***Neoparamoeba/Paramoeba***
(protist: amoeba)

**Overview**

Protists are single-celled organisms with membrane-bound nuclei (eukaryotes). Protists which move and feed using pseudopodia (false feet) are known as amoebae. Rather than forming a monophyletic group, amoebae are divided into three major disparate groups: Heterolobosea (supergroup Excavata), Rhizaria (supergroup SAR) and Amoebozoa (supergroup Amorphea). The latter contains four classes of amoebae differing in their types of pseudopodia and organelles: Tubulinea, Discosea, Gracilipodia and Archamoebae. Discosean amoebae are flattened in profile and form broad lobopodia. Vexilliferid amoebae form digitiform (finger-like) subpseudopodia (dactylopodia) emerging from the cell-wide lobopodium and most species have a fibrillar surface layer (glycocalyx) with embedded scales. Most species do not form cysts and are found as free-living aquatic organisms, although some can be opportunistic pathogens of marine hosts. The paramoebae are characterized by the presence of parasomes (Nebenkorper) which are semi-crystalline folded arrays on minicircle DNA in the kinetoplasts (mitochondria) of eukaryotic endosymbionts. Several species have been linked to disease and death in marine fish and invertebrates. *Neoparamoeba perurans* is currently recognized as the aetiological agent of amoebic gill disease (AGD) principally of salmonids, although other species (*N. pemaquidensis*, *N. branchiphila*) may be associated with diseases.

**Classification**:
Domain: Eukaryota (membrane-bound nucleus)
Supergroup: Amorphea (unikonts with single flagellum, or nonflagellated amoebae)
Phylum: Amoebozoa (locomotion by noneruptive pseudopodia, asexual development)
Subphylum: Lobosa (with lobose pseudopodia)
Class: Discosea/Flabellinea (flattened forms, protoplasmic flow polyaxial)
Order: Dactylopodida (tapering finger-like subpseudopodia (= dactylopodia), most do not form cysts)
Family: Vexilliferidae (long slender subpseudopodia, spiny appearance, many with glycostyles/scales on cell surface, paramoebids with parasomes (Nebenkorper) near nucleus)
Genus: *Neoparamoeba/Paramoeba* (parasitic on gills of fish, trophozoites without scales)
Species: various species (cause amoebic gill disease (AGD), mainly in salmonids)

**Parasite biodiversity and host range:** Protists are unicellular eukaryotes that move using undulipodia (flagella or cilia), pseudopodia (false-feet) or a unique gliding motion. Amoebae form pseudopodia to move and feed. Several types of amoebae are recognized on the basis of differences in their biology and morphology, with recent molecular phylogenetic studies supporting their classification into three major disparate phyla: Heterolobosea (supergroup Excavata), Amoebozoa (supergroup Amorphea) and Rhizaria (supergroup SAR). Most species are free-living in aquatic and terrestrial habitats where they feed on other micro-organisms, but several species have become symbiotic in metazoan organisms as endocommensals or opportunistic-facultative parasites (representatives tabulated below).

| Higher taxonomy | Class/Order | Family | Genus | Hosts | Tissues (disease)* |
|---|---|---|---|---|---|
| Supergroup: Excavata (with conspicuous ventral feeding groove) | | | | | |
| Group: **Discoba** (diverse group supported robustly by molecular studies) | | | | | |
| Phylum: Heterolobosea (amoebo-flagellates, most form cysts) | O: Schizopyrenida (no fruiting bodies) | Vahlkampfidae (eruptive limax pseudopodia, flagellated stages) | *Naegleria* | mammals | central nervous system (PAM) |
| Supergroup: Amorphea (unikonts with single flagellum, or nonflagellated amoebae) | | | | | |
| Phylum: **Amoebozoa** (locomotion by noneruptive pseudopodia, asexual development) | | | | | |
| Subphylum: Conosa (archamoebae & mycetozoa) | C: Archamoebae (amitochondriate, rounded cysts) | Entamoebidae (uninucleate, endozoic) | *Entamoeba* | mammals | colon (dysentery), central nervous system (SAM) |
| Subphylum: Lobosa (lobose amoebae) | C: Discosea (flattened forms, protoplasmic flow polyaxial) | Vexilliferidae (dactylopodia, parasomes) | *Paramoeba* *Neoparamoeba* | fish | gills (AGD) |
| | C: Longamoebea (flattened elongated cells, stellate cysts) | Acanthamoebidae (acanthopodial subpseudopodia) | *Acanthamoeba, Balamuthia* | mammals | central nervous system (GAE) |

| Supergroup: SAR (Stramenopiles + Alveolata + Rhizaria) (3 groups robustly supported by molecular studies) | | |
|---|---|---|
| Group: **Rhizaria** (amoebae with fine pseudopodia in simple, branching or anastomosing patterns) | | |
| Phylum: Cercozoa | Filosa (with filopodia, naked or testate) | free-living (aquatic, terrestrial) |
| Phylum: Endomyxa | heterotrophic amoeboid or plasmodial cells | free-living, some parasitoids |
| Phylum: Retaria | Foraminifera (with reticulopodia), Radiolaria (with axopodia) | free-living (aquatic) |

*PAM = primary amoebic meningoencephalitis; SAM = secondary amoebic meningoencephalitis; GAE = granulomatous amoebic encephalitis; AGD = amoebic gill disease.

Amoebae that move using noneruptive pseudopodia are placed in the phylum Amoebozoa, either in the subphylum Lobosa (with lobose pseudopodia) or Conosa (with microtubular cones). Lobose amoebozoans include the Dactylopodida with finger-like subpseudopodia (= dactylopodia) and the Centramoebida with finely-tapering subpseudopodia (= acanthopodia). Vexilliferids are spiny dactylopodid amoebae most of which do not form cysts. They include the paramoebae which are characterized by the possession of parasomes (Nebenkorper) adjacent to the nucleus. They are naked amoebae (without tests or shells) and have simple life-cycles (without temporary flagellated stages). Paramoebae are found free-living in marine waters throughout the world, particularly in areas with high levels of nutrients such as tidal marshes, intertidal zones and marine sediments. Several species have been found as opportunistic pathogens in fish, particularly in association with amoebic gill disease (AGD) in salmonids. The causative agent of AGD may not be a single species but an assemblage of similar species. Amoebae recovered from affected fish were initially identified morphologically as *Paramoeba pemaquidensis*, and later reassigned to the genus *Neoparamoeba* when it was found they lacked surface scales. Molecular studies using 18S rRNA gene sequences then led to the differentiation and characterization of *N. pemaquidensis* and *N. branchiphila* from the gills of affected fish, but their role in AGD aetiology was presumptive. Recent molecular studies, including fluorescent *in situ* hybridization (FISH), demonstrated the aetiological agent of AGD to be the novel species *N. perurans* and this work has been validated by parasite culture and experimental transmission studies (thus fulfilling Koch's postulates). Infections are found predominantly in cultured salmonids, including sea-caged Atlantic salmon and rainbow trout in Tasmania, pen-reared coho in USA and Canada and chinook salmon in New Zealand. Several *Paramoeba* species have also been found in association with mass mortalities in crabs, sea urchins and lobsters, predominantly in North America.

| Protozoan species | Hosts | Sites | Disease | Geographic location |
|---|---|---|---|---|
| *Neoparamoeba branchiphila* | Salmoniformes: salmonid (Atlantic salmon) | gills | AGD? | Australia |
| *Neoparamoeba pemaquidensis* | Salmoniformes: salmonid (Atlantic salmon, rainbow trout, brown trout), Pleuronectiformes: pleuronectid (flounder), Scombriformes: gempylid (barracouta), Perciformes: serranid (seabass, seabream), Syngnathiformes: syngnathid (seahorses) | gills | AGD? | Australia, North America, Europe |
| *Neoparamoeba perurans* | Salmoniformes: salmonid (Atlantic salmon, coho, chinook, rainbow trout), Pleuronectiformes: pleuronectid (turbot), Osmeriformes: plecoglossid (ayu) | gills | amoebic gill disease (AGD) | Australia, New Zealand, North and South America, Europe |
| *Paramoeba invadens* | Echinodermata: echinoid (sea urchins) | radial nerve/water vascular canal tissues | mass mortality | North America |
| *Paramoeba pernicosa* | Crustacea: portunid (blue crab, rock crab, European green crab) | haemal spaces, connective tissue, organs | gray crab disease | North America |
| *Paramoeba* sp. | Crustacea: nephropid (lobster) | nervous system, muscles, gills | red tail, limp lobster | North America |

**Parasite morphology**: These small lobose amoebae have only been found as trophozoites (cyst formation does not occur) and they lack the well-organized cell surface structures of other vexilliferids (such as hexagonal glycostyles or surface scales). The trophozoites are subspherical measuring from 15-40 μm in length although they can range in size from 10-75 μm. They form numerous (up to 50) digitiform pseudopodia when floating and mammiliform pseudopodia when adherent to substrates. Trophozoites have an extensively flattened hyaline zone with an irregular anterior margin, and occasionally conical pseudopodia projecting forwards. The trophozoites contain a single vesicular nucleus (5-7 μm in diameter) with a large central endosome as well as one or more intracellular perinuclear bodies (2-5 μm) known as parasomes (or secondary nuclei, Nebenkorper, amphisomes) which have recently been found to be folded arrays of minicircle DNA in the kinetoplast of eukaryotic endosymbionts known as *Perkinsiella amoebae*-like organisms (PLOs) related to the marine flagellates *Ichthyobodo*.

**Site of infection**: In fish, amoebae attach themselves to the surfaces of the gills and rarely penetrate the epithelium. In blue crabs, amoebae were found haemal spaces, connective tissues, muscles, hepatopancreas and in heavy infections, in the central nervous system. In sea urchins, amoebae were widespread in all tissues but consistently localized in radial nerve/water vascular canal tissue. In lobster, amoebae were frequently detected in the central nervous system but also in the gills and muscles.

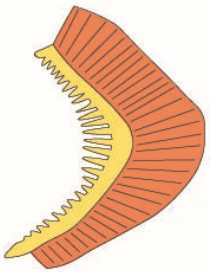
**Pathogenesis**: In fish, the attachment of amoebae to the gill epithelia resulted in extensive epithelial hyperplasia and hypertrophy with proliferation of amoebae in multifocal lesions. Fish became lethargic and displayed respiratory distress with flared opercula and behavioural changes, such as accumulating at the surface, reduced feeding and sometimes reversed postures (ventral side up). There was extensive mucus on the gills (mucoid branchitis) with clubbed necrotic gill filaments developing focal white-grey patches in heavy infections, conditions drastically reducing gill function and possibly predisposing to secondary bacterial infections. Histologically, there was prominent epithelial hyperplasia and fusion of distal portions of the secondary lamellae, often resulting in the formation of large interlamellar vesicles. Mortalities up to 50% have been reported. Fish vary greatly in their susceptibility to AGD, with Atlantic salmon and rainbow trout being most susceptible. Fish with existing gill lesions due to jellyfish damage, clubbing and necrosis gill syndrome are often rapidly colonized by amoebae with severe outcomes. In crabs, clinical signs included tissue displacement and lysis (esp. hemocytes) with significant decreases in haemocyanin contributing to the grayish appearance of the ventral exoskeleton (hence the name gray crab disease). In sea urchins, parasites were associated with significant decreases in coelomocytes and mass mortalities. In lobsters, amoebae were associated with lethargy and coagulopathy (‘limp lobster’) and morbid lobster frequently had red discolouration (gaffkemia) typical of ‘red tail’.

**Developmental cycle and mode of transmission:** The amoebae are normally free-living marine organisms but they can opportunistically infect fish (termed amphizoic). AGD is transmitted horizontally when mucus containing amoebae sloughs off infected gills into the water column and amoebae are then free to colonize the gills of other fish. Attachment to the gills is facilitated by lectin/glycoconjugated bonds and characteristic gill lesions appear 2-4 days later and dramatically increase by day 7. Infections are most prevalent during late summer and autumn, in seawater at temperatures of 8-9°C or above, and in seawater with high salinity (>32 ppt (parts per thousand)), although infections have been found at 7.2 ppt. Infections in crabs may also be transmitted by the consumption of moribund or dead crabs.

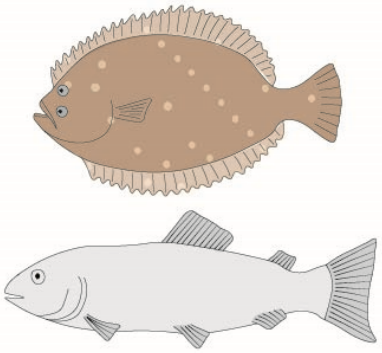
**Differential diagnosis**: AGD is diagnosed by the detection of large numbers of amoebae in fresh wet mounts of gills, although they can be difficult to differentiate from epithelial cells. Amoebae can be found in stained histological sections (especially using periodic acid-Schiff’s (PAS) reagent), but their numbers may be reduced due to detachment from gill surfaces during fixation and processing. Nuclei and parasomes are readily visible using Feulgen DNA stains. Polyclonal antibodies have been developed to immunolabel amoebae in sections, but they exhibit cross-reactivity between different species. Amoebae can be cultured *in vitro* from clinical samples, either by malt yeast agar with seawater overlaid or on salmon embryo cell lines. Different amoebae species are relatively similar morphologically - they are best differentiated by PCR amplification of 18S rRNA gene sequences.

**Treatment and control**: Outbreaks of AGD in tanks and pens have been associated with high stocking densities (overcrowding), microfouling and biofouling (often due to overfeeding and organic enrichment), stagnant water (poor flow), warm water (12-20ºC) and high salinity (>32 ppt). Conventional external treatments of affected fish with formalin, chelated copper, diquat, malachite green and chloramine were not preventive or curative. The most effective treatment was freshwater bathing of diseased fish for 2-6 hours which provided symptomatic relief and substantially reduced the number of amoebae (good for land-based tanks, but difficult for sea-based netpens). Bath treatment with hydrogen peroxide (100-200 ppm for 1-2 hours) also reduced numbers of amoebae but there was a narrow safety margin before toxicity problems arose. Aquaculture enterprises have adopted various management strategies in attempts to prevent and/or control outbreaks of AGD, with some successes attributed to keeping fish cohorts separated at different sites (rather than co-mingled), reducing stocking densities, and positioning sea-cages further apart and in estuarine waters with lower salinity.

# *Neoparamoeba, Parameoba*



gills
(mucoid branchitis,
gill clubbing/necrosis,
amoebic gill disease)



Vertebrate Hosts
(fish, esp.
salmonids)

digitiform
lopopodia

parasome
(Nebenkorper)

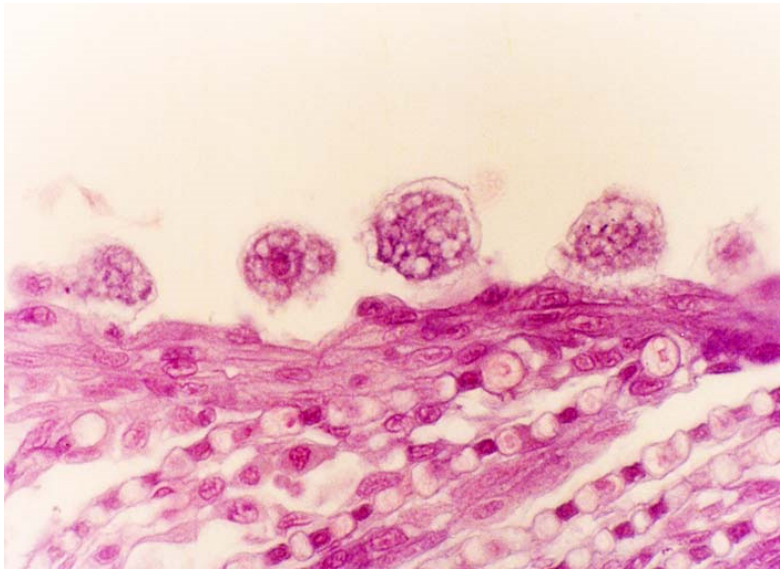
trophozoite
(15-40 µm)

mamilliform
lopopodia

attach to
gill epithelia

no cyst formation

transmission between hosts by transfer of trophozoites
through close contact or contaminated mucus



*Neoparamoeba* trophozoites on fish gills