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Description of *Cochliopodium spiniferum* sp. n., with Notes on the Species Identification within the Genus *Cochliopodium*

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Summary. The new species named *Cochliopodium spiniferum* was isolated from a low-saline habitat at the White Sea (north-western Russia). It is identical in the light-microscopical features to *Cochliopodium* sp. 3 described by Bark in 1973. In contrast, the scales comprising the tectum of these amoebae are different. This is the first evident case when two cochliopodiums are identical in LM, but differ in the scale structure, emphasizing the necessity of both LM and EM study for precise species identification within the genus *Cochliopodium*.

Key words: *Cochliopodium spiniferum* sp. n., scales, species identification, systematics, ultrastructure.

Abbreviations: EM - electron microscopy, GA - "Golgi attachment", LM - light microscopy, MT - presumed microtubule, MTOC - microtubule organizing center, S - spines of the scales in the cross-section.

INTRODUCTION

The genus *Cochliopodium* comprises lens-shaped, flattened lobose amoebae bearing a tectum - a monolayer of scales covering the dorsal surface of the cell. Traditionally, the structure of the scales was considered to be the most reliable feature for characterizing *Cochliopodium* species (Bark 1973). However, recently Kudryavtsev *et al*. (2004) have shown that there is a group of *Cochliopodium* species, which differ in LM features but have very similar scales. In this paper, I describe an opposite situation, when two species of the genus *Cochliopodium* are similar in the LM features, but differ in the structure of scales. These species are "*Cochliopodium* sp. 3", an unnamed strain studied by Bark (1973), and *Cochliopodium spiniferum* sp. n., described in the present paper.

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MATERIALS AND METHODS

Cochliopodium spiniferum was isolated from the bottom sediments of a stream flowing through a periodically flooded marsh near the Marine Biological Station of the St. Petersburg University at Srednii Island (Chupa Inlet, Kandalaksha Bay, the White Sea). Salinity of the site of sampling varies from freshwater to 10-15 ‰, depending on the tidal cycle. It was 6 ‰ at the moment of sampling. Amoebae were cloned and maintained on 1.5% NN agar prepared

with 6 ‰ artificial seawater (Wiegandt GmbH, Germany). For EM, amoebae were fixed with 1% phosphate-buffered OsO_4 for 30 min, dehydrated and embedded in Epon 812 resin. Whole mounts of scales were prepared according to the protocol of Sadakane *et al*. (1996) and stained with a 2 % solution of uranylacetate in 70 % ethanol.

RESULTS

Cochliopodium spiniferum sp. n.

Diagnosis: Length in locomotion, 18-39 μm (mean, 27 μm), breadth, 22-50 μm (mean, 32 μm), L:B ratio, 0.62-1 (mean, 0.84). Fan-shaped, oval or triangular, with numerous subpseudopodia and trailing filaments. One vesicular nucleus 5-7 μm in diameter, large central nucleolus 1.7-3 μm in diameter, with small lacunae. Five-rayed scales composed of a circular base plate, a central column of 5 stalks and a complex top part consisting of a central cone, a peripheral disc and a vertical striated spine. Diameter of the base plate, 0.8-1 μm; of the top disc, 0.6-0.8 μm; height of a scale, 0.8-0.9 μm; diameter of a spine, 0.04-0.07 μm, length of a spine, 1.7-2.2 μm.

Type material: Type strain is deposited with the Culture Collection of Algae and Protozoa (IFE, UK; accession No, CCAP 1537/3).

Habitat: Bottom sediments of a marsh with varying salinity at Srednii Island (Chupa Inlet, Kandalaksha Bay, the White Sea).

Differential diagnosis: Differs from the only named species similar in LM features, *C. bilimbosum*, in the scale structure. Identical in LM features to the unnamed strain "*Cochliopodium* sp. 3 " (Bark 1973) but differs from it in the structure of the scales.

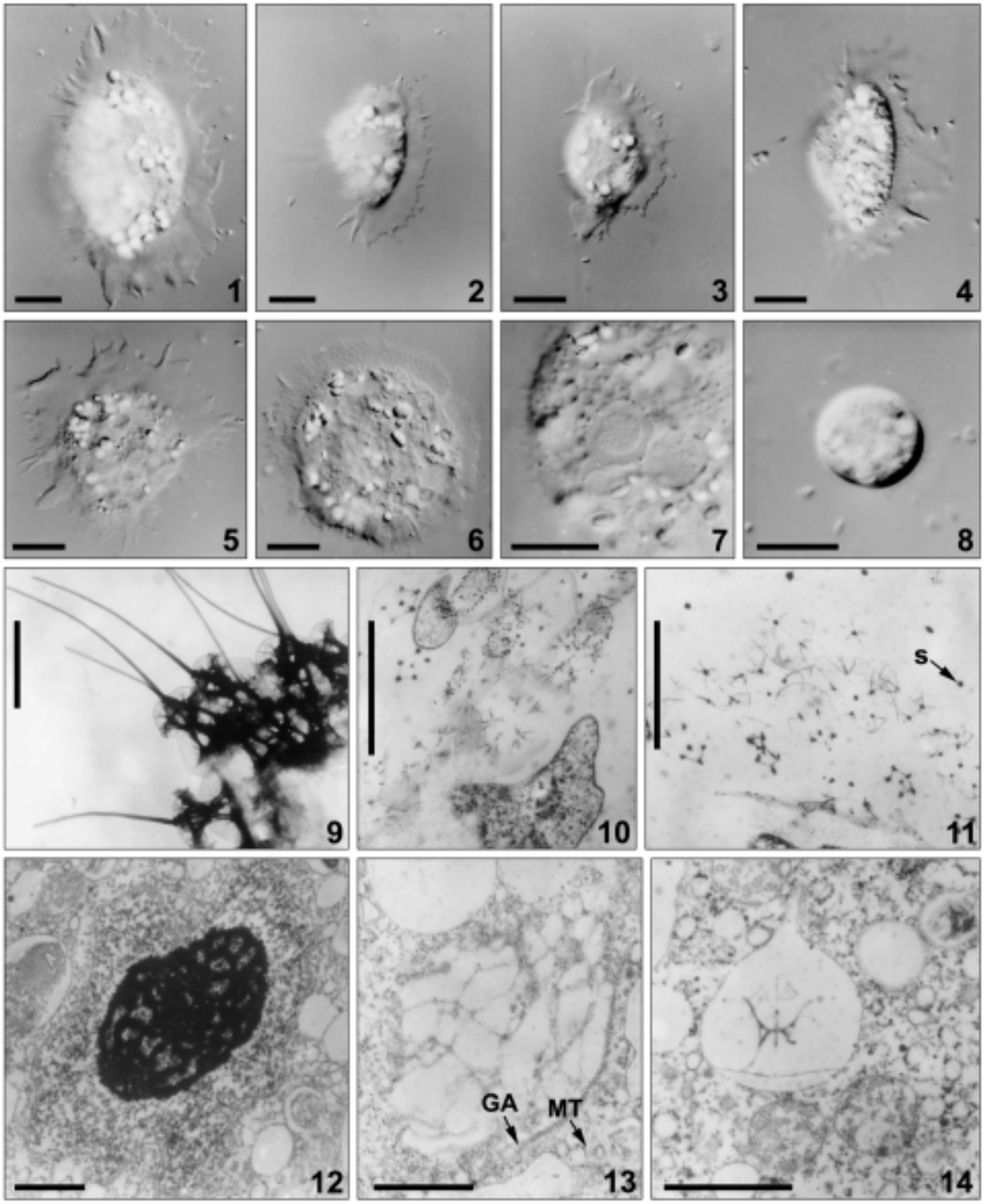
Description: During locomotion the amoebae were oval, fan-shaped or, more rarely, broadly triangular with the base directed anteriorly (Figs 1-4). The leading edge of the hyaloplasm was irregular and very dynamic (Figs 1, 3). The anterior and lateral parts of the hyaloplasm extended far beyond the border of the tectum (Fig. 4). Often, the cell formed several short conical subpseudopodia directed anteriorly. Sometimes, the anterior and antero-lateral hyaloplasm was deeply cleft into a few separate lobes (Fig. 3). These lobes moved posteriorly along the lateral margins of the cell, and retracted after they reached the posterior edge. Sometimes, the tips of these lobes remained adhered to the substratum, leaving trailing filaments behind the moving cell after the retraction of the entire lobe. Besides, numerous trailing filaments were formed along the posterior margin of hyaloplasm, producing a "brush-like" appearance of the rear edge of an amoeba (Fig. 1). The posterior margin of the hyaloplasm was very narrow and often hidden beneath the granuloplasmic hump.

During non-directed movement, the amoebae were irregularly triangular, with numerous long hyaline subpseudopodia (Fig. 5). Some cells were rounded, with a narrow hyaloplasmic veil, but without any subpseudopodia or lobes (Fig. 6). Stationary amoebae were rounded and flattened. Their hyaloplasmic veil was very narrow, sometimes almost completely retracted. Amoebae never floated spontaneously. When detached artificially from the substratum, they adopted a spherical shape and settled down quickly. Sometimes, the cells remained spherical for a while after making contact with the substratum. In this case, the amoeba sometimes formed several long, narrow hyaline pseudopodia, penetrating the tectum.

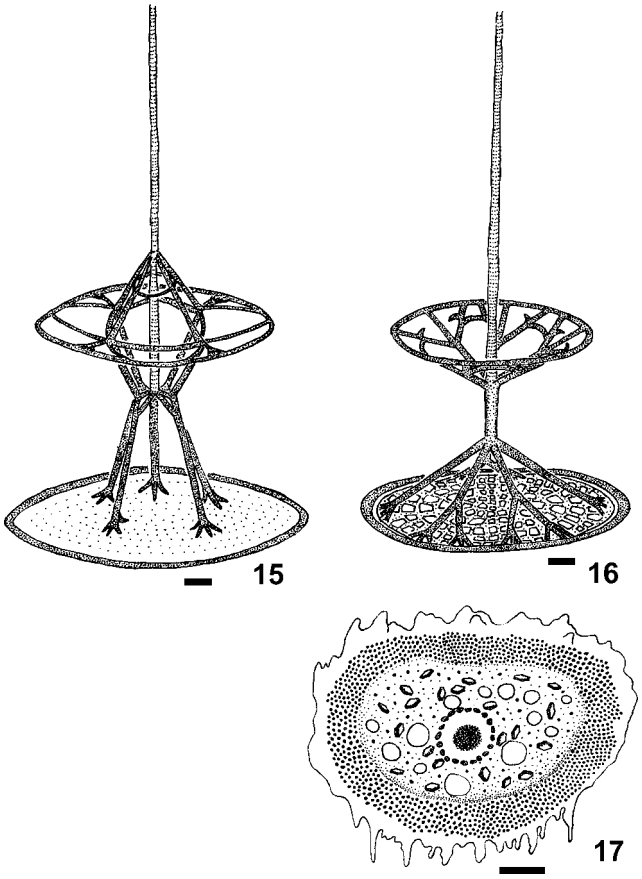
The scales comprising the tectum were clearly seen as fine granules distributed over the dorsal surface of the cell (Figs 4, 6). The optical profile of the tectum was distinctly visible as a cross-striated double line along the margin of the granuloplasmic mass (Fig. 7). When the tectum was observed tangentially at high magnification (1250x) using DIC optics, very short, indistinct "rays" were seen radiating from the surface.

The nucleolus in the living cells (Fig. 7) appeared to be slightly "granulated". A layer of tiny, non-refractile granules could be observed along the periphery of the nucleus. No contractile vacuole was observed. The granuloplasm contained numerous yellowish refractile bipyramidal crystals, 1-3 μm in size (Figs 1-7) and a number of vacuoles of various sizes. Amoebae fed on bacteria and particles of non-living organic matter of similar size. At room temperature, amoebae began to encyst after 2-3 weeks in culture. Cysts (Fig. 8) were spherical or slightly ovoid, 10-20 μm in diameter, with a distinct inner wall. The outer wall was formed by the scales comprising the tectum, and it was not always clearly visible under LM.

The base plate of a scale had a distinct outer rim and appeared to be amorphous both in the whole mounts (Fig. 9) and in the sections (Fig. 10). At the bases of the stalks of the vertical column there were bird-foot-like structures with 3 or 4 digits, one digit being opposed to the others. The top part of the scale consisted of a peripheral funnel, formed by five radial spokes corresponding to the stalks, a fine networked disc, and a central cone 0.25-0.28 μm in height, formed by five



Figs 1-14. *Cochliopodium spiniferum* sp. n. **1-8 -** LM photographs, **9-14 -** EM photographs. **1-4 -** locomotive forms; **5-6 -** non-directly moving amoebae; **7** - nucleus; **8** - cyst; **9** - scales in a whole mount preparation; **10 -** base plates of the scales in tangential section; **11 -** top parts of the scales sectioned at various planes; **S** - spines in the cross-section; **12** - nucleus; **13** - dictyosome showing "Golgi attachment" (GA) and a radiating microtubule (MT); **14** - scale in the scale-forming vacuole and mitochondria. Scale bars 10 μm (1-8); 1 μm (9-14).



Figs 15-17. 15 - outline of the scale structures of *Cochliopodium spiniferum*; **16** - *Cochliopodium* sp. 3 (after Bark 1973, modified); **17** - locomotive form of *Cochliopodium* sp. 3 (after Bark 1973, modified). Scale bars 0.25 μm (15, 16); 10 μm (17 - estimated from the size of amoebae provided in the description of species).

filaments, originating from the spokes of the funnel (Figs 11, 14). The filaments of the cone were cross-connected between each other at two levels. A vertical spine (Figs 9, 11) projected from the center of the top part of each scale. This spine was slightly tapering and cross-striated with a periodicity of about 0.006 μm. Usually, only a few scales were seen in the sections, which might be caused by the loss of scales in the viscous medium during fixation or embedding steps.

Each nucleus observed in the sections was irregularly rounded, with a central electron dense nucleolus, sometimes with small lacunas (Fig. 12). The Golgi complex was usually located near the nucleus. It consisted of one dictyosome formed by numerous dilated vesicles rather than by a stack of flattened cisternae. An elongated cross-striated MTOC-like structure resembling the "Golgi attachment" (Yamaoka *et al.* 1984) was present near the surface of the dictyosome opposite to the nucleus. Sometimes microtubules were seen radiating from it (Fig. 13). The numerous vesicles of various sizes containing scales at different stages of assembly surrounded the dictyosome (Fig. 14). Mitochondria (Fig. 14) were rounded or ovoid with tubular cristae.

DISCUSSION

Identification

Among the known species of the genus *Cochliopodium*, this amoeba is almost completely identical in LM features to a new but unnamed species "*Cochliopodium* sp. 3" (Fig. 17) comprehensively studied by Bark (1973). The only feature, in which these strains differ, is the ability to encyst, known in the strain described here but never observed in Bark's strain. However, TEM evidence reveals considerable differences in the scale structure. The scales comprising the tectum in Bark's amoebae (Fig. 16) consist of complexly structured base plates, a vertical column made up by the stalks converging towards the top to form a single central core. The simple top part is in the shape of a funnel with the spine in the center. In contrast, in the present strain (Fig. 15) the base plate is amorphous, the stalks never fuse together to form a single central core and the top part of the scale consists of a peripheral funnel, formed by 5 radial spokes, a fine networked disc, a central cone, and a vertical spine. Certain similarities in the structure of the scales, in particular, 5-rayed symmetry and the central spine that is identical in both species, suggests that these species might be closely related, but their distinctness is evident. Another unnamed species with the similar scales was studied by Yamaoka (Yamaoka and Kunihiro 1985, Yamaoka *et al.* 1991). The scales of this species differ from those of my strain in the same way as the scales of Bark's "*Cochliopodium* sp. 3". Besides, their spines appear to be much thicker than those of my strain and carry distinct filamentous material (see Yamaoka *et al.* 1991, Figs 2, 3 at page 12, and Fig. A at page 14). Therefore, the species of Yamaoka is also distinct from my strain, but may be closely related to it due to the similar structure of the scales. Among the named species of *Cochliopodium*, the present strain resembles only *C. bilimbosum* in LM features, but has very different scales. Other species and unnamed strains of *Cochliopodium* studied with EM, namely, *C. minus*,

C. larifeili, *C. gulosum*, *C. barki*, and *Cochliopodium* sp. "NYS strain" (Yamaoka *et al.* 1984; Dyková *et al.* 1998; Kudryavtsev 1999, 2000; Kudryavtsev *et al.* in press) have scales, totally different from the present strain. Other species, for which EM data are not available yet (see Bark 1973 for the list of these species), are different from the present strain in the shape of the locomotive form, the appearance of tectum, and (*C. clarum* Schaeffer, 1926) the structure of the nucleus. Thus, the strain described here is evidently a new species, which I name *Cochliopodium spiniferum* (due to the long spine in the center of a scale).

Taxonomic status of "*Cochliopodium* sp. 3" (Bark 1973)

The strain named "*Cochliopodium* sp. 3" is certainly a new species of *Cochliopodium*. Bark (1973) studied its LM and EM morphology but for unknown reason did not name this strain. It is not possible to do this now, since no type material was deposited. Therefore, the Bark's species requires future re-isolation. *C. spiniferum* is identical to this strain in the LM morphology, but differs in the scale structure - the first such case in *Cochliopodium*. These data rise a question of the possibility to re-isolate "older" species of *Cochliopodium* like those described by West (1901) or Penard (1902) which have never been studied with EM. The modern researcher never knows whether an isolated strain, even identical to a 19th century amoeba in LM data, would differ from it in the structure of scales.

Ultrastructural features

A peculiar electron-dense structure associated with the dictyosome in *Cochliopodium spiniferum* appears to be a MTOC, like in *Cochliopodium barki* (Kudryavtsev *et al.*, 2004). Structurally, this MTOC is not similar to those of other amoeboid protists which are mostly trilaminar, consisting of two electron-dense plates and an electron-transparent layer between them (e. g., Grell and Benwitz 1978, Pussard and Pons 1978, Page 1981, Smirnov 1995-1996, Gothe *et al.* 1999). The apparent presence of a MTOC in *Cochliopodium* makes the set of features of these amoebae extremely unusual, i.e., a generally "gymnamoebian" cell type combined with the tectum and non-typical cytoplasmic MTOC. This complicates the placing of the genus together with the naked lobose amoebae, as suggested by Page (1988) since the presence of a MTOC is rather an exception in the latter group than a rule. Bark's (1973) hypothesis of the relatedness of *Cochliopodium* to chrysophyceans and haptophyceans, based solely on the presence of organic scales on the cell surface, appears to be even weaker supported, since the organic scales are known to date in a wide set of very distant or non-related protistan taxa.

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