Protistology 1 (3), 110-112 (2000)
February, 2000

Protistology

The first isolation of *Cochliopodium gulosum* Schaeffer, 1926 (Lobosea, Himatismenida) since its initial description. II. Electron-microscopical study and redescription

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Summary

The results of electron-microscopical study of *Cochliopodium gulosum* Schaeffer, 1926 are presented. Tectum of these amoebae consists of the scales which differ in the details of their structure from those of any of the previously studied species of *Cochliopodium*. It provides another confirmation to the systematic validity of *C. gulosum*. The modified diagnosis of this species which includes the description of the scale structure is proposed.

Key words: ultrastructure, systematics, Lobosea, Himatismenida, *Cochliopodium gulosum*, scales

Introduction

In the previous paper (Kudryavtsev, 1999a) it was reported about the isolation and light-microscopical identification of *Cochliopodium gulosum* Schaeffer, 1926.

The diagnosis of this species was initially composed by Schaeffer (1926) using only light-microscopical characters. However, the combination of light-microscopical characters of amoebae and the pattern of organization of scales constituting tectum was recently shown to be the most reliable specific feature within the genus *Cochliopodium* (Bark, 1973; Dykova et al., 1998; Kudryavtsev, 1999b). Hence, it seems to be necessary to modify the diagnosis of *C. gulosum* which previously was never studied with EM by the addition of the description of scale structure.

In this paper the results of electron-microscopical study of *Cochliopodium gulosum* are presented and the modified diagnosis of this species is proposed.

Material and Methods

For electron-microscopical study amoebae were fixed while adhering to the previously polymerized Epon plates as follows: 0.5% OsO_4 – 10 min at room temperature; 2.5% glutaraldehyde – 40 min at +4°C; 1% OsO_4 – 60 min at room temperature. All the fixatives were prepared with 0.1 M phosphate buffer (pH 7.4). After dehydration in ethanol series specimens were embedded in Epon. Sections were stained with 2% uranyl acetate in 70% ethanol and Reynolds' lead citrate, and examined with Tesla BS-500 electron microscope.

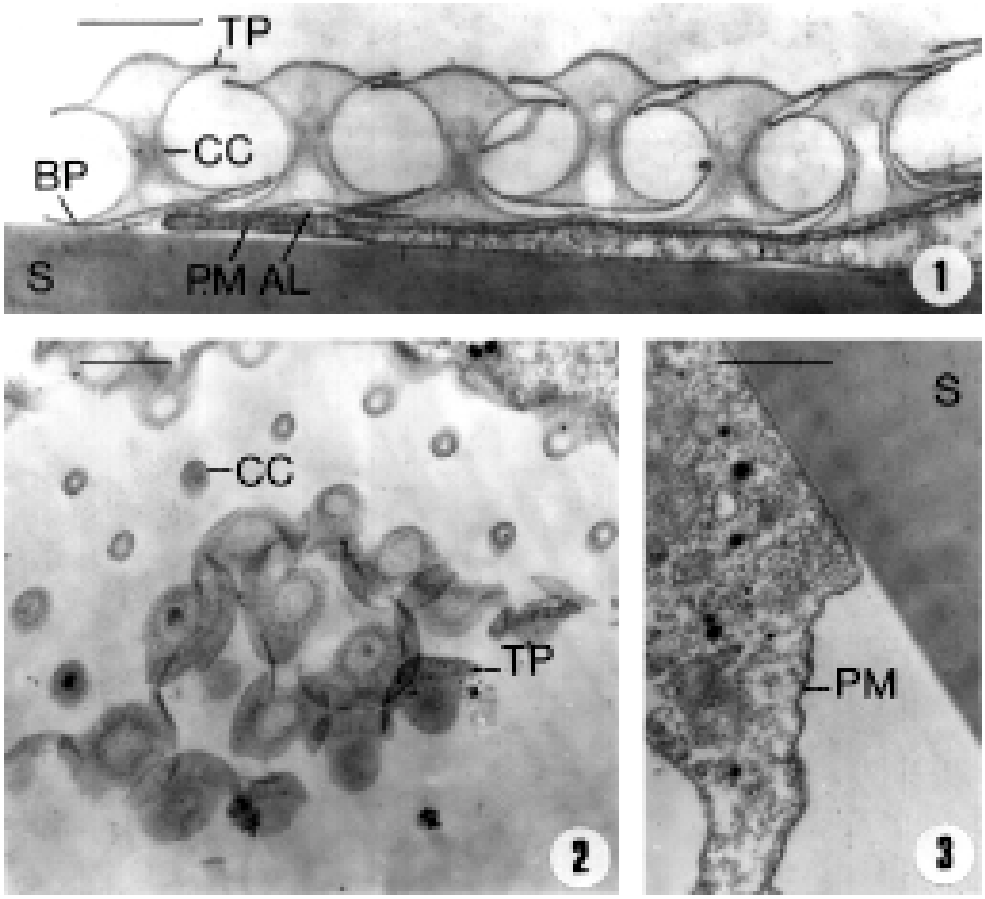
Results and Discussion

Scales constituting tectum had a circular base plate. From the center of the base plate a central column was arising (Fig. 1). It was circular in cross section and widened downwards (Fig. 2). This column possessed an internal cavity, whose surface was covered with honeycomb-like material (Fig. 1). The top part of a scale had a shape of a cone widening upwards (Figs 1, 2). It possessed a central dome-like apical structure and the internal hemispherical cavity almost completely filled with an electron dense hemispherical body.

A scale was composed of densely packed fibrillar matter. The diameter of the base plate of a scale varied from 1.8 to 2 µm, of the top part, from 1.4 to 1.6 µm. Height of a scale varied from 1.1 to 1.3 µm.

Schematic drawing of the pattern of scale organization is represented in Figs. 4.

When amoeba was adhered to the substratum, scales were situated over the thin layer of amorphous material covering the plasma membrane on the dorsal surface of a



Figs 1–3. *Cochliopodium gulosum*. Electron-microscopical photographs. **1**. Vertical section through the margin of an amoeba adhering to the substratum. **2**. Oblique section through tectum. **3**. Vertical section through an amoeba adhering to the substratum showing its ventral surface. BP – base plate of a scale, CC – central column of a scale, TP – top part of a scale, PM – plasma membrane, AL – layer of amorphous material, S – substratum. Scale bars: 1 μm throughout.

cell (Fig. 1). Neither scales nor the layer of amorphous material were observed over the plasma membrane on the ventral surface of a cell (Fig.1, 3).

Scales constituting tectum were overlapping with their base and top parts (Fig. 1), but no connecting structures could be demonstrated between them. A scale could be easily separated from the cell surface (Fig. 1).

These ultrastructural data correspond to the results of the light-microscopical observations of this species made earlier (Kudryavtsev, 1999a) and confirm its inclusion in the genus *Cochliopodium*.

In the pattern of scale organization this species differs from any of the previously studied species and unidentified strains of *Cochliopodium* (Bark, 1973; Nagatani et al., 1981; Dykova et al., 1998; Kudryavtsev, 1999b, our unpublished data). Thus, these observations provide another support to the systematic validity of *C. gulosum* and confirm the idea that within the genus *Cochliopodium* species can be most reliably separated from each other by the combination of light-microscopical characters of amoebae and the scale structure. Therefore we include the description of the scale structure in the diagnosis of *C. gulosum*. Light-microscopical part of this diagnosis initially designed by Schaeffer (1926) should be modified by the exclusion of the characters which are included in the current diagnosis of the genus *Cochliopodium* (see: Kudryavtsev, 1999b) and the characters which are meaningless for the modern systematics of the amoebae (for example, the colour of the cytoplasm and nucleus). Schaeffer's descriptions of certain light-microscopical features are modified according to the results of our study (Kudryavtsev, 1999a).

New type material (neotype and paraneotype) was established because Schaeffer's paper published in 1926 did not contain any indication on the original type material.

Diagnosis

Cochliopodium gulosum Schaeffer, 1926, emend.

Length of the locomotive form, 56–90 μm (mean, 80 μm), breadth, 56–86 μm (mean, 73 μm). In locomotion cells rounded, with length slightly greater than breadth or, rarely, vice versa. The anterior margin of the hyaloplasm bears 2–3 to 10 subpseudopodia, about 10 μm in length.

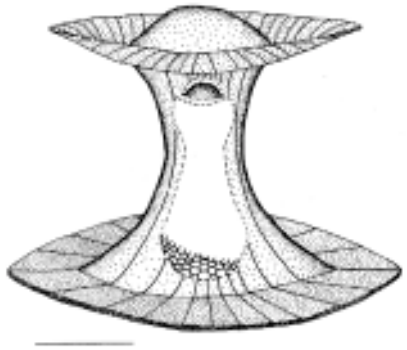


Fig. 4. *Cochliopodium gulosum*. Schematic drawing of the pattern of scale organization. Scale bar: 0,5 µm.

Smooth posterior end. Nucleus single, of vesicular type, with large central nucleolus. Diameter of nucleus, 8–15 µm (mean, 12 µm), of nucleolus, 6–10 µm (mean, 8 µm). Granuloplasm contains small rounded refractile bodies and numerous small transparent vacuoles. Scales consist of a circular base plate, a hollow central column with internal surface covered with a honeycomb-like material and a cone-shaped top part with an internal cavity filled with an electron dense body. Diameter of the base plate of a scale, 1.8–2 µm, of the top part, 1.4–1.6 µm. Height of a scale, 1.1–1.3 µm.

Observed habitats: Cold Spring Harbor, Great South Bay, Long Island, among *Zostera marina* and other submerged seaweeds (Schaeffer, 1926); the Chupa Inlet, Kandalaksha Bay, the White Sea, in the sand of the intertidal zone.

Type material: neotype 1999: N 811, paraneotypes N 812, 813. Type slides are deposited with the collection of preparations of the Laboratory of Invertebrate Zoology, Biological Research Institute, Saint-Petersburg State University, Saint-Petersburg, Russia.

Differential diagnosis: differs from *C. bilimbosum* (Auerbach, 1856) Leidy, 1879, *C. larifeili* Kudryavtsev, 1999 and *C. minus* Page, 1976 in the scale structure, size and shape of the locomotive form; from *C. actinophorum* (Auerbach, 1856) Page, 1976, *C. minutum* West, 1901, *C. digitatum* (Greeff, 1866) Penard, 1902, *C. opalinum* Penard, 1903, *C. ambiguum* Penard, 1904, *C. spumosum* Penard, 1904 and *C. papyraceum* Bovee, 1957 in the size and shape of the locomotive form; from *C. granulatum* Penard, 1890 in the light-microscopical appearance of tectum and the shape of the locomotive form; from *C. vestitum* (Archer, 1871) Archer, 1877 and *C. echinatum* Korotneff, 1879 in the absence of the thin hair-like projections on the surface of tectum; from *C. clarum* Schaeffer, 1926 in the organization of the nucleus, size and shape of the locomotive form.

By now only four species of the genus *Cochliopodium* could be isolated and identified with certainty. These are *C. bilimbosum* (Auerbach, 1856) Leidy, 1879, *C. larifeili* Kudryavtsev, 1999, *C. minus* Page, 1976 and *C. actinophorum* (Auerbach, 1856) Page, 1976. All these species are considered to be freshwater. As a result of the present study we can include in this genus another species – the first marine species which can be isolated and identified with certainty – *C. gulosum*.

Acknowledgements

I am grateful to Dr Alexey V. Smirnov for general supervision over the work, to Dr Andrew V. Goodkov for valuable discussion of the manuscript. Oleg G. Manylov helped me greatly in the preparation of the manuscript. The collecting and preliminary treatment of the material were carried out at the Marine Biological Station of the Saint-Petersburg State University.

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