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

Alexander A. Kudryavtsev
Department of Invertebrate Zoology, Faculty of Biology and Soil Science, St. Petersburg State University, Russia

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% OsO₄ – 10 min at room temperature; 2.5% glutaraldehyde – 40 min at +4°C; 1% OsO₄ – 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
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.
Smooth posterior end. Nucleus single, of vesicular type, with large central nucleolus. Diameter of nucleus, 8–15 \( \mu \text{m} \) (mean, 12 \( \mu \text{m} \)), of nucleolus, 6–10 \( \mu \text{m} \) (mean, 8 \( \mu \text{m} \)). 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 \( \mu \text{m} \), of the top part, 1.4–1.6 \( \mu \text{m} \). Height of a scale, 1.1–1.3 \( \mu \text{m} \).

Observed habitats: Cold Spring Harbor, Great South Bay, Long Island, among \textit{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 \textit{C. bilimbosum} (Auerbach, 1856) Leidy, 1879, \textit{C. larifeili} Kudryavtsev, 1999, \textit{C. minus} Page, 1976 and \textit{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 – \textit{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.

References

Kudryavtsev A.A. 1999a. The first isolation of \textit{Cochliopodium gulosum} Schaeffer, 1926 (Lobosea, Himatismenida) since its initial description. I. Light-microscopical investigation. Protistology. 1, 72–75.

Address for correspondence: Alexander A. Kudryavtsev, Dept. of Invertebrate Zoology, Fac. of Biology & Soil Sci., St. Petersburg State University, 199034, Universitetskaja nab. 7/9, St. Petersburg, Russia. E-mail: aak@ak5341.spb.edu

The manuscript is presented by A.V. Goodkov