ACTA
PROTOZOOLOGICA

Acta Protozool. (2014) 53: 325–333
http://www.eko.uj.edu.pl/ap
doi:10.4467/16890027AP.14.020.1776

Scale Structure of *Cochliopodium actinophorum* (Auerbach, 1856) (Amoebozoa, Cochliopodiidae) and a New Diagnosis of this Species

Alexander KUDRYAVTSEV

Department of Invertebrate Zoology, Faculty of Biology, Saint-Petersburg State University, Saint-Petersburg, Russia

Abstract. *Cochliopodium actinophorum* (Auerbach, 1856) is one of the oldest and most frequently mentioned species of the genus, yet the structure of scales comprising tectum of these amoebae has never been described, and the strain previously used to re-define this species based on light microscopic characters was lost. A new strain identified as *C. actinophorum* using light microscopy was isolated recently, and molecular sequence data of this strain were published, yet without any morphological data. Here, the results of light and electron microscopic study of this strain are presented that confirm its identification as *C. actinophorum* and allow a proposal of a new diagnosis of this species thus linking morphology, scale ultrastructure and available gene sequence data. The newly isolated strain deposited with CCAP (accession number 1537/10) is designated as a neotype.

Key words: Amoebozoa, *Cochliopodium*, morphology, scales, taxonomy, ultrastructure.

Abbreviations: CCAP – Culture Collection of Algae and Protozoa; DIC – differential interference contrast; PJ – Prescott and James medium; SSU rRNA (rDNA) – small subunit ribosomal RNA (ribosomal DNA); TEM – transmission electron microscopy.

INTRODUCTION

Cochliopodium actinophorum (Auerbach, 1856) is one of the "oldest" species of this genus that is believed to be described as one of its two first members by Auerbach (1856) under the name *Amoeba actinophora* almost 20 years before the genus *Cochliopodium* was established (Hertwig and Lesser 1874). Due to its early introduction, the species name had a long taxonomic history being placed in various genera of lobose amoebae, but finally was formally included in *Cochliopodium* (Page 1976, Penard 1902). At present, light microscopic characters and scale fine structure are the most reliable evidence that defines a morphospecies of *Cochliopodium* (Bark 1973; Kudryavtsev 1999, 2004, 2005, 2006; Kudryavtsev and Smirnov 2006; Kudryavtsev *et al*. 2004). However, the most recent reinvestigation and diagnosis of *C. actinophorum* were based on light microscopic data only (Page 1968,

Address for correspondence: Alexander Kudryavtsev, Department of Invertebrate Zoology, Faculty of Biology, Saint-Petersburg State University, Universitetskaya nab., 7/9 199034 Saint-Petersburg, Russia; E-mail: a.kudryavtsev@bio.spbu.ru

1976) and no details of scale ultrastructure have been available. Yet, this is one of the few *Cochliopodium* species included in the keys for amoebae identification (Page 1976, 1988). The strain of *C. actinophorum* studied by Page (1968, 1976) and deposited in the Culture Collection of Algae and Protozoa (CCAP strain 1537/2; e.g. Catalogue of the UK National Culture Collection 2001) was lost several years ago. A new strain identified as *C. actinophorum* was isolated recently, deposited in CCAP (accession number 1537/10), and gene sequences of mitochondrial cytochrome oxidase (Cox1; Nassonova *et al*. 2010), nuclear SSU rDNA and actin (Kudryavtsev *et al*. 2011) were published. However, neither light microscopic nor ultrastructural data for this strain have been published until now. The purpose of this paper is to present the light microscopic and ultrastructural data for this strain and revise a diagnosis of *C. actinophorum* linking a species name to morphology, ultrastructure and available molecular sequences.

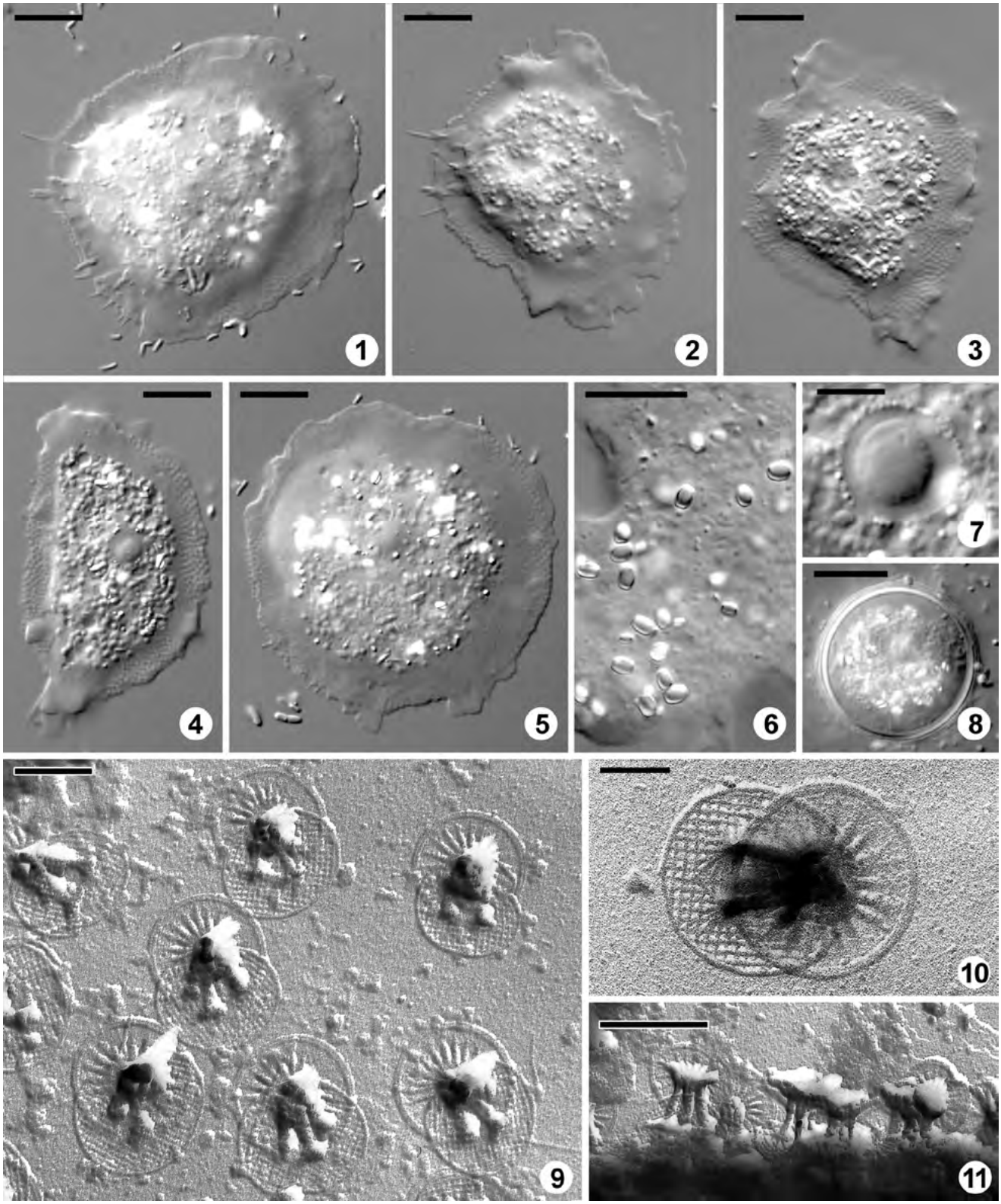
MATERIALS AND METHODS

Amoebae were isolated from the bottom sediments of the Volga river collected near the Institute for Biology of Inner Waters of the Russian Academy of Sciences (Borok Settlement, Central Russia, approximately 58°4′N, 38°16′E). Samples were inoculated in Prescott and James medium (PJ; Prescott and James 1955) with addition of sterilized wheat grains (2–3 per 90-mm Petri dish). Cloning was done by picking single cells with glass capillary pipettes into the dishes with fresh medium. Subsequently, established cultures were transferred into, and further maintained in 0.025% Cerophyl infusion prepared with PJ. Light microscopic study was performed using Carl Zeiss Axiovert 200 inverted microscope and Leica DM2500 upright microscope, both equipped with phase contrast and differential interference contrast (DIC) optics. Living amoebae were observed, photographed and measured when moving on the surface of coverslips. For transmission electron microscopy (TEM) several different fixation protocols were tried; the results presented were apparently obtained by fixation of amoebae with 1% osmium tetroxide prepared with 0.05 M cacodylate buffer (pH 7.4) for 1 hour at + 4°C followed by washing with the same buffer (3 × 5 minutes), dehydration in a graded ethanol series followed by epoxy propane, infiltration and embedding in Araldite M. Sections were prepared with a diamond knife using a Reichert Ultracut E ultramicrotome, double-stained with 2% uranyl acetate in 70% ethanol and Reynolds' lead citrate. Whole mount preparations of scales were made by placing the amoebae on formvar-coated copper grids, rinsing several times with bidistilled water followed by a brief fixation in osmium vapour. Dried grids were shadowed with chromium at an angle of 15–20°. Sections and whole mounts were observed using a Philips EM208 electron microscope operated at 80 kV.

RESULTS

1. Light microscopic study. During locomotion amoebae were rounded, oval or broadly triangular when viewed from above (Figs 1–4). Most of the cells had a length:breadth ratio about 1 during active locomotion, however, some of the amoebae were either oval, with breadth being the greatest dimension, or elongated and drop-shaped, with length greater than breadth. The cytoplasm consisted of a broad, flat peripheral hyaline sheet that completely surrounded a raised granuloplasmic mass located centrally. Anterior and lateral parts of the peripheral hyaline sheet usually had a maximal breadth, and its posterior part was narrower, so that the central granuloplasmic mass looked slightly shifted towards the posterior end of the cell in dorsal view (Figs 1–3). The anterior margin of hyaloplasm had an outline of a smooth broad arc, or possessed several irregularities and hyaloplasmic waves, but never produced subpseudopodia. In some locomotive forms lateral parts of the peripheral hyaline area adhered to the substratum and temporarily produced a lobe-like extension behind the advancing cell (Fig. 4). The posterior margin of the locomotive form was either angulate (Figs 1–3) or almost straight, at right angle to the direction of movement (Fig. 4). Several short trailing filaments were usually located at the posterior end of the locomotive form, originating from the posterior margin of hyaloplasm and areas of the ventral surface of the cell beneath the posterior part of the granuloplasmic mass (Figs 1–2). Tectum was clearly seen on the surface of adhering amoebae in above view as a regular array of granules (best visible in Figs 3–4). Anterior and lateral parts of the hyaline margin were often seen to extend beyond the border of the scale layer. Length of the locomotive form was 25–46 μm (average 34 μm), breadth, 30–78 μm (average 41 μm), length:breadth ratio was 0.54–1.15 (average 0.85) (n = 139).

Non-directly moving amoebae were either rounded and flattened (Fig. 5), or had an irregular shape, with the cell spread over the substratum and separated into several lobes with hyaloplasm at the margins. Stationary amoebae were hemispherical or lens-shaped, rounded in above view. Their peripheral hyaline sheet was much narrower than in the locomotive form and had an equal breadth from all sides. Floating forms were never seen in undisturbed cultures, and were only produced when amoebae were artificially disturbed and detached from the substratum. A floating cell was contracted, with the



Figs 1–11. *Cochliopodium actinophorum*, strain CCAP 1537/10, light micrographs of the living amoebae (**1–8**) and electron micrographs of the whole mounts of scales (**9–11**). **1–4.** Locomotive forms on the glass surface, note scales visible in **3** and **4**. **5**. Amoeba during non-directional movement. **6.** Granuloplasm at a higher magnification showing crystals. **7.** Nucleus. **8.** Cyst. Scale bars: 10 μm in Figs 1–6 and 8, 5 μm in Fig. 7, 0.5 μm in 9, 0.25 μm in 10, and 1 μm in 11.

compact, spherical central cytoplasmic mass consisting of granuloplasm and several (most frequently 3–5) tapering, hyaline pseudopodia that were either straight, or slightly bent at the tips. Length of these pseudopodia was usually several times greater than the diameter of the central cytoplasmic mass. Flotation did not occur for a long time; most of the floating cells settled back to the substratum and resumed locomotion within minutes.

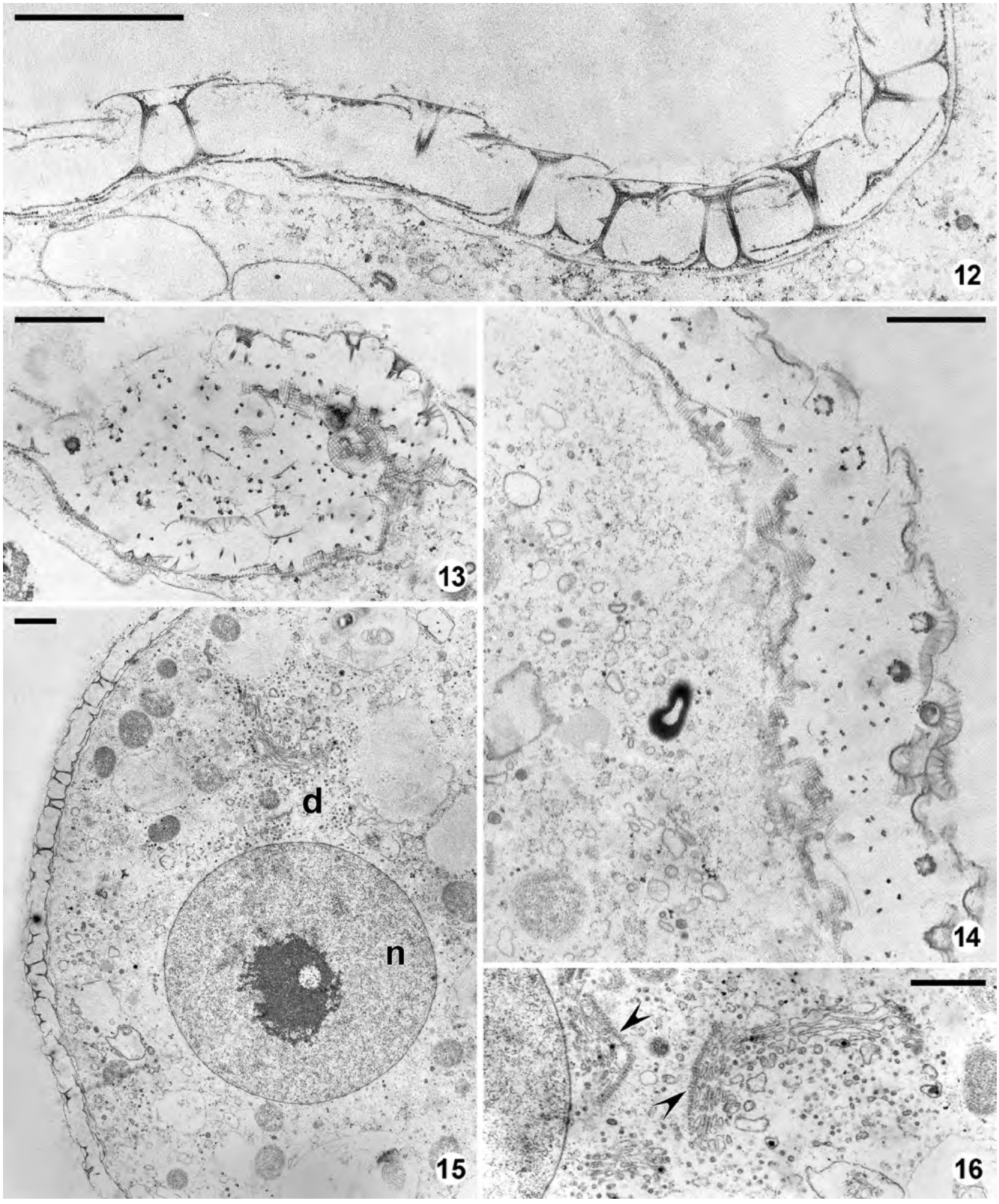
The nucleus (Fig. 7) was spherical, of vesicular type, with the large central nucleolus that had an uneven, slightly undulate margin and might contain several small surface invaginations. The nuclear envelope was conspicuous and surrounded with the coarse layer of yellowish, highly refractile granules. The diameter of nucleus was 5–9.5 μm (average 7.4 μm), of nucleolus, 3–6.3 μm (average 4.3 μm) (n = 51). The granuloplasm contained several peripheral contractile vacuoles that expanded by fusion of numerous small vesicles and worked asynchronously. There were numerous conspicuous yellowish refractile crystals (Fig. 6) numbering 15–30 per cell. These crystals were up to 2–3 μm long; the smaller ones had a bipyramidal shape, while the larger crystals looked more like hexagonal plates that were thicker in the center, therefore, they looked bipyramidal when observed from their narrow side. Other inclusions were food vacuoles and dense spherical or elliptical granules below 1 μm in diameter. Amoebae fed on bacteria and other organic particles of similar size. Encystment occurred regularly and started in 4–7 days after mass inoculation of a fresh medium. Mature cysts (Fig. 8) were spherical, with the nucleus, granular cytoplasm and crystals concentrated in the central part. The cyst wall was made up of two layers, the outer layer being coarser than the inner one and consisting of scales comprising the tectum in a trophic amoeba. Diameter of cysts was 18.7–22.8 μm (average 21 μm) (n = 20).

2. Electron microscopy. Transmission electron microscopic study has shown that amoebae were covered with a tectum consisting of typical scales that can be assigned to the Category 1 following Anderson and Tekle (2013). Scale shape that can be deduced from shadowed whole mounts (Figs 9–11) and ultrathin sections (Figs 12–14) is presented in Fig. 17. The base plate of the scales had a shape of a circular grid (Figs 9, 10, 13) with a square mesh that had a uniform size of 40 nm. Four stalks with conspicuous bird foot-like structures at the bases arose from the base plate. The upper approximately one-third of each stalk was split into two branches that attached to the funnel-shaped apical part of the scale that had a square base and consisted of ca. 18 radial spokes attached to a solid outer rim that in favorable views appeared to consist of two closely adjacent rings. Very delicate and poorly discernible concentric rings were seen making up an apical part in favorable sections. The diameter of a base plate was 690–900 nm (average 767 nm; n = 9), of an apical part, 700–940 nm (average 700 nm; n = 16), height of the scale was 500–673 nm (average 544 nm; n = 36). The preservation quality of the cytoplasm was generally poor, yet some details could be observed. Among them were the nucleus that was rounded in sections and contained an electron-dense nucleolus with uneven margin and several less dense internal spaces (Fig. 15) generally corresponding in structure to what was observed with light microscopy, and a large dictyosome located close to the nucleus. A dictyosome consisted of not less than 6 flattened cisternae surrounded with numerous smaller vesicles (Figs 15–16). A conspicuous Golgi attachment was adjacent to one of the poles of a dictyosome (Fig. 16).

DISCUSSION

The described strain certainly belongs to the genus *Cochliopodium*, and based on its light microscopic characters appears to be identical to an amoeba that was redescribed by Page (1968) under the name *Hyalodiscus actinophorus* and later (Page 1976) renamed *Cochliopodium actinophorum*. An identification and taxonomy of this amoeba has two aspects, both of which have to be considered here: (1) Identity of the presently described strain to Page's one; (2) Identity of this and Page's strains to a species *C. actinophorum* described initially under the same name (Auerbach 1856, as *Amoeba actinophora*) and consistency of the use of this name in subsequent literature (e.g. Cash and Hopkinson 1905, De Saedeleer 1934, Gruber 1882, Penard 1902).

Unfortunately, it was not possible to compare the strain studied here and that of Page, directly. The strain isolated by Page in 1964 from a bog in southwestern Columbia County, in Wisconsin (USA) and described in 1968 (op. cit.) was deposited with CCAP under accession number CCAP 1537/2 (e.g. Catalogue of the UK National Culture Collection 2001), but perished several years ago, before I had a chance to reinvestigate it. Therefore, only light microscopic description



Figs 12–16. *Cochliopodium actinophorum* CCAP 1537/10, transmission electron micrographs. **12–14.** Scales in a vertical section (**12**) and in tangential sections (**13–14**). **15.** Section of an amoeba at lower magnification showing nucleus (**n**) and dictyosomes (**d**). **16.** Dictyosomes at higher magnification showing Golgi attachment (arrowheads). Scale bars: 1 μm throughout.

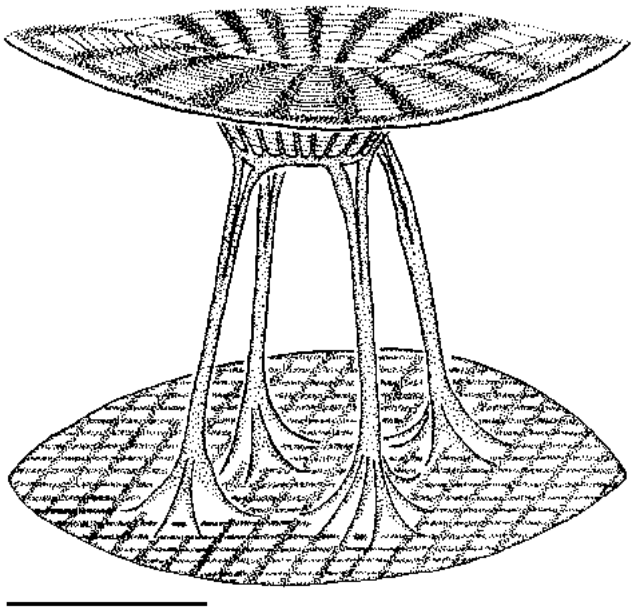


Fig. 17. *Cochliopodium actinophorum* CCAP 1537/10, diagram, illustrating the scale structure as deduced from ultrathin sections and shadowed whole mounts. Scale bar: 0.25 μm.

and line drawings (Page 1968), as well as several light micrographs made later (Page 1976, 1988) are available for comparison. Comparison of the main characters of the locomotive form of the strain studied here, such as its shape in above view, uroidal structures, width of the peripheral hyaloplasmic veil, and absence of subpseudopodia fit the description published by Page (1968). Shape of the floating form, nuclear structure (especially the layer of coarse perinuclear granules), and contents of the granuloplasm are identical in both strains. Cell dimensions are slightly different in the present strain, as the ranges obtained in the present study are generally narrower than reported by Page (1968), yet within the size range of Page's amoebae. Diameter of nucleus, nucleolus and cysts of the present strain were slightly smaller than those described by Page, yet largely overlapping in ranges, indicating that this variation is purely intraspecific and does not warrant different species. This allows a conclusion that the studied strain belongs to the same morphospecies as the one described by Page (1968); morphological differences from the other known *Cochliopodium* spp. will be outlined in a differential diagnosis below.

The second problem of the identification of this strain is its identity to the previously described species under the same name. Page (1968) concluded that his strain was identical to *Amoeba actinophora* Auerbach, 1856 and should belong to the genus *Hyalodiscus* Hertwig et Lesser, 1874. The latter assignment is certainly wrong that was recognized later (Page 1976), and the species should belong to the genus *Cochliopodium*, otherwise *Cochliopodium* and *Hyalodiscus* should be synonymized. Auerbach's (1856) description and drawings of *Amoeba actinophora* show the main light microscopic characters typical of *Cochliopodium* spp. However, there are not many details provided in the description that might allow an unambiguous identification of species. In fact, these are only shape of the floating form, and shape and number of crystals in the granuloplasm. However, Gruber (1882) believed that he isolated exactly the same species as Auerbach, and that it was not identical to *Cochliopodium pellucidum* described by that time (Hertwig and Lesser 1874). Composition and sustainability of the cell coat visible with light microscopy on the surface of amoebae were considered to be the main differences between the two species. Whereas in *C. pellucidum* the latter was considered to be permanent and was described as having a structure similar to the test of *Arcella* spp. (Gruber 1882), the cell coat in *A. actinophora* was thought of as a very delicate and temporary structure representing an outermost layer of the cytoplasm that could disappear as the cell was expanding over the substratum. Yet, Penard (1902) described an amoeba that he named *Cochliopodium actinophorum* from the Lake Leman, and considered it to be identical to the one described by Auerbach, simultaneously including it in the genus *Cochliopodium* for the first time. This point of view was not supported by subsequent researchers (Cash and Hopkinson 1905, Page 1968), and re-examination of Penard's figures is consistent with this opinion. Primarily, Penard's amoebae were essentially smaller than those studied by Auerbach and Gruber (16–18 μm vs. 30–40 μm), but at the same time had a much better visible cell coat, and seemed to have formed subpseudopodia more frequently, also during adhesion to the substratum (Penard 1902, Fig. 1 at p. 189). Cash and Hopkinson (1905) studied another strain of similar amoebae and concluded that their observations were in agreement with those of Gruber (1882), but Penard's species was different. Among subsequent authors who mentioned this species, Sandon (1923) was in agreement with Cash and Hopkinson, whereas De Saedeleer

(1934) and Valkanov (1936) re-introduced the name *Cochliopodium actinophorum* stating that this species was identical to amoebae studied by both, Auerbach (1856) and Penard (1902). Both papers were neither cited, nor discussed by Page (1968), but comparison of his data with those published by De Saedeleer and Valkanov (op. cit.) shows that amoebae studied by Page (1968) were largely similar to those studied by De Saedeleer and Valkanov. Moreover, it was De Saedeleer (1934) who first described and figured a cyst of this species.

Analysis of the above-cited descriptions shows that although the described organisms were definitely similar, it is hard to deduce any specific characters that would allow a modern researcher to distinguish a particular morphospecies. The main problem is that no shape of a cell that could be clearly and unambiguously attributed to the locomotive form was described in earlier works, and other characters, although generally typical of *Cochliopodium*, are not those that can be specifically linked to a particular species name. Some of these characters were illustrated, but not specifically mentioned. For example, Gruber (1882) depicts a layer of granules surrounding nucleus in his amoebae (Plate 9, Fig. 14), but does not mention it in the text. This situation makes it difficult to definitely conclude whether subsequently described organisms were identical to Auerbach's (1856) *Amoeba actinophora*, although Penard's (1902) one was probably not. I therefore suggest a conservation of the name *Cochliopodium actinophorum* with the formal authority of Auerbach (1856) and De Saedeleer (1934) who first created a combination based on at least formally the same organism, and with a set of distinguishing characters described by Page (1968) and in the present paper. As no type material of this species was ever designated, and Page's strain CCAP 1537/2 was lost, I propose a designation of the strain *Cochliopodium actinophorum* CCAP 1537/10 described here as a neotype for this species. The results reported here allow an emendation of the diagnosis of this species, so that now it includes a description of the scale structure to facilitate a more precise identification of this species in the future. Scales of these amoebae correspond to those of Category 1 following Anderson and Tekle (2013) under which also *C. barki*, *C. megatetrastylus*, *C. minus*, *C. minutoidum* and *C. plurinucleolum* fall (Anderson and Tekle 2013, Bark 1973, Kudryavtsev 2006, Kudryavtsev *et al*. 2004). Scales of *C. actinophorum* differ from the mentioned species in being "higher" and having "longer" stalks (i.e. ratio between scale height and diameters of a base plate and apical part is the largest, as well as height from the base plate to the base of an apical funnel is maximal); in addition *C. actinophorum* appears to have the largest mesh size of the base plate (40 nm, while in most other of the mentioned species it comprises about 15 nm; Kudryavtsev 2006). Among other studied strains of *Cochliopodium*, scales of *C. actinophorum* appear to be the most similar to those of an unidentified "NYS strain" (Nagatani *et al*. 1981, Yamaoka *et al*. 1984). These amoebae show similar light microscopic characters to the strains described by Page (1968) and in the present study, and their scales are very similar in appearance to those described here. Minor differences include finer mesh of the base plate (30 nm) larger height (700 nm on average; the scales of the present strain are 500–673 nm high, average 544 nm) and smaller diameter of a base plate (640 nm, whereas in the strain studied here it was 690–900 nm, with the average of 767 nm). At the moment it is impossible to conclude whether these dimensional differences warrant different species, or correspond to intraspecific variation, as there are no statistical data on scale variation in multiple genetically identical strains of *C. actinophorum*, but Page's (1987) suggestion that the NYS strain can also be assigned to *C. actinophorum* cannot be refuted with these data.

Molecular sequence data were previously obtained based on the strain described here; these include SSU ribosomal RNA, actin and Cox1 gene sequences (Geisen *et al*. 2014, Kudryavtsev *et al*. 2011, Nassonova *et al*. 2010). Accession numbers are listed here after the diagnosis in order to link species name, morphology and gene sequence data, and permit molecular identification of this species as well.

TAXONOMIC APPENDIX

Cochliopodium actinophorum (Auerbach, 1856) De Saedeleer, 1934, emend.

Diagnosis. Amoebae generally between 14 and 80 μm large; measured size of the locomotive form in the type strain: length 25–46 μm (average 34 μm), breadth 30–78 μm (average 41 μm), length:breadth ratio 0.54–1.15 (average 0.85) (n = 139). Rounded or oval during locomotion, rarely broadly triangular. Anterior and lateral edges of peripheral hyaline area smooth or uneven, but without subpseudopodia. Uroid with few short trailing filaments produced at the posterior part of a hyaline margin and ventral surface of the cell beneath

the central granuloplasmic mass. Floating form with a spherical central mass of the granuloplasm and tapering hyaline subpseudopodia produced either as a bundle on a local area of the amoeba surface, or radiating separately. Vesicular nucleus irregularly rounded, with a large central nucleolus; nuclear envelope surrounded with a layer of coarse, refractile granules. Diameter of nucleus 5–9.5 μm (average 7.4 μm), of nucleolus 3–6.3 μm (average 4.3 μm) (n = 51). Granuloplasm contains 15–30 crystals of either bipyramidal shape, or, in the largest ones, a shape of hexagonal plates with rounded edges and tapering margins. Size of the crystals up to 2–3 μm. Spherical cysts with a double wall; diameter of cysts 18.7–22.8 μm (average 21 μm) (n = 20). Scales made up of a circular grid-like base plate with a square mesh, 4 stalks arising from the center of a base plate, and a funnel-shaped apical part made up of about 18 radial spokes and fine concentric rings. Base plate diameter 690–900 nm (average 767 nm; n = 9), apical part, 700–940 nm (average 700 nm; n = 16), height of the scale 500–673 nm (average 544 nm; n = 36).

Type material: type culture (neotype) deposited with the Culture Collection of Algae and Protozoa (Oban, UK), accession number CCAP 1537/10.

Observed habitats: freshwater.

Molecular sequences: GenBank accession numbers JF298248-JF298251 (SSU rRNA), JF298259-JF298265 (actin), Kudryavtsev *et al*. (2011); Cox1: GQ354207 (Nassonova *et al*. 2010), KJ173779 (Geisen *et al*. 2014).

Differential diagnosis: Scale structure clearly distinguishes *C. actinophorum* from *C. bilimbosum*, *gallicum*, *gulosum*, *kieliense*, *larifeili*, *maeoticum*, *pentatrifurcatum*, *spiniferum* and *vestitum*. More similar in the scale structure to *C. barki*, *C. megatetrastylus*, *C. minus*, *C. minutoidum* and *C. plurinucleolum*, but differs from all of these species in having "higher" scales with a larger ratio of scale height to diameters of base plates and apical parts, and also longer stalks of the central column relative to the depth of an apical funnel. Additionally, locomotive form is larger than in all of the listed species, with the exception of *C. megatetrastylus*; differs from *C. minus* and *C. minutoidum* in the absence of subpseudopodia and from *C. plurinucleolum*, in the structure of nucleus.

Acknowledgements. Partially supported by the grant 12-04-01835-a from the Russian Foundation for Basic Research, as well as a grant and Core Facility Centers "Culturing of Microorganisms" and "Development of Molecular and Cell Technologies" of the Saint-Petersburg State University. I am grateful to Dr. Anna Kudryavtseva (Engelgardt Institute of Molecular Biology, Russian Academy of Sciences) for collecting the natural sample where the strain was found.

REFERENCES

Anderson O. R., Tekle Y. I. (2013) A description of *Cochliopodium megatetrastylus* n. sp. isolated from a freshwater habitat. *Acta Protozool.* **52:** 55–64

Auerbach L. (1856) Ueber die Einzelligkeit der Amoeben. *Z. Wiss. Zool*. **7:** 365–430

Bark A. W. (1973) A study of the genus *Cochliopodium* Hertwig and Lesser 1874. Protistologica **9:** 119–138

Cash J., Hopkinson J. (1905) The British freshwater Rhizopoda and Heliozoa. The Ray Society, London

Catalogue of the UK National Culture Collection (2001) UKNCC, Ambleside, Oban

De Saedeleer H. (1934) Beitrag zur Kenntnis der Rhizopoden: morphologische und systematische Untersuchungen und ein Klassifikationsversuch. Mem. Mus. R. Hist. Nat. Belg. **60:** 1–112

Geisen S., Kudryavtsev A., Bonkowski M., Smirnov A. (2014) Discrepancy between species borders at morphological and molecular levels in the genus *Cochliopodium* (Amoebozoa, Himatismenida), with the description of *Cochliopodium plurinucleolum* n. sp. *Protist* **165:** 364–383

Gruber A. (1882) Contributions to the knowledge of the amoebae. *Annals Mag. Nat. Hist.* **9:** 106–115

Hertwig R., Lesser E. (1874) Ueber Rhizopoden und denselben nahestehende Organismen. *Arch. Mikr. Anat*. **10 (Suppl.):** 35–238

Kudryavtsev A. A. (1999) 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. *Protistology* **1:** 66–71

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. (2005) Redescription of *Cochliopodium vestitum* (Archer, 1871), a freshwater spine-bearing *Cochliopodium*. *Acta Protozool*. **44:** 123–128

Kudryavtsev A. (2006) "Minute" species of *Cochliopodium* (Himatismenida): Description of three new fresh- and brackish-water species with a new diagnosis for *Cochliopodium minus* Page, 1976. *Eur. J. Protistol.* **42:** 77–89

Kudryavtsev A., Brown S., Smirnov A. (2004) *Cochliopodium barki* n. sp. (Rhizopoda, Himatismenida) re-isolated from soil 30 years after its initial description. *Eur. J. Protistol.* **40:** 283–287

Kudryavtsev A., Smirnov A. (2006) *Cochliopodium gallicum* n. sp. (Himatismenida), an amoeba bearing unique scales from cyanobacterial mats in the Camargue (France). *Eur. J. Protistol.* **42:** 3–7

Kudryavtsev A., Wylezich C., Pawlowski J. (2011) *Ovalopodium desertum* n. sp. and the phylogenetic relationships of Cochliopodiidae (Amoebozoa). *Protist* **162:** 571–589

Nagatani Y., Yamaoka I., Sato N. (1981) Scale structure of the external surface of an amoeba. *Zool. Mag.* **90:** 112–115

Nassonova E., Smirnov A., Fahrni J., Pawlowski J. (2010) Barcoding amoebae: comparison of SSU, ITS and COI genes as tools for molecular identification of naked lobose amoebae. *Protist* **161:** 102–115

Page F. C. (1968) Generic criteria for *Flabellula*, *Rugipes* and *Hyalodiscus* with descriptions of species. *J. Protozool.* **15:** 9–26
Page F. C. (1976) An illustrated key to freshwater and soil amoebae. Freshwater Biological Association, Ambleside
Page F. C. (1987) The classification of 'naked' amoebae (phylum Rhizopoda). *Arch. Protistenkd.* **133:** 199–217
Page F. C. (1988) A new key to freshwater and soil gymnamoebae. Freshwater Biological Association, Ambleside
Penard E. (1902) Faune rhizopodique du Bassin du Léman. Henry Kündig, Libraire de l'Institut, Genève
Prescott D. M., James T. W. (1955) Culturing of *Amoeba proteus* on *Tetrahymena*. *Exp. Cell Res.* **8:** 256–258
Sandon H. (1923) Some protozoa from the soils and mosses of Spitsbergen. *J. Linn. Soc. (Zool.)* **35:** 449–476
Valkanov A. (1936) Versuch einer Revision der Rhizopodengattung *Cochliopodium* Hertw. & Less. *Sitzber. Ges. Naturf. Fr.* 92–101
Yamaoka I., Kawamura N., Mizuno M., Nagatani Y. (1984) Scale formation in an amoeba, *Cochliopodium* sp. *J. Protozool.* **31:** 267–272

Received on 30th December, 2013; revised on 16th January, 2014; accepted on 17th January, 2014