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Platyamoeba pseudovannellida N. Sp., a Naked Amoeba With Wide Salt Tolerance Isolated from the Salton Sea, California

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ABSTRACT. A new species of naked amoeba, *Platyamoeba pseudovannellida* n.sp., is described on the basis of light microscopic and fine structural features. The amoeba was isolated from the Salton Sea, California, from water at a salinity of ca. 44‰. Locomotive amoebae occasionally had a spatulate outline and floating cells had radiating pseudopodia, sometimes with pointed tips. Both these features are reminiscent of the genus *Vannella*. However, the surface coat (glycocalyx) as revealed by TEM indicates that this is a species of *Platyamoeba*. Although salinity was not used as a diagnostic feature, this species was found to have remarkable tolerance to fluctuating salinity levels, even when changes were rapid. Amoebae survived over the range 0‰ to 150‰ salt and grew within the range 0‰ to138‰ salt. The generation time of cells averaged 29 h and was not markedly affected by salt concentration. This is longer than expected for an amoeba of this size and suggests a high energetic cost of coping with salinity changes. The morphology of cells changed with increasing salinity: at 0‰ cells were flattened and active and at the other extreme (138‰) amoebae were wrinkled and domed and cell movement was very slow. At the ultrastructural level, the cytoplasm of cells grown at high salinity (98‰) was considerably denser than that of cells reared at 0‰.

Key Words. Euryhaline, generation times, taxonomy, ultrastructure.

THE Salton Sea is the largest inland lake in California with an average salinity of ~ 44‰ although this varies depending on location, depth, and meteorological conditions. Today, it is a highly productive water body that supports impressive fish stocks and provides an over-wintering site for many migrating birds. However, there is concern that the lake biota is under threat from increasing salinity levels. This increase is due to the intense irrigation of adjacent agricultural lands of the Coachella and Imperial Valleys. The subsequent runoff of waters containing trace salts into this closed water body and the high surface evaporation rates in this desert region compound the salinity problem. Since 1907, salinity has increased from 3.6‰ to its current level (González et al. 1998).

In a recent biotic survey of the sea, the waters were found to be rich in naked amoebae: about 45 different morphospecies were recorded and 40% of these are probably new to Science (Rogerson and Hauer 2001). Moreover, in some of the hypersaline ponds around the perimeter of the sea, 6 morphospecies of amoebae were isolated from water with salinities around 160‰. Such tolerance by amoebae to elevated salinities raises the question of how tolerant are they in general to salinity fluctuations. This is of interest for two reasons. Firstly, saline waters represent a vast habitat. According to Williams (1998), the total volume of inland saline water in the world is more or less equivalent to the combined volume of inland freshwater lakes and rivers. Secondly, amoebae inhabiting coastal waters are frequently subjected to rapid salinity fluctuations after heavy rains or as a consequence of surface evaporation.

Despite the importance of this environmental variable, there have been relatively few detailed studies on salinity tolerance in amoebae. Most studies focus on extreme environments and simply document the presence of unidentified amoebae in these hypersaline regions. For example, Post et al. (1983) commonly found 4 amoebae in a hypersaline lagoon containing water over 150‰ salt. Likewise, unidentified amoebae were found at 230‰ in the Great Salt Lake (Vorhies 1917), in Hutt Lagoon, Australia, at salinities between 125–210‰ (Post et al. 1983), and from solar ponds in Sinai (Wilbert and Kahon 1981).

The present study describes a new species of amoeba isolated from the main body of the Salton Sea where salinities average 44‰. The Salton Sea was formed within the last 100 yrs thus all species introductions are recent.

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MATERIALS AND METHODS

Isolation and laboratory cultures. Amoebae were isolated from water at 44‰ collected in 1999 from the shore of the Salton Sea, California. Aliquots (~ 5 ml) were added to Petri dishes containing seawater (32‰) adjusted to a salinity of 44‰ using NaCl, Na_2SO_4, $MgSO_4$ and KCl (8.2 g, 1.8 g, 1.8 g and 0.2 g, respectively). This adjusted sea water will hereafter be referred to as Artificial Salton Seawater (ASSW). According to González et al. (1998), addition of these four salts best mimics the ionic composition of Salton Sea water. Adding a sterile rice grain to nourish the attendant bacterial prey stimulated the growth of amoebae. Cultures were established by taking small drops of these mixed cultures and placing them on a malt/yeast agar plate made with ASSW (0.1 g malt extract, 0.1 g yeast extract, 15 g non-nutrient agar in one-liter adjusted seawater). A streak of *Escherichia coli* was added to provide prey for amoebae. After incubation, clonal cultures were established by transferring individual amoebae on a small agar block with a sterile scalpel to wells of a 24-well microtiter plate (Falcon, Becton Dickinson Labware, Franklin, Lanes, NJ) each containing 1 ml of ASSW. The well with the most luxuriant growth of amoebae was chosen as the clonal culture for further studies. Subsequent subcultures were made weekly into adjusted seawater in small Petri dishes (55-mm diam.) containing a rice grain.

To test for cyst formation, healthy cultures were left to dry out at room temperature. Several days later, these cultures were rehydrated with distilled water and dishes were searched for the presence of cysts and trophic amoebae.

Light and electron microscopy. Light microscopical observations were made with an Olympus IX 70 inverted microscope. Morphological observations, morphometrics, and determination of locomotive rates were made on amoebae attached to the base of Petri dishes. Light micrographs were recorded using the high-resolution optics offered by a short working-distance condenser. In this case, cells were observed under coverglasses but observation times were short to ensure that the behavior of cells was not affected.

Permament slides were made using Nissenbaum's fixative and Kernechtrot staining according to the methods of Page (1988). These preparations were used for nuclear measurements and for the holotype slide (deposited with the British Museum of Natural History, London).

Amoebae growing at 0‰ and 98‰ were prepared for transmission electron microscopy. Cells were harvested from exponentially growing cultures using gentle centrifugation. After

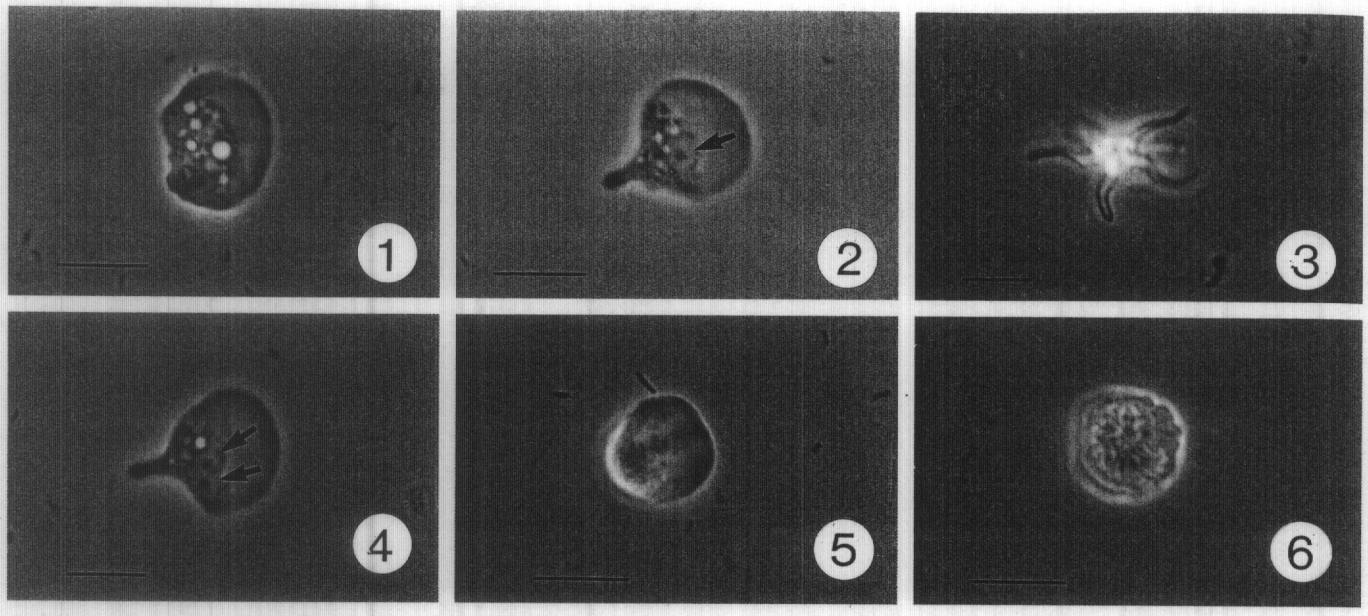

Fig. 1–6. Light micrographs of *Platyamoeba pseudovannellida* n.sp. at salinities between 0 and 138‰. **1.** Normal locomotive form in seawater adjusted to 44‰. **2.** Occasional spatulate form at 44‰ showing nucleus with central nucleolus (arrow). **3.** Floating form of the cell showing blunt, and occasional tapered, radiating pseudopodia. **4.** Binucleate (arrows), spatulate cell at 0‰ salt. **5.** Typical domed-shaped morphology of amoeba grown at 98‰ salt. **6.** Typical domed, and wrinkled morphology of cells grown at 138‰ salt. Scale bar 10 μm throughout.

drawing off the supernatant, amoebae were resuspended in 2% (w/v) glutaraldehyde prepared in 0.05 M cacodylate buffer (pH 7.2), fixed for 20 min at 4 °C, harvested by gentle centrifugation, and post-fixed in 2% (w/v) osmium tetroxide in 0.05 M buffer for 50 min at 4 °C. After pelleting, the amoebae were enrobed in 0.8% warm agar (~ 42 °C) and immediately centrifuged at 1,500 *g* to concentrate the cells before the agar set. Full details are given in Anderson et al. (1996). Small cubes of agar-enrobed, fixed cells were dehydrated through an acetone series (30%—100%), and embedded in Spurr's low viscosity resin. Ultrathin sections, obtained with a Porter-Blum MT-2 ultramicrotome fitted with a diamond knife, were stained with Reynold's lead citrate and examined with a Philips 201 TEM operated at 60 kV.

Effect of salinity on survival and growth. The salinity tolerance range of the *Platyamoeba* isolate from the Salton Sea was examined using two approaches. Experiments either used a gentle shock approach (incremental changes never more than 10‰) or a rapid shock approach where amoebae were transferred from 44‰ into a wide range of salinities without acclimatization. In both approaches, growth rate and culture yield were measured to assess the effect of salinity change on this amoeba. Since culture stress might induce division in cells, positive growth was only measured if cells underwent at least 3 cell divisions.

Salinities tested ranged from 0‰ (i.e. amoeba saline, Page 1988) to 160‰. For salinities greater than 32‰, appropriate concentrations of the four salts, NaCl, Na_2SO_4, $MgSO_4$ and KCl, were added to natural seawater in proportions given by González et al. (1998). For salinities less than 32‰, seawater was diluted with amoeba saline. For the rapid shock experiments, 0.1 ml of inoculum of amoebae (~ 50 cells) growing at 44‰ was added to Petri dishes containing 10 ml of medium adjusted to the following salinities: 0, 8, 16, 24, 32, 42, 52, 62, 72, 82, 92, 102, 112, 122, 132, 142, 152, and 160‰. The number of amoebae in 10 random fields of view on the base of the Petri dish were counted initially (T_0) and three times daily for about 1 wk. In all cases, three replicate dishes were set up.

Counts of cells per field of view were converted to numbers of cells per Petri dish ($\log_{10}$) and growth curves were drawn and regressions calculated for the exponential phase of growth. The maximum yield of cells per treatment was extrapolated from the graphs. The slopes were used to compute the growth rate constant (k) according to the formula given by Stanier et al. (1976):

$$k = \frac{\log_{10} N_t - \log_{10} N_0}{0.301t}$$

where N_t is the final number of cells, N_0 is the initial number of cells, and t is time in h. The generation time in hours was calculated as 1/k.

Gentle shock experiments were conducted in the same manner except that growth rates were calculated in cultures never given more than a 10‰ shock. In these experiments, amoebae were adapted to growth at a specific salinity and then shocked

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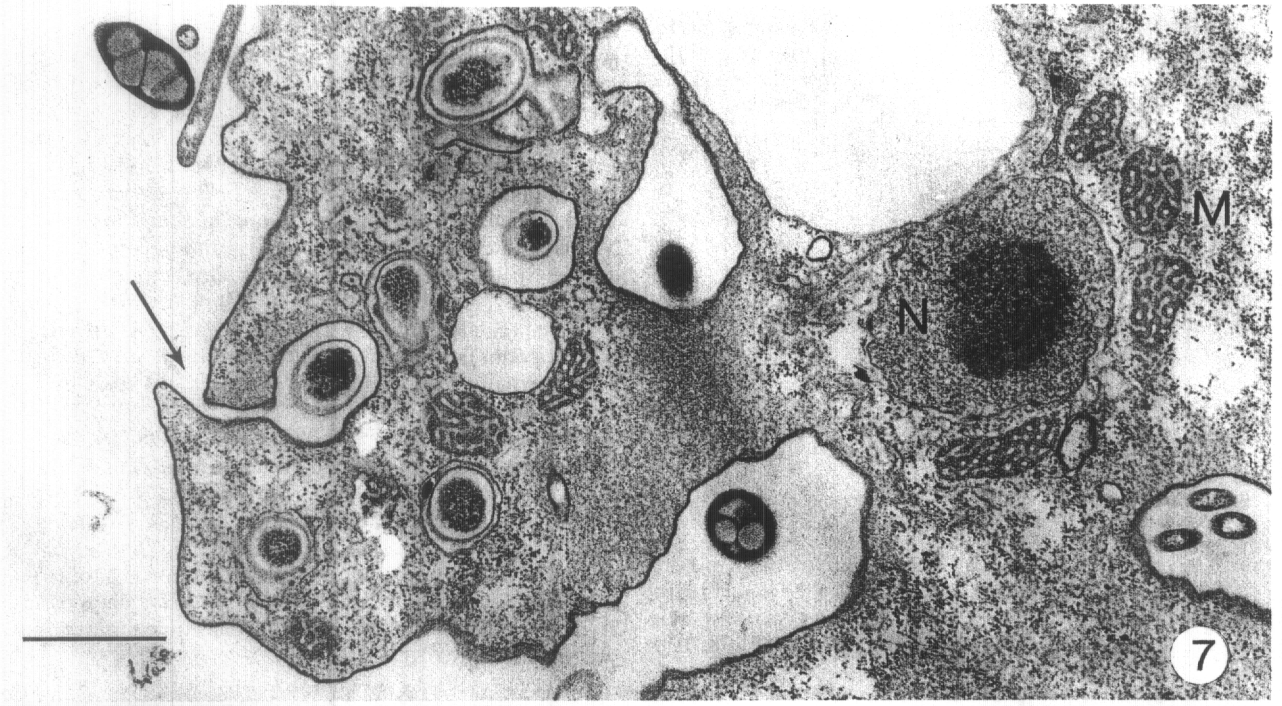
Fig. 7–11. Electron micrographs of *Platyamoeba pseudovannellida* n.sp. cultures at 2 salinities (0‰ and 98‰). **7.** Overview of the cytoplasm of a cell grown at 0‰ showing nucleus (N), mitochondria (M) with branched, tubular cristae (see also Fig. 8 and 9) and recently formed food vacuole (arrow). Scale bar = 1 μm. **8.** Detail of cytoplasm and mitochondria (M) of cell grown at 0‰. Scale bar = 0.5 μm. **9.** Detail of cytoplasm and mitochondria (M) of cell grown at 98‰. Scale bar = 0.5 μm. **10.** Glycocalyx (arrow) of amoeba grown at low salinity (0‰) showing thin arrangement of segmented-like structures in series on the surface, typical of the genus *Platyamoeba*. Scale bar = 50 nm. **11.** Glycocalyx of amoeba grown at high salinity (98‰) showing typical arrangement of surface structures (arrow) with one of the long glycofilaments only found at high salinity (*). Scale bar = 50 nm.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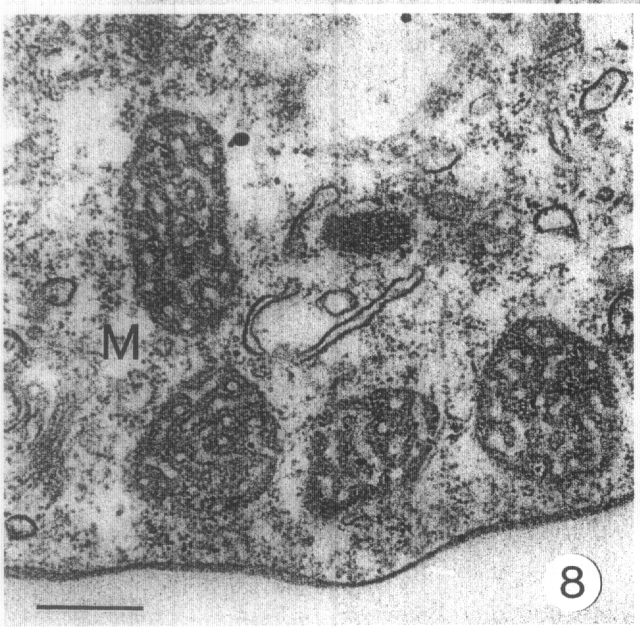
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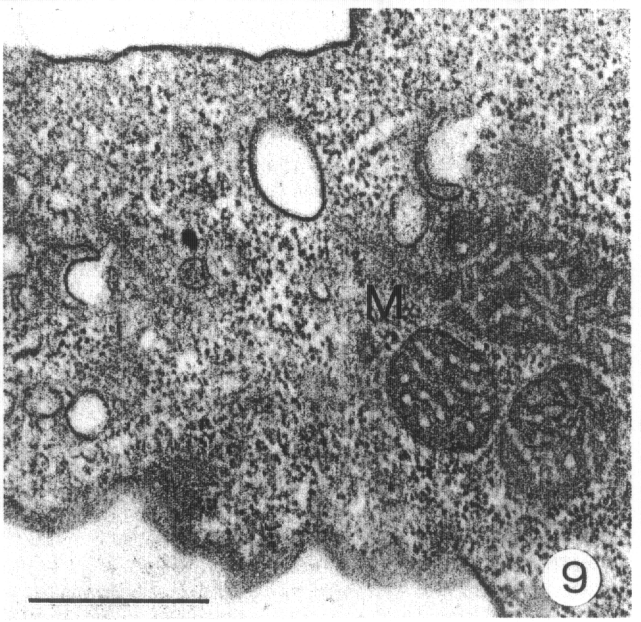
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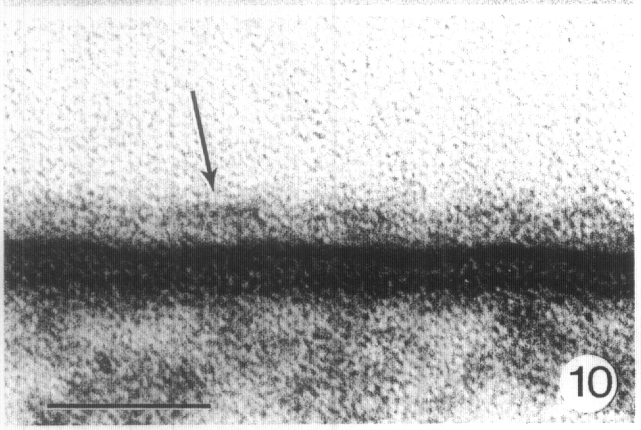
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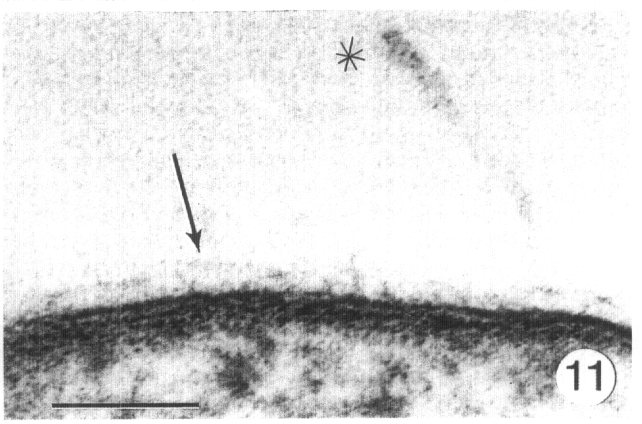
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Table 1. Dimensions of *Platyamoeba pseudovannellida* n. sp. as influenced by salinity (‰).

Salinity	Length	Breadth
0	16.0 (2.6)*	13.0 (3.3)
8	13.4 (2.9)	14.7 (2.7)
16	15.0 (2.6)	13.1 (2.4)
24	14.6 (2.5)	15.8 (2.6)
32	16.3 (3.9)	14.3 (1.2)
48	12.6 (2.5)	15.0 (2.1)
64	14.3 (2.5)	15.7 (2.3)
80	15.0 (1.6)	12.8 (1.9)
96	14.6 (2.1)	13.4 (2.6)
98	11.9 (1.6)	12.3 (2.1)
108	12.6 (2.0)	12.4 (3.8)
138**	9.4 (1.4)	10.2 (2.4)
150**	10.4 (1.8)	9.7 (1.8)

* SD in parenthesis (n = 10).

** Cells markedly rounded and inactive at these salinities. Overall means (less data for 138‰ and 150‰) were 14.2 μm for length and 13.9 μm for breadth (l:b ratio = 1.02).

± 10‰. The following salinity levels were used for the gentle shock experiments: 0, 8, 18, 28, 38, 48, 58, 68, 78, 88, 98, 108, 118, 128, 138, 148, and 158‰.

RESULTS

Platyamoeba pseudovannellida n. sp. Hauer, Rogerson and Anderson (Fig. 1—11)

Description. Locomotive amoebae ovate with a mean length (l) of 14.2 (8.5–25.5) μm and breadth (b) of 13.9 (6.8–25.5) μm giving a l:b ratio of 1.02 (Table 1, Fig. 1). Nucleus spherical (mean diam. 2.1 μm; S.D. 0.4, n = 10) with a distinct central nucleolus (mean diam. 0.9 μm; S.D. 0.2, n = 10) (Fig. 2, 7). Locomotive rate was 0.34 μm sec^{-1} (20.4 μm h^{-1}) when grown in media at the same salinity as the Salton Sea (44‰). Floating form with radiate pseudopodia (usually 8.5–10 μm long but up to 15 μm) that terminated in a blunt tip (Fig. 3). Some cells had more tapered radiate pseudopodia, reminiscent of those found in floating *Vannella*. Anterior hyaloplasm typically occupying one-half to one-third of cell length in amoebae at normal salinity (44‰). Posterior of the cell often flat or slightly concave but in ~ 5% of cells, outline was spatulate. This morphology was much more common in fast moving cells at salinities less than 64‰ (Fig. 2, 4). Mitochondria (~ 1 μm length) were elongate with branched tubular cristae (Fig. 8, 9). Glycocalyx layer fuzzy, ~ 10 μm thick, and in favorable sections consisting of arrangements of segmented-like structures in series across the surface (Fig. 10, 11).

Dehydrated cultures could be revived with relative ease because this species formed cysts which were spherical, wrinkled in appearance (regardless of salinity) with a diameter of ~ 10 μm.

Habitat. Marine, living on suspended particulates in water at salinity levels ~ 44‰.

Specimen deposited. A holotype specimen (Kernechtrot stained permanent slide, registration number 2001:6:25:1) has been deposited with the British Museum of Natural History, London.

Etymology. *Platyamoeba pseudovannellida* n.sp. is named to denote the similarity with *Vannella*. The spatulate appearance of some cells is a feature normally associated with *Vannella*.

Remarks. Amoebae with flattened locomotive forms that are fan-shaped (flabellate), ovate or oblong generally belong to either the genus *Vannella* or the genus *Platyamoeba*. The most rigid diagnostic feature is arguably the nature of the glycocalyx, which is organized into tower-like glycostyles in *Vannella* and arranged as filamentous material in *Platyamoeba* (Page 1988). At the light microscope level, locomotive cells of *Vannella* are frequently flabellate although some have a spatulate shape with a long posterior tail. Floating amoebae usually have radiating pseudopodia that taper to fine tips. *Platyamoeba*, on the other hand, is usually flabellate or oblong in shape but not spatulate. Suspended cells often have blunt, radiating pseudopodia.

The species described here has characteristics at the light microscope level reminiscent of *Vannella*, notably the occasional spatulate posterior and the occasional tapered radiating pseudopodia in some floating forms. However, the surface coat characteristics are in line with *Platyamoeba* as is the ability of this genus to form cysts.

Clydonella (Sawyer 1975) is a genus with features intermediate between *Vannella* and *Platyamoeba* (Page 1988). However, the surface coat features of this genus are unknown at this time. Because of the importance of the nature of the glycocalyx in identification, we have assigned this new species to the genus *Platyamoeba*. The isolate shows remarkable salinity tolerance and although this character was not used as a diagnostic feature, it does help to set this amoeba apart from other fan-shaped amoebae.

Effect of salinity on cells. Salinity levels markedly affected the locomotive rates of *P. pseudovannellida*. In freshwater, cells were active moving at 1.05 ± 0.46 μm sec^{-1}. As salinity increased there was a concomitant decrease in locomotion. At 44‰ movement slowed to 0.34 ± 0.09 μm sec^{-1} and at 108‰ to 0.11 ± 0.04. At the highest salinity supporting growth (i.e. 138‰) movement was below detection. Salinity of the culture medium markedly influenced the morphology of the amoebae. At 0‰ and 44‰, cells were flattened and the posterior cytoplasmic mass was distinct and granular with many vesicles and vacuoles; some of these were contractile vacuoles which were evident in amoebae growing in cultures at 0, 8, 16, and 24‰. Amoebae at 0 g l^{-1} were sometimes spatulate (Fig. 2, 4) whereas only a few cells were spatulate at 44‰ and none adopted this morphology at the very highest salinities (above 100‰). Some amoeba growing in 0‰ were binucleate (Fig. 4) a condition never observed at higher salinities.

As salinity increased above about 60‰, amoebae became increasingly domed and thicker. The posterior cytoplasm, which occupied half of the cell, was raised such that cytoplasmic detail was obscured (Fig. 5, at 98‰). The cell surface resembled the theca of a smooth, thecate amoeba. At 138‰, the cell was domed and wrinkled (Fig. 6) and usually inactive, although cell growth was possible at this salinity if amoebae were acclimatized by gradual change. Amoebae survived in culture up to 152‰, although at these levels no true growth was measured (i.e. some cells divided in the first 72 h, but subsequent divisions were not sustained).

The size of cells was not affected by salinity over the range 0–108‰ (Table 1). At the highest salinities, when cells became rounded and generally less active, the dimensions were smaller. At the ultrastructural level, the effects of salinity were subtle. The most obvious feature was an increased density of cytoplasm at high salinity (98‰, Fig. 9) relative to the cytoplasm of cells grown at 0‰ (Fig. 8). Mitochondria were larger in cells reared in the low salinity compared to those grown in high salinity medium. There were also slight differences in the glycocalyx as a result of salinity. The basic glycocalyx layer was about 10 nm thick, and this was found in both high and low salinity-reared cells. But in the case of cells grown at 98‰, there were also long (100–150 nm) glycofilaments extending

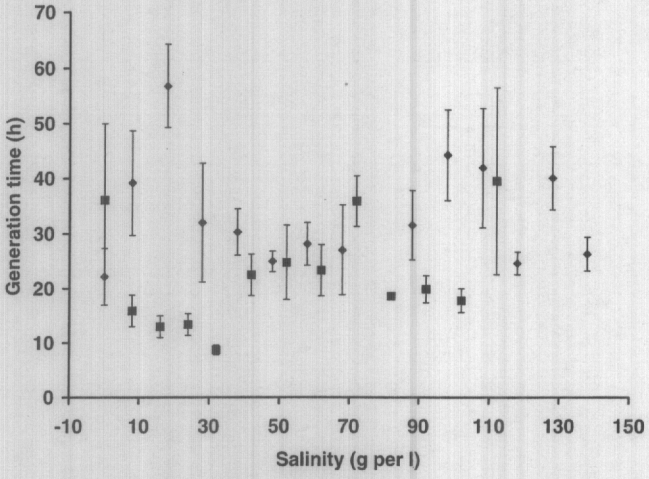

Fig. 12. Effect of salinity on the generation times of *Platyamoeba pseudovannellida* n.sp. Amoebae were either subjected to a rapid salinity change (■) or a gentle change of never more than 10‰ salt (◆). Means ± S.E (n = 3).

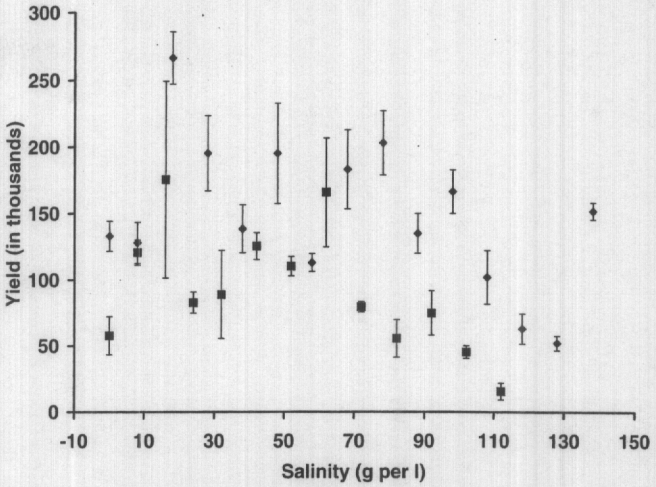

Fig. 13. Effect of salinity on the yields (thousands of cells per Petri dish) of *Platyamoeba pseudovannellida* n.sp.. Amoebae were either subjected to a rapid salinity change (■) or a gentle change of never more than 10‰ salt (◆). Means ± S.E. (n = 3).

from the cell surface (Fig. 11). These were never found in cells grown at 0‰ (Fig. 10).

Effect of salinity on survival and growth. *Platyamoeba pseudovannellida* n.sp. showed remarkable tolerance to salinity fluctuations, even when the changes were dramatic. The results of the rapid change experiment, where cells were taken from cultures at 44‰ and inoculated into a wide range of salinities, showed that growth was possible over the range 0–112‰ salt. However, overall there was no discernible trend (Fig. 12). The mean generation time of *Platyamoeba* across all salinities was 23.9 ± 16.6 h (S.D). Although some salinities promoted more rapid growth, such as the shorter generation times across the lower salinities 16–32‰ (mean generation time 11.7 h), there was no consistent trend across the breadth of salinities tested when the different generation times were compared by paired *t*-tests ($p \leq 0.05$). In other words, the higher and lower salinities examined did not produce significantly longer generation times.

The gentle shock experiments, where salinity changes were never more than 10‰, extended the range of *Platyamoeba* slightly (0–138 g l^{-1}; Fig. 12). Once again, the generation time was more or less constant over this entire range averaging 33.6 ± 9.7 h (S.D.). Comparing the range of means of the rapid and gentle shock treatments by ANOVA showed that generation times from these two treatments were not significantly different ($F = 2.829$; $p = 0.105$).

The only significant difference between the rapid shock and gentle shock conditions was found with the yield data (i.e. total number of cells per Petri dish). In most cases, higher yields were found with the gentle shock treatments (Fig. 13) indicating that although amoebae were replicating at the same rate in both treatments they underwent more divisions with the gentle shock treatment. An ANOVA ($F = 8.212$; $p = 0.0081$) comparing the mean yields across the salinities tested showed that the gentle shock yields were significantly higher (an overall mean of 117,202 cells per Petri dish) when compared with the rapid shock yields (an overall mean of 71,510 cells/Petri dish).

DISCUSSION

Justification for the new species. Although *Platyamoeba pseudovannellida* n.sp. shares features with *Vannella*, notably the occasional spatulate shape, the ultrastructural evidence clearly indicates that this is a species of *Platyamoeba*. This genus is perhaps the most widely distributed of all the marine amoebae (Page 1988) and there are several named species that must be considered. *Platyamoeba australis*, *P. bursella*, *P. calycinucleolus*, *P. flabellata*, and *P. mainensis* are all larger than *P. pseudovannellida* n.sp.with mean sizes in the 20—30 μm range (see Page 1983). *Platyamoeba plurinucleolus* is smaller (8–34 μm). However, its nucleus is unique with a parietal, rather than central nucleolus (Page 1974). Several other platyamoebae are similar in size to *P. pseudovannellida* n.sp. but have significant distinguishing characteristics. *Platyamoeba leei* has a mean length of 20 μm, but is linguiform and lacks radiating pseudopodia (Sawyer 1975). *Platyamoeba murchelanoi* has a mean length of 11 μm but again lacks radiating pseudopodia in the floating form (Sawyer 1975). *Platyamoeba douvresi* (mean length 13 μm), *P. langae* (mean length 9 μm) and *P. weinsteini* (mean length 12 μm) are all close in size to *P. pseudovannellida* n.sp. and have l:b ratios close to 1.0. However, they all have a straight or slightly convex posterior edge; a spatulate shape was never reported. Moreover, they all have nuclei slightly larger than that of *P. pseuodovannellida* (3.0, 2.5, 3.5 μm, respectively). The locomotive rates of *P. douvresi* and *P. weinsteini* further set them apart. Their rates were around 40 μm min^{-1} (Sawyer 1975), twice as fast as the rate of *P. pseudovannellida* n.sp. in seawater. The locomotive rate of *P. langae* was similar to *P. pseudovannellida* n.sp. However, the floating form of this amoeba was quite different. Cells of *P. langae* adopted a peg or rod-shaped appearance without radiating pseudopodia (Sawyer 1975).

Sawyer (1975) described three discoid, flattened species of *Clydonella*, a genus with light microscopical features intermediate between *Vannella* and *Platyamoeba* (Page 1983). However, none of these species had spatulate forms and all had locomotive rates around 35–40 μm min^{-1}.

Since the Salton Sea is a relatively recent water body that has only undergone dramatic salinity shifts within the last 100 yrs, it is possible that *P. pseudovannellida* n.sp. is a freshwater isolate that invaded and adapted to the changing salinities. However, it is considerably smaller than *Platyamoeba schaeferrii*, which is 27–29 μm long. Although closer in size to *P. placida* and *P. stenopodia* (15 μm to 35 μm), both these freshwater species have smooth cysts (Page 1988) quite unlike the wrinkled cysts seen in *P. pseudovannellida* n.sp.

Although the ability to tolerate a wide range of salinities was

not used as a diagnostic feature, it does set *P. pseudovannellida* apart from most other amoebae. When describing the marine amoebae *P. douvresi*, *P. weinsteini* and *P. langae*, Sawyer (1975) documented the lowest salinity for growth as 22.5, 7.5 and 7.5‰ salt, respectively. Such modest tolerance to salinity change has been noted for other species. For example, Anderson (1994) examined salinity tolerance in *Vexillifera telmathalassa* isolated from coastal regions of Barbados and found it to exhibit normal locomotion and shape down to 16‰. Decreasing salinity further slowed the rate of locomotion until cells were immobilized at 12‰. Moreover, a recent study of benthic amoebae in the Baltic Sea (Garstecki and Arndt 2000), which is a brackish area with salinities between 2–12‰, reported that isolates were more or less equally split between freshwater and marine forms.

Thus, the pattern emerging is one whereby many marine and freshwater amoebae readily tolerate salinity changes over modest ranges. So for now, it seems sensible to retain the distinction between freshwater and marine amoebae, unless brackish waters are being sampled. But the ability of *P. pseudovannellida*, *Ovulinata parva* (Anderson et al. 1996, 1997), *Vannella simplex* (Smirnov, pers. comm.), *Flabellula citata* (Page 1983), and *F. calkinski* (Page 1983), to grow in both 0‰ water and 32‰ seawater suggests that the distinction between freshwater and marine water is increasingly grey and less robust as a diagnostic feature.

Hypersaline amoebae and *P. pseudovannellida*. Kinne (1964) defined hypersaline waters as those between 40–300‰. A more elaborate set of definitions was offered by Por (1980) who categorized hypersaline waters into 4 groups. Alpha (α) hypersaline waters range from ~ 40–100‰, beta (β) hypersaline waters are those exhibiting a dramatic reduction in diversity, between ~ 100–140‰. Increasing salinity levels further simplifies the ecosystem through gamma (γ) waters, at salinities from ~ 140 to the upper 200‰, to delta (δ) hypersaline waters ~ 300‰. Adopting this terminology, it is clear that *P. pseudovannellida* n.sp. can survive and grow in waters spanning freshwater to β-hypersaline waters.

Platyamoeba pseudovannellida n.sp. is certainly not unique among protists in its ability to tolerate wide salinity fluctuations. Several amoebae have been shown to have a wide tolerance to salinity, but *P. pseudovannellida* n.sp. seems unique in its ability to grow in β-saline conditions all the way down to freshwater. Smirnov (pers. commun.) recently described a new species of *Vannella* capable of growing over a slightly narrower salinity range (6–90‰) than *P. pseudovannellida* n.sp. (0–138‰). Butts (1935) conducted salinity experiments on the marine amoeba *Flabellula mira* (= *Vannella mira*) and noted that it could tolerate salinities from ~ 6.5 to 48‰. Preliminary data gathered by the authors suggest that freshwater amoebae may be less tolerant of salinity change than marine amoebae, and to illustrate this point the freshwater *Thecamoeba osseosaccus* could only tolerate salinity ranges from 0% to 40% (i.e. up to 12.8‰; Oshima et al. 1986).

Over the range of salinities examined, *P. pseudovannellida* n.sp. displayed different morphologies at different salinities. This is not surprising since several workers (Gaievskaia 1925; Pack 1919; Reddy 1972; Sawyer 1970, 1971) have all shown that the morphology of some ciliates and amoebae can be greatly altered by changing the salinity of the growth medium. What is surprising is that growth measured as generation time was more or less consistent across all salinities. Regardless of whether amoebae were acclimatized or not, the overall generation time was 29 h at 20 °C. This is longer than the 16-h theoretical generation time for this small amoeba. This was estimated using conversions given in Rogerson et al. (1994) and Butler and Rogerson (1996). Even allowing for errors inherent in these conversions, the long generation time implies a high energetic cost of adapting to different salinities.

It was expected that there would be an optimum growth rate at normal salinity (around 44‰) but because of the scatter in the growth data, this was not apparent (Fig. 12). Likewise, acclimatization had little effect on the growth rate of this species, although yields were consistently higher when cells were not given rapid salinity shocks. In other words, when acclimatized, amoebae did not grow any faster but did undergo more divisions before entering stationary phase. The reasons for the consistency in growth across such a wider range of salinities is unknown at this time, but from an ecological standpoint it shows that this species is euryhaline and is unlikely to be endemic to the Salton Sea. Rather, it is expected that this species should be widely distributed in many freshwater and saline environments.

The main problem faced by halophiles is control of osmotic pressure. Without the ability to control water movement, cells would lose water to the surrounding saline environment. Very little is understood about the mechanisms for tolerating high external salt concentrations. The hypersaline alga, *Dunaliella salina*, synthesizes intracellular glycerol to balance osmotic pressure (Ben-Amotz and Avron 1972) while *Paramecium calkinsi* responds to salinity stress by dramatically altering cellular free amino acids (Cronkite et al. 1991). The concentration of intracellular amino acids in *Acanthamoeba castellanii* is also directly related to the tonicity of the growth medium (Drainville and Cagnon 1973). The flagellate, *Ochromonas malhamensis*, adjusts the intracellular concentration of carbohydrates (e.g. isofloridoside, metabolically derived from glucose) in response to increasing external osmotic pressure (Kauss 1977) in addition to increases in amino acids, mainly alanine (Aaronson 1980). We examined cells grown at two salinity extremes (0‰ and 98‰) to look for ultrastructural effects. Only three features were evident and while interesting, none of these sheds light on the mechanism of salt tolerance at this time. Firstly, mitochondria were larger in cells reared in low salinity media. This is perhaps not surprising since the cell cytoplasm presumably comes to an osmotic balance with the surrounding medium. In freshwaters, this would result in the mitochondria taking up water from the cytoplasm. Conversely, in high salinity culture, the cells probably increase their cytoplasmic osmolytes such that the mitochondria may lose water to the cytoplasm. Secondly, amoebae at high salinity (98‰) had a glycocalyx, typical of *Platyamoeba*, but with additional long glycofilaments. Finally, amoebae grown at 98‰ had a much denser and more compactly granular cytoplasm than amoebae at 0‰. If the ribosomal density is greater, this may reflect the manufacture of a proteinaceous material to help balance osmotic pressure or alternatively may be a consequence of increased water loss from the cell. The denser cytoplasm helps to explain the slow rate of movement of cells in high salinity media. More studies will be required to elucidate salinity tolerance mechanisms in amoebae, but *P. pseudovannellida* n.sp. with its ability to be grown with ease over a wide salinity range, could be an ideal model organism for future work. There are, of course, amoebae that can tolerate much higher salinity levels, such as the two species noted by Post (1977) in water collected from the Great Salt Lake (330–350‰). But these are probably obligate halophiles and less amenable to laboratory cultivation and investigation.

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