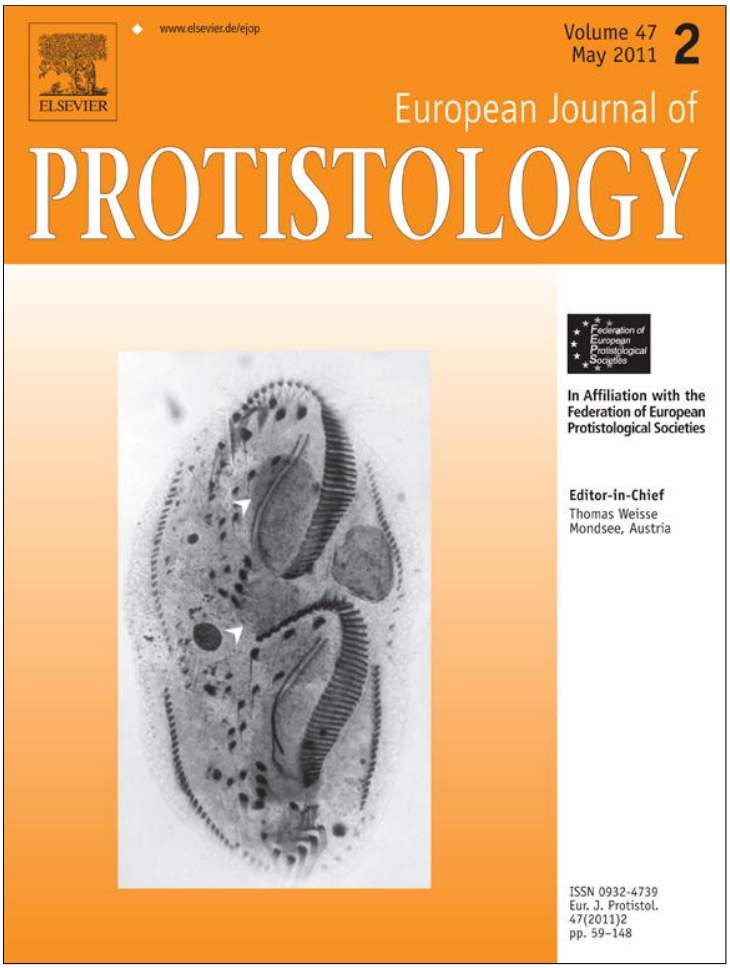

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Dermamoeba algensis n. sp. (Amoebozoa, Dermamoebidae) – An algivorous lobose amoeba with complex cell coat and unusual feeding mode

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Abstract

The genus *Dermamoeba* unifies oblong, flattened amoebae of lingulate morphotype, possessing a thick multilayered cell coat. It includes two species, *D. granifera* and *D. minor*. In this paper we describe a third species of this genus, *D. algensis* n. sp. This species is algivorous; engulfing a large algal cell, it destroys part of the cell coat liberating the plasma membrane, which forms the food vacuole. Thus the glycocalyx never appears inside the phagosome. This observation confirms that some of the thick-coated amoebae may use this way to avoid energetically costly digestion of their own glycocalyx. Studies of the physiology of this organism show that it feeds most actively at a temperature of 22–25 °C. Below and above this temperature the feeding intensity drastically decreases. The new species can survive NaCl concentrations up to 5%, which roughly corresponds to 50 ppt salinity. Accordingly, *D. algensis* has a wide range of salinity tolerance.

Keywords: Amoebozoa; Systematics; Ultrastructure; Biology

Introduction

The genus *Dermamoeba* was established by Page and Blakey (1979) for a single amoeba species, formerly known as *Thecamoeba granifera*, described by Greeff (1866). This oblong, flattened amoeba, never forming discrete pseudopodia, has much in common with other thecamoebids except for the fact that its dorsal surface is always smooth, without any ridges or wrinkles (the latter may be occasionally observed close to the uroid and extend only at a short distance anteriorly). Ultrastructural investigations of this species (Page and Blakey 1979) showed that it possesses a very thick cell coat, consisting of a thick layer of fibrous material, underlined and covered by thinner vertically structured layers. This kind of a cell coat was termed "cuticle" (Page and Blakey 1979) and the new genus *Dermamoeba*, belonging to the family Thecamoebidae Schaeffer 1926, was established to accommodate this species. Further a strain formerly recognized as "*Thecamoeba granifera* ssp. *minor*" (Pussard et al. 1979), was raised to a species rank by Page (1988).

The third species of the genus *Dermamoeba*, *D. algensis*, described in the present paper, was found in the year 1999. Light-microscopic (LM) and transmission electron microscopic (TEM) studies were performed at the same time, and sequences of the 18S rRNA gene and actin-coding gene were done (Fahrni et al. 2003). The culture was deposited with CCAP (UK), where it was maintained for several years before it was lost. The phylogenetic analysis by Fahrni et al.

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(2003), as well as a number of later studies (Michel et al. 2006; Brown et al. 2007; Tekle et al. 2008) did not recover a relationship of *D. algensis* with other amoebae of the family Thecamoebidae *sensu* Page (1987). Instead, it formed an independent branch of unclear affinities (Smirnov et al. 2005), close to amoebae of the order Vannellida (Kudryavtsev et al. 2005) or sister to the single sequenced representative of the genus *Mayorella* (Pawlowski and Burki 2009; Smirnov et al. 2007, 2008). When the fourth genus of thecamoebids, the genus *Paradermamoeba* (Smirnov and Goodkov 1994, 2004) was included in the phylogenetic analysis, it became clear that *Dermamoeba algensis* robustly groups with *Paradermamoeba*, and all this assemblage, including *Mayorella* (Smirnov et al. in prep.).

Despite numerous references to this species in the phylogenetic papers, the systematic description of *Dermamoeba algensis* was delayed by a number of technical reasons. In the present paper we describe this species, paying special attention to its unusual feeding mode, related with the engulfment of rather large food objects – cells of unicellular algae.

Materials and Methods

Amoebae were isolated by Prof. B.V. Gromov in 1999 from a small freshwater pond close to Kirovsk village (North-Western Russia, 33 km East of Saint Petersburg).

Cultures were maintained in glass tubes or in the 90 mm Petri dishes filled with the mineral medium No. 1 (Gromov and Titova 1991) under daylight lamps (light intensity 25 μM photons m^{-2} s^{-1}) and a constant temperature of 24 °C. A culture of the alga *Tribonema gayanum* Pasch. CALU–20, maintained on the agar medium No. 3 (Gromov and Titova 1991), was used as a food source. This culture was deposited to CCAP and kept there for some time before it was lost.

Light-microscopic images were made in the years 2000 and 2001, using Olympus BH2 with DIC optics and inverted Nikon Diaphot photomicroscope equipped with phase contrast optics. 100 cells were measured on the object slide to obtain morphometric data. For TEM analyses, amoebae were placed in 40 mm Petri dishes filled with culture medium. A suspension of *Tribonema gayanum* was added to these dishes. After 2–2.5 h, amoebae started intensive feeding. At this moment the culture was pre-fixed with osmium tetroxide (final concentration in the Petri dish 0.025%), transferred to glass tubes, concentrated by centrifugation at 2000 rpm and washed one time with 0.05 M sodium cacodylate buffer. Amoebae were then fixed with 2.5% glutaraldehyde with 0.5 μg/ml of tannin for 1 h, washed 3× 5 min with 0.05 M sodium cacodylate buffer, postfixed with 1% osmium tetroxide, washed again 3× 5 min with 0.05M natrium cacodylate buffer and embedded in agar blocks (2.5% agar Difco). The blocks were cut into cubes of ca 1 mm^3 each, dehydrated in 30%, 40%, 50%, 60%, 70%, 80%, 96% and 100% ethanol, acetone/ethanol mixture 1:1 and in pure acetone, and finally embedded in Spurr resin. Sections were stained with uranyl acetate and Reynold's lead citrate (Reynolds 1963) and examined using a JEM 100C electron microscope.

To prepare permanent preparations, amoebae were fixed with Bouin's solution, stained with the iron haemotoxylin, dehydrated and embedded in Canada balsam.

To check the chemical nature of the cell coat, during the preparation of the TEM embeddings a 0.3% Ruthenium red solution was added to the osmium tetroxide solution used for postfixation at a final concentration of 0.075%. The postfixation time was extended to 1.5 h. This method is intended to stain mucopolysaccharides (see Waller et al. 2004).

To test for food preferences, a number of algal strains maintained in the collection of algae of the Biological Research Institute of St. Petersburg State University were used (Table 1). To study the relative intensity of feeding under different temperatures, a known number of amoebae cells and a known number of algal cells, equal in each experiment, were inoculated into the tubes containing 6 ml of the medium. The tubes were incubated for two weeks under constant light but at different temperatures.

Results

Light microscopy of trophozoites and cysts

Amoebae in locomotion were of lingulate morphotype, i.e. smooth, oblong or ovoid cells, without folds or wrinkles on the dorsal surface (Figs 1–7). The hyaloplasm formed a deep antero-lateral crescent (Figs 2, 3, 5). Cells had no differentiated uroidal structures. The length of the locomotive form was 50–100 μm (average 75 μm); breadth 30–50 μm (average 37 μm); length/breadth ratio was 1.4–2.8 (average 2.0). When changing the direction of locomotion, the cell usually produced a wide, rounded lateral pseudopodium, which rapidly became a leading one and the cell began to move in a new direction. As a variant of this behavior, some cells formed not a single large pseudopodium, but a number of smaller pseudopodia, progressing in different directions; one of them soon became a leading one. Finally, an amoeba could change direction of locomotion by bending its body during motion, without the loss of the locomotive morphology. Stationary cells were rounded, with several short, wide pseudopodia or hyaline lobes surrounding the main cytoplasmic mass. When a stationary cell resumed locomotion, it produced a number of short, rounded pseudopodia, aimed in different directions, and moved rather chaotically. After some time of such behavior, one of the pseudopodia took a leading function and the cell gradually adopted a usual locomotive form. The floating form was of irregular shape and resembled a rounded or oblong mass. Some of the floating cells produced a few short conical hyaline pseudopodia with rounded tips.

The vesicular nucleus (Fig. 8) was often located close to the single contractile vacuole. Both were at the posterior part of the cell. Most amoebae had one nucleus, however some were binucleate, but these nuclei were never closely apposed

Table 1. Test of the food preferences of *Dermamoeba algensis*.

Species of algae	Strain number	Repeats of the test		
		1	2	3
1. *Ankistrodesmus braunii* Brunnth	80	+	+	+
2. *A. convolutus* Corda	252	−	−	−
3. *A. falcatus* Ralfs	1	+	+	+
4. *A. falcatus* Ralfs var. stipitatus (Chod.) Korsh.	253	−	−	−
5. *A. rotundus* Korsh.	364	+	+	+
6. *Bracteacoccus grandis* Bisch. et Bold	927	+	+	+
7. *B. minor* (Chodat) Petrova	825	+	+	+
8. *Chlorella paramecii* Loefer str. Loefer	189	+	+	+
9. *Ch. protothecoides* Kruger str. Kruger	284	−	−	−
10. *Ch. pyrenoidosa* Chick str. Emerson	168	+	+	+
11. *Ch. sacchorophila* Kruger str. Tschermak-Woess	833	+	+	+
12. *Ch. sorokiniana* Shih. et Krauss srt. Lewin	826	+	+	+
13. *Ch. terricola* Hollerb. str. Gromov	130	+	+	+
14. *Ch. variegata* Beijer. str. Beijerinck	188	+	+	+
15. *Ch. vulgaris* Beijer. var. viridis Chod. str. Chodat	184	+	+	+
16. *Chlorococcum hypnosporum* Starr str. Starr	473	−	−	−
17. *Chl. lacustre* Archib. et Bold str. Strelkova	886	−	−	−
18. *Chl. minutum* Starr. str. Gromov	746	+	+	+
19. *Chl. polymorfum* Bisch. et Bold str. Bischoff	478	−	−	−
20. *Chl. punctatum* Arce et Bold str. Arce	476	+	+	+
21. *Chl. rugosum* Archib. et Bold	938	−	−	−
22. *Kirchneriella obesa* (West) Schmidle str. Gromov	28	−	−	−
23. *Pediastrum boryanum* (Turp.) Menegh.	386	+	+	+
24. *Scenedesmus acuminatus* (Lagerch) Chodat	251	−	−	−
25. *Sc. acuminatus* (Lagerch) Chodat str. Gromov	411	+	+	+
26. *Sc. acutus* Meyen str. Kvitko	65	−	−	−
27. *Sc. basilensis* Chodat str. Vischer	203	+	+	+
28. *Sc. bijugatus* (Turp.) Kutz.	12	−	−	−
29. *Sc. ecornis* (Ralfs) Chod. str. Gromov	160	−	−	−
30. *Sc. obliquus* (Turp.) Kutz.	13	+	+	+
31. *Sc. quadricauda* (Turp.) Breb. str. Gromov	418	−	−	−
32. *Tribonema gayanum* Pasch. str. Gromov	20	+	+	+
33. *T. vulgare* Pasch. str. Chudjakov	890	−	−	−

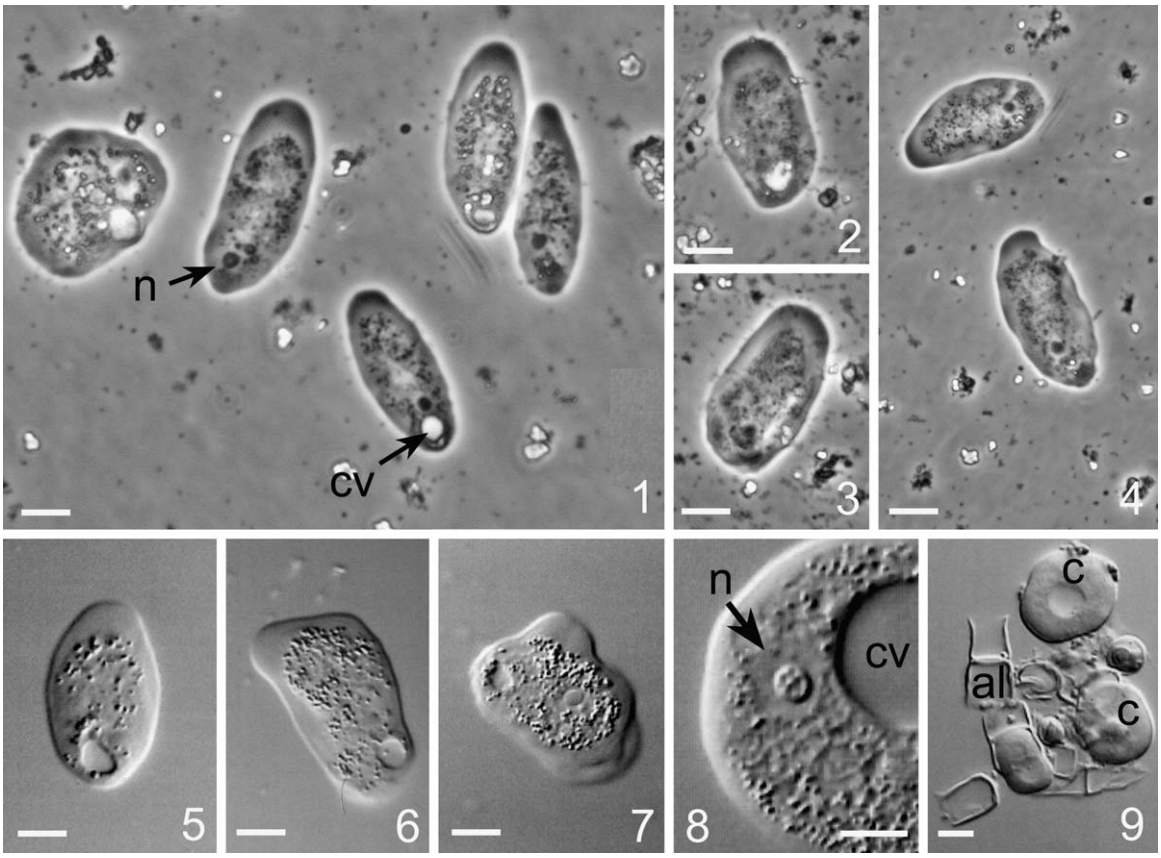
(+) amoebae exhibit growth; (−) no growth of amoebae. Numbers of algal strains are provided according to the catalog of the collection of algae of the Biological Research Institute of St. Petersburg State University.

to each other such as, e.g. in *Sappinia diploidea*. The nucleolus was centrally located, rounded and had a number of lacunae of different size, clearly visible with LM and confirmed with TEM (Figs 10, 18). Because of these lacunae, the nucleolus looked foamy in DIC optics under higher magnifications (Fig. 8). The diameter of the nucleus was 2.9–5.7 μm (average 4.7 μm), that of the nucleolus 1.4–4.6 μm (average 3.2 μm). The inclusions in the cytoplasm were not numerous. Mostly they were dark granules and refractive bodies. Some of them were rounded, while others had irregular shape. Some cells contained food vacuoles filled with the remnants of algal material.

In our cultures amoebae readily formed rounded, single-walled cysts (Fig. 9). The diameter of the cyst was 47–70 μm (average 58 μm). Using light microscopy the cyst wall looked rather thin, smooth and relatively closely apposed to the cell membrane. There was no evidence of cyst pores.

Electron microscopy of trophozoites and cysts

The nucleus in TEM sections was of vesicular type, with the single central nucleolus (Fig. 10). The nucleolus was more or less compact but had a number of lacunae inside, corresponding to those visible in LM (Fig. 8). Around the nucleolus there were small patches of dense material (chromatin?) distributed in the karyoplasm. There was no evidence for nuclear lamina of any sort (Fig. 12). Some of the sectioned cells were binucleate; nuclei were located at some distance from each other and never were tightly apposed. The cytoplasm between the nuclei had usual appearance, i.e. there was no evidence for any specific structures in this area (Fig. 11). Mitochondria were rounded or oval, they had an electron-dense matrix and tubular cristae (Fig. 13). The dictyosomes were not numerous; they consisted of 4–6 flattened cisternae, surrounded by numerous vesicles (Fig. 15).



Figs 1–9. Light microscopy of *Dermamoeba algensis*. **(1–4)** Live amoebae in the mineral medium on the plastic surface of the culture dish. Resting and locomotive forms, phase contrast, inverted microscope. **(5)** Locomotive form on the glass surface, DIC. **(6, 7)** Amoeba, changing direction of movement. **(8)** The cell was heavily flattened with the coverslip to show the nucleus. A number of lacunae are visible in the nucleolus. **(9)** A few amoeba cysts among the remnants of algal food. Bars = 20 μm. n, nucleus; cv, contractile vacuole; al, algal cells; c, amoebae cysts.

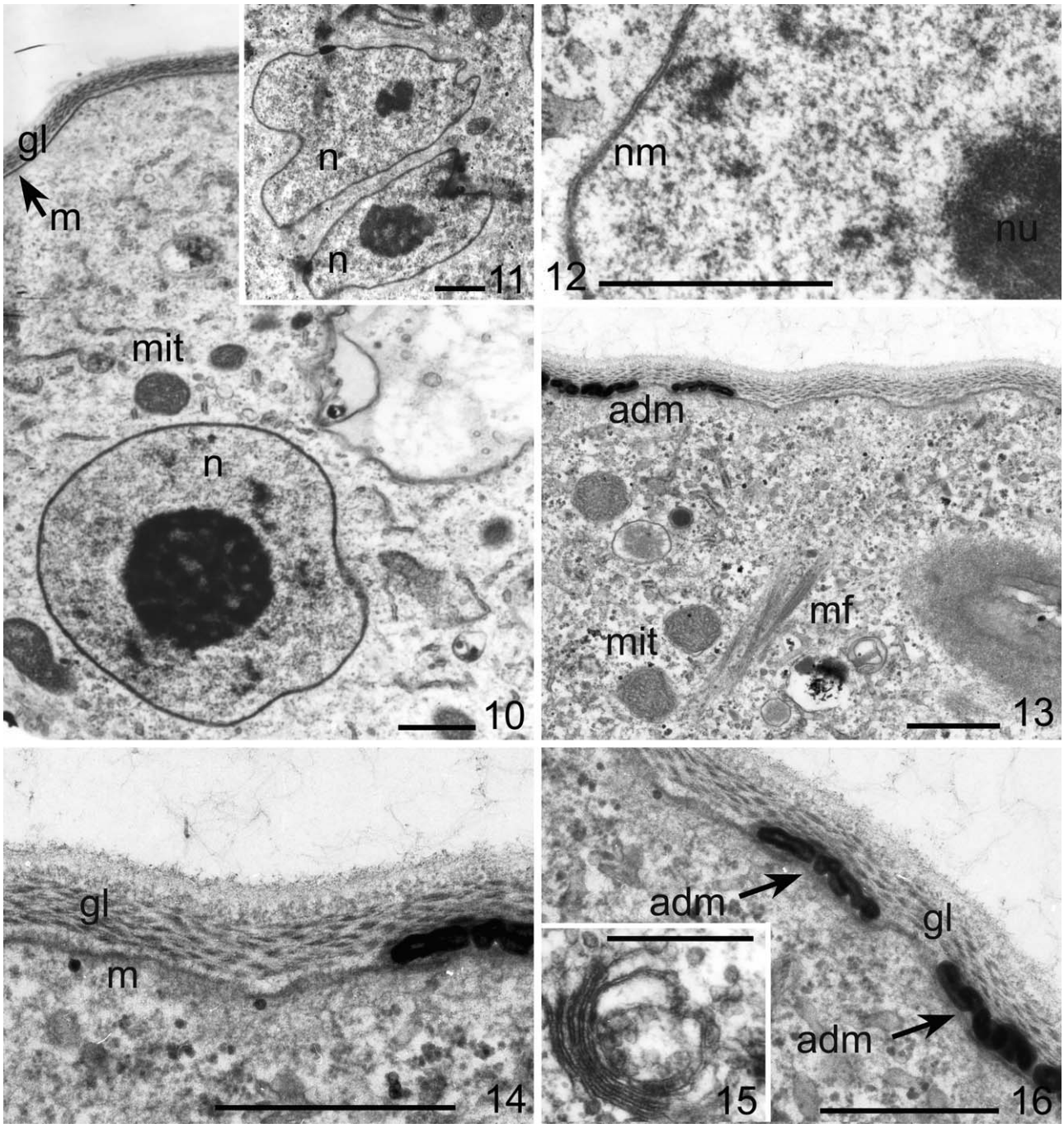
The cell coat was rather thick (ca. 500 nm) and consisted of three layers (Figs 13, 14, 16). The basal layer in TEM sections was a little distant from the plasma membrane and consisted of spindle-like fibrillar structures parallel to the plasma membrane. The space between this layer and the cell membrane was filled with very fine, hardly visible vertically oriented filamentous material. In some sections in this area there were rounded patches of an electron-dense material, often closely connected in chain-like groups (Figs 14, 16). The layer next to the basal one was much more loosely structured and consisted mostly of patchy filamentous material, oriented perpendicularly to the plasma membrane. Finally, the top layer of the glycocalyx was amorphous (perhaps a result of the destruction of the top area of the cell coat under fixation). When cells were stained with ruthenium red, only the top layer of the glycocalyx was densely stained (not illustrated), thus confirming its polysaccharide nature. Bundles of microfilaments were found in different areas of the cytoplasm, but there were no cytoplasmic microtubules.

Cysts observed by TEM were rounded, single-walled (Figs 17, 18). The cyst wall had a heterogeneous structure, consisting of a mixture of a dense, patchy material embedded into a more transparent foamy matrix. The cell surface was always widely separated from the cyst wall; the glycocalyx of the cell remained intact inside the cyst (Fig. 17). In the cysts, we never saw the arrangements of a dense material between the cell membrane and the glycocalyx.

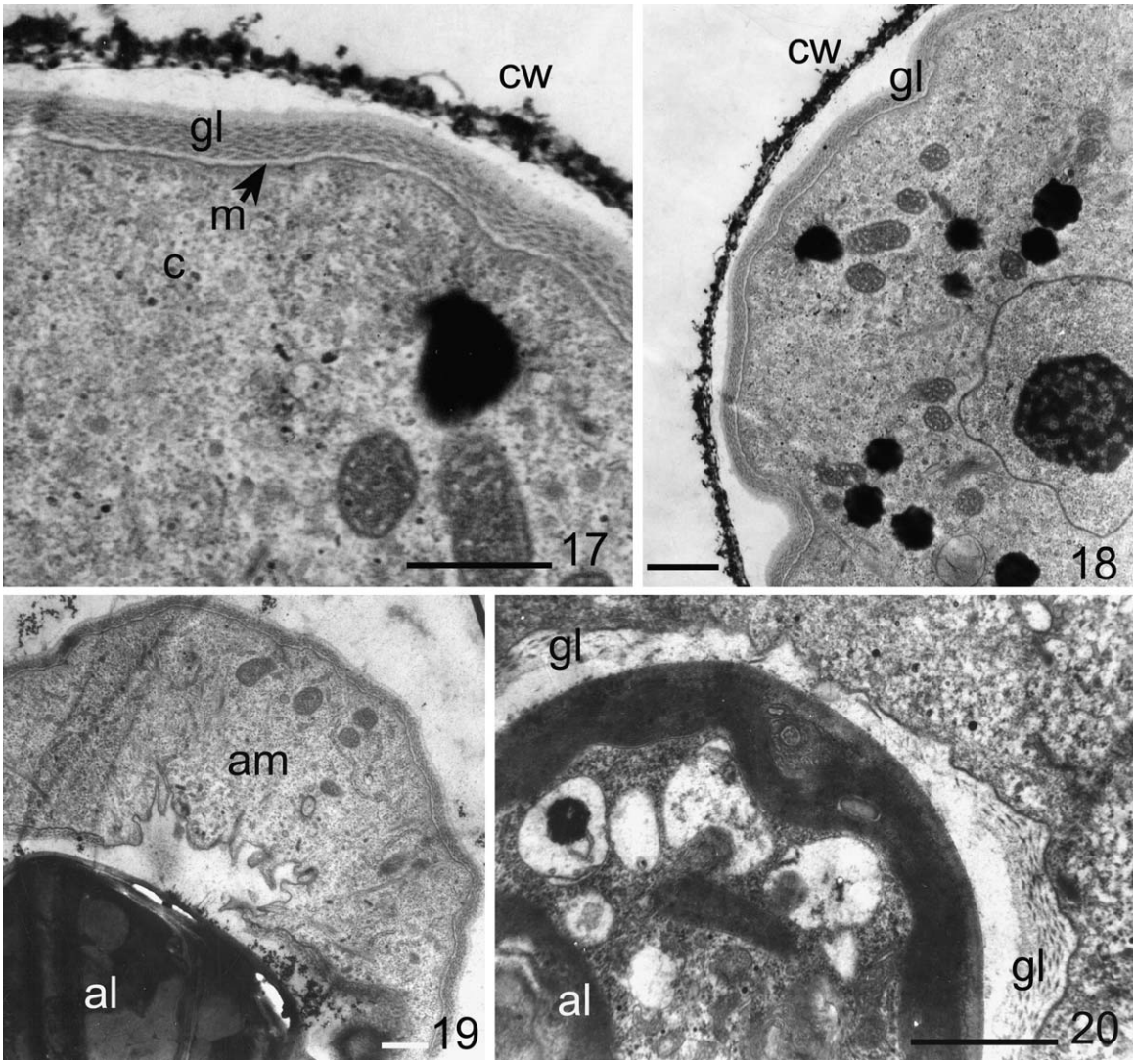
Feeding

Dermamoeba algensis is a thick-coated organism, feeding on relatively large objects, i.e. algal cells, which are often comparable in size to the entire amoeba. It can utilize both unicellular and filamentous algae. The phagosome, formed to engulf the food object, is very large. It comprises a considerable portion of the amoeba cell volume. Thus, this species has evolved a very specific mode of feeding, related with the reorganization and partial destruction of the cell coat, in order to avoid digestion of a large portion of its own glycocalyx.

When the amoeba cell contacts the algal prey, it assumes a characteristic lens-like form. The ventral part of the lens adheres to the food object (Fig. 19). The cell forms an invagination at the centre of the area of contact. The process of the destruction of the cell coat starts in this invagination and progresses to the periphery (Fig. 20). The process results



Figs 10–16. Transmission electron microscopy of *Dermamoeba algensis* trophozoites. **(10)** Overall view of a cell, including the nucleus with a number of small lacunae in the nucleolus. **(11)** Two nuclei in one section of a binucleate cell. **(12)** Higher magnification of the nucleolus, showing the absence of any nuclear lamina under the nuclear membrane and numerous tiny patches of the nucleolar material distributed in the karyoplasm. **(13)** A section through the cell showing mitochondria with tubular cristae; bundles of microfilaments and the characteristic arrangement of a dense material over the cell membrane but under the glycocalyx. **(14)** Higher magnification of the cell coat, showing the cell membrane and a bilayered glycocalyx. **(15)** Dictyosome of the Golgi complex. **(16)** Higher magnification of the area of the arrangement of dense material; it is clearly visible that this material is located between the cell membrane and the glycocalyx. Bars = 1 μm. gl, glycocalyx; m, cell membrane; mit, mitochondria; n, nucleus; nm, nuclear membrane; nu, nucleolus; mf, microfilaments; adm, arrangement of dense material.

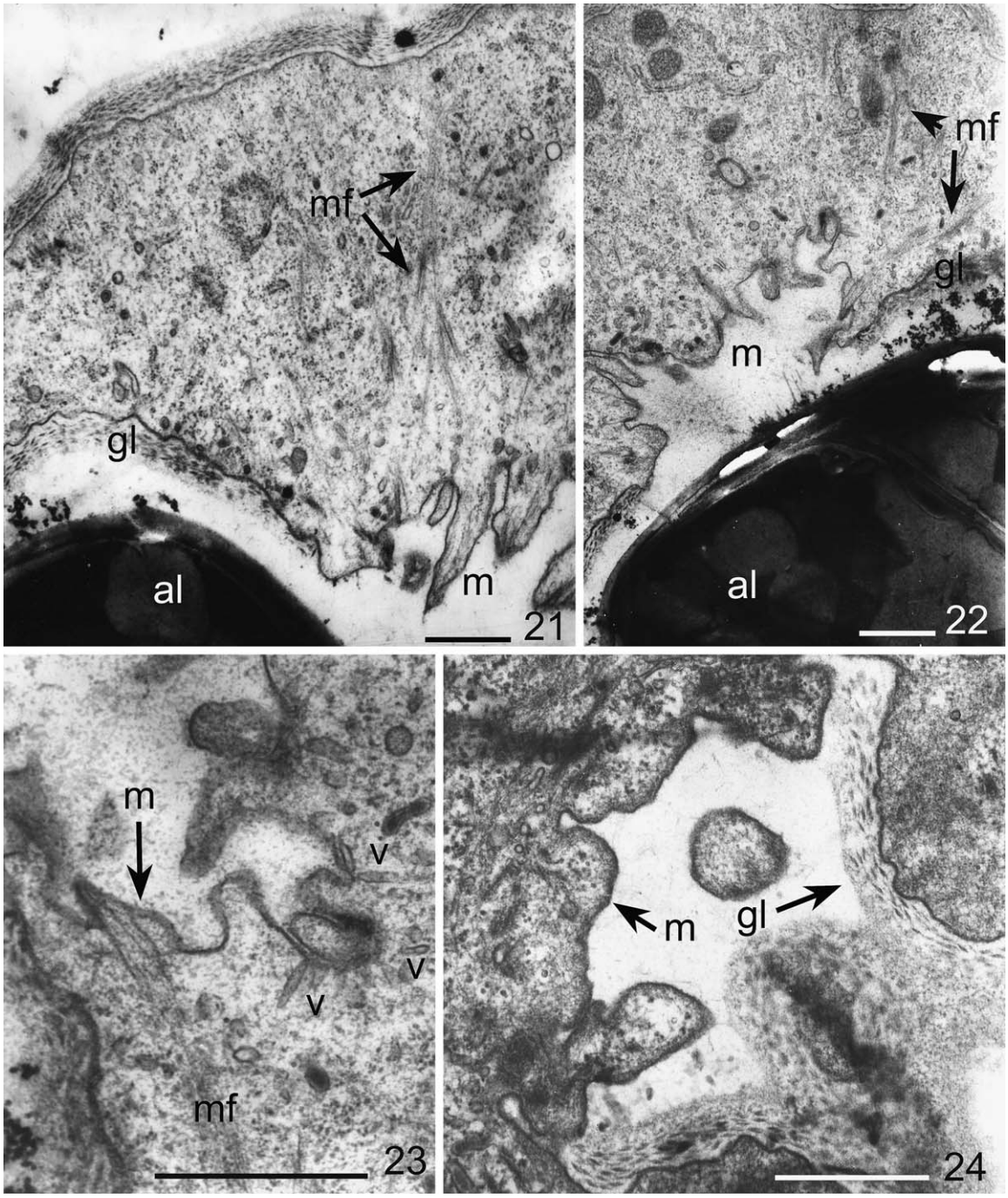


Figs 17–20. Cyst of *Dermamoeba algensis* and feeding of this species. **(17, 18)** Section through the cyst. **(19)** Overall view of the feeding cell. Note the absence of a glycocalyx in the centre of the invagination. **(20)** Higher magnification of the central part of the invagination of the developing food cap. It is clearly visible that the cell liberates the cell membrane; the glycocalyx is becoming destroyed in this area. Bars = 1 μm. gl, glycocalyx; m, amoeba cell membrane; c, cytoplasm; cw, cyst wall; am, amoeba cell; al, algal cell.

in complete disappearance of the glycocalyx in the centre of the area of amoeba contact with the food object. Fig. 19 shows numerous fine filamentous structures connecting the plasma membrane of an amoeba with the algal surface. These filaments may derive from the remnants of the glycocalyx material. The central invagination becomes increasingly deep. The cell forms extra depressions at the bottom of this invagination, the shape of the food cap gets very complex (Figs 21, 22). In TEM sections of the food cap, numerous tiny vesicles and channels are visible under the cell membrane at the area of these depressions (Figs 23, 24). The lateral parts of the amoeba progressively surround the algal cell, while the destruction of the glycocalyx continues. As a result, no glycocalyx appears in the food vacuole. A remarkable feature at this stage of the phagocytosis is the bundles of microfilaments penetrating the entire cell, from the dorsal surface to the plasma membrane of the forming food cap (Fig. 21). In some TEM images it looks like these bundles are connected directly to the plasma membrane of the food cap (Fig. 23). Fig. 24 shows a section through the marginal area of the half-closed food vacuole. It is clearly visible that the bottom of the food vacuole is completely free from the cell coat while it still persists at the entrance to the food vacuole. The process of feeding is shown schematically in Fig. 25.

Feeding specificity and salinity tolerance

The experiments on the food preferences of *D. algensis* indicated that this species cannot grow on an agar surface supplied with *E. coli*. However, it is able to utilize a wide range of algae, both unicellular and filamentous, and may be



Figs 21–24. Feeding of *Dermamoeba algensis*. **(21)** Section through the amoeba forming a food cap; the bundles of microfilaments go across the entire cell, from the invagination of the food cap to the other side of the cell. The glycocalyx remains intact only at the most lateral area of the invagination, its gradual destruction is visible closer to the centre of the food cap. **(22)** Higher magnification of the central area of a food cap. The cell membrane is free from the glycocalyx; it looks like this is an area of active pinocytosis. **(23)** Higher magnification of the central area of a food cap. Numerous tiny vesicles are formed beneath the cell membrane; the bundles of microfilaments span directly from the cell membrane, which has even no traces of the glycocalyx. **(24)** A part of a half-enclosed area of a food cap clearly showing the absence of the glycocalyx at the bottom of a cap. Bars = 1 μm. al, algal cell; m, amoeba cell membrane; v, vesicles; mf, microfilaments.

considered as specifically algivorous (Table 1). In the experiments, it exhibited growth on 19 species of algae (among 33 tested), including algae from very different systematic groups (Table 1). The intensity of feeding increased with increasing temperature (Table 2), reaching a peak at 22–24 °C. However, despite its algal diet, this species never has been reported to establish a symbiotic association with unicellular algae.

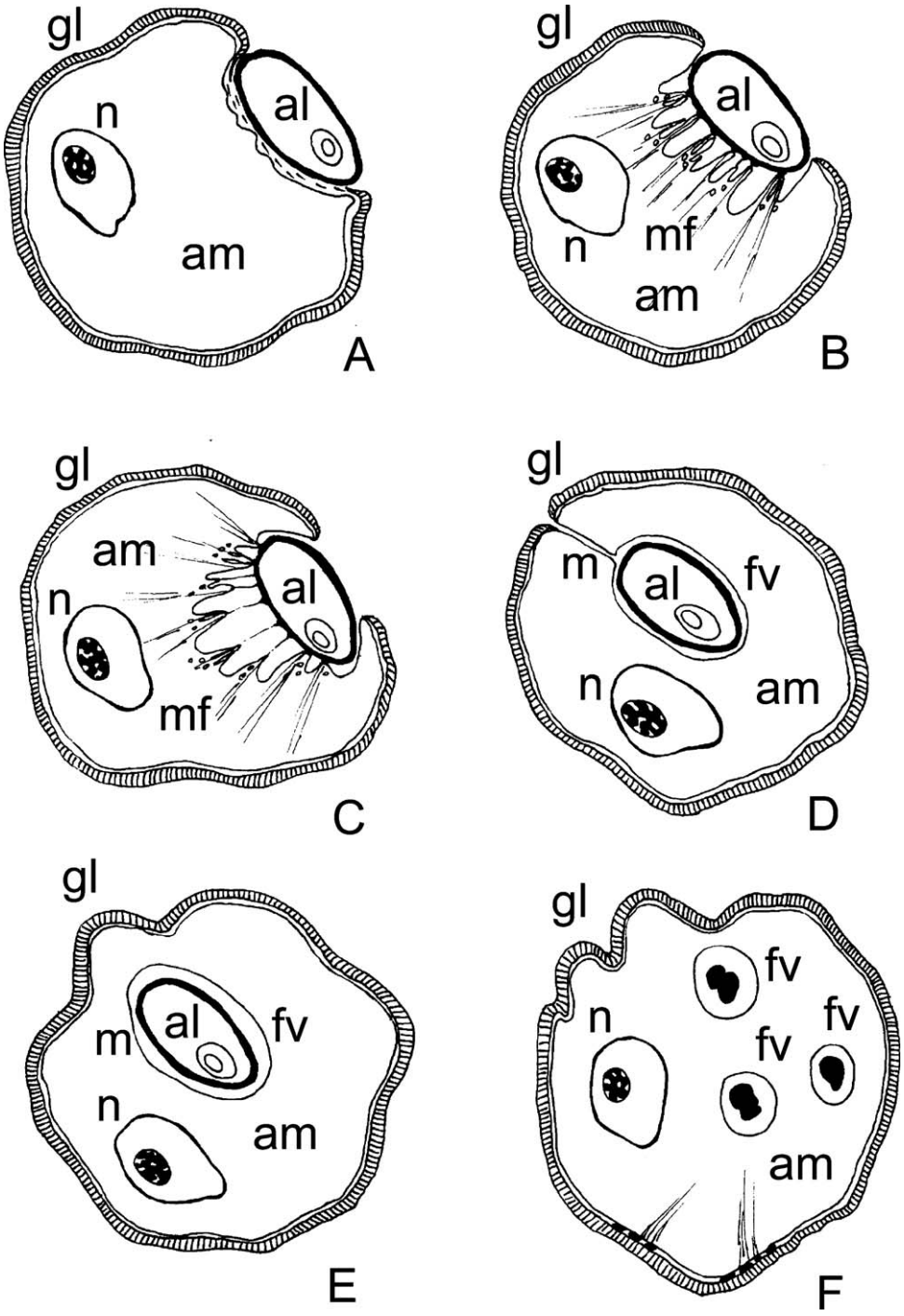


Fig. 25. Feeding of *Dermamoeba algensis* (scheme). (A) The cell adheres to the food object and assumes a lens-like shape; the destruction of the amoeba cell coat begins from the centre of the area of adhesion. (B, C) The amoeba forms a food cap and engulfs the algal cell; this process is related to the formation of the deep depressions at the bottom of the food cap and formation of numerous bundles of microfilaments going across the entire cell; the impression is that the cell "draws" the algal food into the phagosome. (D, E) Closing of the phagosome; there is no glycocalyx inside the food vacuole. (F) Fragmentation of the food vacuole and digestion of the algal material. Not to scale. al, algal cell; am, amoeba cell; n, nucleus of the amoeba cell; gl, glycocalyx of amoeba; mf, bundles of microfilaments; fv, food vacuole; m, membrane of the food vacuole.

The experiments revealed that *D. algensis* can feed on heat-killed (by heating to 80 °C) algal cells of *Tribonema gayanum*. Moreover, when the *Tribonema* culture was autoclaved, which resulted in the destruction of cells, and a non-filtered extract was introduced to *D. algensis* as a food source, the amoebae were able to live and multiply feeding on algal debris and, perhaps, dissolved organic matter.

Table 2. Feeding intensity of *D. algensis* under different temperatures. The table shows the percent of algal cells of *Tribonema gayanum*, eaten by amoebae after two weeks of cultivation. Values were estimated after counting the number of algal cells remaining in culture.

Temperature (°C)	Percent of eaten cells of *Tribonema gayanum*			Average
	Repeats of the experiment			
	1	2	3	
6	20%	15%	23%	19.3%
10	25%	40%	50%	38.3%
15	60%	61%	70%	63.6%
22	100%	90%	95%	95.0%
25	90%	95%	95%	93.3%
32	85%	85%	78%	81.0%

The experiments on the salinity tolerance, performed in the culture medium with addition of NaCl, showed that amoebae can grow and multiply up to 5% NaCl content, which corresponds to approximately 50 ppt salinity, hence differing substantially from natural seawater ion concentrations.

Discussion

Identification and diagnosis of the new species

The isolate described in the present paper based on all LM and TEM characters must be classified as a member of the genus *Dermamoeba* Page et Blakey 1979. This genus comprises only two species: *D. minor*, which is much smaller than the present isolate (max. 50 μm in length, with an average of 41 μm), and *D. granifera*. The latter species reaches 85 μm in maximal length (average 63 μm), which is smaller than the present isolate (50–100 μm, average 75 μm). The nucleus of *D. granifera* contains two closely apposed endosomes (Page and Blakey 1979; Page 1988), which is not a characteristic of the present isolate. Cysts are not known for *D. granifera*, but this character is weak since it may be an artifact of culturing. Page (1988) mentioned that the cytoplasm of *D. granifera* is always filled with numerous yellowish or brownish spheres, probably – lipid globules. There is nothing like this in the cytoplasm of the present isolate. Fungi are the primary diet of *D. minor*. Page (1988) suggests that the same is true for *D. granifera*. In contrast, the present isolate is algivorous. All these characters warrant the description of the present isolate as a new species, named here *D. algensis*.

Diagnosis. Dermamoeba algensis sp. n: Amoeba of lingulate morphotype; length in locomotion 50–100 μm (average 75 μm); breadth 30–50 μm (average 37 μm); length/breadth ratio 1.4–2.8 (average 2.0). Single vesicular nucleus 2.9–5.7 μm in diameter (average 4.7 μm) with the single central nucleolus 1.4–4.6 μm in diameter (average 3.2 μm). The nucleolus contains a number of lacunae. Cell coat ca. 500 nm in thickness. Cysts rounded, single-walled, 47–70 μm diameter (average 58 μm); cyst wall is smooth, no cyst pores.

Type material: Stained preparations No. 715 (holotype), 716, 717 (paratypes) deposited with the museum of slides of the Laboratory of Cytology of Unicellular Organisms, Institute of Cytology of the Russian Academy of Sciences. The strain described here was deposited with CCAP (UK) for several years, but now it is lost. The 18S rRNA sequence of the type strain is deposited with the GenBank under the accession number AY294148.1; actin gene sequence accession number is AAQ55806.

Type location: small freshwater pond close to Kirovsk village (North-Western Russia).

Etymology: algensis = feeding on algae (Latin)

Feeding behavior of the amoebae and evolution of their cell coat

The feeding behavior of *Dermamoeba algensis* is adapted to the ingestion of large food objects, related to the invagination and internalization of large portions of the cell membrane. This species has a thick, differentiated cell coat. If ingested within the food vacuole, it perhaps could: (1) represent a serious problem for the digestion of the engulfed algal food and (2) require the cell to perform an energetically low-efficient process digesting the cell coat and its subsequent reassembly at the cell surface. In addition, the cell coat may impede the adhesion of the food to the cell surface during food capture and phagocytosis.

We obtained only LM and TEM data on the feeding of *D. algensis*. To conclude on the nature of the process of the destruction of the cell coat during the feeding of this species it would be desirable to have histochemical data, showing when the enzymatic attack on the food object starts during the phagocytosis, but these data are unavailable. Hence we have to analyze two possibilities: (1) enzymatic attack of the algal cell starts after the food vacuole is formed. In this case the destruction of the cell coat is an interesting adaptation to the ingestion of large food objects or (2) enzymatic attack of the algal cell starts immediately after the amoeba adheres to its surface. In this case, the destruction of the cell coat is a follow-up of the early enzymatic attack of the prey.

In the alternative (1) it is possible to suggest that the destruction of the cell coat at the area of a forming food cap is a reversible process aimed (a) to avoid the irreversible destruction and further digestion of the cell coat material; (b) to improve the adhesion of the cell to the prey object and (c) to increase the efficiency of the enzymatic attack of the algal cell in a newly formed phagosome. The thick, multilayered cell wall of algae is not readily digested, thus this adaptation may, among other factors, minimize the amount of enzymes required to digest the food object. It seems logical to suggest that the cell coat is getting disassembled up to the level of the certain building units which may be easily used by the cell to restore it whenever necessary. This hypothesis requires a cytochemical analysis of the content of the numerous vesicles observed beneath the cell membrane within the area of the forming phagosome.

We may also suggest that the destruction of the cell coat is not a preventive measure, but the follow-up of the enzymatic attack, which starts immediately after the adhesion of an amoeba to the algal cell (alternative 2). Fig. 20 shows that the surface of the algal cell, half-enclosed in the food cap, looks slightly degraded, with traces of some material radiating from it to the cell membrane. This may be interpreted as an evidence of a starting enzymatic attack, thus we may assume that the cell coat is less stable and gets destroyed first, before the visible destruction of the algal cell wall. In Figs 19–21, a number of small vesicles, some with dense material while others with electron-transparent content are visible, but we cannot conclude about the dynamics and direction of this process.

Among the naked lobose amoebae, the feeding-related liberation of part of the cell membrane is known only in amoebae of the genus *Pellita* (Smirnov and Kudryavtsev 2005). However, details of this process in *Pellita* are very different from those in *D. algensis*. *Pellita catalonica* never disassembles the cell coat, instead it liberates an area of the cell membrane by a process that looks like "pushing away" the glycostyles. This may be achieved either by the mobility of the glycostyles along the cell membrane (perhaps related with the partial destruction of the basal layer of the glycocalyx), or by synthesis of a new membrane, free of glycostyles in the particular areas (perhaps, using the source of membrane material, deposited in the cytoplasmic vesicles). *Dermamoeba algensis* disassembles the cell coat only for feeding. When the cell moves, the cell coat remains intact at the ventral surface. In contrast, *Pellita*, possessing a much thicker cell coat, must penetrate it with short subpseudopodia to adhere to the substratum (Smirnov and Kudryavtsev 2005).

The order Dermamoebida, to which *D. algensis* belongs, includes three representative amoebae genera, all possessing a thick, well-developed cell coat. Among them, the cell coat of *Mayorella* spp. is the thinnest one, reaching a maximum of 280 nm in *M. cantabrigiensis* (Page 1988). Amoebae of this genus show a typical mode of adhesion and endocytosis. Although the latter process was never specifically studied, in the published TEM pictures of *Mayorella* the remnants of the cell coat are visible inside young phagosomes. This was never specifically illustrated, but portions of two young phagosomes with the remnants of glycocalyx inside are visible in Smirnov (1999, Fig. 7a). The cell coat of *Paradermamoeba valamo* may reach up to 520 nm in thickness (Smirnov and Goodkov 1994, 2004). The cell coat does persist and it is intact on the ventral surface of the moving cell. We never have studied endocytosis in *Paradermamoeba*. However, its cell coat consists of glycostyles, not embedded in any matrix, in contrast with *Dermamoeba* spp., where it is a solid layer.

So, among the thick-coated amoebae we propose a kind of morphological continuum. In amoebae of the genus *Mayorella* the cell coat mediates both the adhesion and the endocytosis, thus behaving as a usual glycocalyx. In *D. algensis* the cell coat mediates the cell adhesion but not phagocytosis. Finally, in *Pellita*, it is even thicker (up to 1 μm) and can mediate neither cell adhesion nor feeding.

The above continuum is an additional argument in favor of the idea that when the cell coat of an amoeba reaches some critical thickness, density and complexity, it becomes too large of a problem for cell adhesion (cannot mediate it any more) and phagocytosis (getting too costly to digest and restore it). Thus, cells acquire adaptations to avoid the involvement of the cell coat as a mediator of the relationships of the cell with the environment. This process may go in parallel in the different phylogenetic branches of Amoebozoa, so it is merely a biological adaptation, not an evolutionary marker. Perhaps that is why there is no known amoeba (yet?) possessing, e.g. a 5 μm cell coat. Probably further evolution is possible only in the direction of a complete separation of the coating structures from the cell membrane and formation of the extracellular test.

Another interesting point observed in *D. algensis* is the observation of the bundles of microfilaments in the cytoplasm of the feeding cell during the ingestion of the algal prey. These bundles are especially well-developed; they penetrate the entire cell, from the dorsal surface to the membrane of the food cap. In some images (e.g. Fig. 21) it looks like they contact with the membrane of the food cup. The arrangements of the microfilaments around the forming food caps are usual for amoeboid protists, especially for algivorous amoebae (e.g. Patterson et al. 1987), but in no one studied species these bundles are so developed and oriented as they are in *D. algensis*. From the observed pattern it appears that the primary role of these bundles is the ingestion of the prey into the forming phagosome. In TEM images it looks like there is some filamentous material radiating from the surface of the algal cell to the membrane of the food vacuole (Fig. 20). This may be evidence of the adhesion of the prey to the cell membrane. The overall impression from the TEM images is that there is some tension in this area of the cell. It may be that these bundles force the ingestion of the algal cell, helping in the deepening of the food cup. This process probably is another adaptation of *D. algensis* to the ingestion of large food objects.

Salinity tolerance of *Dermamoeba algensis*

Another interesting point of the biology of *D. algensis* is the high level of tolerance of this species to NaCl observed in the experiments, suggesting a high level of salinity tolerance. It is generally considered that the same amoeba species cannot inhabit both marine and freshwater habitats (Page 1988), but freshwater and marine species may co-occur in the brackish Baltic Sea (Garstecki and Arndt 2000; Schmöller 1961, 1964) and several "marine" amoebae species can tolerate freshwater conditions (Hauer et al. 2001; Page 1983; Sawyer 1975). Leidy (1879) noted the ability of *Cochliopodium* spp. to tolerate a wide range of conditions, from freshwater to marine; the same was noted for a strain of *Vannella* (Bovee 1965 as *V. mira*). However, molecular studies indicated that a brackish water strain identified at the morphological level as *Vannella simplex* (Smirnov et al. 2002) appeared to be genetically distinctive, and this strain was named *V. danica* (Smirnov et al. 2007). Overall, it appears that the ability to tolerate a wide salinity range as observed, e.g. in *Platyamoeba pseudovannellida* (Hauer et al. 2001) and *Vannella arabica* (Smirnov 2001) does not necessarily indicate that these freshwater species regularly colonize marine habitats and vice versa. Thus far, *D. algensis* is known only from continental freshwater.

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