 μm (\bar{x} 1.2 μm).

Isolate 8 comprised flagellates with a kinetoplast associated with the flagellar base, and 2 tubular mitochondria (Fig. 1X), and was assigned to the order Kinetoplastida (Frolov et al. 2001). This kinetoplastid isolate was composed of epimastigote flagellates with a single recurrent flagellum (Fig. 1W) and undulating membrane (Fig. 1X), and was assigned to the family Trypanosomatidae (Maslov et al. 2001). However, the presence of a large kinetoplast (Fig. 1X), 868 nm in diameter, suggested a bodonid lineage (Maslov et al. 2001). This flagellate isolate may represent an incertae sedis, as it is generally believed that trypanosomatids descended from within the bodonids (Lukeš et al. 2004). The greatest dimensions of this isolate were 3 to 4 μm (\bar{x} 3 μm), L:B 1.0 to 1.5 μm (\bar{x} 1.2 μm).

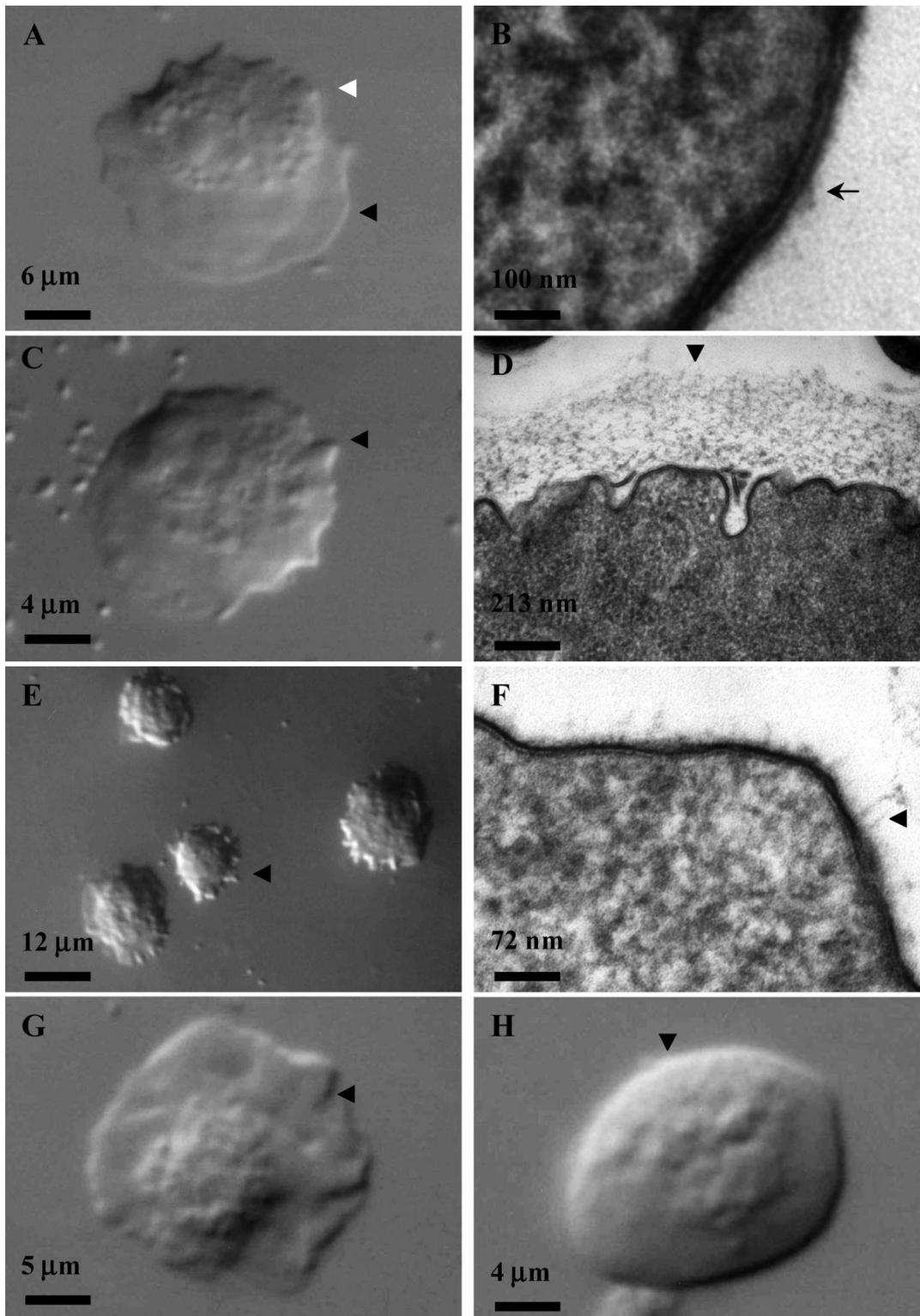


Fig. 1. (above and next 2 pages) Isolates from gills of salmon smolts *Salmo salar*. (A) *Platyamoeba* sp. (scanning electron microscopy [SEM], ► = hyaloplasm, ▷ = granuloplasm); (B) *Platyamoeba* sp. showing a thin anorphous cell surface (transmission electron microscopy [TEM], arrow = filamentous elements); (C) *Mayorella* sp. (SEM, ► = hyaloplasm); (D) *Mayorella* sp. showing a thin complex cuticle (TEM, ► = glycoalyx); (E) *Vexillifera* sp. (SEM, ► = conical subpseudopodia); (F) *Vexillifera* sp. showing a thin cuticle (TEM, ► = subunits of glycoalyx); (G,H) *Flabellula* sp. (G: SEM, ► = hyaline clef; H: SEM, cylindrical form, ► = limax-like crest)

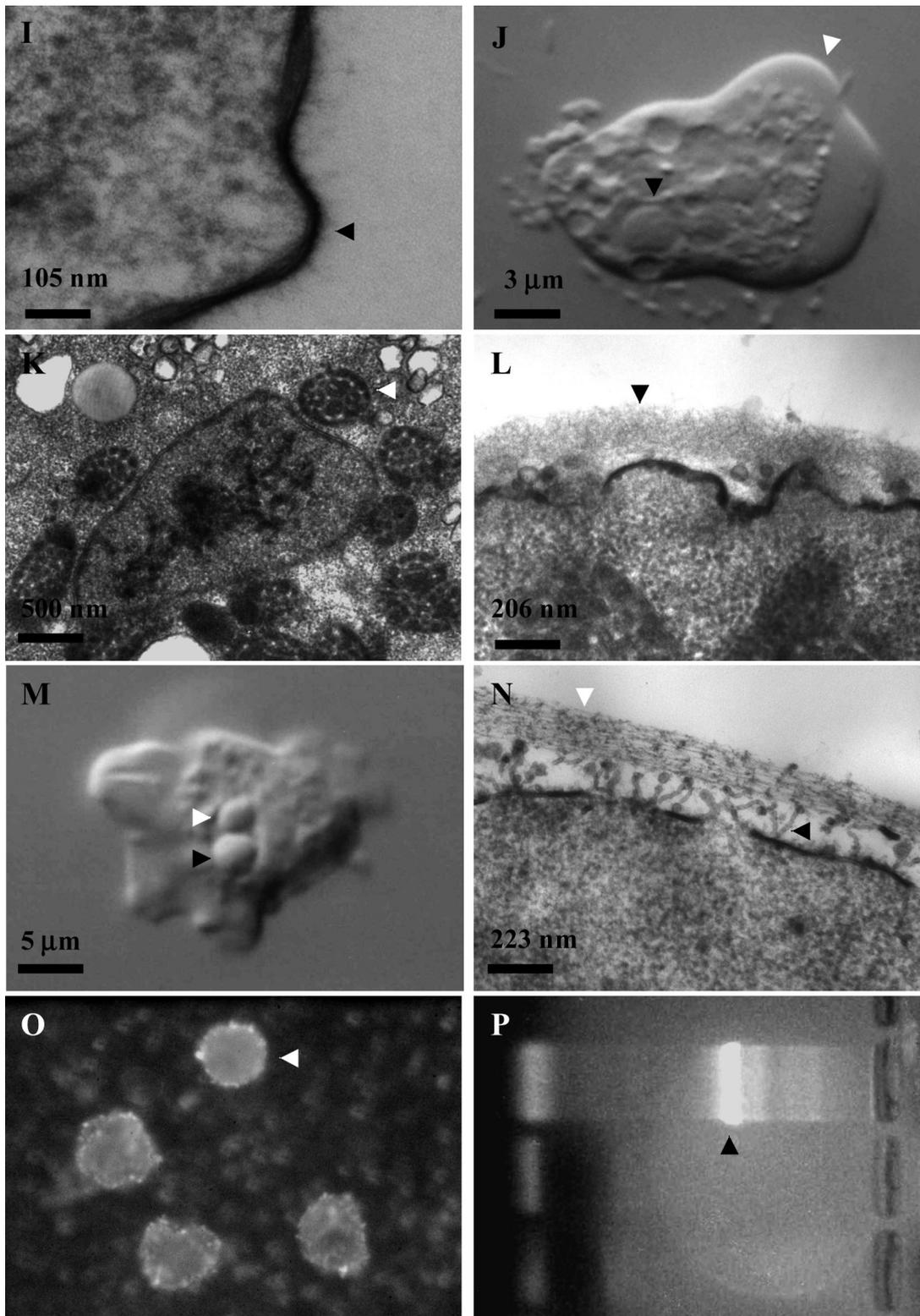


Fig. 1 (continued). (I) *Flabellula* sp. showing a thin amorphous glycocalyx (TEM, \blacktriangleright = glycocalyx); (J) *Nolandella* sp. (SEM, \blacktriangleright = nucleus, \triangleright = hyaloplasmic bulge); (K,L) *Nolandella* sp. (K: TEM, \triangleright = tubular mitochondrion; L: TEM, \blacktriangleright = thick glycocalyx); (M) *Neoparamoeba* sp. (SEM, \blacktriangleright = nucleus, \triangleright = parasome); (N) *Neoparamoeba* sp. showing a thick glycocalyx with hair-like glycostyles covered by a secondary fibrillar matrix (TEM, \blacktriangleright = glycostyle, \triangleright = fibrillar matrix); (O) *Neoparamoeba* sp. (\triangleright), specific immunofluorescent antibody (IFAT); (P) 18 S rDNA amplicon (\blacktriangleright); positive and negative controls are not shown

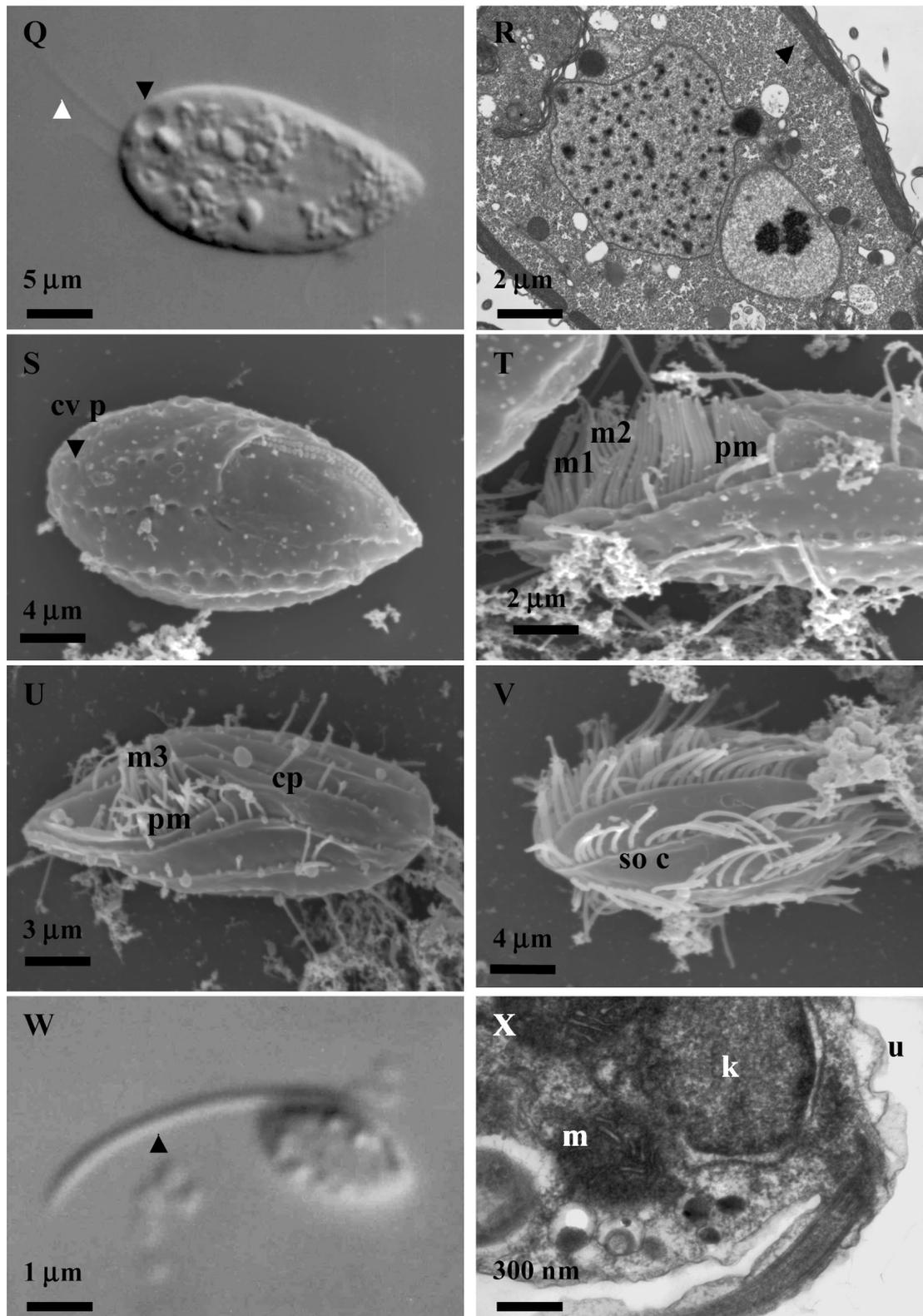


Fig. 1 (continued). (Q) *Paranophrys* sp. (SEM, \blacktriangleright = contractile vacuole, \blacktriangleright = caudal cilium); (R) *Paranophrys* sp. (TEM, \blacktriangleright = mucocyst); (S to V) *Paranophrys* sp. (SEM, cv p = contractile vacuole pore, m1–3 = membranoids 1–3, pm = paroral membrane, cp = cytoproct, so c = somatic cilia); (W) trypanosomatid (SEM, \blacktriangleright = flagellum); (X) trypanosomatid (TEM, k = kinetoplast, m = plate-like mitochondrial cristae, u = undulating membrane)

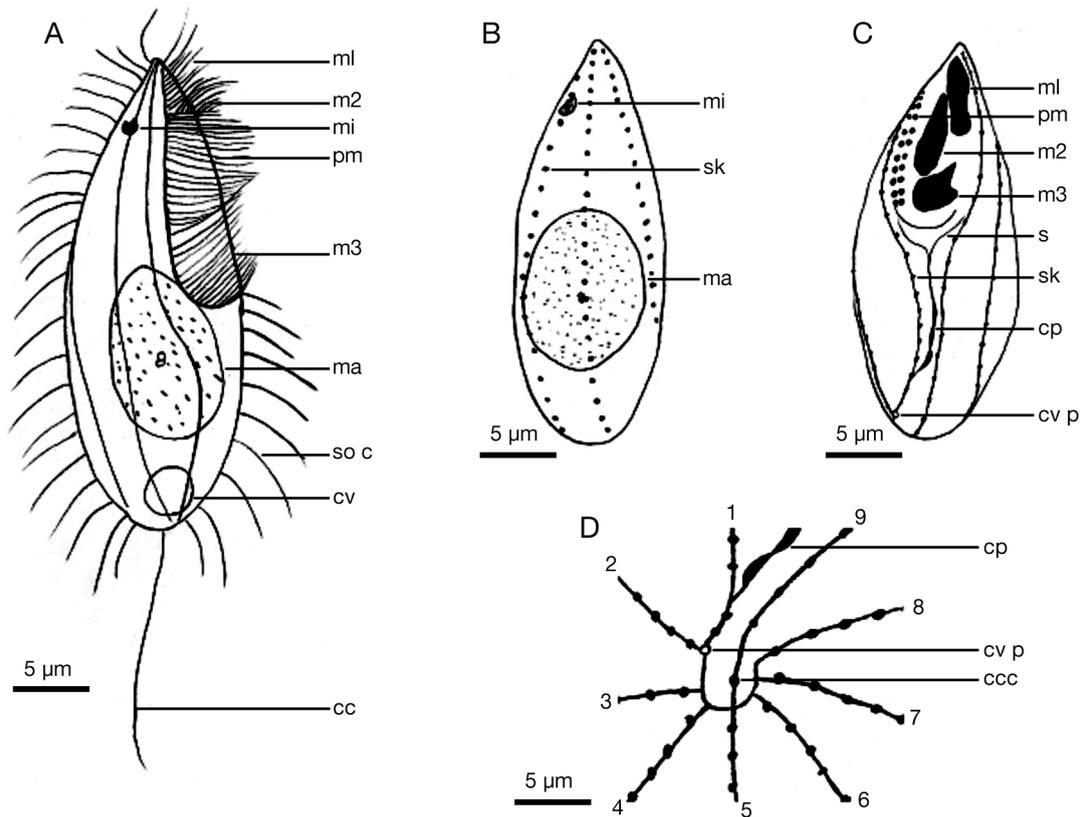


Fig. 2. *Paranophrys* sp. (A) *in vivo* and (B–D) after protargol impregnation: (B) dorsal; (C) ventral; (D) caudal view. cc = caudal cilium; ccc = caudal cilium complex; cp = cytoproct; cv = contractile vacuole; cv p = contractile vacuole pore; m1–3 = membranoids 1–3; ma = macronucleus; mi = micronucleus; pm = paroral membrane; s = scutica; sk = somatic kinety; so c = somatic cilia

DISCUSSION

Only 8 of the 92 protozoan isolates cultured from the smolt gills were examined in detail, due to time constraints. The significance of the 84 undetermined isolates is unknown, and needs further resolution. Isolate selection was on the basis of isolation frequency, relative abundance within the gill microfaunal community over time as seen in the monthly/twice monthly samples, and light microscopical features of free and cultured isolates. The majority of isolates prepared belonged to the genus *Neoparamoeba*. This is in contrast to earlier findings where *Platyamoeba* and *Vannella* were the most common amoebae genera isolated from the gills of both cultured Atlantic salmon and turbot with or at risk of AGD (Dyková et al. 1999, Howard 2001).

Isolate 1 was identified as a vannellid amoeba of the genus *Platyamoeba* (order Euamoebida). *Platyamoeba* is the most commonly isolated marine euamoebid genus (Page 1983). Amoebae belonging to the genus *Platyamoeba* were the most commonly isolated amoebae from the gills of Atlantic salmon with, and at risk of, AGD in Tasmania (Howard 2001). Furthermore,

species belonging to the genus *Platyamoeba* have been reported from turbot with AGD in Spain (Leiro et al. 1998). Simultaneous isolation of *Platyamoeba* sp. and *Neoparamoeba* sp. and the association of this latter genus with gill disease raises the question of the possible pathogenic role of *Platyamoeba* sp. in the gills of cultured Atlantic salmon in Ireland.

Isolate 2 was identified as a paramoebid amoeba of the genus *Mayorella*. *Mayorella* is a widely distributed euamoebid genus in both marine and freshwater systems (Page 1983). There have been no reports that this genus is pathogenic. However, the paramoebid *Paramoeba eilhardi* accompanied *Neoparamoeba* sp. in the gills of Atlantic salmon with, and at risk of, AGD in Tasmania (Howard 2001). Isolation of this genus in conjunction with *Neoparamoeba* sp. and other protozoa raises the question of its contributory role in the progression of gill disease in cultured salmonids in the Irish situation.

Isolate 3 was identified as a vexilliferid amoeba of the genus *Vexillifera*. The euamoebid genus *Vexillifera* has been isolated from both marine and freshwater systems (Page 1988). *Vexillifera* sp. has also accompanied *Neoparamoeba* sp. in the gills of Atlantic salmon

with AGD in Tasmania (Howard 2001). Furthermore *V. bacillipedes* has been reported as the causative agent of seasonal epizootics of systemic amoebiasis in hatchery-reared rainbow trout in Italy (Sawyer et al. 1978). The genus *Vexillifera* has therefore, not only demonstrated an ability to colonise gills, but has also demonstrated pathogenic competence.

Isolate 4 was identified as a flabellulid amoeba of the genus *Flabellula*. The euamoebid genus *Flabellula* has only been described from marine systems (Page 1983). *Flabellula* sp. was thought to be involved in AGD in Tasmania, as *Flabellula* sp. alone were isolated from the gill of cultured Atlantic salmon with severe AGD (Howard 2001). *Flabellula* sp. has also been isolated from turbot with AGD (Dyková et al. 1999). This genus has not only demonstrated an ability to colonise gills, but has also been associated with disease.

Isolate 5 was identified as a limax hartmannellid amoeba of the genus *Nolandella*. A single marine species of the euamoebid genus *Nolandella* has been described (Page 1983); it has not been reported as pathogenic. However, the ability of other limax amoebae to colonise fish and elicit disease has been established. For example, an undetermined hartmannellid amoeba has been associated with amoebiasis in goldfish (Voelker et al. 1977). Furthermore, a limax vahlkamfiid, *Heteramoeba* sp. was found to accompany *Neoparamoeba* sp. in the gill of cultured Atlantic salmon with severe AGD in Tasmania (Howard 2001). A free-living hartmannellid *Saccamoeba limax* has also been isolated from the gills of Atlantic salmon (Dyková et al. 2002).

Isolate 6 was identified as a vexilliferid amoeba of the genus *Neoparamoeba*. *Neoparamoeba* sp. is a ubiquitous marine euamoebid that has been isolated and cultured from the marine environment in many parts of the world, including biofouling communities on salmon sea cages, and marine and estuarine sediments (Tan et al. 2002, Crosbie et al. 2003). *N. pemaquidensis* is the causative agent of AGD in finfish culture, in France, Ireland, Spain, United States, Tasmania and New Zealand (Nowak et al. 2002). *N. branchiphila* has also been isolated from gills turbot in Spain, and Atlantic salmon with clinical AGD in Tasmania (Dyková et al. 2005). *N. invadens* and *N. perniciosus* are associated with systemic infection of the green sea urchin *Strongylocentrotus drobachiensis* and the blue crab *Callinectes sapidus*, respectively (Sprague et al. 1969, Jones 1985). The ability of this genus to colonise gills and effect disease has been clearly demonstrated. However, the pathogenicity of this genus in the Irish situation remains unconfirmed, as *Neoparamoeba* sp. has been found colonising smolt gills throughout the year; however, its abundance does not correlate with gill lesion frequency or severity, and it appears to be out com-

peted by accompanying protozoans (Bermingham & Mulcahy 2004). Nevertheless, *Neoparamoeba* sp. has been established as a primary pathogen in Tasmania, where it attaches to healthy gill epithelium, resulting in subsequent lesion development (Adams & Nowak 2004). Therefore, the pathogenicity of this genus in the Irish situation needs resolution.

Isolate 7 was identified as an orchitophryid scuticociliate of the genus *Paranophrys* (order Philasterida). The subclass Scuticociliatia occurs abundantly in coastal areas, particularly in eutrophic mariculture waters; for example, *P. marina* is an ectocommensal within the mantle cavity of farmed bay scallop *Argopecten irradians* (Song et al. 2002). It is known that some free-living scuticociliates can infect fish and shellfish under still unknown conditions (Dragesco et al. 1995). *Paranophrys* sp. is an opportunistic secondary parasite of cultured fleshy prawn *Penaeus chinensis*, in which it colonises pre-existing wounds, and subsequently invades the haemolymph and damages various organs including the gills (available at: www.pac.dfo-mpo.gc.ca/sci/shelldis/pages/cildsp_e.htm). The scuticociliate *Philasterides dicentrarchi* has also caused severe infection of the gills and other organs of cultured turbot and European sea bass (Dragesco et al. 1995, Paramá et al. 2003). Furthermore, undetermined scuticociliates accompanied *Neoparamoeba* sp. in the gills of turbot in Spain, sharp snout sea bream and European sea bass cultured in the Mediterranean (Dyková & Novoa 2001) and Atlantic salmon in Ireland (Bermingham & Mulcahy 2004). The ability of members of this subclass to colonise their host and elicit disease has been clearly demonstrated.

Isolate 8 was identified as a trypanosomatid-bodonid incertae sedis (order Kinetoplastida). Free-living kinetoplastids are a main and permanent component of aquatic ecosystems (Arndt et al. 2000). The bodonids have a variety of life cycles ranging from free-living to parasitic, the latter being well-represented ectoparasites of fish gill and skin, for example, *Cryptobia branchialis* and *Ichthyobodo necator* (Callahan et al. 2002, Kuperman et al. 2002). However, trypanosomatids are obligatory endoparasitic organisms; *Trypanosoma burresoni*, for example, is a haemoflagellate of the American eel *Anguilla rostrata* (Jones & Woo 1993). *Ichthyobodo*-like flagellates have been associated with, and isolated from, gills of diseased cultured Atlantic salmon smolts in Ireland (Bermingham 2004). The ability of kinetoplastids to colonise fish and elicit disease has been demonstrated.

Neoparamoeba together with 5 other genera of amoebae comprising *Platyamoeba*, *Mayorella*, *Vexillifera*, *Flabellula* and *Nolandella*, a scuticociliate of the genus *Paranophrys*, and trypanosomatids (trypanoso-

matid-bodonid incertae sedis) were isolated from the gills. A number of these genera of amoeba, including *Neoparamoeba*, *Platyamoeba*, *Vexillifera*, *Flabellula* have been implicated in disease of cultured teleosts (Leiro et al. 1998, Dyková et al. 1999, Howard 2001). Similarly, some scuticociliates and kinetoplastids are proven agents of local and systemic disease in cultured teleosts (Callahan et al. 2002, Paramá et al. 2003). The pathogenic role of these protozoa in smolt gills needs to be determined. While *Neoparamoeba* sp. plays a primary role in AGD in Tasmania (Adams & Nowak 2004), this does not appear to be the case, at least universally, in Ireland (Bermingham & Mulcahy 2004). This raises the question of the role of the other protozoans, isolated in conjunction with *Neoparamoeba* sp., in gill disease of cultured salmon smolts in Ireland.

Experiments are now in progress to determine the pathogenicity of the cultured isolates in the gills of marine reared Atlantic salmon smolts.

Acknowledgements. Funding was from Marine Harvest Ireland, Fanad County Donegal. We are grateful for the help and advice of the staff of 'Marine Harvest' throughout this study, to Dr. P. Dockery, Dr. D. O'Leary, B. Riedewald, M. Heapes and M. Cotter for EM training and advice, and for the use of EM facilities.

LITERATURE CITED

- Adams MB, Nowak BF (2004) Experimental amoebic gill disease of Atlantic salmon, *Salmo salar* L.: further evidence for the primary pathogenic role of *Neoparamoeba* sp. (Page 1987). *J Fish Dis* 27:105–113
- Arndt H, Dietrich D, Auer B, Cleven E, Gräfenhan T, Weitere M, Mylnikov A (2000) Functional diversity of heterotrophic flagellates in aquatic ecosystems. In: Leadbeater BSC, Green JC (eds) *The flagellates*. Taylor & Francis, London, p 240–268
- Bermingham ML (2004) The multifactorial aetiology of amoebic gill disease (AGD) in Ireland. PhD thesis, National University of Ireland, Cork
- Bermingham ML, Mulcahy MF (2004) Environmental risk factors associated with amoebic gill disease in cultured salmon, *Salmo salar* (L.) smolts in Ireland. *J Fish Dis* 27:555–571
- Buchmann K, Nielsen T, Sigh J, Bresciani J (2004) Amoebic gill infections of rainbow trout in freshwater ponds. *Bull Eur Assoc Fish Pathol* 24:97–91
- Callahan HA, Litaker RW, Noga EJ (2002) Molecular taxonomy of the suborder Bodonina (order Kinetoplastida), including the important fish parasite, *Ichthyobodo necator*. *J Eukaryot Microbiol* 49:119–128
- Chatton E (1909) Une amibe, *Amoeba mucicola* n. sp., parasite des labres, associée a une trichodine. *C R Séances Soc Biol* 67:690–692
- Crosbie PBB, Nowak BF, Carson J (2003) Isolation of *Neoparamoeba pemaquidensis* Page, 1987 from marine and estuarine sediments in Tasmania. *Bull Eur Assoc Fish Pathol* 23:241–244
- Dragesco A, Dragesco J, Coste F, Gasc C, Romestand B, Raymond JC, Bouxi G (1995) *Philasterides dicentrarchi* n. sp., (Ciliophora, Scuticociliata), a histophagous opportunistic parasite of *Dicentrarchus labrax* (Linnaeus 1758), a reared marine fish. *Eur J Protistol* 31:327–340
- Dyková I, Lom J (2004) Advances in the knowledge of amphizoic amoebae infecting fish. *Folia Parasitol* 51:81–97
- Dyková I, Novoa B (2001) Comments on diagnosis of amoebic gill disease (AGD) in turbot, *Scophthalmus maximus*. *Bull Eur Assoc Fish Path* 21:40–44
- Dyková I, Lom J, Machááková B, Pecková H (1998) *Vexillifera expectata* sp. and other non-encysting amoebae isolated from organs of freshwater fish. *Folia Parasitol* 45:7–26
- Dyková I, Figueras A, Novoa B (1999) Epizoic amoebae from the gills of turbot *Scophthalmus maximus*. *Dis Aquat Org* 38:33–39
- Dyková I, Figueras A, Peric Z (2000) *Neoparamoeba* Page, 1987: light and electron microscopic observations on six strains of different origin. *Dis Aquat Org* 43:217–223
- Dyková I, Veverková M, Fiala I, Machááková B (2002) A free-living amoeba with unusual pattern of mitochondrial structure isolated from Atlantic salmon, *Salmo salar* L. *Acta Protozool* 41:415–419
- Dyková I, Nowak BF, Crosbie PBB, Fiala I, Pecková H, Adams MB, Machááková B, Dvofíáková H (2005) *Neoparamoeba branchiphila* n. sp., and related species of the genus *Neoparamoeba* Page, 1987: morphological and molecular characterization of selected strains. *J Fish Dis* 28:49–64
- Elliott N, Wong F, Carson J (2001) Detection and abundance of *Neoparamoeba pemaquidensis* in the environment. In: Battaglene EC, Cobcroft JM (eds) *The first scientific conference of the Atlantic salmon subprogram handbook*. CSIRO Marine Laboratories, Hobart, Tasmania, p 12–20
- Foissner W (1991) Basic light and scanning electron microscopic methods for taxonomic studies of ciliated Protozoa. *Eur J Protistol* 27:313–330
- Frolov AO, Karpov SA, Mylnikov AP (2001) The ultrastructure of *Proccryptobia sorokini* (Zhukov) comb. nov. and rootlet homology in kinetoplastids. *Protistology* 2:85–95
- Howard T (2001) Paramoebiasis of sea-farmed salmonids in Tasmania: a study of its aetiology, pathogenicity, and control. PhD thesis, University of Tasmania, Hobart
- Jones DP (1985) The role of oxygen concentration in oxidative stress: hypoxic and hyperoxic models. In: Sies H (ed) *Oxidative stress*. Academic Press, Orlando, FL, p 151–195
- Jones SRM, Woo PTK (1993) Biology of *Trypanosoma burresoni* n.sp. from the American eel, *Anguilla rostrata*. *J Parasitol* 79:542–547
- Kent ML, Sawyer TK, Hedrick RP (1988) *Paramoeba pemaquidensis* (Sarcocystidophora: Paramoebidae) infestation of the gills of coho salmon *Oncorhynchus kisutch* reared in sea water. *Dis Aquat Org* 5:163–169
- Kuperman BL, Matey VE, Barlow SB (2002) Flagellate *Cryptobia branchialis* (Bodonida: Kinetoplastida), ectoparasite of tilapia from the Salton Sea. *Hydrobiologia* 473:93–102
- Leiro J, Paniagua E, Ortega M, Paramá A, Fernández J, Sanmartín ML (1998) An amoeba associated with gill disease in turbot, *Scophthalmus maximus* (L.). *J Fish Dis* 21: 281–288
- Lukeš J, Guilbride JL, Votýpka J, Zíková A, Benne R, Englund PT (2004) The kinetoplast DNA network: evolution of an improbable structure. *Eukaryot Cell* 1:495–505
- Maslov DA, Podlipaev SA, Lukeš J (2001) Phylogeny of the Kinetoplastida: taxonomic problems and insights into the evolution of parasitism. *Mem Inst Oswaldo Cruz* 96: 397–402
- Munday BL, Foster CK, Roubal FR, Lester RGJ (1990) Paramoebic gill infection and associated pathology of

- Atlantic salmon, *Salmo salar*, and rainbow trout, *Salmo gairdneri*, in Tasmania. In: Perkins FO, Cheng TC (eds) Pathology in marine science. Academic Press, San Diego, CA, p 215–222
- Nowak BF, Carson J, Powell MD, Dyková I (2002) Amoebic gill disease in the marine environment. Bull Eur Assoc Fish Path 22:144–147
- Page FC (1983) Marine Gymnamoebae. Institute of Terrestrial Ecology, Culture Centre for Algae and Protozoa, Cambridge
- Page FC (1988) A new key to fresh water and soil Gymnamoebae. Freshwater Biological Association, Ambleside
- Palmer R, Carson J, Ruttledge M, Drinan E, Wagner T (1997) Gill disease associated with *Paramoeba*, in sea reared Atlantic salmon in Ireland. Bull Eur Assoc Fish Path 17: 112–114
- Paramá A, Iglesias R, Álvarez MF, Leiro J, Aja C, Sanmartín ML (2003) *Philasterides dicentrarchi* (Ciliophora: Scuticociliatida): experimental infection and possible routes of entry in farmed turbot, *Schophthalmus maximus*. Aquaculture 217:73–80
- Sawyer TK, Ghittino P, Andruetto S, Pernin P, Pussard M (1978) *Vexillifera bacillipedes* Page, 1969, an amphizoic amoeba of hatchery rainbow trout in Italy. Trans Am Microsc Soc 97:596–600
- Song W, Honggang MA, Mei W, Mingzhuang Z (2002) Comparative studies on two closely related species *Uronemella filificum* (Kahl, 1931) and *Uronema elegans* (Maupas, 1883) with redescription of *Paranophrys marina* (Thompson et Berger, 1965) (Ciliophora: Scuticociliatida) from China Seas. Acta Protozool 41:263–278
- Sprague V, Beckett RL, Sawyer TK (1969) A new species of *Paramoeba* (Amoebida, Paramoebidae) parasitic in the crab, *Callinectes sapidus*. J Invertebr Pathol 14:167–174
- Tan CKF, Nowak BF, Hodson SL (2002) Biofouling as a reservoir of *Neoparamoeba pemaquidensis* (Page 1970) the causative agent of amoebic gill disease in Atlantic salmon. Aquaculture 210:49–58
- Taylor PW (1977) Isolation and experimental infection of free-living amoebae in fresh water fishes. J Parasitol 63:232–237
- Voelker FA, Anver MR, McKee AE, Casey HW, Breuniman GR (1977) Amebiasis in goldfish. Vet Pathol 14:247–255

Editorial responsibility: Dieter Steinhagen,
Hannover, Germany

Submitted: June 23, 2006; Accepted: November 16, 2006
Proofs received from author(s): June 22, 2007