

## Redescription of *Cochliopodium vestitum* (Archer, 1871), a Freshwater Spine-bearing *Cochliopodium*

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**Summary.** A freshwater lobose rhizopod *Cochliopodium vestitum* (Archer, 1871) (Himatismenida) was re-isolated and studied. New diagnosis for this species, based on the light- and electron-microscopic data is provided. The most peculiar feature of this species is fine spines visible with light microscopy, which originate from the top of some of the scales comprising the tectum, as revealed with electron-microscopy. Comparison of *C. vestitum* with the other spine-bearing cochliopodiums (*C. echinatum* and *C. longispinum*) suggests synonymy of these species, and *C. vestitum* remains the only valid spine-bearing *Cochliopodium*.

**Key words:** amoebae, *Cochliopodium*, Himatismenida, scales, systematics, ultrastructure.

### INTRODUCTION

At the border of 19-20th centuries, four species of *Cochliopodium*, carrying long thin radial spines on the cell surface were described. These were *C. vestitum* (Archer, 1871) Archer, 1877, initially *Amphizonella vestita*; *C. pilosum* Hertwig et Lesser, 1874; *C. echinatum* Korotneff, 1879; and *C. longispinum* West, 1901. Later Archer (1877) proved the species

name *C. pilosum* to be a junior synonym of *C. vestitum*. Other species were relatively well recognized and mentioned as distinct species (Leidy 1879, Penard 1902, Awerintzew 1906). However, Cash and Hopkinson (1909), for example, considered *C. echinatum* and *C. longispinum* to be synonyms. Descriptions of these four species do not contain a complete set of light-microscopic features and none of them has been re-isolated and studied using electron microscopy. Thus, the taxonomic status of these species remains ambiguous. The nature of spines visible with light microscopy (LM) on the surface of these amoebae remains unclear. This paper reports on the study of two spine-bearing strains of *Cochliopodium*, identified as *C. vestitum*. Rede-

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scription of this species and its comparison with the other spine-bearing *Cochliopodium* spp. are provided.

## MATERIALS AND METHODS

Two strains of *Cochliopodium vestitum* were isolated from the upper layer of the bottom sediments in Leshchevov Lake (Valamo Archipelago, Ladoga Lake, North-Western Russia) in September 1998 and from the upper layer of bottom sediments and washings of *Sphagnum* sp. at the bank of an unnamed lake at Srednii Island (Chupa Inlet, Kandalaksha Bay, The White Sea) in September 1999-2001. Samples were inoculated in the 90 mm Petri dishes with Prescott and James medium (Prescott and James 1955) and one wheat grain per dish. Amoebae found in the samples survived several transfers into the fresh Prescott and James medium, while attempts of cloning were not successful in all cases. For transmission electron microscopy (TEM) amoebae were fixed at room temperature while adhering to the surface of the previously polymerized Epon 812 resin with 0.5% phosphate-buffered  $\text{OsO}_4$  for 10 min, followed by 2.5% glutaraldehyde for 20-30 min and 1%  $\text{OsO}_4$  for 1 h. After dehydration, the specimens were embedded in Epon 812 resin. Sections were stained with uranyl acetate and Reynolds' lead citrate. Whole mounts of the scales were made by treatment of the cells with 0.5% Triton X-100, followed by several washes with distilled water and placement on formvar-coated grids.

## RESULTS

### *Cochliopodium vestitum* (Archer, 1871) emend.

**Diagnosis:** Length in locomotion 39-70  $\mu\text{m}$  (mean 59  $\mu\text{m}$ ), breadth 48-74  $\mu\text{m}$  (mean 65  $\mu\text{m}$ ), length : breadth ratio 0.6-1.08 (mean 0.9); in locomotion rounded to oval, with numerous subpseudopodia at the anterior margin of hyaloplasm. Very high dome-shaped granuloplasmic mass. One nucleus of vesicular type, 13-15  $\mu\text{m}$  in diameter (mean 14  $\mu\text{m}$ ), nucleolus 6-8  $\mu\text{m}$  in diameter (mean 7  $\mu\text{m}$ ). Scales consist of a quadrangular base plate, 4 vertical stalks cross-connected at two levels and a wide funnel-shaped top part formed by 4 concentric rings and 24 radial spokes originating from the vertical stalks. Some of the scales with a sharp spine (10-14  $\mu\text{m}$  in length) originating from the center of a top part. Side of the base plate 0.6  $\mu\text{m}$ , diameter of the top part 0.8-0.9  $\mu\text{m}$ , height of the scale 0.71  $\mu\text{m}$ .

**Observed habitats:** Freshwater pools in Counties Westmeath and Tipperary, Great Britain (Archer 1871); freshwater Absecom pond (New Jersey) and China Lake (Wyoming), North America (Leidy 1879); lakes in Novgorod and Tver' regions, central Russia (Awerintzew 1906); bottom sediments of a freshwater Leshchevov

Lake at the Valamo Archipelago, Ladoga Lake, bottom sediments and patches of *Sphagnum* sp. at the bank of a freshwater lake at Srednii Island (Chupa Inlet, Kandalaksha Bay, the White Sea), North-Western Russia.

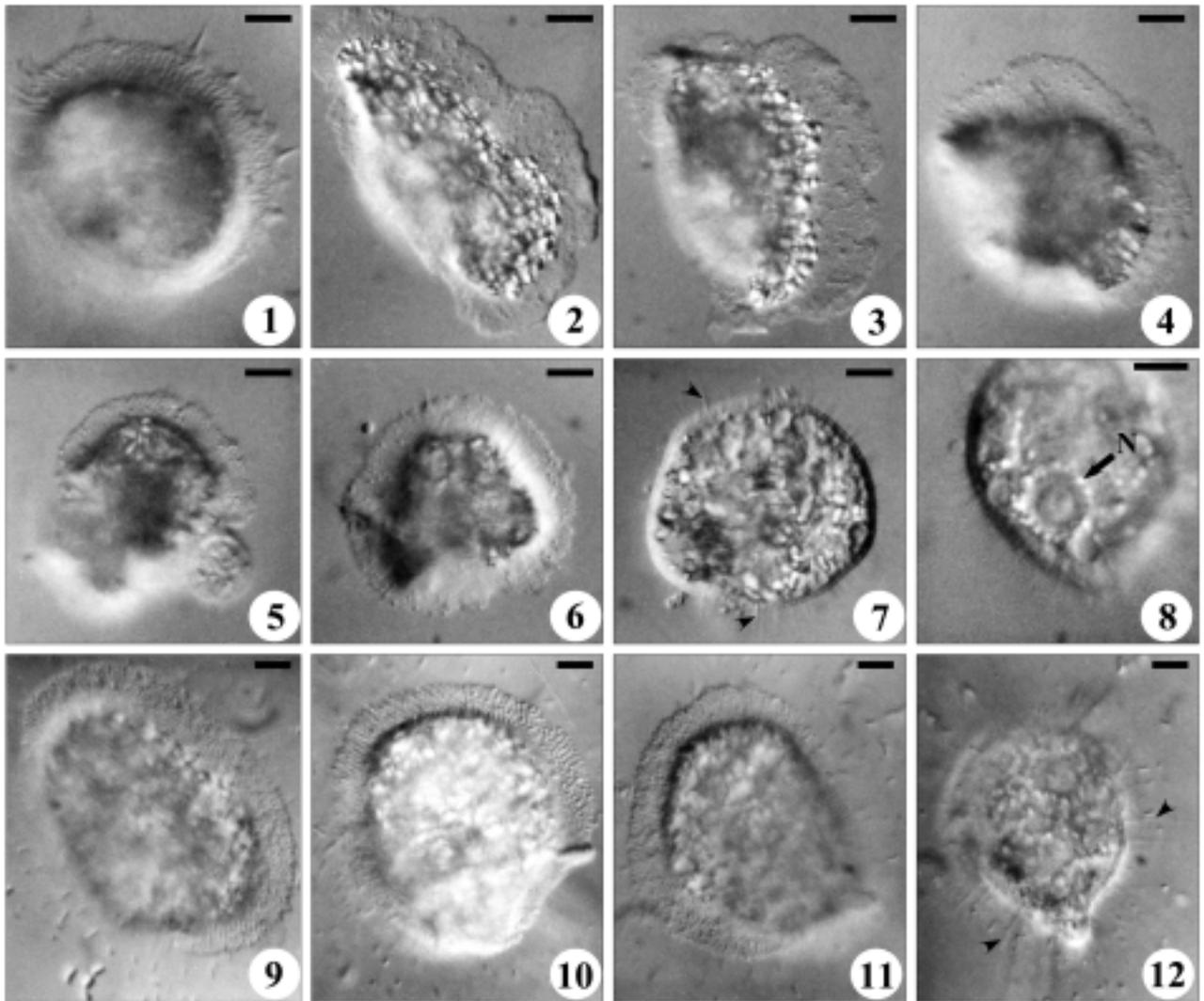
**Type material:** Since neither Archer nor later researchers made slides of this species, I establish the neotype. Type slides are deposited with the collection of preparations of the Biological Research Institute of the Saint-Petersburg State University, accession numbers 953 (neotype) and 954 (paraneotype).

**Differential diagnosis:** None of the *Cochliopodium* spp. known to date possesses scales with the spines easily visible with LM.

**Description:** Both Ladoga and the White Sea strains were identical in LM features. Amoebae were covered by the tectum, with scales distinctly seen as the large granules on the dorsal surface of hyaloplasm (Figs 1-4, 9-11). Anterior and lateral margins of the hyaloplasm often extended beyond the border of scales. Numerous thin hair-like spines were seen radiating from the surface (Figs 7, 12). In adhering amoebae these spines were seen only when the granuloplasm was in focus (Fig. 12).

The locomotive form was rounded, oval or rarely drop-shaped (Figs 1-4, 9). Central granuloplasmic mass surrounded with the flattened hyaloplasmic veil was normally shifted back to the posterior end of the body, "hiding" narrow posterior margin of the hyaloplasm when amoebae were observed from the top. Hyaloplasm had a uniform width in anterior and lateral parts, narrowing posteriorly. Anterior and lateral margins of hyaloplasm were very dynamic and formed flattened or conical subpseudopodia 8-10  $\mu\text{m}$  in length (Fig. 1). While the amoeba was advancing, subpseudopodia moved to, and finally retracted at the posterior end of the cell. Often subpseudopodia split or fused together from the tips to the bases. Posterior part of the hyaloplasm was very narrow, with the straight or slightly curved edge, sometimes with few trailing filaments. When the cell changed the direction of locomotion, part of the posterior edge formed broad adhesive expansion. In slower movement amoebae were rounded, with almost smooth margin of hyaloplasm (Figs 5, 10).

Non-directly moving amoebae were more rounded than locomotive, sometimes irregularly triangular (Figs 6, 11). The granuloplasm occupied a central position, and the hyaloplasm was wider than in the locomotive cells. Stationary amoebae were hemispherical or bell-shaped, rounded in above view, with the hyaloplasmic veil completely retracted (Fig. 7). In culture dishes amoebae



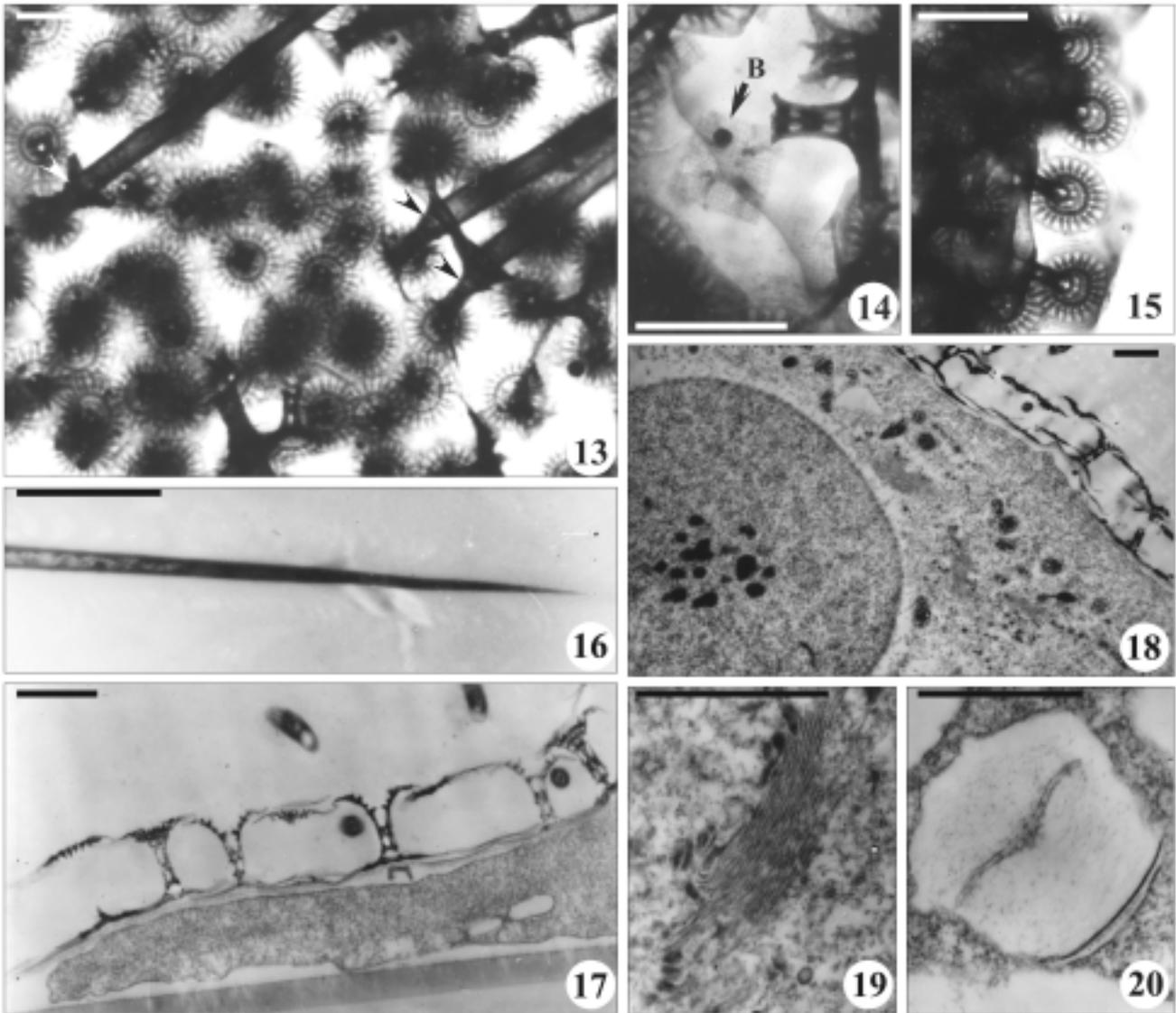
**Figs 1-12.** *Cochliopodium vestitum*, LM micrographs (1-8, White Sea strain; 9-12, Valamo strain). 1-4, 9 - locomotive forms; 5, 10 - amoebae during slower movement; 6, 11 - non-directly moving amoebae; 7 - stationary amoeba; 8 - nucleus (N) in a living cell; 12 - granuloplasm of the adhering amoeba showing spines on the surface of tectum. Spines in Figs 7 and 12 arrowheaded. Scale bars 10  $\mu$ m.

floated spontaneously. Floating form was spherical, with several long slender hyaline pseudopodia, formed in a compact bundle from the small scale-free area on the cell surface (Fig. 21). Pseudopodia slowly bent, but were never seen to branch or anastomose.

Nucleus (Fig. 8) was spherical, with the layer of refractile perinuclear granules and a large central nucleolus, which in living and stained amoebae often looked pale and non-homogeneous. The granuloplasmic crystals had a shape of the hexagonal plates about 5  $\mu$ m in size (Figs 2, 3, 7). Numerous transparent vacuoles of various sizes were seen close to the ventral surface of the cell. Amoebae fed on bacteria and small eukaryotes (flagel-

lates, unicellular algae, and amoebae). Encystment was never observed in cultures.

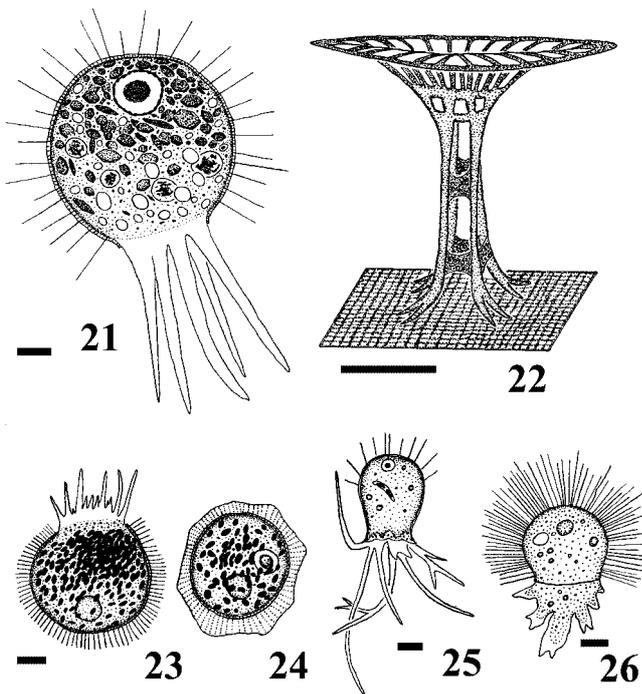
The base plates of the scales (Fig. 22) were grid-shaped (Fig. 14) with the mesh size about 30-40 nm. The stalks of the central column were almost straight, slightly curving outwards at the point of attachment of the top part (Figs 14, 17). Every stalk attached to the base plate with a bird-foot structure having two digits. Near the base and in the middle neighboring stalks were joined together with wide connectives. At the top, stalks continued into the top part of a scale (Figs 13, 15, 17), which started from the first concentric ring. The stalks were attached to this ring, and above it each stalk gave 2



**Figs 13-20.** *Cochliopodium vestitum*, White Sea strain, TEM micrographs. **13-15** - whole mounts of the scales (B - base plate of a scale in Fig. 14); **16** - tip of the spine in a whole mount preparation; **17** - tectum in the section through the hyaloplasmic veil of an adhering amoeba; **18** - overview of the nucleus and cytoplasm; **19** - dictyosome; **20** - scale under formation in a Golgi-derived vesicle. Spine-bearing scales in Fig. 13 are marked with arrowheads. Scale bars 1  $\mu$ m.

branches, altogether forming a basket of 8 radial spokes. These spokes were attached to the second concentric ring, above which every spoke branched into 3, altogether forming a wide funnel of 24 radial spokes cross-connected with two more concentric rings, the outermost one and the one in the middle. In the whole mounts some of the scales were seen to have such a typical structure, but possessed a long sharp-ended electron dense spine, originating from the center of a top funnel (Figs 13, 16). Diameter of the base of a spine was 0.3  $\mu$ m.

Scales on the surface of the plasma membrane densely overlapped between each other with their bases and top parts. The space between the plasma membrane and the scale layer (ca 100 nm) was filled with the fuzzy material, which was never seen on the ventral surface of the cell (Fig. 17). Nucleus in the sections had a central nucleolus, consisting of numerous irregularly rounded electron-dense granules of various sizes (Fig. 18). A well-developed Golgi complex was seen near the nuclear envelope, lying dorsally to the nucleus (Fig. 18). It consisted of several dictyosomes, each composed of



**Figs 21-24.** Drawings of *Cochliopodium vestitum*. **21** - floating form, Valamo and White Sea strains; **22** - scale, White Sea strains; **23** - floating form after Archer (1871); **24** - adhering form after Archer (1871). Scale bars 10  $\mu\text{m}$  (21, 23, 24); 0.25  $\mu\text{m}$  (22).  
**Figs 25, 26.** Drawings of: **25** - *Cochliopodium echinatum* after Korotneff (1879); **26** - *C. longispinum* after West (1901). Scale bars 10  $\mu\text{m}$  (25); 20  $\mu\text{m}$  (26).

several tens of flattened cisternae (Fig. 19). Dictyosomes were surrounded with the numerous vesicles containing various materials, and sometimes scale under formation (Fig. 20). In contrast to some other species of *Cochliopodium* (e. g. Yamaoka *et al.* 1984, Kudryavtsev 2004, Kudryavtsev *et al.* 2004), the “Golgi attachment”, an electron-dense dictyosome-associated structure resembling a MTOC was never seen in the sections. Mitochondria had electron-dense matrix and tubular cristae.

## DISCUSSION

### Identification

This *Cochliopodium* is comparable only with those species, which were reported to carry LM-visible spines on the surface of tectum, since it is impossible, that anyone observing this species could have overlooked this feature. Among such species the present strains mostly

resemble *C. vestitum* (Archer, 1871) Archer, 1877. These strains are similar to this species in the size and the shape of adhering and floating amoebae (Figs 23, 24). Archer (1871) stated that in his species “hair-like processes” (spines) were up to 20  $\mu\text{m}$  in length and had “very variable degree of development” (*op. cit.*, p. 115). Therefore Bark (1973) suggested that Archer studied more than one species. However, in the studied strains the spines were also poorly seen in locomotion, and their observed length depended on the angle of view. The nature of the surface spines in Archer’s amoebae seems to be identical to that of my strains, since Archer (1871) observed the spines detached from the surface of amoebae “with slightly capitate lower extremity” (*op. cit.*, p. 123), the same was observed in my strains. “Irregularly scattered, generally elliptic, or rounded ... grayish, or somewhat purple colored, sharply and darkly bounded, and clear and shiny bodies” (Archer 1871, p. 112) of *C. vestitum* may correspond to the crystals of my strains. Chlorophyll granules in the cytoplasm of *C. vestitum* considered as a very characteristic feature of this species, and never observed in my strains, might have originated from the ingested food. Archer (1877) rejected this idea, describing at the same time feeding of his amoebae on unicellular green algae and reporting the observation a strain which did not contain chlorophyll (Archer 1871). Later descriptions of *C. vestitum* mostly agree with that made by Archer (Leidy 1879, West 1901, Penard 1902, Cash and Hopkinson 1909), except the length of the spines, which is normally indicated as being 3.7-5  $\mu\text{m}$  (West 1901, Cash and Hopkinson 1909) and diameter of the cell in Penard’s (1902) strain indicated as 35  $\mu\text{m}$ . However, comparison of the length of spines in the figures with the cell diameter in descriptions suggests that they were longer than indicated in the text, at least as long as 10  $\mu\text{m}$ . My strains fit these later descriptions as well; therefore, it is possible to identify them as *C. vestitum*.

### Synonymy of the spine-bearing *Cochliopodium* spp.

As mentioned earlier, *C. pilosum* Hertwig et Lesser, 1874 is certainly the synonym of *C. vestitum*. Hertwig and Lesser (1874) only cite Archer’s (1871) description and provide a new name for a species, which they transfer to a new genus. This mistake has already been corrected (Archer 1877, Leidy 1879), and *C. pilosum* is not a valid species since 1877.

*Cochliopodium echinatum* has initially been described without any data on the cell dimensions and locomotive morphology (Korotneff 1879). After that it

was observed by Penard (1902), who provided the diameter of the floating cell. Measurements of the figures made by Korotneff (Fig. 25) and Penard show that the spines of *C. echinatum* were approximately 10–15 µm in length, which is identical to *C. vestitum*. My strains of *C. vestitum* are highly similar to *C. echinatum*, at least in those features which were described by the researchers mentioned (*op. cit.*). West (1901) described another spine-bearing species, *C. longispinum* (Fig. 26), for which Cash and Hopkinson (1909) suggested a synonymy to *C. echinatum*. Both West, and Cash and Hopkinson state that *C. longispinum* differs from *C. vestitum* in the more delicate “shell”, wider “shell mouth”, broader pseudopodia, lack of chlorophyll granules in the cytoplasm and longer spines (23–29 µm). However, the thickness of tectum observed with bright field LM may depend on the conditions of observation. “Width of the shell mouth” is outstandingly variable in *Cochliopodium* amoebae, given the nature of their “shell” (tectum). Breadth of the pseudopodia is also variable, depending on the state of an amoeba, and the presence of chlorophyll granules in the cytoplasm depends on the diet of the cell. Thus, the only stable difference between *C. vestitum* and *C. longispinum* is the length of the spines, but this seems to be not enough for reliable identification of the species. Therefore, I propose a synonymy of *C. vestitum*, *C. echinatum* and *C. longispinum*, and *Cochliopodium vestitum* (Archer, 1871) remains the only valid species by priority.

**Acknowledgements.** This work was partially supported by the Ministry of Education of Russia (grant E02-6.0-78).

## REFERENCES

- Archer W. (1871) On some fresh-water Rhizopoda, new or little known. Fasc. II. On *Amphizonella vestita* (sp. nov.), *Acanthocystis spinifera* (Greeff) and *Plagiophrys sphaerica* (Clap. et Lachm.). *Quart. J. Micr. Sci.* **11**: 107–151
- Archer W. (1877) Resume of recent contributions to our knowledge of “freshwater Rhizopoda”. Part IV. *Quart. J. Micr. Sci.* **17**: 330–353
- Awerintzew S. (1906) Freshwater Rhizopoda. *Proc. Saint-Petersburg Nat. Soc.* **36**: 121–351 (in Russian)
- Bark A. W. (1973) A study of the genus *Cochliopodium* Hertwig and Lesser 1874. *Protistologica* **9**: 119–138
- Cash J., Hopkinson J. (1909) The British Freshwater Rhizopoda and Heliozoa. London: The Ray Society, London, 2
- Hertwig R., Lesser E. (1874) Über Rhizopoden und denselben nahestehende Organismen. *Arch. Mikr. Anat.* **10** (Suppl.): 35–243.
- Korotneff A. (1879) Etudes sur les Rhizopodes. *Arch. Zool. Exp. Gen.* **8**: 467–482
- Kudryavtsev A. (2004) Description of *Cochliopodium spiniferum* sp. n., with notes on the species identification within the genus *Cochliopodium*. *Acta Protozool.* **43**: 345–349
- Kudryavtsev A., Brown S., Smirnov A. (2004) *Cochliopodium barki* n. sp. (Rhizopoda, Himatizmenida) re-isolated from soil 30 years after its initial description. *Europ. J. Protistol.* **40**: 283–287
- Leidy J. (1879) Freshwater Rhizopods of North America. Washington: U. S. Geol. Survey of the Territories, Washington
- Penard E. (1902) Faune Rhizopodique du Bassin de Leman. Geneva: Henry Kundig, Geneva
- Prescott D. M., James T. W. (1955) Culturing of *Amoeba proteus* on *Tetrahymena*. *Exp. Cell. Res.* **8**: 256–258
- West G. S. (1901) On some British freshwater Rhizopods and Heliozoa. *J. Linn. Soc. (Zool.)* **28**: 309–342
- Yamaoka I., Kawamura N., Mizuno M., Nagatani Y. (1984) Scale formation in an amoeba, *Cochliopodium* sp. *J. Protozool.* **31**: 267–272

Received on 7th August, 2004; revised version on 7th January, 2005; accepted on 12th January, 2005