Protistology 1 (2), 66-71 (1999)
July, 1999

Protistology

Description of *Cochliopodium larifeili* n. sp. (Lobosea, Himatismenida), an amoeba with peculiar scale structure, and notes on the diagnosis of the genus *Cochliopodium* (Hertwig and Lesser, 1874) Bark, 1973

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Summary

Cochliopodium larifeili n. sp., was isolated from bottom sediments of a freshwater lake at the Valamo Archipelago (Lake Ladoga) and studied using both light and electron-microscopical methods. Tectum of this species is composed of cubical scales which differ in general pattern of organization from those, characteristic for other electron-microscopically studied members of the genus. A scale consists of a quadrangular base plate with honeycomb-like appearance, four vertical columns arising from the corners of the base plate and an arc-shaped quadrangular top part. These data necessitate some corrections in the current diagnosis of the genus *Cochliopodium* created by Bark (1973) which included the description of scale structure.

Key words: amoebae, systematics, Lobosea, Himatismenida, *Cochliopodium*, tectum

Introduction

The genus *Cochliopodium* (Hertwig and Lesser, 1874) Bark, 1973 comprises lobose amoebae which are flattened and disc-shaped during locomotion and partially covered by a flexible layer of scales termed "tectum" (Bark, 1973). Bark (op. cit.) studied three strains of this genus with TEM, only one of which was identified as a previously described species – *Cochliopodium bilimbosum* (Auerbach, 1856) Leidy, 1879, and found out that all of them had scales of a similar general pattern of organization. He concluded that this scale structure is a characteristic feature of the genus and included it in the generic diagnosis. This was accepted in several subsequent publications (Nagatani et al., 1981; Dykova et al., 1998).

During the study of amoebae fauna from freshwater lakes of Valamo Archipelago (North-Western Russia) a new species of tectum-bearing amoebae was found. This organism doubtlessly belongs to the genus *Cochliopodium* although its tectum is composed of scales with non-typical pattern of organization. The description of this species is presented here, and the taxonomic value of the scale structure within the genus is discussed.

Material and Methods

The amoebae were isolated from the upper layer of bottom sediments from Lake Konevskoye (Valamo Archipelago, Lake Ladoga) in September, 1995 taken at a depth of about 1 m. Samples were inoculated in 100 mm Petri dishes with freshwater overlay and two rice grains in each dish. After one week of incubation clones were initiated by transferring single cells into 40 mm Petri dishes with 1,5% non-nutrient agar covered with Prescott-James medium (Prescott, James, 1955). Permanent preparations were stained with iron haematoxylin. For electron microscopy amoebae were fixed while adhering to agar surface as follows: 0,5% OsO_4 – 10 minutes at room temperature; washed in buffer three times; 2,5% glutaraldehyde – 1,5 hours at +4°C; washed in buffer three times; 1% OsO_4 – 1 hour at +4°C. All the fixatives were prepared with 0,1 M phosphate buffer (pH 7,4). After dehydration in ethanol series, specimens were embedded in Epon.

Results

Locomoting amoebae were rounded (fig. 1–2), oval (fig. 3, 5) or sometimes drop-shaped (fig. 4) with breadth

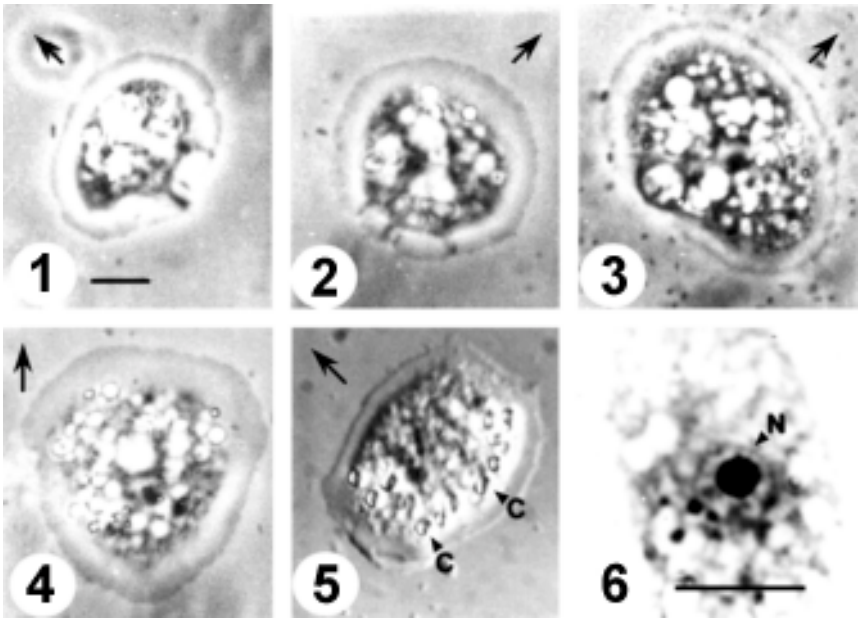

Fig. 1–6. *Cochliopodium larifeili*. Light microscopical photographs. **1–4.** Locomotive forms on glass surface (phase contrast). **5.** Locomotive form on glass surface (differential interference contrast). **6.** Haematoxylin stained preparation, bright field. Arrows in figs 1–5 indicate the direction of locomotion. C – crystals, N – nucleus. Scale bars 10 μm throughout.

usually greater than length. Central granuloplasmic hump was usually oval (when viewed from above) or triangular, narrowing from anterior to posterior end (fig. 4). It was surrounded by a thin clear hyaloplasmic veil, slightly narrowing from lateral to posterior parts. In rapid locomotion, lateral parts of hyaloplasmic veil were often broader than anterior ones. Sometimes broadening of the hyaloplasm at one of its sides occurred during locomotion (fig. 5). The anterior margin of hyaloplasm was almost smooth and arc-shaped, sometimes with slight irregularities in outline. Subpseudopodia were never formed.

In locomotion amoebae mostly had several short posterior "projections" of the granuloplasmic hump (fig. 1–2). They were never seen to extend behind the border of the hyaloplasmic veil. Sometimes cells also formed hyaloplasmic trailing filaments (not illustrated). Because of the minute size of scales, tectum was seen only using differential interference contrast as a double line along the margin of granuloplasmic mass. Length of the locomotive form varied from 18 to 40 μm (mean 30 μm), breadth, from 28 to 54 μm (mean 38 μm), length/breadth ratio, from 0,41 to 1,14 (mean 0,8).

Stationary amoebae usually had disc-like shape with narrow hyaloplasmic border surrounding central granuloplasmic mass. Sometimes spherical cells slightly adhering to the substratum occurred. Differentiated floating form was observed neither in cultures nor when amoebae were disturbed and suspended in a drop of medium.

Amoebae usually had one spherical or somewhat irregular nucleus of vesicular type with a large central nucleolus (fig. 6). The diameter of nucleus was 4 μm. The most characteristic cytoplasmic inclusions were highly refractive cubical or bipyramidal yellow crystals 1–3 μm in size numbering 5 to 15 per cell (fig. 5). Rounded transparent vesicles and food vacuoles with bacteria were seen as well. Cysts were never observed in cultures.

The tectum covered the upper surface of an adhered amoeba (fig. 12). A thin layer of amorphous material was always seen between the plasma membrane and tectum and on the surface of plasma membrane free from tectum (fig. 11).

The base plate of the scale had a quadrangular form with rounded corners. Sometimes in sections it appeared triangular (fig. 9), which was caused by oblique sectioning as shown in fig. 8. When observed from above, the base plate had a honeycomb-like appearance with an electron-dense rim along the perimeter (fig. 9). Four "columns" circular in cross-section rose vertically from the corners of the base plate ending with an arc-shaped quadrangular top part with four rounded apertures (fig. 9, 10). Length of the side of both base and top plates was about 0,5 μm. Height of the scale was about 0,6 μm. Schematic drawing of the pattern of scale organization is represented in figure 7.

The only nucleus studied electron-microscopically (fig. 13) was spherical with rounded central nucleolus which had a central lacuna. A well developed dictyosome (fig. 14) consisted of about 10 flattened cisternae filled with electron-dense material and surrounded by numerous vesicles, indicative of high functional activity of the Golgi complex.

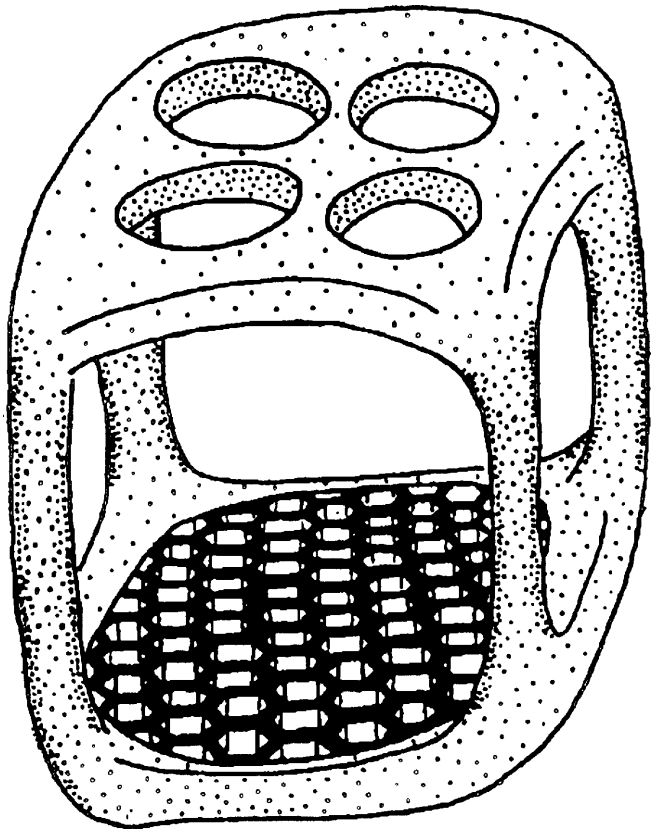

Fig. 7. Pattern of the scale organization of *Cochliopodium larifeili*.

Discussion

In the diagnosis of the genus *Cochliopodium* Bark (1973, p. 136) stated that scales constituting tectum "...have the following structure, a circular base plate composed of an open grid, a column consisting of a number of legs arising perpendicularly from base plate, a funnel shaped or wheel shaped capital surmounting column, in some species a spine arising from centre of capital...". Thus every new tectum-bearing species which has the general scale pattern differing from that described by Bark, should not be included in the genus *Cochliopodium* even if all other characters of the organism correspond to the diagnosis of the genus. The generic position of the majority of species currently belonging to the genus also remains uncertain, because only two out of about twenty named species included in the genus have been studied electron-microscopically (Bark, 1973; Dykova et al., 1998). The diagnosis proposed by Bark was based on the investigations of only three strains, and so, seems to be excessively detailed.

The features of the strain studied here confirm this point of view. The general scale pattern of our species differs from that, described by Bark. However, in the organization of locomotive form and the presence of tectum, this amoeba corresponds to the diagnosis of the genus *Cochliopodium*.

Thus, we propose the modified diagnosis of this genus that does not contain the general scale pattern. The presence of tectum becomes the only electron-microscopical character important for justifying the generic position of an amoeba. Nevertheless, the scale structure remains important for species identification within the genus. However, further electron-microscopical investigations may probably reveal several groups in the genus *Cochliopodium* clearly separated from each other with respect to the general scale pattern.

Cochliopodium (Hertwig and Lesser, 1874) Bark, 1973, emend.

Amoebae covered by tectum. In locomotion somewhat flattened and disc-like, with hyaloplasm extending as a veil surrounding raised central or post central granuloplasmic mass.

The strain studied here cannot be identified with any of known species within the genus *Cochliopodium*. In general shape and size of amoebae during locomotion this isolate most closely resembles *C. minus* Page, 1976 (for description see Page, 1968; as *Hyalodiscus actinophorus* var. *minor*). But amoebae of the present isolate did not have a "single layer of yellowish, highly refractive granules" (Page, 1968, p. 19) surrounding the nucleus, a characteristic feature of *C. minus*. Moreover, recent light- and electron-microscopical investigations of *C. minus* (Dykova et al., 1998; our unpublished data) show that this

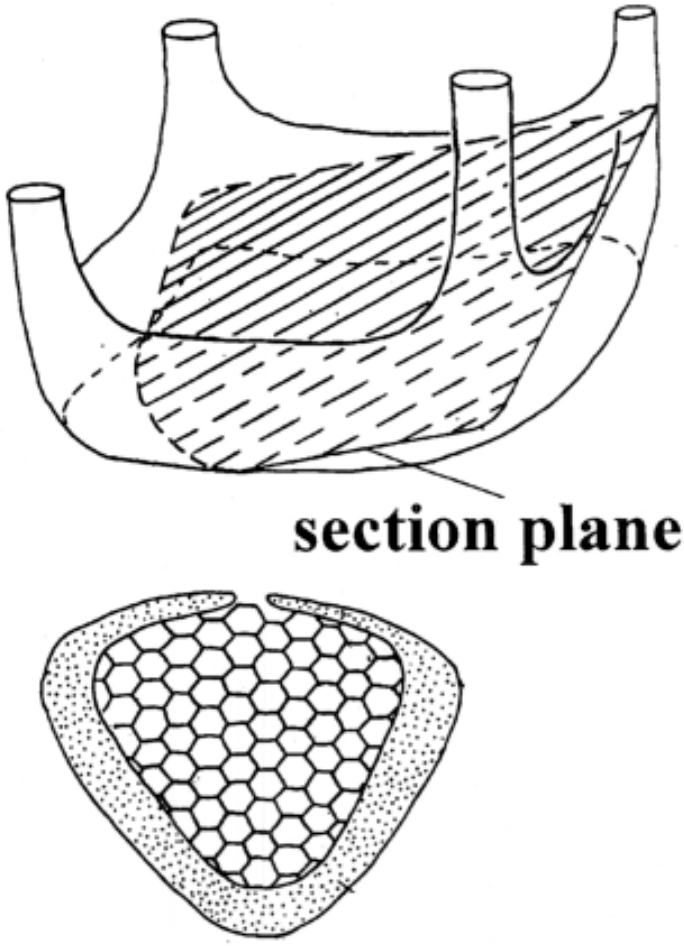

Fig. 8. Scheme of the oblique section through the base plate of the scale (above), showing why triangular base plates (below) can be seen at the electronograms.

species differs greatly from the strain described here in the shape of locomotive form and structure of scales and the shape of cytoplasmic crystals. Therefore this strain cannot be identified as *C. minus*.

Another species to which our strain bears some resemblance is *C. minutum* West, 1901. This species, like our isolate, has no scales visible with light microscope, most probably due to their size. However, amoebae studied by West (1901) were much smaller than amoebae described here (their size was 12,4–13,5 μm), they had smooth posterior end and were characterized by the presence of "somewhat irregular, usually attenuated at the extremity" pseudopodia (West, 1901, p. 312). Our amoebae often had posterior "projections" of the granuloplasm, sometimes trailing hyaloplasmic filaments and no pseudopodia.

The scale structure and light-microscopical characters of our strain show that it is not identical to any of the unidentified strains studied by now (Bark, 1973; Nagatani et al., 1981). Therefore, our amoebae should be regarded as belonging to a new species of the genus *Cochliopodium* – *Cochliopodium larifeili*.

By now only three named freshwater species belonging to the genus *Cochliopodium* could be isolated and identified with certainty (Bark, 1973; Page, 1976, 1988). These are *C. actinophorum* (Auerbach, 1856) Page, 1976 (studied with LM only but still well described and can be easily identified; see Page, 1976, 1988), *C. bilimbosum* (Auerbach, 1856) Leidy, 1879, and *C. minus* Page, 1976. Now we can include in the genus *Cochliopodium* the fourth freshwater species which can be reliably isolated and identified using both light- and electron-microscopical features – *C. larifeili*.

Diagnosis: *Cochliopodium larifeili* n. sp.

Smooth anterior margin that sometimes possesses slight irregularities and no subpseudopodia. Short posterior hyaloplasmic filaments or granuloplasmic projections, never extending behind the border of hyaloplasm usually present. Length of the locomotive form 18–40 μm (mean 30 μm), breadth, 28–54 μm (mean 38 μm), L/B ratio, 0,41–1,14 (mean 0,8). Single nucleus about 4 μm in diameter, with central nucleolus. 5–15 highly refractive cubical or bipyramidal crystals 1–3 μm in size present in granuloplasm. Scales cubical, their height is 0,6 μm, length

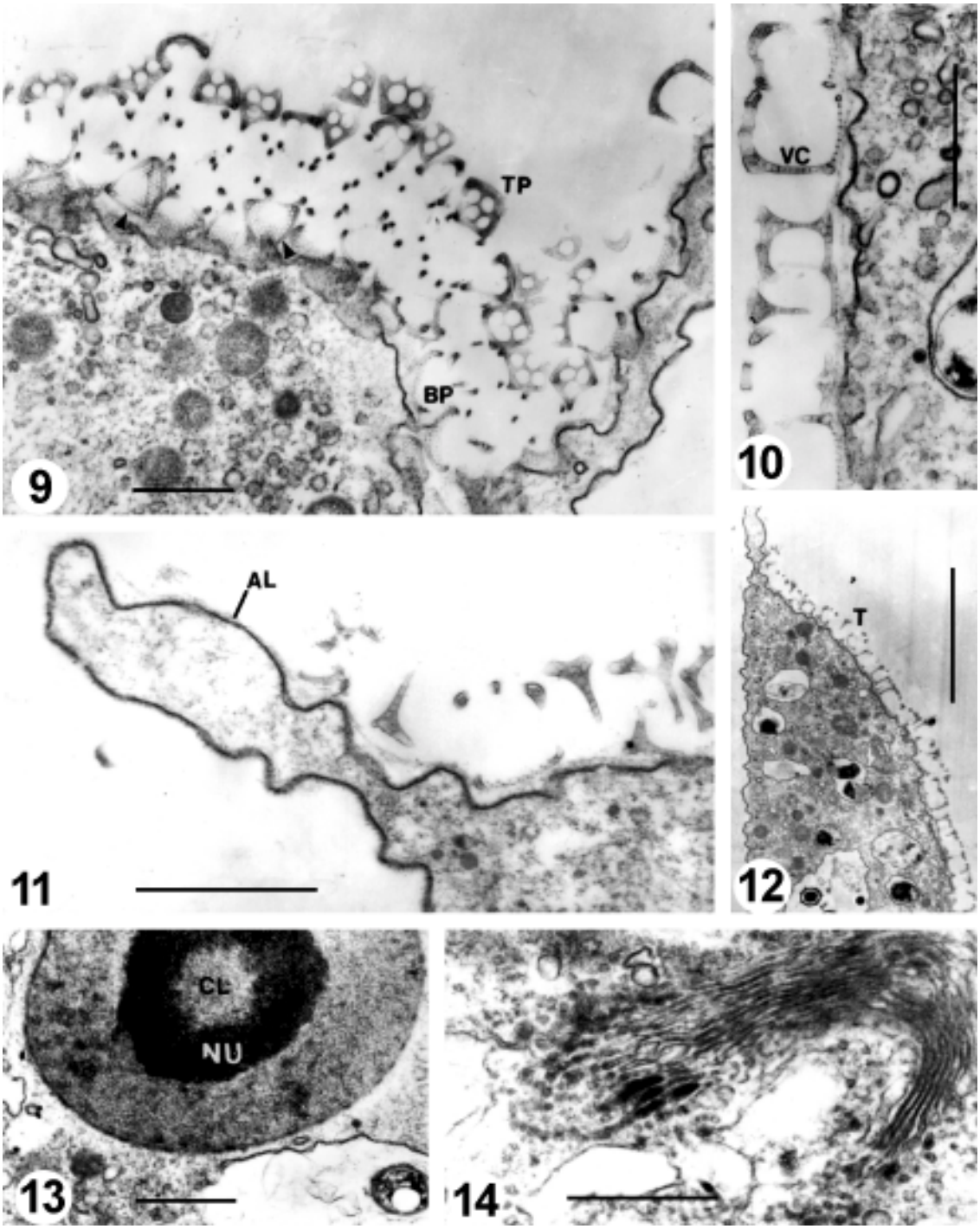

Fig. 9–14. *Cochliopodium larifeili*, electron-microscopical photographs. **9.** Oblique section through tectum showing scales sectioned at different levels (arrowheads indicate triangle-shaped base plates). **10.** Vertical section through tectum. **11.** Section through the cell margin showing a thin layer of amorphous material on the surface of plasma membrane. **12.** Section through the cell adhered to the substratum. **13.** Nucleus. **14.** Golgi complex. TP – top plate of the scale, BP – base plate of the scale, VC – vertical column, AL – layer of amorphous material, T – tectum, NU – nucleolus, CL – central nucleolar lacuna. Scale bars: 12 – 5 μm; other figures – 1 μm.

of the side is 0,5 μm. They consist of quadrangular base plate with honeycomb-like structure, four vertical columns arising from the corners of the base plate and arc-shaped quadrangular top with four apertures. Cysts unknown. Freshwater.

Observed habitat: Lake Konevskoye (Valamo Archipelago, Lake Ladoga, North-Western Russia).

Type material: holotype N 923; paratype N 924. Type slides are deposited with the collection of preparations of the Biological Research Institute, St. Petersburg, Russia.

Differential diagnosis: This species differs from *C. minus* in size and shape during locomotion, the shape of cytoplasmic crystals and the scale structure and from *C. minutum* in size and shape during locomotion.

Acknowledgements

I am most thankful to Dr Alexey V. Smirnov for bringing *Cochliopodium* to my attention and general supervision of the work; to Dr Andrew V. Goodkov for valuable discussion of the manuscript. I am grateful to Oleg G. Manylov and Natalia V. Lentsman for assistance during the preparation of the manuscript. The collecting and preliminary treatment of the material were carried out at the field station of the Research Centre "Valamo" of the St. Petersburg Society of Naturalists.

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