

New Freshwater Species of Centrohelids *Acanthocystis lyra* sp. nov. and *Acanthocystis siemensmae* sp. nov. (Haptista, Heliozoa, Centrohelea) from the South Urals, Russia

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Abstract. Two new species of centrohelids *Acanthocystis lyra* sp. nov. and *A. siemensmae* sp. nov. from the Pismenka River in the South Urals, Russia, have been studied with scanning electron microscopy. Cells of these species have both long and short spine scales with hollow shafts and circular basal plates. *A. lyra* has the long spine scales divided into two curved S-shaped branches possessing small teeth on their inner surface. The short spine scales have primary and secondary bifurcations. Every secondary branch ends with two teeth. *A. siemensmae* has both long and short scales with funnel-like apices, which possess small teeth. Based on the scale morphology *A. lyra* has been attributed to the *A. turfacea* species group, whereas *A. siemensmae* has been attributed to the *A. pectinata* species group, both according to classification proposed by Mikrjukov, 1997. Similarities and differences of the new species with other members of the genus *Acanthocystis* have been discussed.

Key words: Heliozoa, Centrohelids, *Acanthocystis*, protists, SEM, taxonomy.

Abbreviations: SEM – scanning electron microscopy

INTRODUCTION

Centrohelid heliozoans are ubiquitous non-ciliate unicellular phagoheterotrophs with flat mitochondrial cristae and slender radiating axopodia for trapping prey. According to the most recent revision of the classifi-

cation of this group (Cavalier-Smith *et al.* 2015), the subphylum Heliozoa is included in the phylum Haptista and contains a sole class Centrohelea. Most centrohelids possess axopodia, bearing concentric core extrusomes, and supported with hexagonally arranged microtubules nucleated by a single centrosome (Siemensma 1991). Centrohelids have a cosmopolitan distribution. They inhabit benthos and periphyton in both marine and freshwater ecosystems, where they are predators on other microorganisms (Mikrjukov 2002).

Centrohelids are characterized by conservatism of inner structure, whereas the morphology of an outer

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coat composed of spine and plate scales is highly varying (Mikrjukov 2002, Siemensma 1991). The main elements of the outer coat are scales, which can be visualized well with electron microscopy (Febvre-Chevalier and Febvre 1984, Mikrjukov 2002). The structure of the scales reflects the evident morphological and taxonomic diversity of centrohelids. Structural and dimensional characteristics of scales are the most important diagnostic signs serving for identification of centrohelid heliozoans according to morphological systems of their classification (Siemensma 1991, Mikrjukov 2002). A clearly expressed diversity of forms and sizes of the siliceous scales underlies taxonomy of centrohelids (Mikrjukov 2002). Moreover, morphology of the scales is used for evolutionary and phylogenetic assumptions and hypotheses (Mikrjukov 2002, Zlatogursky 2016).

Acanthocystis Claus, 1867 is the most diverse centrohelid genus, which probably contains a lot of undescribed species (Cavalier-Smith and Heyden 2007), though approximately 30 species have been already established on the basis of their morphology (Zlatogursky 2014). Generally, at present the taxonomic diversity of centrohelids is considered to be understudied (Cavalier-Smith and Heyden 2007, Zlatogursky 2014). In this aspect findings of new species are valuable for estimation of current centrohelidian biodiversity and for their taxonomy.

In this paper two new species of the genus *Acanthocystis* have been described based on SEM of scale structure.

MATERIALS AND METHODS

Sampling site and collection. A sample of water with the top layer of bottom sediment was collected 11.05.2014 into sterile 300 ml container from the Pismenka River in the South Urals (South-East of European part of Russia), grid references 51°15'41N, 57°28'42E. Salinity and pH at this location were 0.01‰ and pH 7.3, respectively.

Microscopy. The samples were transported to the laboratory, and viewed in Petri dishes using a light microscope Axioscope (Carl Zeiss, Germany) equipped with phase-contrast optics. Diameter of individual cells was measured with subsequent calculation of the mean and standard error (Table 1). For SEM, the cells were individually collected on the coverslip with a Pasteur pipette, air-dried, and washed in distilled water. Then the coverslips were attached to the specimen stubs and gold-palladium coated. The specimens were examined with a scanning electron microscope Jeol JSM 6510 (Germany). Scales were measured in EM images. Mean, maximal, and minimal sizes, as well as standard errors, were calculated for each scale type separately (Table 1).

RESULTS

Acanthocystis lyra sp. nov. (Figs 1, 3 A–C)

Diagnosis: Cells are 21.0–30.0 µm (mean 26.0) in diameter. A cell coat consists of plate scales of a single type, as well as long and short spine scales. The spine scales have cylindrical shafts, branching apices and circular basal plates with a clearly expressed marginal rim. The long spine scales have a length 5.1–14.0 µm. The scale apices are divided into two pointed, deflected S-shaped branches 0.47–1.42 µm long. Both branches due to their curves form a lyrate distal end. The inner edge of each branch has 6–8 short teeth, whereas the outer edge is smooth. Basal plates of the long spine scales are 0.6–1.5 µm. The short spine scales are 1.9–3.7 µm long, broadly bifurcate at their apices. The scales possess marked primary and secondary bifurcations. Each apex of the secondary branch has two short teeth. Basal plates of the short spine scales are 0.6–1.2 µm in diameter. The plate scales are elliptical 2.4–4.1 × 1.4–2.5 µm, ornamented with an axial thickening in the central part.

Comparisons with similar species: *A. cornuta* Dürschmidt, 1987 is similar to *A. lyra* in the structure of its long spine scales. The spine scales of *A. cornuta* are divided into two branches. Each branch has 2–4 teeth on the inner surface, whereas branches of *A. lyra* have 6–8 teeth on the inner surface. In addition, *A. cornuta* has spine scales of one type and plate scales ornamented with an axial thickening and radial ribs unlike *A. lyra*.

Branching spine scales are also known in *A. bicornis* Dürschmidt, 1987. Long scales of *A. bicornis* are divided into two branches. Each branch has a tooth on the inner surface, whereas branch of *A. lyra* has 6–8 teeth. Short scales of *A. bicornis* with broadly bifurcate branches have two teeth of different length on the apices of each branch. In contrast to *A. bicornis*, the short scales of *A. lyra* possess secondary bifurcation and two teeth at apices of the secondary branches. Plate scales of both *A. bicornis* and *A. lyra* have similar structure.

Etymology: The species group name *lyra* (from the Latin *lyra*; lyre, a stringed musical instrument) refers to the apex of long spine scale looking like lyre.

Type locality: Bottom sediment from the Pismenka River, Kidryasovo settlement, the South Urals, South-East of European part of Russia, 51°15'41N, 57°28'42E. Collected 11.05.2014.

Hapantotype: Preparation #37 (Fig. 1) has been deposited in the Laboratory of Microbiology, Institute for

Table 1. Morphometry of *Acanthocystis lyra* sp. nov. and *Acanthocystis siemensmae* sp. nov. Abbreviations: Min – minimum, Max – maximum, Mean – arithmetic mean, SE – standard error of the mean, n (i) – number of individuals, n – number of measurements.

| Species | Parameter | Min (µm) | Max (µm) | Mean unweighted±SE (µm) | n (i) | n |
|--|----------------------------|----------|----------|-------------------------|-------|-----|
| <i>Acanthocystis lyra</i> sp. nov. | Cell diameter | 21.0 | 30.0 | 26.0±3.9 | 4 | 4 |
| | Long spine scales | | | | | |
| | Length | 5.1 | 14.0 | 7.8±1.71 | 4 | 42 |
| | Length of branches | 0.47 | 1.42 | 0.84±0.2 | 4 | 70 |
| | Diameter of basal plates | 0.6 | 1.5 | 0.83±0.16 | 4 | 38 |
| | Short spine scales | | | | | |
| | Length | 1.9 | 3.7 | 2.7±0.47 | 4 | 65 |
| | Length of primary branches | 0.5 | 3.1 | 0.97±0.3 | 4 | 107 |
| | Diameter of basal plates | 0.6 | 1.2 | 0.85±0.14 | 4 | 69 |
| | Plate scales | | | | | |
| | Length | 2.4 | 4.1 | 2.9±0.32 | 4 | 26 |
| | Width | 1.4 | 2.5 | 1.8±0.3 | 4 | 30 |
| <i>Acanthocystis siemensmae</i> sp. nov. | Cell diameter | 17.0 | 21.0 | 19.0±2.0 | 3 | 3 |
| | Long spine scales | | | | | |
| | Length | 6.5 | 10.8 | 8.3±1.26 | 3 | 28 |
| | Diameter of basal plates | 0.9 | 1.2 | 1.02±0.08 | 3 | 18 |
| | Short spine scales | | | | | |
| | Length | 1.65 | 2.9 | 2.2±0.26 | 3 | 76 |
| | Apex diameter | 1.2 | 1.9 | 1.55±0.18 | 3 | 64 |
| | Diameter of basal plates | 0.6 | 1.0 | 0.83±0.09 | 3 | 80 |
| | Plate scales | | | | | |
| | Length | 2.5 | 4.4 | 3.17±0.38 | 3 | 45 |
| | Width | 1.2 | 2.7 | 1.96±0.27 | 3 | 36 |

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***Acanthocystis siemensmae* sp. nov. (Figs 2, 3 D–F)**

Diagnosis: Cells are 17.0–21.0 µm (mean 19.0) in diameter. A cell coat consists of plate scales of a single type and spine scales of two types. The long spine scales have narrow, hollow shafts 6.5–10.8 µm long. The shaft is slightly branching towards the apex and has 14–16 teeth. Extended part of the apex contains four symmetrically oriented longitudinal ridges; each ridge is ended by four teeth of different length. Two central teeth of the ridge usually are longer than lateral ones. Basal plates of the long spine scales are circular, 0.9–1.2 µm in diameter. The short spine scales are 1.6–2.9 µm long with

narrow shafts and expanded cup-shaped apices up to 1.9 µm in diameter. The apex looks like a maple leaf due to division onto 3–5 ridges of irregular shape. Each ridge has a few teeth of different length. Basal plates of the short spine scales are circular, 0.6–1.0 µm in diameter. The plate scales are plane, patternless, elliptical or pear-shaped with a thin marginal rim. Length of the plate scales is 2.5–4.4 µm, width 1.2–2.7 µm.

Comparisons with similar species: *A. siemensmae* is slightly similar to *A. pectinata* Penard, 1889, *A. polymorpha* Dürschmidt, 1985 and *A. nichollsi* Siemensma et Roijackers, 1988, in structure of the cup-shaped apex of the short spine scales. The short spine scales of *A. pectinata* have 4–6 short teeth; the long spine scales have 2–3 teeth. In contrast, *A. siemensmae* has the short spine scales with 3–5 ridges; each of them is ended by a few teeth. Its long scales possess 4 ridges and 14–16

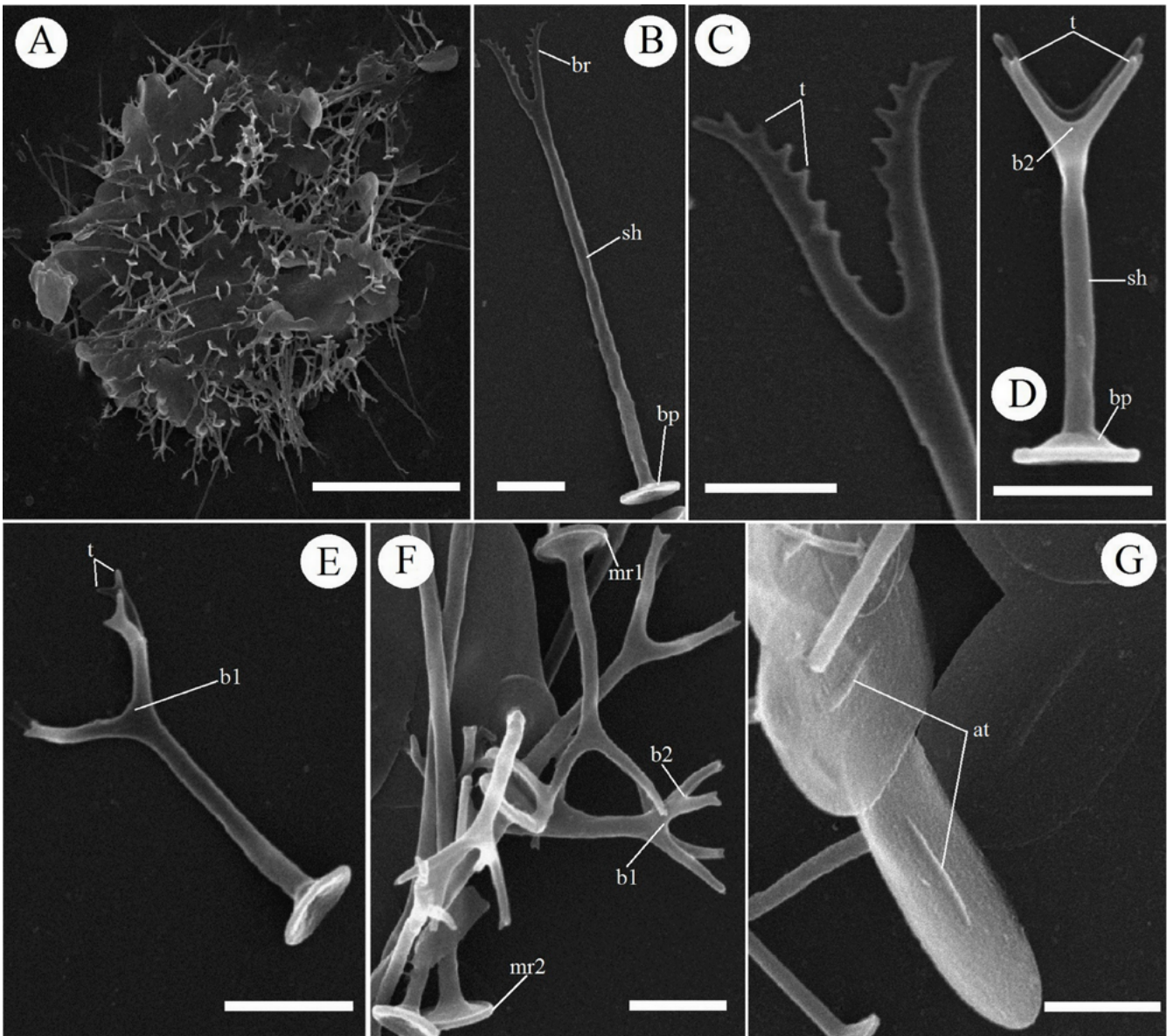


Fig. 1. SEM of *Acanthocystis lyra* sp. nov. **A** – General view of the scales. **B** – Long spine scale. **C** – Lyrate distal end of long spine scale with teeth on inner edge. **D** – Short spine scale with secondary bifurcation. **E** – Short spine scale with primary bifurcation and teeth. **F** – Short spine scales with primary and secondary bifurcations. A marginal rim is seen on basal plates of short and long spine scales. **G** – Plate scales ornamented with an axial thickening. Abbreviations: at – axial thickening; b1 – primary bifurcation; b2 – secondary bifurcation; bp – basal plate; br – branch of furca; mr1 – marginal rim on basal plate of short spine scale; mr2 – marginal rim on basal plate of long spine scale; sh – shaft; t – teeth. Scale bars: A – 10 μm ; B, D, E, F, G – 1 μm ; C – 0.5 μm .

teeth. In addition, plate scales of *A. pectinata* are ornamented by granules, whereas those of *A. siemensmae* are patternless.

The short spine scales of *A. polymorpha* possess 5–8 ridges ended with one tooth, while *A. siemensmae* has the short spine scales with 3–5 ridges, each of them with a few teeth. The long spine scales of *A. polymorpha*

have slightly dilated distal ends with teeth. In contrast, the distal ends of the long spine scales of *A. siemensmae* reveal four ridges with terminal teeth.

Short spine scales of *A. nichollsi* have a flared terminus consisting of six spine-like ribs joined by a membrane with a scalloped margin, whereas the short spine scales of *A. siemensmae* are ended by 3–5 ridges with

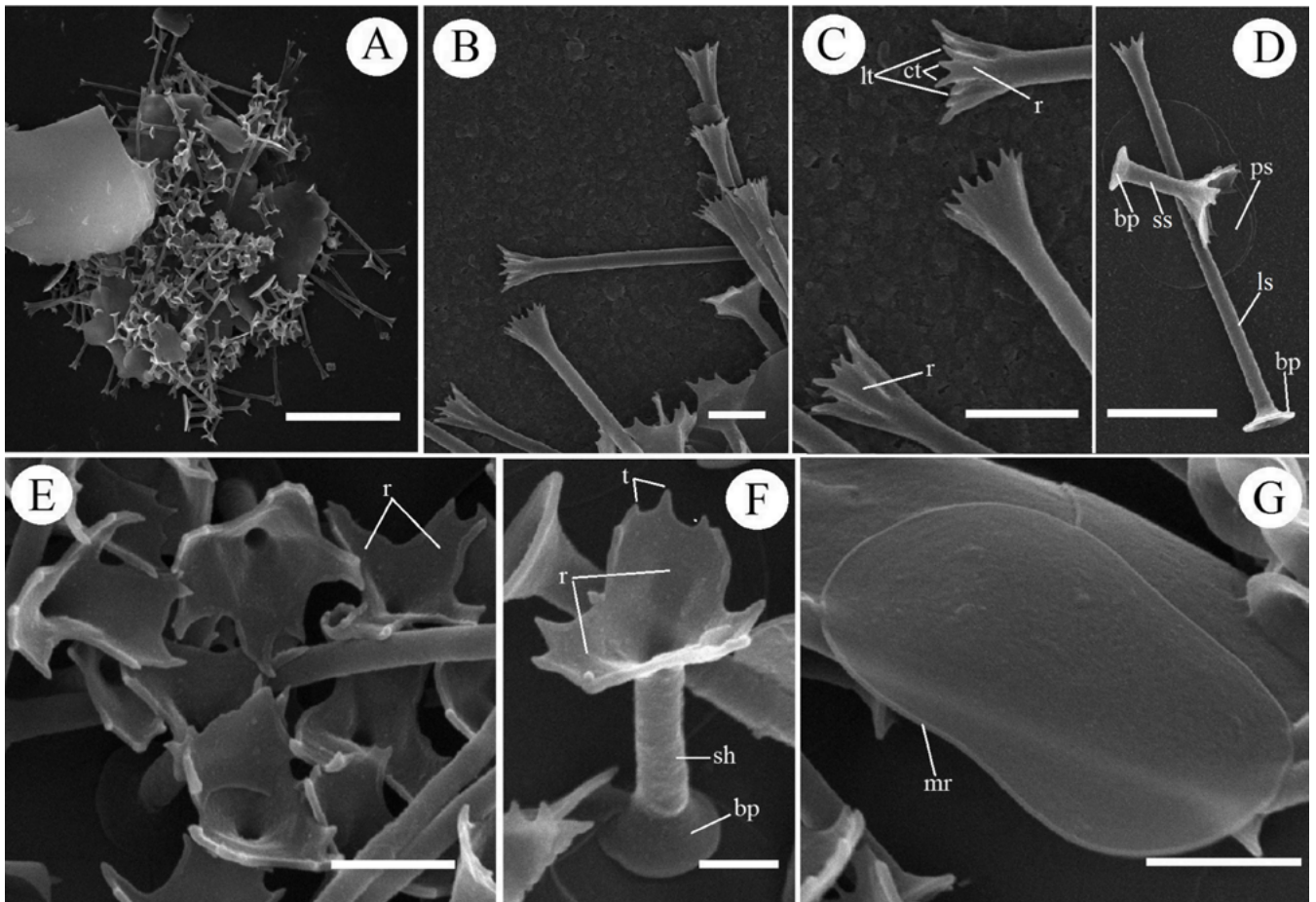


Fig. 2. SEM of *Acanthocystis siemensmae* sp. nov. **A** – General view of the scales. **B** – Long spine scales. **C** – Apices of long spine scales with ridges. **D** – Plate scale, long and short spine scale with basal plate. **E** – Apices of short spine scales with ridges. **F** – Ridges with teeth at the apex of short spine scale. **G** – Plate scale with marginal rim. Abbreviations: bp – basal plate; ct – central tooth; ls – long spine scale; lt – lateral tooth; mr – marginal rim; ps – plate scale; r – ridge; sh – shaft; ss – short spine scale; t – teeth. Scale bars: A – 10 μm ; B, C, E, G – 1 μm ; D – 2 μm , F – 0.5 μm .

a few apical teeth. The long spine scales of *A. siemensmae* have 4 ridges with teeth, while ones of *A. polymorpha* have slightly dilated distal ends with teeth. The plate scales of *A. nichollsi* are elliptical and ornamented with a medial thickening, radial ribs and a marginal rim, whereas the only ornamentation on the plate scales of *A. siemensmae* is the marginal rim.

Etymology: The species group name *siemensmae* is proposed in honor of an authority on several groups of amoeboid and heliozoan protists Ferry Siemensma.

Type locality: Bottom sediment from the Pismenka River, Kidryasovo settlement, the South Urals, South-East of European part of Russia, 51°15'41N, 57°28'42E. Collected 11.05.2014.

Hapantotype: Preparation #50 (Fig. 2) has been deposited in the Laboratory of Microbiology, Institute for Biology of Inland Waters of Russian Academy of Sciences (Borok, Russia).

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DISCUSSION

Two new freshwater species of centrohelid heliozoans, *Acanthocystis lyra* n. sp. and *Acanthocystis siemensmae* n. sp., have been described in this paper based on the unique structure of the scales covering the cells as

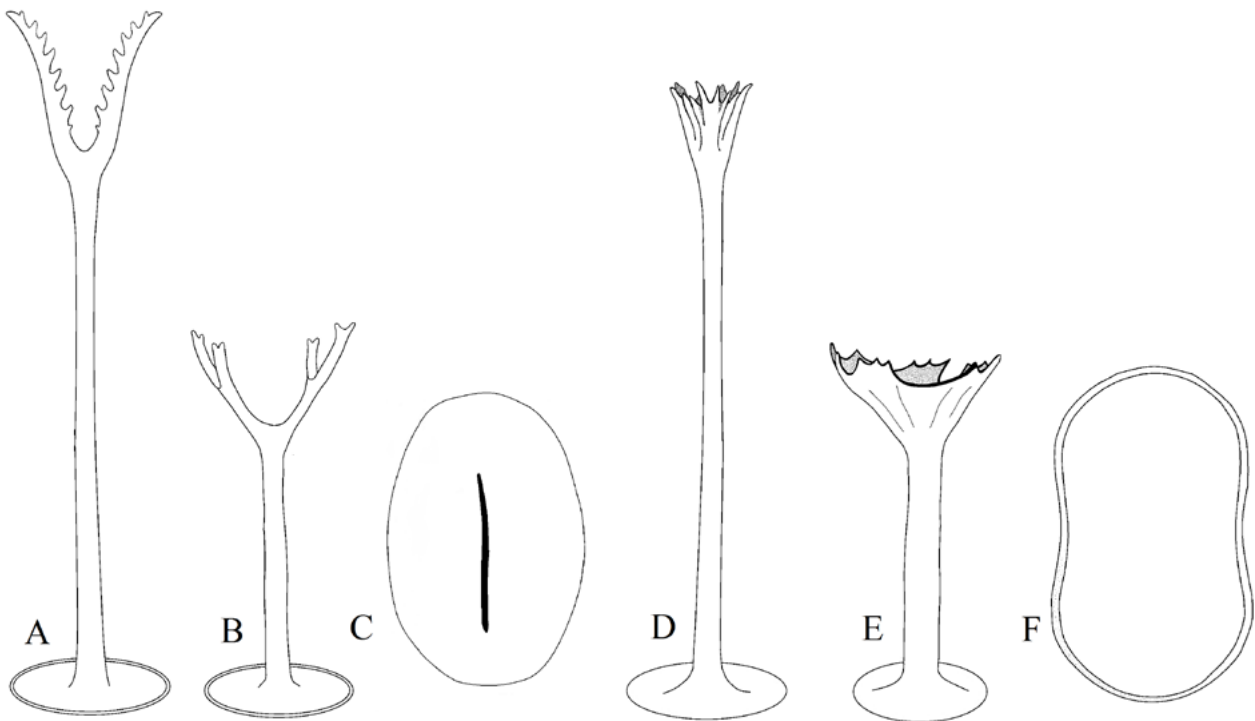


Fig. 3. Line drawings of scales of *Acanthocystis lyra* sp. nov. (A–C) and *Acanthocystis siemensmae* sp. nov. (D–F): A, D – long spine scales; B, E – short spine scales; C, F – plate scales.

revealed by SEM. At present, morphological characteristics of scales are applied as the main criteria for species identification of centrohelid heliozoa (Cavalier-Smith and Heyden 2007; Mikrjukov 2002; Zlatogursky 2010, 2014). Based on morphological characteristics only, a centrohelid classification system composed of 3 families and 15 genera was proposed by Mikrjukov (2002).

The genus *Acanthocystis* was divided into four morphological groups based on the structure of the scale apex (Mikrjukov 1997): *A. wiasemskii* (pointed or obtuse apex), *A. penardi* (truncated apex with a few small marginal teeth), *A. pectinata* (apex with three or more branches) and *A. turfacea* (dichotomously branched apex). *A. lyra* has dichotomously branched spine scales as well as secondary bifurcation in the short spine scales. Based on these morphological features *A. lyra* has been placed in the *A. turfacea* group. Spine scales of *A. siemensmae* in their morphology are similar to *A. pectinata*, *A. polymorpha* and *A. nichollsi*, included in the *A. pectinata* group (Mikrjukov 1997). This fact seems sufficient to put *A. siemensmae* into the *A. pectinata* group.

Because the scales are poorly visible by light microscopy, the most exact method for identification of centrohelids is SEM. SEM is a traditional method for examining the ultrastructure of centrohelidian scales. This method provides high quality images of spine and plate scales and so it is applied for taxonomic descriptions of centrohelidian species. Lately, a number of new species of the centrohelid heliozoa from different habitats was described using SEM: *Acanthocystis antonkomolovi*, *A. mikrjukovi*, *A. mylnikovi*, *A. olgashelestae*, *A. elenazhivotovae* (Leonov 2010); *Choanocystis antarctica* (Tikhonenkov and Mylnikov 2010); *A. crescenta*, *A. kirilli*, *C. minima* (Zlatogursky 2010); *Raphidiophrys heterophryoidea* (Zlatogursky 2012); *A. dentata* (Leonov and Mylnikov 2012). Atomic force microscopy (AFM) was shown to be an alternative to SEM in some circumstances with the morphology of *Heterophrys marina*, *Polyplacocystis ambigua*, *Raineriophrys erinaceoides* having been described successfully by Plotnikov *et al.* 2013. Exact morphometric data, profiles of spine and plate scales, and their three-dimensional images were obtained using AFM. How-

ever, as compared to SEM, AFM was shown to have some disadvantages, such as longer scanning time and low-contrast images (Plotnikov *et al.* 2013).

Along with the morphological criteria, sequences of the 18S rRNA gene have been applied to heliozoan phylogenetics and systematics (Sakaguchi *et al.* 2005; Cavalier-Smith and Heyden 2007; Cavalier-Smith and Chao 2012; Zlatogursky 2014, 2016). The most recent studies, based on phylogenomic analysis, strongly confirm phylogenetic position of Centrohelea as a sister group to Haptophytina (Cavalier-Smith *et al.* 2015, Burki *et al.* 2016).

Based on the latest gene sequencing data (Cavalier-Smith and Heyden 2007; Cavalier-Smith and Chao 2012; Cavalier-Smith *et al.* 2015; Zlatogursky 2014, 2016), it is apparent that the morphological system of classification proposed by Mikryukov (2002) underrepresents the actual phylogeny of the group. Perhaps the greatest problem standing in the way of a true system of centrohelid classification is an apparent lack of morphological data on the full range of biodiversity existing in nature (Cavalier-Smith and Heyden 2007, Zlatogursky 2014). So, every description of new centrohelidian species gives valuable information, leading to a resolution of the classification problem.

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