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A Description of Seven Antarctic Marine Gymnamoebae Including a New Subspecies, Two New Species and a New Genus: *Neoparamoeba aestuarina antarctica* n. subsp., *Platyamoeba oblongata* n. sp., *Platyamoeba contorta* n. sp. and *Vermistella antarctica* n. gen. n. sp.

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ABSTRACT. Seven marine gymnamoebae were isolated from different environments of seawater, slush (pack ice meltwater), and sediment in the Ross Sea area of Antarctica. All amoebae were isolated and maintained at temperatures below 4 °C. Growth, rate of locomotion, and general morphology were observed at an environmentally appropriate temperature (1 °C) and at room temperature (~25 °C). Molecular (srDNA sequences) and microscopical techniques were used to identify the gymnamoebae and establish their phylogenetic affinities. Three isolates (S-131-2, SL-200, and W4-3) were assigned to a psychrophilic subspecies of *Neoparamoeba aestuarina*, *N. aestuarina antarctica* n. subsp., one isolate (S-205) was assigned to a new species of *Platyamoeba*, *P. oblongata* n. sp., two isolates (W51C#4 & W51C#5) were also assigned to a new species of *Platyamoeba, P. contorta* n. sp., and one isolate (S-241) was a novel psychrophilic gymnamoeba *Vermistella antarctica* n. gen. n. sp. Molecular and morphological results revealed that *V. antarctica* was not related to any described family of gymnamoebae. Strains S-205, W51C#4, and W51C#5 were capable of locomotion at room temperature, while strains SL-200, S-131-2, W4-3, and S-241 exhibited locomotion only below ~ 10 °C. Our results imply that the Antarctic environment is host both to cosmopolitan gymnamoebae that have acquired adaptations for existence at low environmental temperature and to apparently novel psychrophilic amoebae described here for the first time.

Key Words. Amoebae, cold water, microplankton, nanoplankton, protist, protistan community, psychrophilic, Ross Sea.

AMOEBAE are increasingly recognized as significant members of the microbial community in most environments globally. In temperate marine environments, they have been reported as abundant, taxonomically diverse, and dynamically linked to environmental factors, such as seasonal cycles, salinity regimes, and other physico-chemical variables (Anderson 1998; Butler and Rogerson 1995; Davis, Caron, and Sieburth 1978; Sawyer 1975). Information on their occurrence in cold-water ecosystems is scarce, although there have been some reports of cold-water gymnamoebae at North Atlantic locations near Scotland (e.g. Anderson and Rogerson 1995; Anderson, Rogerson, and Hannah 1997; Butler and Rogerson 1996). Among the few studies of Antarctic gymnamoebae, Penard (1911) noted the presence of rhizopod amoebae during the British Antarctic Expedition in 1907–1909. Dillon, Walsh, and Bierle (1968), providing some of the earliest observations, isolated gymnamoebae from meltwater ponds and soil from several locations on Ross Island and the nearby exposed mainland of Antarctica, and identified six genera and eight species from three known families (i.e. Chaidae, Mayorellidae, and Thecamoebidae). Smith (1978) examined the distribution and ecology of terrestrial protozoa of sub-Antarctic and maritime Antarctic islands and identified eight microbial communities, some containing gymnamoebae, including *Metachaos*, *Vahlkampfia*, *Mayorella*, *Flabellula*, *Naegleria*, *Tetramitus*, and *Vexillifera*. However, flagellates, ciliates, and testate amoebae were reported to be the most abundant taxa, and gymnamoebae were noted in only four of the communities. More recently, Hara et al. (1986), Kopylov and Sashin (1988), and Scott and Marchant (2005) have also reported the presence of gymnamoebae in the Antarctic marine environment, while Mayes et al. (1997, 1998) reported rates of bacterivory and the temporal distribution of some Antarctic gymnamoebae.

Gymnamoebae tend to be less studied than other protists in most habitats. Some of this paucity of research with gymnamoebae can be attributed to general difficulties in isolating, culturing, and identifying these amoebae. Taxonomic identifications are especially difficult because, at present, they are based on features determined by light microscopical observation of living specimens. Thus, samples obtained using plankton nets or bottle casts and preserved with standard fixatives do not contain identifiable gymnamoebae due to disfigurement and loss during collection or fixation. Gymnamoebae collected from extremely cold ecosystems, such as the Antarctic, pose additional problems because some of these species may require very low temperatures to culture them successfully.

The use of DNA sequence information has proven to be a valuable augmentation to the morphological characters that have traditionally been used to identify and classify protists (Caron, Countway, and Brown 2004; Coyne et al. 2001; Gast and Byers 1995; Knauber, Berry, and Fawley 1996). The use of these modern genetic methods brings us closer to the establishment of a molecular taxonomy (Adl et al. 2005), but molecular data alone cannot be used to address all evolutionary and ecological questions. Rather, morphological and experimental information, combined with corresponding molecular data, provide the best approach for understanding the evolutionary relationships among these species, and their ecological and physiological roles in natural ecosystems. Classification schemes are changing as research using the combined approach of molecular and morphological methods progresses. The general term gymnamoebae as used for this research describes naked amoebae with locomotive pseudopodia. However, the more recent classification scheme by Adl et al. (2005) is used in the formal diagnosis of the novel Antarctic amoebae.

The goals of the research reported here were two-fold. First, we have isolated and described seven marine gymnamoebae from the Ross Sea, Antarctica. Second, we combined traditional morphological and fine structural descriptions with modern molecular phylogenetic analyses based on small subunit ribosomal RNA gene sequences. This study thereby describes new species and genera of gymnamoebae from the harsh environment of Antarctica, increasing our meager knowledge of psychrophily among these protists and providing insight into the phylogenetic breadth of these species.

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MATERIALS AND METHODS

Amoebae culture enrichment and isolation. Natural samples were collected from three different environments (W, water; SL, slush/meltwater; and S, sediment) of the Ross Sea, Antarctica during a 1999 Life in Extreme Environments (LExEn) cruise on board the R/V Nathaniel B. Palmer. Mixed enrichment cultures were started from the natural samples using inorganic F/2+Si (Guillard 1975) or organic media (Table 1). Seven clonal or uniprotistan amoeba cultures were established (Table 1) from the mixed enrichments by micropipetting or serial dilution. Cultures and isolation media were kept chilled on ice at all times. All cultures were grown at 1 °C under continuous lighting or a 14:10 light:dark cycle.

DNA isolation, PCR amplification, cloning, and sequencing of small subunit ribosomal RNA genes. Amoeba cultures were collected by filtration onto a 0.8-μm polycarbonate filter. The collected cells were resuspended in 200 μl of 2× lysis buffer (100 mM Tris pH 8.0, 40 mM EDTA, 100 mM NaCl, 1% (w/v) SDS) and processed using a hot detergent protocol (Gast, Dennett, and Caron 2004). Small subunit ribosomal RNA genes (srDNA) were amplified using primers Euk A and Euk B (Medlin et al. 1988) in 50-μl reactions with 1 μl template DNA, 5 μl of 25 mM $MgCl_2$, 1 μl of 100 ng primers, 4 μl of 2.5 mM nucleotides, and 0.25 μl of T*aq* DNA polymerase (Promega, Madison, WI). The PCR amplification had an initial denaturation of 95 °C for 5 min, followed by 30 cycles of 95 °C for 45 s, 65 °C for 45 s, 72 °C for 3 min. A final extension at 72 °C for 7 min was run to complete extension products. Products were band isolated with a Zymoclean™ gel DNA recovery kit (Zymo Research, Orange, CA). Three microliters of purified product were used in cloning with a pGEM®-T Easy Vector System kit (Promega). Positive clones were picked and grown, and plasmid DNA was recovered using a Genemachines© RevPrep™ Orbit II automated workstation (Genomic Solutions, Ann Arbor, MI). Inserts of one to three clones from each amoeba isolate culture were sequenced in both directions using internal srDNA primers (Weekers et al. 1994) and ABI Prism® BigDye™ Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA). Chromatograms were analyzed using the Sequencher™ editing program (Gene Codes Corporation, Ann Arbor, MI).

Molecular phylogeny. BLAST (Altschul et al. 1997) searches were conducted to establish preliminary taxonomic affiliations. Based upon these results, sequences were retrieved from GenBank (Bilofsky and Burks 1988) for phylogenetic analyses. In addition, a minimum of one sequence from each family or group of gymnamoebae (Adl et al. 2005; Fahrni et al. 2003; Peglar et al. 2003; Rogerson and Patterson 2000) was aligned with the Antarctic isolates in a preliminary analysis. Only full-length sequences with clear taxonomic affiliation were included in the final alignments. Full-length srDNA sequences from seven Antarctic amoeba isolates were aligned with 54 full-length srDNA sequences of amoebae from GenBank using GCG and Seqlab (Bioinfo 4U, Valdosta, GA). Three datasets were analyzed: one with all seven isolates plus related amoebae (Fig. 1), one of the *Neoparamoeba* subset (Fig. 2), and one of the *Vannella/Platyamoeba* subset (Fig. 3). A total of 30 sequences was used in the overall phylogenetic analysis, 23 sequences in the *Neoparamoeba* group analysis, and 33 sequences in the *Vannella/Platyamoeba* group analysis.

The full-amoeba dataset was analyzed using Modeltest (Posada and Crandall 1998) to identify the evolutionary model for maximum likelihood analyses. The result indicated that the general time-reversible model with a proportion of invariable sites and γ distribution (GTR+I+G) would be most appropriate. Maximum likelihood analysis was run with the following parameters: base frequencies A = 0.3129, C = 0.1558, G = 0.2491, T = 0.2822; substitution rate A-C = 1.3407, A-G = 2.9912, A-T = 1.3688, C-G = 0.7838, C-T = 5.5073, G-T = 1; invariable sites = 0.2562; γ = 0.5885. The *Neoparamoeba* data set Modeltest result indicated that HKY+I+G would be the most appropriate model. The maximum likelihood run parameters for this dataset were: base frequencies A = 0.3041, C = 0.1472, G = 0.2054, T = 0.3433; substitution rates A-C = 0.9544, A-G = 4.7141, A-T = 1.6963, C-G = 0.4313, C-T = 6.6423, G-T = 1; invariable sites = 0.5086; γ = 0.4719. The *Vannella/Platyamoeba* dataset Modeltest result indicated that GTR+I+G would be the most appropriate model. The maximum likelihood run parameters for this dataset were: base frequencies A = 0.3145, C = 0.1465, G = 0.2696, T = 0.2694; substitution rates A-C = 1.4410, A-G = 2.7526, A-T = 2.0179, C-G = 0.7791, C-T = 5.3570, G-T = 1; invariable sites = 0.1463; γ = 0.5545. All phylogenetic reconstructions were run using PAUP* (Swofford 1999). One-hundred maximum likelihood bootstrap replicates were accomplished for each dataset. All trees were unrooted, but *Phaeocystis globosa* and *Emiliania huxleyi* were used as the outgroup for the full-amoeba dataset, *Korotnevella hemistylolepis* and *K. monoacantholepis* were used as the outgroup for the *Neoparamoeba* dataset and two *Clydonella* sp. were used as the outgroup for the *Vannella/Platyamoeba* dataset.

Light microscopy. Live cells were initially observed with a Leica MZ125 dissecting microscope (Leica, Bannockbum, IL)

Table **1.** Antarctic amoebae isolates, collection locations, and growth medium.

Antarctic isolate	Collection location	Medium
S-131-2 *Neoparamoeba aestuarina antarctica*	Sediment at approximately 500 m depth near the Antarctic continent (76°35.96′S, 165°00.16′W)	Modified ATCC medium 1525 using filtered Sargasso seawater (salinity = 38 ppt)
SL-200 *Neoparamoeba aestuarina antarctica*	Slush (meltwater layer between pack ice and surface snow) in the Ross Sea pack ice (71°00.32′S, 135°03.83′W)	0.45 μm filtered autoclaved Vinyard Sound seawater with a final concentration of 0.02% yeast extract and several sterile rice grains (salinity = 30 ppt)
W4-3 *Neoparamoeba aestuarina antarctica*	Water sample from combined depths at the far north edge of the Ross sea pack ice (65°14.5′S, 165°13.5′W)	0.45 μm filtered autoclaved Sargasso seawater with a final concentration of 0.02% yeast extract and several sterile rice grains (salinity = 38 ppt)
S-205 *Platyamoeba oblongata*	Sediment at approximately 3800 m depth (71°59.54′S, 134°58.44′W)	Modified ATCC medium 1525 using filtered Sargasso seawater (salinity = 38 ppt)
W51C#4 *Platyamoeba contorta*	Water sample at 10 m depth from the same station as W4-3	Modified ATCC medium 1525 using filtered Sargasso seawater (salinity = 38 ppt)
W51C#5 *Platyamoeba contorta*	Water sample at 10 m depth from the same station as W4-3	Modified ATCC medium 1525 using filtered Sargasso seawater (salinity = 38 ppt)
S-241 *Vermistella antarctica*	Sediment at approximately 290 m depth near the Ross ice shelf (76°53′S, 154°14′W)	Modified ATCC medium 1525 using filtered Sargasso seawater (salinity = 38 ppt)

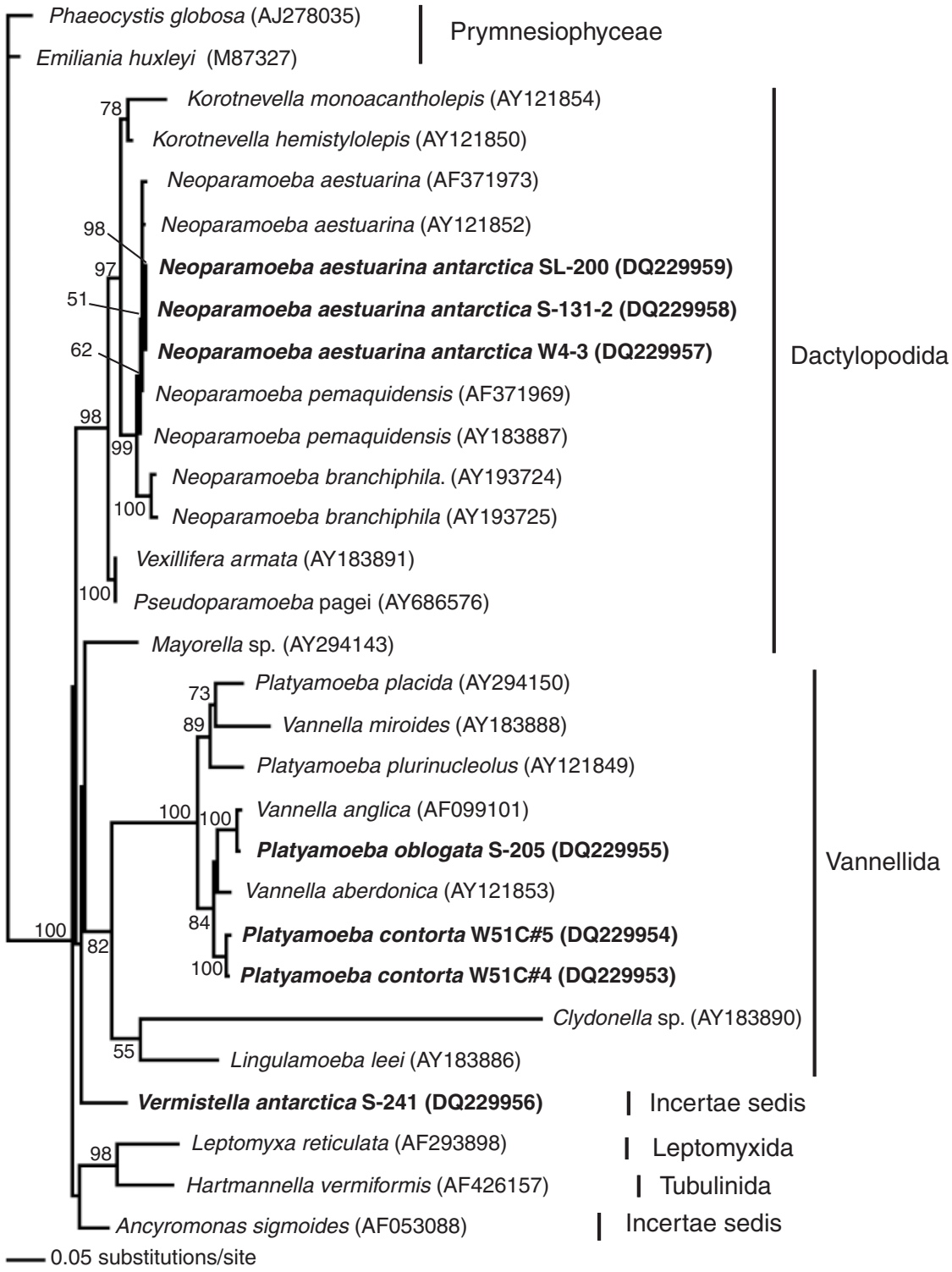


Fig. **1.** Maximum likelihood phylogenetic reconstruction of the taxonomic affiliations of Antarctic gymnamoebae. Numbers are percentages that represent the support of each node based upon 100 maximum likelihood bootstrap replicates. The tree is unrooted, but was drawn in respect to the outgroup containing *Phaeocystis globosa* and *Emiliania huxleyi*.

and then at higher magnifications with a Zeiss Axiovert S100 inverted compound (Zeiss, Thornwood, NY). The Zeiss Axiovert was equipped with a Linkam Peltier stage temperature controller (Waterfield, UK) and a Hamamatsu C4742-95 digital camera (Bridgewater, NJ). Measurements of amoebae and their rates of locomotion were recorded at 1 °C and at room temperature. All observations and measurements ($n = 20$) were done using a Palmer Maloney chamber slide. Openlab software (Improvision, Coventry, UK) was used to capture images and measure locomotion rates of amoebae.

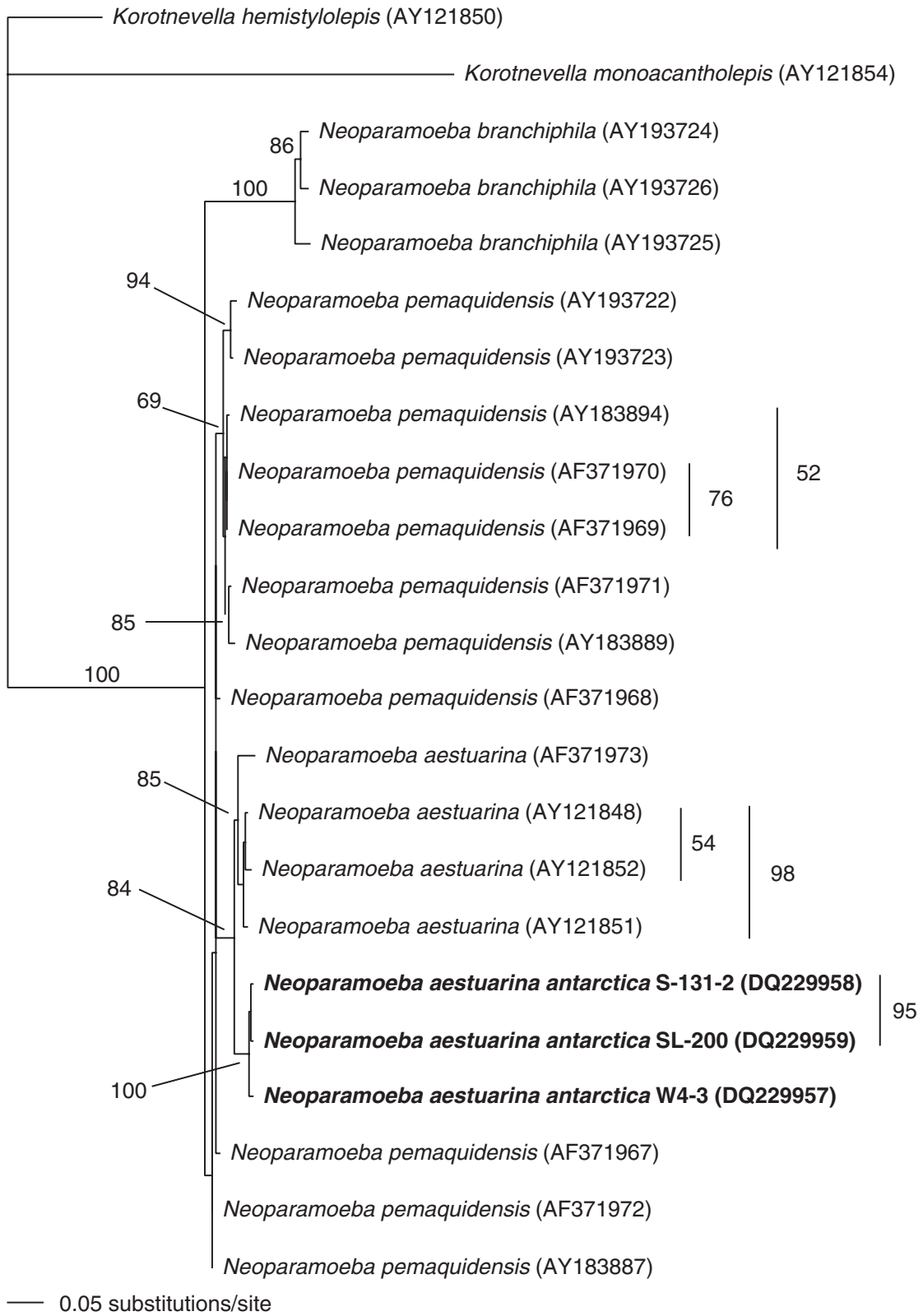


Fig. **2.** Maximum likelihood phylogenetic reconstruction of the taxonomic affiliation of *Neoparamoeba* species. Numbers are percentages that represent the support of each node based upon 100 maximum likelihood bootstrap replicates. The tree is unrooted, but was drawn in respect to the outgroup containing *Korotnevella hemistylolepis* and *Korotnevella monoacantholepis*.

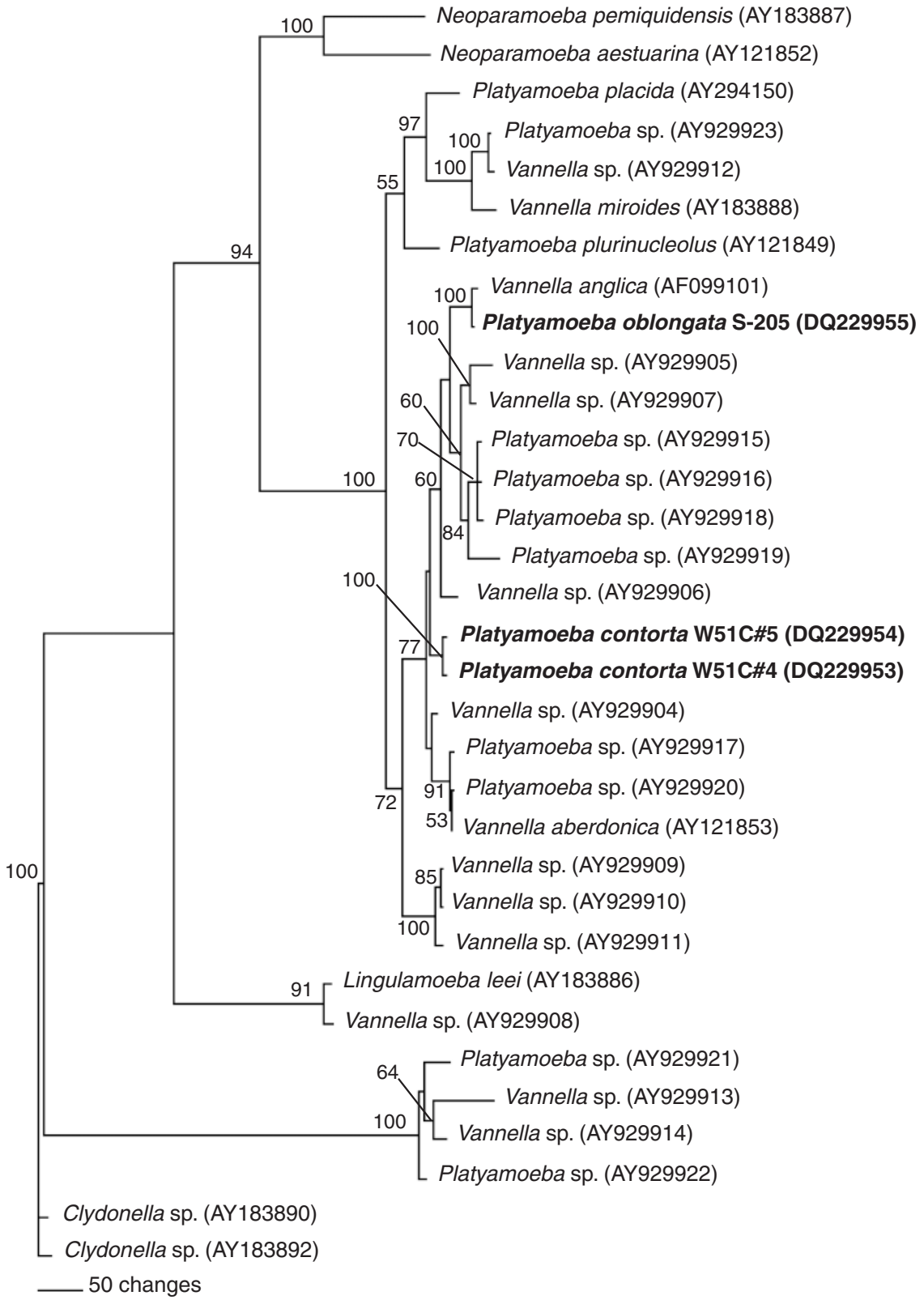


Fig. **3.** Maximum likelihood phylogenetic reconstruction of the taxonomic affiliation of *Vannella*/*Platyamoeba* species. Numbers are percentages that represent the support of each node based upon 100 maximum likelihood bootstrap replicates. The tree is unrooted, but was drawn in respect to the outgroup containing two *Clydonella* species sequences.

Table 2. Antarctic amoebae isolates identifications, sequence length, and most similar available complete srDNA sequence.

Antarctic isolate	Sequence length (bp)	Closest BLAST	% similarity to closest full-length BLAST
S-131-2 *Neoparamoeba aestuarina antarctica*	2099	*N. aestuarina* AY121851	94.49
W4-3 *Neoparamoeba aestuarina antarctica*	2098	*N. aestuarina* AY121848	94.54
SL-200 *Neoparamoeba aestuarina antarctica*	2098	*N. aestuarina* AY121848	94.16
S-205 *Platyamoaba oblongata*	1964	*Vannella anglica* AF099101	97.77
W51C#4 *Platyamoeba contorta*	1987	*Vannella* sp. AY929904	92.33
W51C#5 *Platyamoeba contorta*	1989	*Vannella* sp. AY929904	92.38
S-241 *Vermistella antarctica*	1939	*Ancyromonas sigmoides* AF053088	79.99

Transmission electron microscopy. Samples were prepared using the method of Anderson et al. (1997). The cells were fixed at 4 °C in 2% (w/v) electron microscopic grade glutaraldehyde in a 0.05 M cacodylate buffer (pH = 7.8) prepared using culture medium. The fixed cells were sedimented by centrifugation, enrobed in agarose, and small cubes, approximately 2 mm^3, were rinsed in the cacodylate buffer, and post-fixed for 1 h in 2% (w/v) osmium tetroxide solution prepared in the same cacodylate buffer. The osmium-fixed cells were rinsed in distilled water, dehydrated in a graded ethanol/aqueous series, embedded in Mollenhauer (1964) resin medium, and polymerized at 60 °C for 18 h. Ultrathin sections were cut using a Porter-Blum MT-2 ultramicrotome (Sorvall, Inc., Newtown, CT) fitted with a diamond knife, collected on uncoated 200-mesh copper grids, post-stained with Reynold's lead citrate, and observed with a Philips TEM 201 transmission electron microscope (Philips Electron Optics, Eindhoven, the Netherlands) operated at 60 kV.

RESULTS

Sequences, BLAST results, and alignment. Full-length small subunit ribosomal RNA gene sequences were recovered from seven Antarctic gymnamoeba isolates (Table 2). Sequence lengths ranged from 1939 bp to 2099 bp and no introns were detected. BLAST results for the isolates S-131-2, SL-200, and W4-3 indicated strong affiliation to *N. aestuarina* with sequence similarities of 94.16%–94.54% (Table 2). The closest BLAST result to isolate S-205 was to *Vannella anglica* with 97.77% similarity (Table 2). However, as noted below, TEM observations placed S-205 in the genus *Platyamoeba*. Isolates W51C#4 and W51C#5 had sequences 99.0% similar to each other and showed affiliation to the Vannellida with sequence similarities of 92.33%–92.38% (Table 2). The closest BLAST results for S-241 were to a partial sequence (913 bp) of an unidentified environmental isolate (Accession #AY8355683) with 95.6% similarity, to a partial sequence (1463 bp) of the fungus *Olpidium brassicae* (Accession #DQ32224) with 79.6% similarity, and to the complete sequence of *Ancyromonas sigmoides* with 79.8% similarity. The closest gymnamoebae to S-241 in the BLAST searches were *Vexillifera armata* (Accession #AY183891) and *Pseudoparamoeba pagei* (Accession #AY686576) both with 60.3% similarity. The unidentified sequence and fungus were not included in our phylogenic analysis.

Molecular phylogeny.

Strains S-131-2, SL-200, and W4-3. Maximum likelihood results for the overall gymnamoebae phylogenetic analysis showed that isolates SL-200, S-131-2, and W4-3 grouped strongly with *N. aestuarina* (Fig. 1). However, BLAST results had shown the three Antarctic *N. aestuarina* isolates to be 94.16%–94.54% similar to all available sequences of *N. aestuarina*. Therefore, a genera-specific *Neoparamoeba* alignment was constructed (Fig. 2). The *Neoparamaoba* specific analysis showed that the three Antarctic strains formed a subgroup within the *N. aestuarina* clade.

Strains S-205, W51C#4, and W51C#5. Maximum likelihood results showed that isolates S-205, W51C#4, and W51C#5 grouped within the Vannellida (Fig. 1). However, *Vannella* and *Platyamoeba* genera affiliations within the Vannellida were unresolved (paraphyletic). Therefore, a group Vannellida alignment was constructed. Maximum likelihood results showed that S-205 grouped strongly with *V. anglica* (Fig. 3), whereas isolates W51C#4 and W51C#5 grouped separately from other *Vannella* or *Platyamoeba* species in the alignment (Fig. 3). Because the molecular phylogeny of *Vannella* and *Platyamoeba* genera is unclear (paraphyletic) at this time, the Antarctic isolates were morphologically described below to further clarify their taxonomic affiliation.

Strain S-241. The most divergent sequence recovered from our analysis was isolate S-241. It did not associate with any groups in the molecular phylogeny suggesting that it represented a new genus (Fig. 1), and morphological analyses below provide a description for this organism.

Morphology, fine structure, and diagnostics.

Strains S-131-2, SL-200, and W4-3. Light and electron microscopic observations supported molecular data showing that the above strains were closely related to *N. aestuarina*. At room temperature S-131-2, SL-200, and W4-3 cells contracted, forming 8–15 μm flattened spheres or ovals, and were not observed in locomotion. The morphology of live cells observed at 1 °C and of preserved cells was representative of *N. aestuarina* showing presence of one to two parasomes per cell generally <4 μm in length, a nuclear diam. <5 μm, absence of scales, and a mean cell length $<$ 22 μm (Page 1970). Mean cell size at 1 °C during locomotion was $L = 19.6$ μm (15.0–27.5 μm) $\times$ $B = 11.5$ μm (10.0–15.0 μm); $L{:}B = 1.7$. The locomotive rate at 1 °C was 3.9 $\pm$ 1.8 μm/min.

Strain S-205. Light, fluorescence, and TEM observations supported molecular data to the extent of showing that S-205 is a member of the Vannellida (Page 1980). However, molecular data showed S-205 to be closely related (97.7% sequence similarity) to *V. anglica* while TEM observations showed S-205 to be a *Platyamoeba*. Locomotive trophonts were readily observed at room temperature and at 1 °C. At room temperature, during locomotion, mean cell size was $L = 19.0$ μm (13.8–25.0 μm) $\times$ $B = 22.5$ μm (12.7–31.8 μm); $L{:}B = 0.85$. At 1 °C, during locomotion, mean cell size was $L = 20.8$ μm (17.2–25.8 μm) $\times$ $B = 20.6$ μm (14.3–25.6 μm); $L{:}B = 1.0$. Cell locomotion was 20.7 $\pm$ 7.4 μm/min at room temperature and 13.2 $\pm$ 4.9 μm/min at 1 °C. Floating forms generally displayed several blunt pseudopodia ranging in size from 5 to 22 μm (Fig. 4). Mean central mass diameter of the floating form was 10.2 μm and the length of pseudopodia was often twice the central mass diameter. S-205 frequently exhibits folds and wrinkles when transitioning from the floating form to the locomotive form. During rapid locomotion, cells were flattened and flabellate (Fig. 5), oval, semi-circular, or occasionally spatulate (Fig. 6) or triangular as is typical of *Platyamoeba* spp. The anterior hyaline zone was generally one to three times longer than the more densely granular, thickened posterior zone. Frequently, the anterior hyaline zone would extend around one or both sides of the granular

posterior zone creating posterior protrusions (Fig. 7). Occasionally, the posterior protrusions were observed forming vacuoles.

The nucleus was ovate to oblong with a mean size of $L = 5.4\,\mu m$ (3.2–6.4 μm) × $B = 2.7\,\mu m$ (1.9–2.6 μm) and usually located on one side of the anterior portion of the thickened granular mass (Fig. 8–10). Occasionally, the nucleus was up to one-third of the size of the thickened granular mass and located at the anterior center (Fig. 11). The nucleus occasionally had undulating margins (Fig. 12). The parietal nucleolus was ovate (Fig. 11) or elongated (Fig. 13).

The fine structure of a trophont showed an ovate nucleus (5 × 2 μm) with undulating margins and an elongated parietal nucleolus (3 × 1 μm) (Fig. 14–15). Mitochondria had tubular cristae (Fig. 16) and there were several juxtanuclear Golgi bodies (Fig. 14 and 16). The glycocalyx (15 nm thick, including the dense basal lamina) was characteristic of *Platyamoeba*, consisting of filamentous elements (Fig. 17).

Strains W51C#4 and W51C#5. Morphological observations supported molecular data showing that strains W51C#4 and *W*51C#5 were within the group Vannellida but were not represented by a described species within that group. Locomotive cells could be observed at room temperature or at 1 °C after a settling period of at least 6 h. After 1 day of settling, approximately 20% of the cells were locomotive while the remainder were in the stationary contorted phase. After 3 days of settling, approximately 90% of the cells were locomotive and 10% remained in the stationary contorted phase. Mean cell size during locomotion was $L = 16.0\,\mu m$ (10.0–25.2 μm) × $B = 13.9\,\mu m$ (8.6–23.7 μm; L: $B = 1.2$ at room temperature and $L = 17.5\,\mu m$ (12.5–29.1 μm) × $B = 15.8\,\mu m$ (12.0–23.0 μm); L:$B = 1.1$ at 1 °C. Locomotive rates were 15.6 ± 5.5 μm/min at room temperature and 8.8 ± 3.6 μm/min at 1 °C. During rapid locomotion typical of *Platyamoeba* spp., the cells were discoidal to somewhat ovate and flattened with an anterior hyaline zone that was typically equal to, or occasionally somewhat longer than, the more densely granular, thickened posterior zone (Fig. 18–19). Motile cells also formed temporary prolonged posterior extensions (Fig. 20) that sometimes adhered to the substrate, then rapidly released. Motile cells could readily be observed forming feeding vacuoles (Fig. 21). The hyaline anterior zone was sometimes reflexed and folded backward when changing direction during locomotion (Fig. 22). Cultured individuals observed by light microscopy exhibited some unusual features for a *Platyamoeba* at both room temperature and 1 °C. They were usually less motile and more contracted (~ 12 μm) with wide, broad lobes (Fig. 23), or occasionally with more elongated finger-shaped lobes (Fig. 24). The latter were approximately equal in length to the size of the granular mass. While stationary, they roiled and continuously extended and withdrew the pseudopodia (Fig. 23–38), which is not typical of *Platyamoeba* spp. Although these stationary cells were still attached to the substratum, they might be antecedent to floating forms. Some stationary forms, that were frequently observed while roiling, were comma shaped (Fig. 25 and 26), or in some cases they extended an elongated pseudopodium (Fig. 27), in many cases with a bulge approximately at mid-length (Fig. 28). Micrographs taken approximately 20 s apart showed a stationary cell contorting (Fig. 29–38). Floating forms generally displayed several blunt pseudopodia ranging in length from 2 to 20 μm (Fig. 39, 40). However, floating forms were occasionally observed as contracted cells (12 μm) lacking any extended pseudopodia. Cysts occurred individually or clustered in groups; cyst sizes ranged between 8 and 18 μm in diam. (Fig. 41–43).

The fine structure of a trophont (Fig. 44) showed a round to oval nucleus (3 μm) with a centrally located prominent nucleolus (2 μm). Mitochondria had tubular cristae (25 nm diam.) and were located mainly in the more granular posterior region surrounding the nucleus. The mitochondria were frequently surrounded by a halo of free ribosomes (Fig. 45). A few clear vacuoles were scattered throughout the cytoplasm, and the endoplasmic reticulum was sparse. The glycocalyx was characteristic of *Platyamoeba* consisting of a dense lamina that adheres closely to the plasma membrane. However, it was thinner (10–20 nm) than reported for most characterized *Platyamoeba* species, and in our preparations lacked the typical prominent filamentous projections that are normally observed in *Platyamoeba* spp. (Fig. 45). The cyst (8–15 μm) was rounded and enclosed by a thin fibrous organic wall (~ 0.1 μm) surrounding a contracted cell with an undulating margin and a few shallow clefts. The centrally located nucleus and the surrounding mitochondria appeared to be similar to those of the trophonts. Only a few vacuoles persisted, either larger and ovate, or smaller and more elongated (Fig. 46).

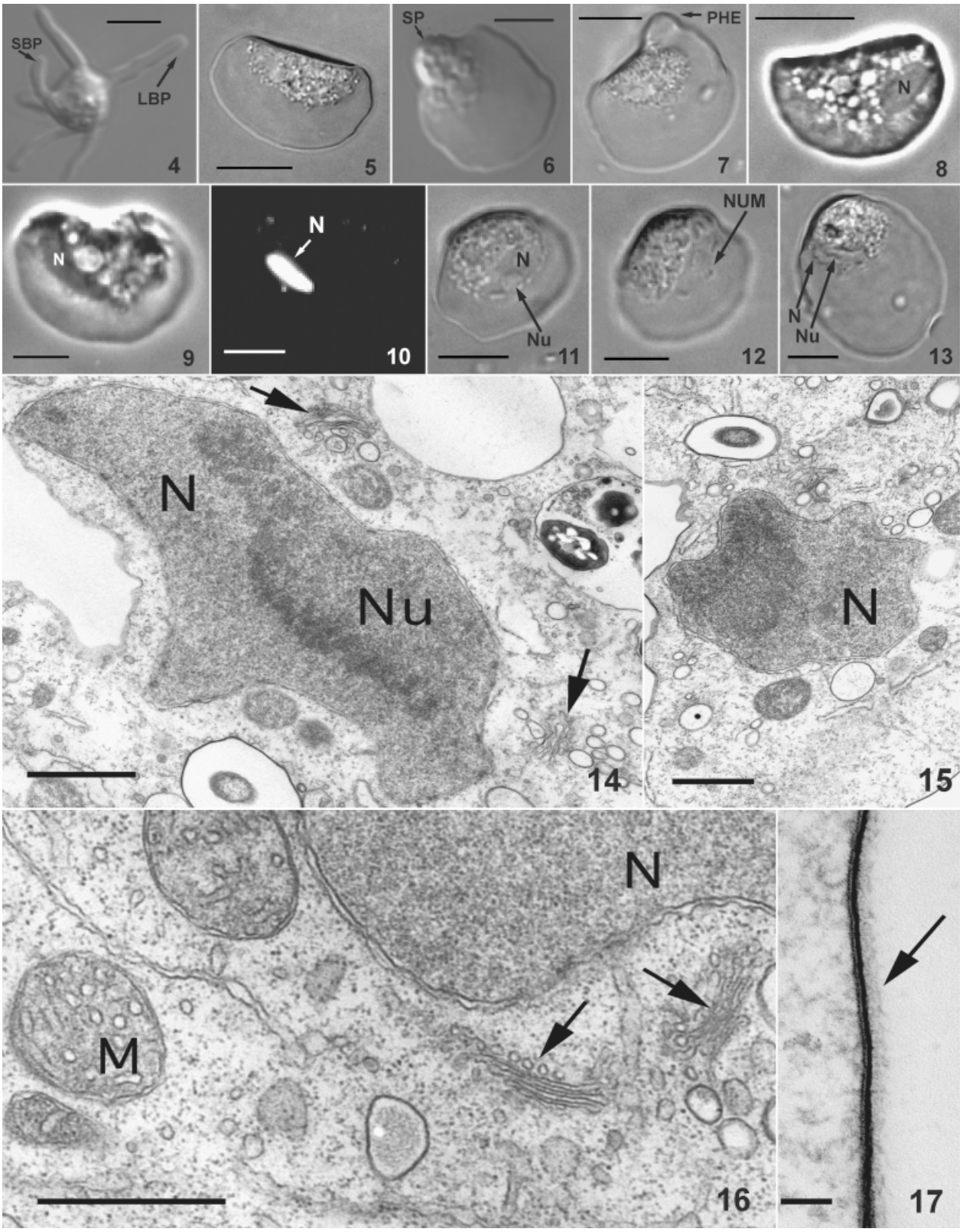
Strain S-241. Morphological observations supported molecular data indicating that S-241 was not related to any described genus of gymnamoebae or heterolobosea. Locomotive cells were observed at 1 °C but could not be observed at room temperature. Cells contracted and formed a 5 μm spherical or stellate morphology at room temperature. At 1 °C, the locomoting cells were usually limax with a mean cell size of $L = 18.9\,\mu m$ (15.1–24.5 μm) × $B = 1.8\,\mu m$ (1.0–2.6 μm); L:$B = 11.4$. Rate of locomotion at 1 °C was 5.6 ± 1.6 μm/min. Locomotive cells were most typically elongated with a rounded posterior and tapered anteriorly to a less rounded tip (Fig. 50). During locomotion or when contracted into a more spherical shape, the cells frequently extended a very long and thin acicular pseudopodium, often with a waving motion or bent at an angle up to 180 °C (Fig. 51, 52). The long, acicular pseudopodium occasionally bent at the tip (Fig. 53). The cell body frequently exhibited a distinctive angular or bent transition region where the elongated pseudopodium emerged, especially when the cell was undergoing a transition from a limax to an acicular form (Fig. 54). Acicular forms could be up to 5 μm longer than the range of limax forms (Fig. 55). Locomotive cells also may be sinusoidal (Fig. 56) or branched (Fig. 57–61). Occasionally, cells were observed with short-blunt subpseudopodia (Fig. 62), but we have not observed pairs of subpseudopodia connected by a hyaline web as reported for *Oscillosignum* spp. (Bovee 1953). Limax cells were observed forming vacuoles at both the anterior and posterior ends of the cell (Fig. 63, 64). Contracted, non-motile cells (5 μm) were most commonly observed at suboptimal temperatures (i.e. above 5 °C) and occasionally observed at ambient temperature of 1 °C. These contracted cells exhibited a more stellate appearance with several short, lobed, or blunt-tipped pseudopods extending from the perimeter (Fig. 65, 66). Floating forms were most often contracted (5 μm) with a few to several short and/or long blunt pseudopodia ranging in length from 2 to 7 μm (Fig. 67). However, floating forms were occasionally observed having short-blunt subpseudopodia (Fig. 68) or one to several fine tapered pseudopodia. No cysts were observed in culture. The rounded nucleus (~ 2 μm) contained a central nucleolus (Fig. 47). There was a prominent Golgi apparatus, typically with four layers of cisternae. The glycocalyx lacked a continuous lamina and consisted of closely spaced pyramidal structures with an irregularly square base and a height of ~ 3–5 nm (Fig. 48). Mitochondria (0.5 μm) were somewhat ovate and had flattened cristae (20 nm wide) that were often curved (Fig. 49) and in some cases the distal portion was dilated forming a swollen or sac-like protrusion.

DISCUSSION

Taxonomy. Molecular and microscopical analyses of our seven isolates of Antarctic gymnamoebae revealed a new subspecies (*N. aestuarina antarctica* n. subsp.), two new species (*P. oblon-*

gata n. sp. and *P. contorta* n. sp.), and a new genus (*Vermistella antarctica* n. gen., n. sp.).

Strains S-131-2, SL-200, and W4-3. Molecular phylogenetic data grouped strains S-131-2, SL-200, and W4-3 within the *N. aestuarina* clade (Fig. 1, 2). Molecular data indicated that although these three Antarctic strains were very closely related to previously identified strains of *N. aestuarina*, they were still genetically distinct from other strains of this species. The srDNA se-



quences for the three Antarctic *N. aestuarina* were 97.6%–97.88% similar to each other, whereas the Antarctic *N. aestuarina* were 94.16%–94.54% similar to the closest *N. aestuarina* and 92.47%–93.03% similar to the closest *N. pemaquidensis* used in the phylogenetic analysis. In turn, the temperate *N. aestuarina* strains had a corresponding intraspecific similarity of 97.32%–97.78%. This finding is similar to the reports of morphological similarities yet minor, consistent differences in srDNA sequences found among different isolates of *Neoparamoeba* spp. (Dykova et al. 2005a; Fiala and Dykova 2003). Dykova et al. (2005a) also showed that variations of multicopy gene alleles within a clone were similar to intraspecific variation ($\sim$ 0.7%–3.5%) within a clade.

Fiala and Dykova (2003) found no discernable morphological differences among strains of *Neoparamoeba* sp. and *N. branchiphila* to *N. pemequidensis* or *N. aestuarina*, but did find that a number of these isolates formed a specific sequence-based clade (*N. branchiphila*), which were 96.42%–98.67% similar to each other and only 91.34%–91.45% similar to the srDNA sequence of *N. pemaquidensis* (American Type Culture Collection (ATCC) 30735), the closest *Neoparamoeba* sp. used in their analysis. The research of Dykova et al. (2005a) showed that molecular characteristics were more reliable than morphological characteristics in identifying different species of *Neoparamoeba*. Therefore, *N. branchiphila* was designated a new species based solely on molecular characteristics. Our Antarctic strains clearly form a distinct subclade within the *N. aestuarina* clade. The Antarctic strains also show less sequence variation in relation to other *Neoparamoeba* spp. than the *N. branchiphila* sequences, supporting their classification as a subspecies rather than a new species.

Light microscopy and TEM observations for S-131-2, SL-200, and W4-3 were consistent with the published description for *N. aestuarina* (Page 1970, 1987). The morphological characteristics distinguishing *N. aestuarina* from *N. pemaquidensis* are based on cell size, nuclear diameter, number of parasomes, and presence or absence of longitutidal ridges and anterior hyaloplasm projections. Where *N. aestuerina* mean cell size is $<22\,\mu$m, nuclear diam. is $<5\,\mu$m, there are low numbers of parasomes ($<4\,\mu$m) present, there is presence of anterior edge hyaloplasm projections, and an absence of longitutidanal ridges (Page 1970, 1987).

Physiological difference between the Antarctic and temperate strains of *N. aestuarina* were found to exist. We have been unable to grow the Antarctic *N. aestuarina* strains at temperatures above 15 °C (unpubl. data) or readily observe locomotive cells at room temperature. In contrast, *Neoparamoeba* spp. have been commonly grown and locomotion has been observed at room temperature (Dykova, Figueres, and Peric 2000; Page 1983). Also, *N. pemaquidensis* isolates from salmon farms near Washington State were found to exhibit their highest growth rates at 20 °C (Kent, Sawyer, and Hedrick 1988). It is not surprising that we recovered several isolates, as *Neoparamoebae* spp. are commonly isolated from the marine environment. However, our work indicates that the Antarctic isolates were psychrophilic. Also notable is the similarity between the three isolates despite the range of sample types from which they were isolated (sediment vs. slush vs. water). In our previous molecular assessments of community structure in the Antarctic marine environment, we found these microhabitats dominated by relatively distinct microbial taxa (Gast et al. 2004).

Thus, based on molecular and physiological differences along with morphological similarities, we designate strains S-131-2, SL-200, and W4-3 as *N. aestuarina antarctica* n. subsp., a psychrophilic subspecies of *N. aestuarina*.

Amoebozoa
Tubulinea
Dactylopodida

Neoparamoeba aestuarina antarctica n. subsp. Moran and Anderson.

Diagnosis. Psychrophilic subspecies of *N. aestuarina*. Morphology is typical of *N. aestuarina* as described by Page (1970). Subspecies cannot be maintained in culture above 15 °C. Locomotive trophonts need to be observed at temperatures below 10 °C. The srDNA sequence is 5.46%–5.8% different from the srDNA sequence of temperate *N. aestuarina*.

Etymology. The subspecies name is based on psychrophily and type locality of Antarctica.

Type locality. Subspecies S-131-2 was collected from sediment at approximately 500 m depth near the Antarctic continent (76°35.96′S, 165°00.16′W). Subspecies SL-200 was collected from slush (meltwater layer between pack ice and surface snow) in the Ross Sea pack ice (71°00.32′S, 135°03.83′W). Subspecies W4-3 was collected from a water sample of combined depths at the far north edge of the Ross Sea pack ice (65°14.5′S, 165°13.5′W).

Deposition of type material. Type cultures have been deposited at the ATCC and will be assigned Accession numbers upon successful cryopreservation.

Gene sequence data. Complete srDNA sequence data are deposited in GenBank under Accession numbers DQ229958 (subspecies S-131-2), DQ229959 (subspecies SL-200), and DQ229957 (subspecies W4-3).

Strain S-205. Strain S-205 was clearly associated with the group Vannellida based on molecular and morphological data. Molecular data showed S-205 to be closely related to *V. anglica* with 97.7% similarity. However, morphological data showed S-205 to be a *Platyamoeba* primarily based on the presence of a filamentous element glycocalyx structure typical of *Platyamoeba* spp. (Fig. 17). The glycocalyx structure is the defining morphological characteristic that separates *Vannella* spp. from *Platyamoeba* spp. (Page 1983). However, molecular data have not been congruous with this morphological assessment showing the two genera to be paraphyletic (Dykova et al. 2005b; Sims, Aitken, and Rogerson 2002). The glycocalyx morphology was used to identify S-205 as a *Platyamoeba*, and not *V. anglica*, as it lacked the characteristic tower-like glycostyles observed in TEM preparations of *Vannella* spp. S-205 had unique nuclear and nucleolar morphology that was not previously described in any *Platyamoeba* or

Fig. **4–17.** Light, fluorescent, and transmission electron microscope images of *Platyamoeba oblongata* n. sp. **4.** Floating form with long (LBP) and short (SBP) blunt pseudopodia. Scale bar = 10 μm. **5.** Locomotive flabellate form. Scale bar = 10 μm. **6.** Locomotive form with spatulate posterior (SP). Scale bar = 5 μm. **7.** Locomotive form with posterior hyaloplasm extension (PHE). Scale bar = 10 μm. **8.** Fixed cell with ovate nucleus (N). Scale bar = 10 μm. **9.** Fixed cell with oblong nucleus (N). Scale bar = 5 μm. **10.** DAPI stained nucleus of *P. oblongata* cell shown in Fig. 9 (N). Scale bar = 5 μm. **11.** Locomotive form with anterior nucleus (N) and parietal nucleolus (Nu). Scale bar = 5 μm. **12.** Locomotive form showing nucleus with undulating margin (NUM). Scale bar = 5 μm. **13.** Locomotive form showing nucleus (N) with elongated parietal nucleolus (Nu). Scale bar = 5 μm. **14.** Transmission electron micrograph of an oblique section through the somewhat elongated nucleus (N), parietal nucleolus (Nu), and two juxtanuclear golgi bodies (arrows). Scale bar = 1 μm. **15.** A cross-section through the nucleus (N) and parietal nucleolus exhibiting the undulating margin of the nucleus and its approximate ovate shape in cross-section. Scale bar = 1 μm. **16.** A higher magnification view of the cytoplasm near the nucleus (N) containing mitochondria (M) with tubular cristae and a pair of juxtanuclear Golgi bodies (arrows). Scale bar = 0.5 μm. **17.** A high magnification view of the plasma membrane and glycocalyx (arrow) exhibiting a typical fine structure of *Platyamoeba*. Scale bar = 40 nm.

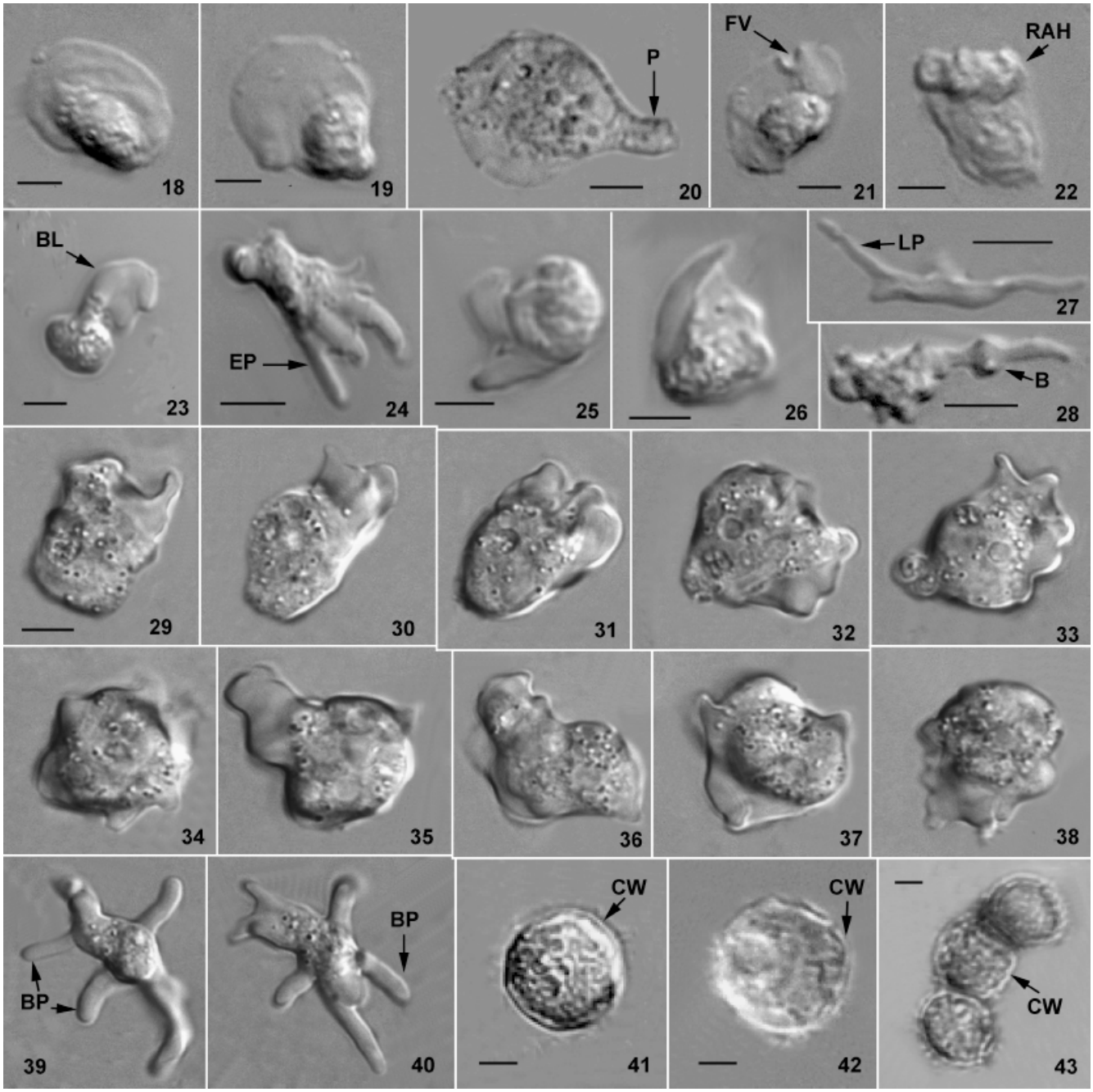


Fig. **18–43.** *Platyamoeba contorta* n. sp. **18, 19.** Motile trophonts. **20.** Motile trophont showing ''panhandle'' posterior (P). **21.** Motile trophont showing feeding vacuole (FV). **22.** Motile trophont changing direction showing reflexed anterior hyaline zone (RAH). **23.** Stationary trophont with wide broad lobe (BL). **24.** Stationary trophont with extended pseudopodia (EP). **25, 26.** Stationary trophonts in a commonly observed ''comma'' form. **27.** Stationary trophont with long thin pseudopodia (LP). **28.** Stationary trophont with commonly observed bulge in extended pseudopodia (B). **29–38.** Stationary contorting forms. Photos taken in an ordered series at 20-s intervals. **39, 40.** Floating forms showing extended blunt pseudopodia (BP). **41–43.** Cysts showing cell wall (CW). All scale bars = 5 μm. Fig. 29–40 are on the same scale.

Vannella species. The nucleus is ovate to elongate (Fig. 8–14). The nucleolus is parietal and ovate to oblong (Fig. 11, 13, 14). It should be noted that *P. nucleolilateralis* (Anderson, Nerad, and Cole 2003) and *P. plurinucleolus* (Page 1974) also have a parietal nucleolus. However, *P. nucleolilateralis* has a spherical nucleus and the floating form has tapered pseudopodia that are not observed in strain S-205. *Platyamoeba plurinucleolus* contains patches of nucleolar material dispersed throughout the nucleoplasm, a feature also not observed in strain S-205. In addition, *P. plurinucleolus* is not closely related to S-205 based on srDNA phylogeny (Fig. 3). Presently, there is no available sequence data for *P. nucleolilateralis*. S-205 is designated as *P. oblongata* n. sp. based on its unique nucleus and nucleolus morphology.

Amoebozoa
Tubulinea
Vannellida

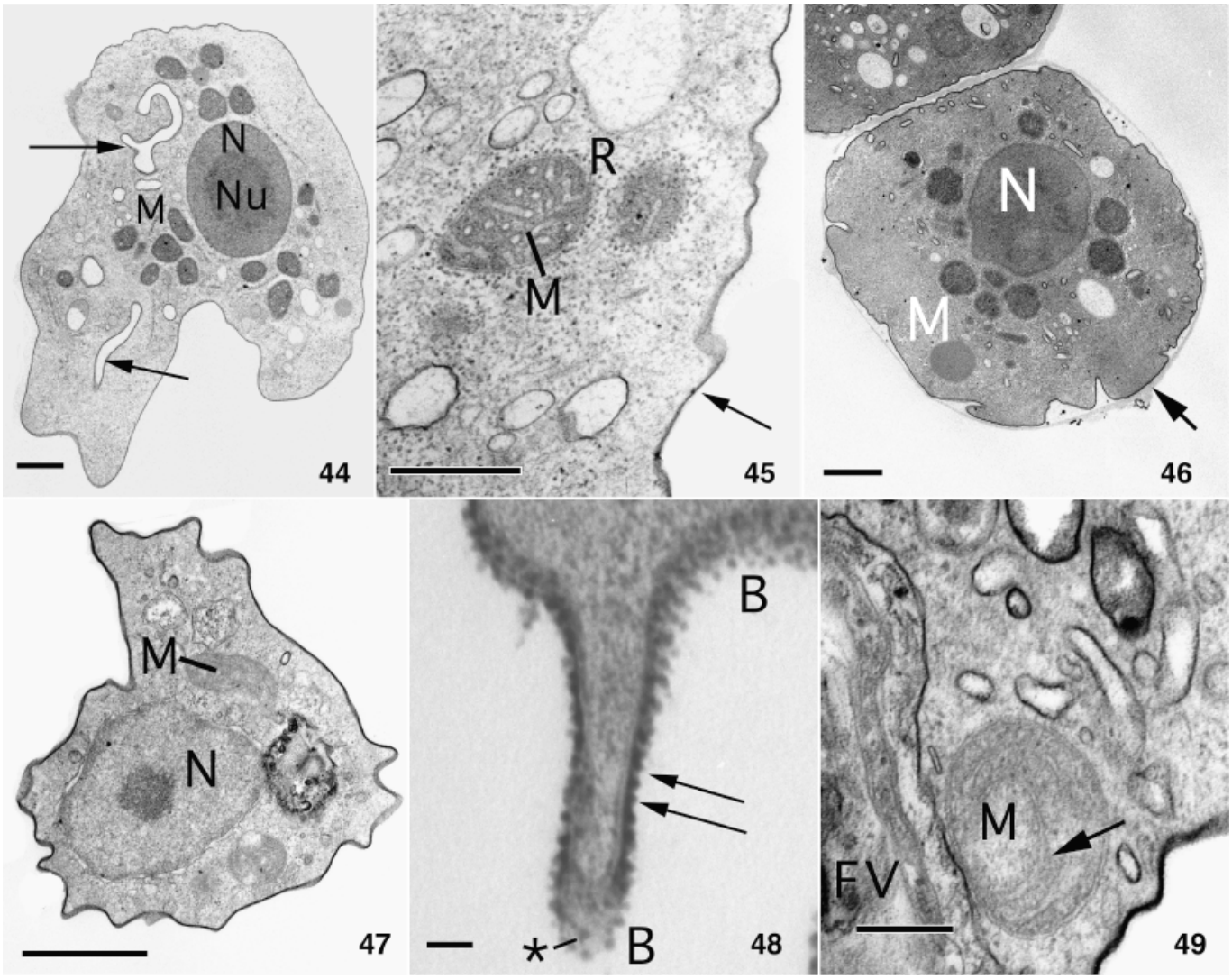


Fig. **44–49.** Electron micrographs of *Platyamoeba contorta* n. sp. (Fig. 44–46) and *Vermistella antarctica* n. gen., n. sp. (Fig. 47–49). **44.** Ultrathin section of *P. contorta* showing the nucleus (N) with central nucleolus (Nu) and mitochondria (M) in the denser cytoplasm surrounding the nucleus. Invaginations (arrows) of the cell surface, exhibiting an electron-dense glycocalyx lining the plasma membrane, may be due to the contortion of the cell as observed by light microscopy. Scale bar = 1 μm. **45.** A higher magnification of the peripheral cytoplasm showing a tubulocristate mitochondrion (M) surrounded by a layer of ribosomes (R). The cell is coated by a rather thin, electron-dense lamina of the glycocalyx (arrow). Scale bar = 0.5 μm. **46.** A cyst, surrounded by a relatively thin organic wall (arrow), showing the undulating cell margin with occasional clefts, a centrally located nucleus (N) and scattered mitochondria (M). Scale bar = 1 μm. **47.** Ultrathin section of *V. antarctica* showing the nucleus (N) with a central nucleolus and sparsely scattered mitochondria (M) in the cytoplasm. Scale bar = 1 μm. **48.** A high magnification view of the glycocalyx composed of pyramidal-shaped glycostyles shown in vertical profile view (arrows) with electron-dense bases (B) that appear irregularly square (asterisk) when viewed in transverse section. Scale bar = 0.02 μm. **49.** A detailed view of a mitochondrion (M) with flattened, sometimes curved cristae (arrow), a portion of a food vacuole (FV), and smooth membrane vesicles that are scattered throughout the cytoplasm. Scale bar = 0.2 μm.

Platyamoeba oblongata n. sp. Moran and Anderson (Fig. 4–14)

Diagnosis. Amoebae during locomotion are flattened and flabellate, semi-circular, and occasionally spatulate or triangular; $L = 20.8\,\mu m$ (17.2–25.8 μm) × $B = 20.6\,\mu m$ (14.3–25.6 μm); *L:B* 1.0. A hyaline leading edge is approximately one to three times the length of the posterior granular mass. The hyaline zone frequently extends around one or both sides of the granular mass creating posterior protrusions. Floating forms generally display several blunt pseudopodia ranging in size from 5 to 22 μm. The nucleus is ovate to oblong (5.0 μm × 2.0 μm) with occasional undulating margins and is generally located along an anterior side of the thickened granular mass. The nucleolus is parietal and ovate to oblong (3.0 μm × 1.0 μm). Mitochondria are tubulocristate. The cell surface is coated with a glycocalyx (15 nm), consisting of filamentous elements attached to a dense basal lamina.

Etymology. The species name is based on the oblong shape of the nucleus and nucleolus.

Type locality. Strain S-205 was recovered from sediment collected at approximately 3,800 m depth in the Ross Sea, Antarctica (71°59.54′S, 134°58.44′W).

Deposition of type material. Type cultures have been deposited at the ATCC and will be assigned accession numbers upon successful cryopreservation.

Gene sequence data. The complete srDNA sequence data of *P.oblongata* (strain S-205) is deposited in GenBank under Accession number DQ229955.

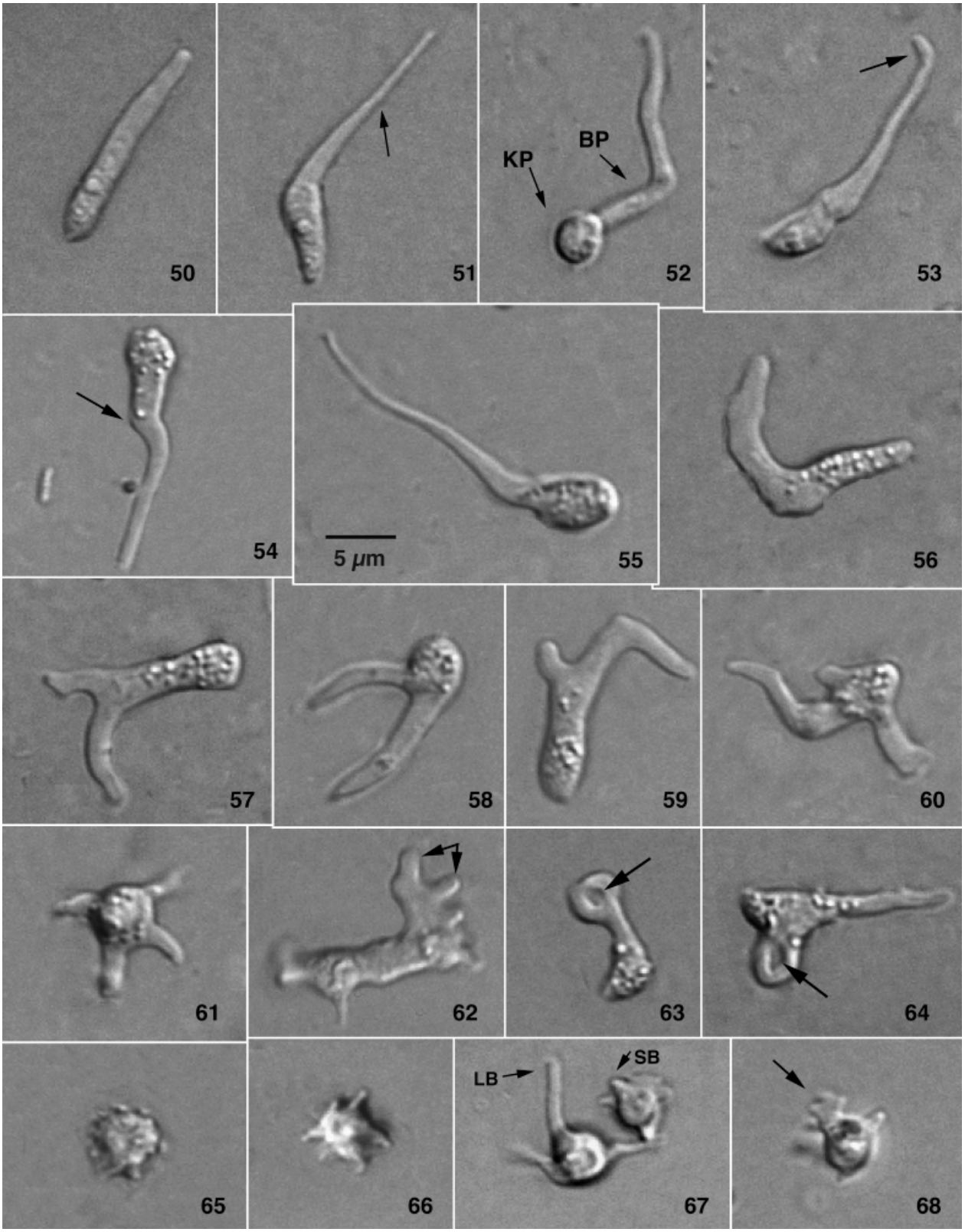


Fig. **50–68.** *Vermistella antarctica* n. gen., n. sp. **50.** Limax form. **51.** Wand-like stiff pseudopod (arrow). **52.** Knob-like posterior (KP) and sharply bent pseudopod (BP). **53.** Bent pseudopod tip (arrow). **54.** Limax vermiform to stiff pseudopod transition (arrow). **55.** Long-tapered pseudopod. **56.** Sinusoidal form. **57–61.** Branched forms. **62.** Blunt digitiform subpseudopodia (arrows). **63, 64.** Characteristic rounded vacuoles formed by fusion of the tips of pairs of pseudopodia (arrows). **65.** Contracted trophozoite. **66.** Contracted stellate trophozoite. **67.** Floating forms with long blunt (LB) and short-blunt (SB) pseudopodia. **68.** Floating form with short blunt subpseudopodia (arrow). Scale bar = 5 μm. Fig. 50–68 are all on the same scale.

Strains W51C#4 and W51C#5. Strains W51C#4 and W51C#5 could not be resolved to genus level with sequence data because the molecular lineages of *Vannella* and *Platyamoeba* species are paraphyletic (Dykova et al. 2005b; Sims et al. 2002). Light microscopy and TEM showed that the morphologies of these strains were consistent with the published description for the genus *Platyamoeba* (Page 1983). However, the morphology of W51C#4 and W51C#5 was not consistent with characteristics published for *Platyamoeba*. These strains were characterized by a unique combination of size (*L*:*B*) and locomotive morphology, especially the peculiar roiling motion of the sedentary cells observed at the level of light microscopy (Fig. 23–38). Fine structure of W51C#4 and W51C#5 was also dissimilar to that of any other published *Platyamoeba* species. The surface lamina of the glycocalyx was thinner and there were no surface filamentous hexagonal elements (Fig. 45), which typically occur in this genus. Furthermore, the peculiar roiling motion illustrated the plasticity of this species, as there was no other published description of this motility for *Platyamoeba* spp. However, there have been published observations of similar behavior in the heteroloboseans. To the best of our knowledge, there has been no published explanation for the adaptive or physiological value of this behavior. In addition, W51C#4 and W51C#5 are the first described marine *Platyamoeba* shown to form cysts (Fig. 41–43, 46) (Page 1983). Thus, based on the sequence analysis, light microscopy, and fine structure, we designate strains W51C#4 and W51C#5, as *P. contorta* n. sp.

Amoebozoa
Tubulinea
Vannellida

Platyamoeba contorta n. sp. Moran and Anderson (Fig. 18–46).

Diagnosis. Amoebae typically are stationary ($\sim$ 12 μm), contorted and roiling in motion with wide, broad lobes or occasionally with more elongated finger-shaped lobes (4–12 μm in length). During locomotion, the cells are discoidal to somewhat ovate and flattened; $L = 17.5$ μm (12.5–29.1 μm) $\times$ $B = 15.8$ μm (12.0–23.0 μm); L:$B = 1.1$ at 1 °C. A hyaline leading edge is approximately equivalent in width to the length of the posterior granular mass. Floating forms generally display several blunt pseudopodia ranging in length from 2 to 20 μm. However, floating forms are occasionally observed as contracted cells (12 μm) lacking any extended pseudopodia. The nucleus is 3 μm in diam. with a centrally located 2-μm broad nucleolus. Mitochondria are tubulocristate. The cell surface is coated with a relatively thin, dense lamina (10–20 nm) without prominent glycostyles, or fine filaments. Cysts 8–18 μm are spherical to slightly oval with a thin fibrous organic wall (0.1 μm) enclosing a contracted cell with undulating margins and occasional shallow clefts. Diagnosis measurements were carried out on amoebae cultured at ambient temperature (1 °C).

Etymology. The species name is based on a commonly observed behavior of the amoeba: it exhibits a roiling or contorted morphology when stationary.

Type locality. Strains W51C#4 and W51C#5 were collected from water 10 m deep located at the far north edge of the Ross Sea pack ice (65°14.5′S, 165°13.5′W).

Deposition of type material. Type cultures have been deposited at the ATCC under Accession numbers PRA-217 (*P. contorta* W51C#4) and PRA-218 (*P. contorta* W51C#5).

Gene sequence data. The complete srDNA sequence data of *P. contorta* is deposited in GenBank under Accession numbers DQ229953 (strain W51C#4) and DQ229954 (strain W51C#5).

Strain S-241. Both molecular phylogeny and microscopy indicated that S-241 was not related to any described genus or group within the Gymnamoebae or Heterolobosea. We found no published srDNA sequences exhibiting similarities high enough to indicate taxonomic identity. We inferred moderate affiliation with the gymnamoebae based on phylogenetic reconstructions, but these analyses did not yield a strong relationship with any particular group (Fig. 1). The morphology of this strain showed a limax locomotive form (Fig. 50), similar to hartmannellids. However, S-241 was more tapered anteriorly than most hartmannellids, and the tip occasionally extended as a long thin pseudopodium (Fig. 51–55) reminiscent of some vexilliferids. Fine structural data clearly indicated that this organism was unlike either the hartmanellids or vexillifeids, especially as the elements of the glycocalyx (Fig. 48) were different in morphology and size than published for either of these genera (Page 1983). Moreover, no hartmannellid has been identified with a long anterior acicular pseudopod as observed in our isolate (Fig. 55). The mitochondria, with flattened curved cristae (Fig. 49), were not characteristic of other gymnamoebae or Amoebozoa. Some Heterolobosea (Excavata) have flattened to discoidal cristae, but it was clear from the molecular phylogenetic analyses and other fine structural features that this isolate was not a heterolobosean. The light microscopic morphological features showing non-eruptive locomotion and no evidence of a flagellated phase were also not similar to other amoeboid heteroloboseans (Patterson, Rogerson, and Vors 2000). The gross morphology suggested some affinity with the gymnamoebae *Subulamoeba* (Bovee 1953) or *Oscillosignum* (Bovee 1953). *Subulamoeba* has a similar locomotive morphology, but lacks the elongated anterior pseudopodium, and has been reported to contain cytoplasmic crystals never observed in our isolate. *Oscillosignum* has a similar morphology of the anterior pseudopodia, but the cell shape of our isolate was different (i.e. lacking short paired web-connected pseudopodia). Furthermore, the sizes of both of these genera were much larger than our isolate (Rogerson and Patterson 2000). S-241was psychrophilic and was not observed in locomotion at temperatures above 10 °C, nor could it be maintained at 20 °C. Thus, based on sequence analysis, light microscopy, and fine structure, we designate strain S-241 as a type species of a new genus assigned the name *V. antarctica* n. gen., n. sp. Although *V. antarctica* shares most of its taxonomic characteristics with the Amoebozoa, it does not have tubular mitochondrial cristae which is presently a defining characteristic of the Amoebozoa. Therefore, there is no current group which *V. antarctica* can be assigned to and is classified as incertae sedis.

Incertae sedis
Vermistella Moran and Anderson, n. gen.

Diagnosis. Amoebae during locomotion are limax-shaped to vermiform with a broader rounded posterior and an awl-shaped body tapering anteriorly to a narrower rounded tip. The tip frequently elongates as a slender, pointed (acicular) pseudopodium equivalent to the cell length or longer. The pseudopodium is either straight, or from time-to-time waved like a wand, or bent at an angle up to 180°. Sedentary cells, less commonly observed ($\sim$ 10%–20%) at ambient temperature, are contracted (rounded to angular) with several peripheral, blunt-lobed to finger-shaped pseudopodia arranged in a stellate configuration. However, these ''stellate cells'' are more likely to be observed at higher temperatures or under suboptimal culture conditions and, although still attached to the substratum, may be antecedent to floating.

Etymology. The genus name was based on the peculiar characteristic of the cell shape that was usually elongated and tapered (vermiform) during locomotion, infrequently sinusoidal, and sometimes contracted with radiating peripheral pseudopodia (stellate).

Vermistella antarctica Moran and Anderson, n. sp. (Fig. 47–68).

Diagnosis. Amoebae with characteristics of the genus, $L = 18.9$ μm (15.1–24.5 μm) $\times$ $B = 1.8$ μm (1.0–2.6 μm);

$L{:}B = 11.4$. Rate of locomotion at 1 °C is 5.6 ± 1.6 μm/min. When changing direction of locomotion, the cells either send out a branched lateral pseudopodium that becomes the dominant axis of locomotion, or they simply curve to one side and establish a new direction of movement. Floating forms are spherical (5 μm), either lacking pseudopodia or with up to seven fine-tapered pseudopodia ranging in length from 2 to 7 μm. The nucleus contains a centrally located nucleolus. Mitochondria, sparsely distributed in the cytoplasm, are somewhat ovate with flattened, often curved cristae. A prominent Golgi apparatus typically has four layers. The glycocalyx lacks a continuous lamina and consists of closely spaced pyramidal structures with a wide base and a height of ~ 3–5 nm. Diagnosis measurements were carried out on amoebae cultured at ambient temperature (1 °C).

Etymology. The species name is based on the geographic type locality of Antarctica.

Type locality. Strain S-241 was collected from sediment at 290 m depth near the Ross Ice Shelf (76°53′ S, 154°14′W).

Deposition of type material. The type culture for *V. antarctica* (strain S-241) has been deposited at the ATCC under Accession number PRA-216.

Gene sequence data. The complete srDNA sequence data of *V. antarctica* (strain S-241) is deposited in GenBank under Accession number DQ229956.

Psychrophily and ecology. It has been suggested that psychrophilic amoebae may optimize metabolic functions for existence at low environmental temperatures (Mayes et al. 1997). It appears that some of these optimizations may occur within cosmopolitan genera, such as *Neoparamoeba*, *Platyamoeba*, and *Vannella* (Mayes et al. 1997; Rogerson, Polne-Fuller, and Gibor 1992). The Antarctic marine environment also appears to be a source for novel amoebae, such as *V. antarctica*. In our study, members of the Vannellida isolated from the Antarctic were not obligate psychrophiles, whereas the *N. aestuarina antarctica* of the group dactylopodida and *V. antarctica* could not grow above 10 °C. This indicates that there may be physiological differences between groups of amoebae.

In addition to the amoebae isolated in this study, we have observed heliozoans, testate amoebae with filopodia and a variety of gymnamoebae including Mayorellids in our mixed enrichment cultures. Some of the highest and most variable densities of gymnamoebae have been found in the Antarctic marine environment (Hara et al. 1986; Kopylov and Sashin 1988; Mayes et al. 1998). These species may play multiple roles in the Antarctic marine environment as they can function as herbivores or bacterivores depending on genera and/or cell size (Mayes et al. 1998; Rose and Caron, unpubl. data). The amoebae isolated in this study existed in cultures as bacterivores. However, larger Antarctic amoebae, such as *Mayorella* sp., are voracious consumers of diatoms (Mayes et al. 1998; pers. observ.), and there are reports of grazing of temperate gymnamoebae on cyanobacteria and algae (Bunt 1970; Laybourn-Parry, Jones, and Holdich 1987). There is still much Antarctic protistan ecology and physiology to be explored, and our Antarctic isolates can serve as tools for future research.

In this study, we have combined molecular (srDNA sequence) and morphological data to describe gymnamoebae from the Ross Sea, Antarctica. These methods complemented each other particularly well in this case because these species possess relatively few, and somewhat variable, morphological features. Taken together, these data provided more taxonomic information and historical reference than either could alone. Sequence information can provide phylogenetic affinity, and ultimately information on species distribution and abundance. However, without a morphological ''face,'' these sequences are of limited use in ecological studies. Morphology (and the establishment and study of cultures of these species) contributes important information on aspects of the physiology and trophic interactions of the amoebae. Together, the identified amoebae and their molecular signatures can now be utilized more effectively in a wide range of evolutionary, ecological, and physiological studies.

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