

Centrohelid heliozoans (Haptista: Centroplasthelida) from mires in the North Caucasus, Russia

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SUMMARY

At present, little is known about the diversity and functioning of microbial communities in mire ecosystems. Among protists, centrohelid heliozoans remain the least studied components of mire microbial communities. The aim of the present work was to determine the species diversity of centrohelid heliozoans at five mountain mires (peatlands) in North Ossetia and Kabardino-Balkaria (North Caucasus, Russia) for the first time. The morphology of cell coverings (siliceous scales) was observed using scanning and transmission electron microscopy for species identification. Thirteen species from four families and six genera were recorded. One new species (*Pterocystis anglocollegarum* sp. n.) was described. *Acanthocystis nigeriensis* was found for the first time since its original description. Four species were new records for Russia: *Acanthocystis nigeriensis*, *Pseudoraphidiophrys veliformis*, *Pterocystis anapoda* and *Pterocystis anglocollegarum* sp. n. The most common species were *Acanthocystis nigeriensis*, *A. trifurca*, *A. turfacea*, and *Pterocystis pinnata*. Above-water microtopographical levels (hummock and carpet) were characterised by significantly higher species richness than mire water bodies (in-mire lakes, hollow-pools, and inter-hummock spaces). Among the microhabitat types studied, moss extracts were characterised by the highest species richness. There was great similarity between the communities of bottom sediments and water surface. Our results also showed that water temperature and pH significantly influenced the species richness of the studied biotopes.

KEY WORDS: Kabardino-Balkaria, North Ossetia, protists, *Pterocystis anglocollegarum* sp. n., *Sphagnum*

INTRODUCTION

Little is known about the functioning of microbial cenoses in mire ecosystems at present. However, the microbial component of mire waters accounts for about 50 % of the total metabolism of the mire ecosystem (Gilbert *et al.* 1998). Centrohelid heliozoans play an important role in the aquatic food web since they feed on small protists and bacteria and thereby are able to control their numbers (Siemensma 1991, Arndt 1993, Stensdotter-Blomberg 1998). Despite this important role, centrohelids remain the least studied components of aquatic ecosystems, including mire waters.

Heliozoans form a polyphyletic group of protists with a spherical cell body, radially diverging axopodia, and an external cell covering (siliceous or organic scales and, less often, organic capsules). Among these organisms, the largest monophyletic taxon is the centrohelid heliozoans (Centroplasthelida Febvre-Chevalier & Febvre, 1984), related to the Haptista (Adl *et al.* 2019). Centrohelids are distinguished by the presence of siliceous or (rarely) organic scales, with the

exception of one genus, *Oxnerella*, which lacks them. The morphology of centrohelids began to be actively studied with the spread of electron microscopy (Nicholls 1983, Dürschmidt 1985, 1987a, 1987b; Croome 1986, 1987; Siemensma & Roijackers 1988; Mikrjukov 1993a, 1993b, 1999, 2001, etc.) and continues to this day. Only about 100 morphospecies of centrohelid heliozoans are known, and it is assumed that only 10 % of centrohelid heliozoan diversity has been studied thus far (Cavalier-Smith & von der Heyden 2007).

The diversity of the centrohelids has been studied unevenly, with more intensive exploration in some regions than in others. The species composition of centrohelids in mires has been poorly investigated. In European Russia, mires were studied only in the Northern region (Arkhangelsk, Vologda and Yaroslavl Regions and the Republic of Karelia, 27 observed species) (Leonov & Plotnikov 2009, Leonov 2010a, 2010b, Leonov & Mylnikov 2012, Prokina *et al.* 2017c) and in the Central region (Voronezh Region, 18 observed species) (Prokina *et al.* 2018). To the best of our knowledge, centrohelids from mountain mires, including the Caucasus, are

completely undescribed. Most of the information on diversity of centrohelic heliozoans in mires lacks detailed description of the mire type, the vegetation associated with the biotope, the type of bottom sediments, and the hydrochemical parameters. These factors are extremely important for understanding the distribution patterns of microbial eukaryotes.

The aim of this work was to identify the species composition and morphology of the centrohelic heliozoans from five mires of the North Caucasus, and to analyse their biotopic distribution.

METHODS

Study area

The study area is located in the central part of the northern slope of the Main Caucasus Range. Five mountain mires (peatlands) were studied (Figure 1): Kubus and Chifandzar (Irafsky District of North Ossetia), Ushtulu, Konskoe and Zayachye (Cherekskiy District of Kabardino-Balkaria).

Kubus Mire (Figure 1a) is located on Kubus Mountain. It is a small oligotrophic *Sphagnum* mire with an even or slightly hummocky structure. *Sphagnum* and *Carex-Sphagnum* communities predominate in the vegetation cover. There are several *Carex-Sphagnum* hollows and a small (~2 m²) hollow-pool. The mire is intensively frequented by tourists.

Chifandzar Mire (Figure 1b) is located in the upper reaches of the Kharesidon River in Digorskoe Gorge (2290 m a.s.l.). This large mire is located on a flat raised terrace on the left bank of the river. The mire has hummocky microtopography and can be categorised as belonging among the eutrophic *Carex* and *Carex-Sphagnum* mires. The vegetation is dominated by *Carex* species, while *Sphagnum* species are few and scarce. The hydrographic network is represented by inter-hummock spaces, small *Sphagnum* hollows, laggs turning into mire streams, as well as passing streams which partly disappear into peat deposits. Slopes surrounding the mire and part of the mire itself are used for cattle grazing. Chifandzar and Kubus Mires are located within the national park “Alania”.

Ushtulu Mire (Figure 1c, 1d) is located within the Kabardino-Balkaria State High-Mountain Reserve, in the upper reaches of the Balkarskiy Cherek River (1995 m a.s.l.). It is a eutrophic *Carex* and partially *Carex-Sphagnum* slope mire with springs of mineral (“Narzan”) groundwater. The vegetation is dominated by *Carex rostrata* Stokes. Mire water bodies are presented by grass, grass-Hypnales, grass-*Sphagnum* inter-hummock spaces and a series of

secondary hollow-pools (0.5–2.0 m in depth, 10–100 m² in area).

Konskoe Mire (Figure 1e) and Zayachye Mire (Figure 1f) are located near the village of Verkhnyaya Balkaria in the inter-ridge space of the Northern (Jurassic) Depression (1776 and 1810 m a.s.l.). The names of these mires are not official since their area does not exceed 0.0025 km² and local people call them simply “lakes” or “mires”. The mires are of limnogenous origin (Busch 1932). In the middle of each of them, there is a residual flowing in-mire lake surrounded by *Carex*, *Carex-Sphagnum* and *Sphagnum* communities. The mires can be characterised as oligotrophic, partially meso-oligotrophic *Sphagnum* mires. On the mires there are traces of burning, which are indicated by *Molinia coerulea* (L.) Moench. Both mires are recommended for protection (Prokin *et al.* 2019).

Additional information on mires of the studied area are presented in the following articles: Busch (1932), Knyasev *et al.* (1992), Doroshina & Nikolajev (2018), Doroshina & Yakimov (2019), Prokin *et al.* (2019), including data on protists (testate amoebae and heterotrophic flagellates) (Tarnogradsky 1959, Prokina & Philippov 2017).

Field methods and data collection

Field survey was carried out in June and September 2018 according to a standard technique (Philippov *et al.* 2017). For each sample plot, geobotanical relevés, photo and video recordings were made, and hydrological measurements (groundwater level, water temperature (t, °C), pH and total dissolved solids (TDS, ppm)) were made using portable HI 98201 and Combo HI 98129 devices (Hanna Instruments). Sixteen moss samples were taken from different microhabitats (Table 1). These were wrung out by hand and the resulting extracts (‘moss extracts’ hereafter) were placed into 15 ml plastic tubes and transported to the laboratory at 4 °C along with other samples from the bottom sediments and water surface.

Laboratory methods

In the laboratory, samples were enriched with a suspension of *Pseudomonas fluorescens* Migula, 1895 bacteria and *Bodo saltans* Ehrenberg, 1838 flagellate cell culture, and placed in Petri dishes. Samples were kept at 22 °C in the dark and observed for ten days to establish the cryptic species diversity (Vørs 1992). An AxioScope A1 light microscope (Carl Zeiss, Germany), with DIC and phase contrast and water immersion objectives (total magnification ×1120), was used for observations of living cells. Specimens for electron microscopy were prepared

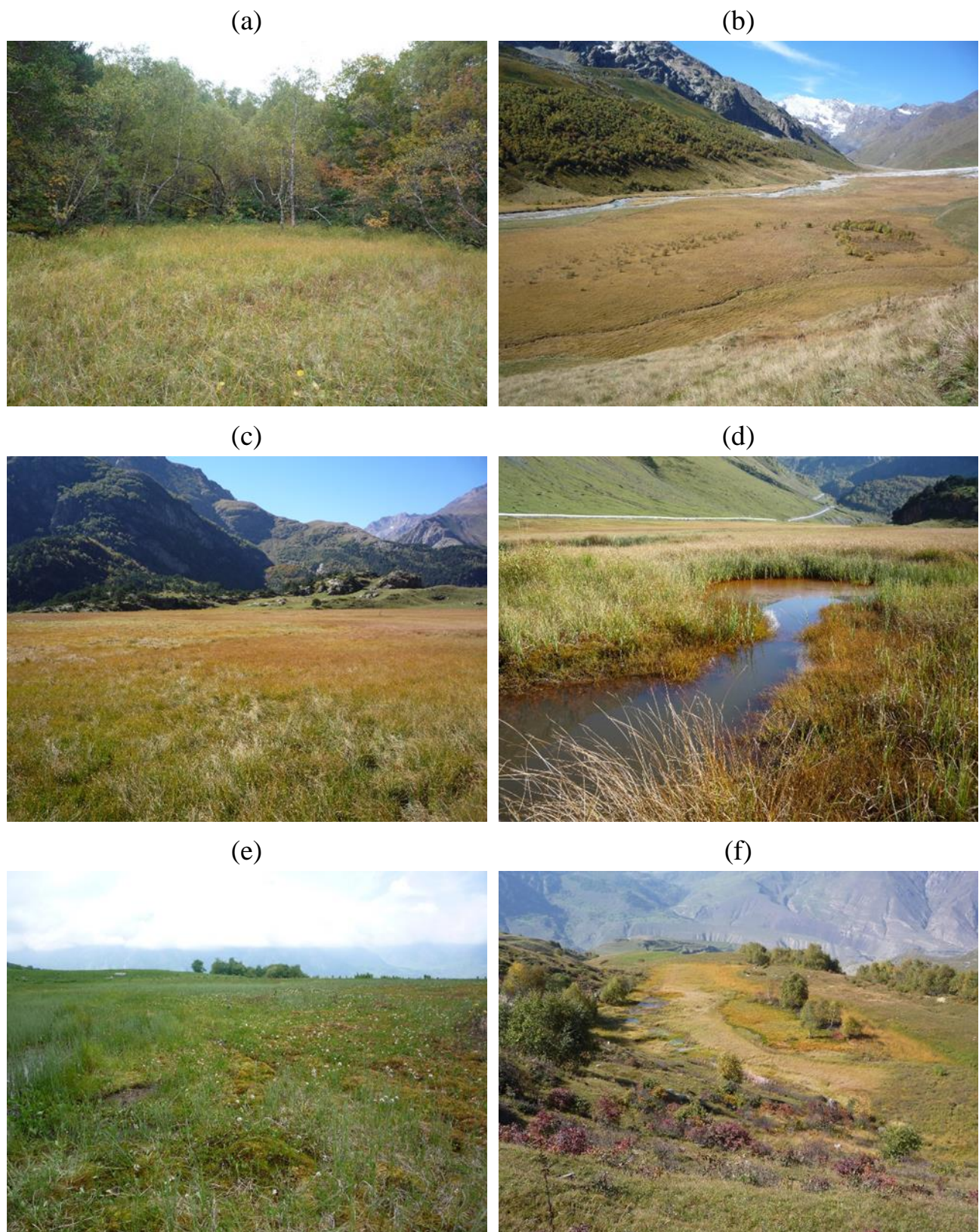


Figure 1. Mires of the North Caucasus: (a) Kubus; (b) Chifandzar; (c) and (d) Ushtulu; (e) Konskoe; (f) Zayachye. Photos: D.A. Philippov.

Table 1. Characteristics of the studied mires.

Name of the mire	Biotope	Coordinates	Water level (cm)	pH	T (°C)	TDS (ppm)	No. samples	Microhabitat	Date
Kubus	Hollow - pool	42°53'35" N 43°34'42" E	+25 to +40	6.1	18	7	1	Bottom sediments (resuspension)	15 Sep 2018
							2	Water surface	
Chifandzar	<i>Carex rostrata</i> - Hypnales inter-hummock space	42°55'12" N 43°30'55" E	-2 to +2	5.7	13	6	3	Bottom sediments (resuspension)	03 Jun 2018
			-2 to +2	6.7	12	8	4	<i>Campyllum stellatum</i> (moss extract)	18 Sep 2018
	5	Water surface							
	<i>Nardus strictum</i> - <i>Carex</i> spp. - <i>Sphagnum magellanicum</i> hummock	42°55'08" N 43°30'48" E	-15 to -20	4.9	15	20	6	<i>Sphagnum magellanicum</i> (moss extract)	03 Jun 2018
<i>Carex</i> spp. - <i>Nardus strictum</i> - <i>Sphagnum teres</i> hummock	42°55'08" N 43°30'51" E	-15 to -17	6.7	14	45	7	<i>Sphagnum teres</i> (moss extract)	03 Jun 2018	
Ushtulu	<i>Carex rostrata</i> - <i>Sphagnum squarrosum</i> hummock	42°58'29" N 43°19'57" E	-7 to -12	4.5	20	35	8	<i>Sphagnum squarrosum</i> (moss extract)	21 Sep 2018
	<i>Carex rostrata</i> - <i>Sphagnum teres</i> hummock	42°58'29" N 43°19'57" E	-6 to -7	5.6	20	40	9	<i>Sphagnum teres</i> (moss extract)	09 Jun 2018
	<i>Carex rostrata</i> inter-hummock space	42°58'26" N 43°20'03" E	+5 to +10	5.6	19	50	10	Water surface	09 Jun 2018
	<i>Carex rostrata</i> - <i>Utricularia intermedia</i> inter-hummock space	42°58'26" N 43°20'03" E	+2 to +5	6.7	16	35	11	Bottom sediments (resuspension)	21 Sep 2018
	Hypnales carpet around a mire pool	42°58'27" N 43°20'03" E	-1 to +2	6.4	17	70	12	<i>Hamatocaulis vernicosus</i> (moss extract)	21 Sep 2018
	<i>Carex irrigua</i> - <i>Calliergon richardsonii</i> carpet around a mire pool	42°58'26" N 43°20'03" E	-1 to -2	5.5	18	65	13	<i>Calliergon richardsonii</i> (moss extract)	09 Jun 2018
	<i>Carex irrigua</i> - <i>Hamatocaulis vernicosus</i> carpet around a mire pool	42°58'26" N 43°20'03" E	-1 to -2	5.6	19	60	14	<i>Hamatocaulis vernicosus</i> (moss extract)	09 Jun 2018
Konskoe	<i>Carex</i> spp. - <i>Sphagnum flexuosum</i> + <i>S. obtusum</i> carpet around an in-mire lake	43°06'03" N 43°29'02" E	-2 to +1	6.0	20	25	15	<i>Sphagnum flexuosum</i> (moss extract)	06 Jun 2018
Zayachye	In-mire lake	43°05'53" N 43°28'42" E	+30 to +80	5.2	18	40	16	Water surface	22 Sep 2018

according to previously described methods (Moestrup & Thomsen 1980, Mikrjukov 2002) and observed on a JEM-1011 (JEOL, Japan) transmission electron microscope (TEM) and a JSM-6510 LV (JEOL, Japan) scanning electron microscope (SEM). Heliozoans were picked from the samples cell by cell by micropipette for SEM preparations. Concentrated samples were used for TEM preparations. A dendrogram showing the similarity of the water bodies by species composition was drawn on the basis of the Bray-Curtis similarity index using the single linkage algorithm in the PAST software package (Hammer *et al.* 2001). The current centrohelid taxonomic system (Adl *et al.* 2019) is used.

RESULTS

Thirteen species of centrohelid heliozoans from four families (Acanthocystidae, Pterocystidae, Raphidocystidae and Choanocystidae) and six genera (*Acanthocystis*, *Choanocystis*, *Pterocystis*, *Raineriophrys* and *Raphidocystis*) were observed. Data on the distribution of centrohelid species amongst water bodies and habitat types are presented in Table 2; morphometric characteristics of the observed species and number of observed cells per sample site are provided in the Appendix. The

morphological characteristics of investigated siliceous scales having an important diagnostic value are listed below.

DIAPHORETICKES Adl *et al.*, 2012

*Haptista Cavalier-Smith, 2003

**Centroplasthelida Febvre-Chevalier & Febvre, 1984

***Panacanthocystida Shishkin & Zlatogursky, 2018

****Acanthocystidae Claus, 1874

Acanthocystis nigeriensis Wujek *et al.*, 2007

(Figure 2a–2c)

Description: Spine scales consist of hollow cylindrical shaft and a circular flattened base. Shaft is 3.20–8.68 μm in length, 0.15–0.23 μm in diameter, base is 0.58–0.86 μm in diameter. Tip of shaft is 0.16–0.25 μm in diameter, divided on 2–3 pointed furcae (0.11–0.22 μm in length). Triangular plate scales are 2.23–3.33 \times 1.75–2.62 μm , with dense margins and a short axial ridge (0.27–0.52 μm in length).

Remarks: This species has been previously noted only once, from freshwater habitats in Nigeria (Wujek *et al.* 2007). The morphology of observed scales corresponds with the original descriptions, except the length of spine scales in type material is shorter (1.5–3.0 μm).

Table 2. Species diversity of centrohelid heliozoans in the studied mire biotopes. Samples numbers correspond with Table 1.

Centrohelid species	Sample number															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
<i>Acanthocystis nigeriensis</i>	–	+	+	–	–	–	–	–	–	–	–	–	–	–	+	+
<i>A. penardi</i>	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–
<i>A. trifurca</i>	–	–	–	–	–	–	+	+	–	–	–	–	+	+	–	–
<i>A. turfacea</i>	+	+	–	–	+	–	–	–	+	–	–	–	–	–	–	–
<i>Raphidocystis symmetrica</i>	–	–	–	–	–	–	–	–	–	+	+	+	–	–	–	–
<i>Choanocystis aculeata</i>	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
<i>Pseudoraphidiophrys veliformis</i>	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–
<i>Pterocystis anapoda</i>	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
<i>P. anglocollegarum</i> sp. n.	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–
<i>P. pinnata</i>	–	–	+	+	–	–	+	–	–	–	+	–	–	–	–	–
<i>P. tropica</i>	–	–	–	–	–	–	–	–	–	–	–	+	–	+	–	–
<i>Raineriophrys erinaceoides</i>	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–
<i>R. kilianii</i>	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–
Total number of species	1	3	2	1	1	1	2	1	2	1	2	4	2	2	1	1

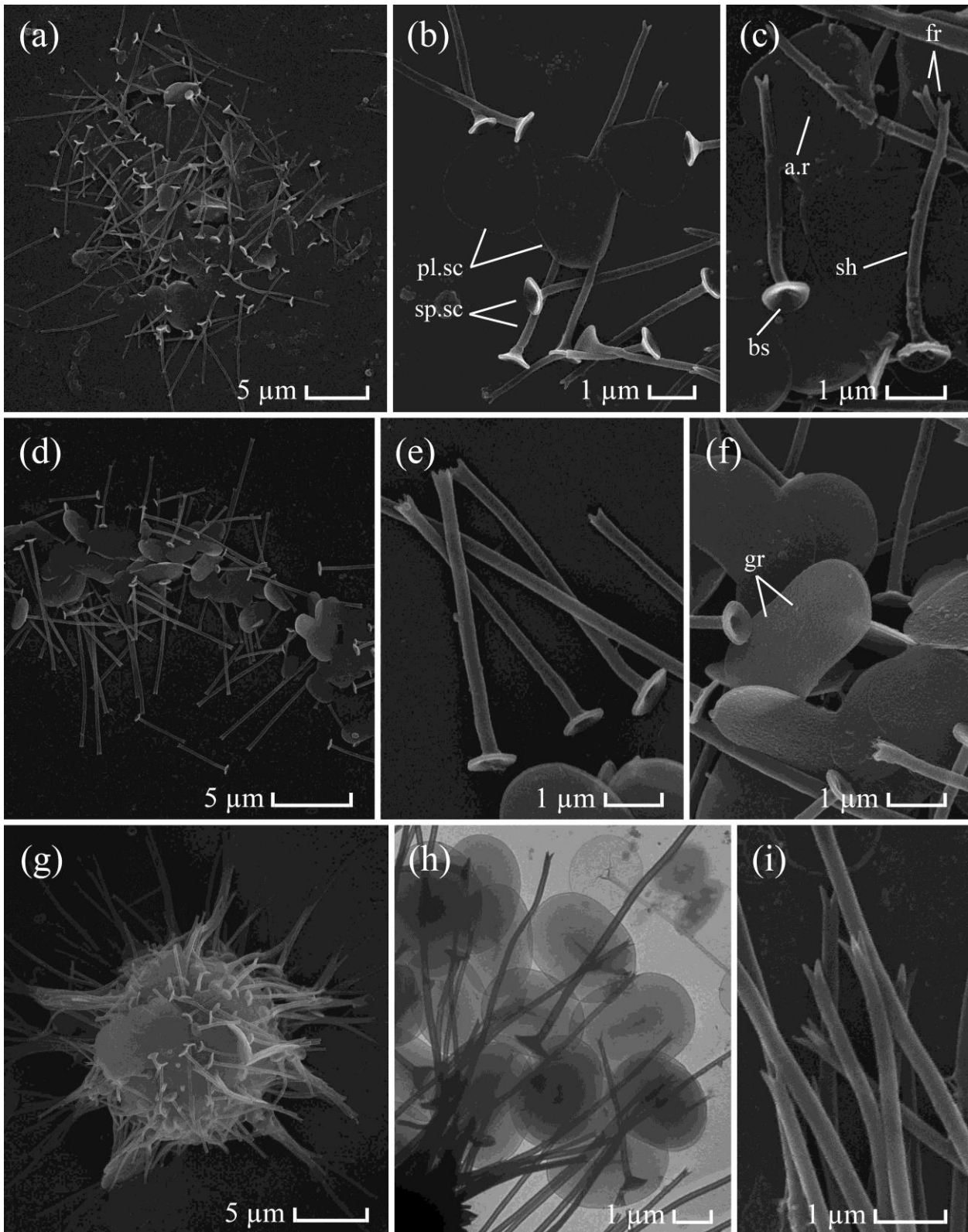


Figure 2. Morphology of observed scales of centrohelid genus *Acanthocystis*, (a)–(g) and (i) SEM, (h) TEM. (a)–(c) *A. nigeriensis*: (a) scales of a single cell; (b)–(c) spine and plate scales. (d)–(f) *A. penardi*: (d) scales of single cell; (e) spine scales; (f) plate scales. (g)–(i) *A. trifurca*: (g) scales of single cell; (h) spine and plate scales; (i) tips of spine scales. Abbreviations: a.r = axial ridge; bs = base of spine scale; fr = furcae; gr = granules; pl.sc = plate scales; sh = shaft of spine scale; sp.sc = spine scales.

Acanthocystis penardi Wailes, 1925 [syn.: *Acanthocystis heterospina* Nicholls, 1983; *A. dresscheri* Siemensma & Roijackers, 1988] (Figure 2d–2f)

Description: Spine scales consist of hollow cylindrical shaft and a circular flattened base. Shaft is 3.79–14.88 µm in length, 0.27–0.37 µm in diameter, base is 0.89–1.39 µm in diameter. Shaft tips are 0.33–0.48 µm in diameter, possess 5–7 furcae (0.12–0.16 µm in length). Oval plate scales are 3.45–4.28 × 2.32–2.54 µm, with dense margins and concentrically oriented granules.

Distribution: Freshwaters of the European part of Russia (Mikrjukov 1993a, Leonov 2009, 2010a, 2012; Leonov & Plotnikov 2009, Leonov & Mylnikov 2012, Zlatogursky 2014, Prokina *et al.* 2017c, 2018); Ukraine ([as *A. dresscheri*] Gaponova 2008); Estonia (Mikrjukov 1993b); Netherlands, Sweden (Siemensma & Roijackers 1988); Mongolia (Kosolapova & Mylnikov 2015); Ethiopia (Prokina *et al.* 2017b); Canada (Nicholls 1983); Chile (Dürschmidt 1987a, Prokina & Mylnikov 2019); Malaysia (Dürschmidt 1987a).

Acanthocystis trifurca Nicholls, 1983 [syn.: *Acanthocystis myriospina* Penard, 1890 sensu Dürschmidt, 1985] (Figure 2g–2i)

Description: Spine scales consist of hollow cylindrical shaft and a circular flattened base. Shaft is 2.41–10.90 µm in length, 0.14–0.23 µm in diameter, base is 0.55–1.0 µm in diameter. Tip of shaft is 0.20–0.32 µm in diameter, divided on 3 pointed furcae (0.12–0.33 µm in length). Oval plate scales are 2.19–3.21 × 1.49–2.11 µm, with axial ridge (0.56–0.69 µm in length) and dense margins. Some plate scales possess rare thin granules.

Distribution: Freshwaters of European part of Russia (Mikrjukov 1999, Leonov & Plotnikov 2009, [as *A. myriospina*] Leonov 2010a, Plotnikov & Ermolenko 2015, Prokina *et al.* 2017c, 2018); Switzerland (Zlatogursky 2014); Ethiopia (Prokina *et al.* 2017b), Australia ([as *A. myriospina*] Croome 1986); Chile ([as *A. myriospina*] Dürschmidt 1985); Canada (Nicholls 1983). Brackish waters of European part of Russia, 2 ‰ ([as *A. myriospina*] Plotnikov & Gerasimova 2017). Black Sea, 16–18 ‰ ([as *A. myriospina*] Leonov 2010a, Prokina *et al.* 2019).

Acanthocystis turfacea Carter, 1863 (Figure 3)

Description: Spine scales of two types, consisting of hollow cylindrical shaft and a circular flattened base. Tip of shaft divided into two furcae, connected by

membrane, which is often poorly developed or almost completely reduced. Shaft is 0.29–0.46 µm in diameter. Long spine scales are 11.56–29.50 µm in length, furcae are 0.90–3.0 µm in length, distance between furca tips is 1.48–1.79 µm. Base is 0.80–2.28 µm in diameter. Short spine scales are 2.87–7.43 µm in length, furcae are 0.70–2.37 µm in length, distance between furca tips is 1.04–3.63 µm. Base is 0.80–1.44 µm in diameter. Oval or almost circular plate scales are 2.22–5.09 × 1.78–3.17 µm, with axial ridge and dense margins on some scales.

Remarks: We observed two variations of cells, “*gigas*” and “*viridis*”, as noticed by Leonov (2012) but never officially described. The first variation (“*gigas*”) (Figure 3e–3h) differs from the second (“*viridis*”) (Figure 3a–3d) by having significantly larger scales (long spine scales are 21.82–29.50 µm, short spine scales are 3.27–7.43 µm, plate scales are 2.22–5.09 µm), the presence of 2–4 small teeth on furca tips of spine scales, and the smooth surface of plate scales without axial ridge and dense margins.

Distribution: Freshwaters of the European part of Russia (Mikrjukov 1993a, Leonov & Plotnikov 2009, Leonov 2010a, Plotnikov & Ermolenko 2015, Prokina *et al.* 2017c, 2018); Sweden (Zlatogursky 2014); Japan (Takamura *et al.* 2000); Canada (Nicholls 1983); Australia (Croome 1986); Chile (Dürschmidt 1985); Baltic Sea, 5–6 ‰ (Vørs 1992); White Sea, 24 ‰ (Leonov 2010a); marine waters of Antarctica, ~35 ‰ (Croome *et al.* 1987); saline inland waters of European part of Russia, 30–42 ‰ (Plotnikov & Gerasimova 2017); Black Sea, ~18 ‰ (Prokina *et al.* 2019).

****Raphidocystidae Zlatogursky in Zlatogursky *et al.*, 2018

Raphidocystis symmetrica (Penard, 1904) Zlatogursky in Zlatogursky *et al.*, 2018 [bas.: *Raphidiophrys symmetrica* Penard, 1904; syn.: *Polyplacocystis symmetrica* (Penard, 1904) Mikrjukov, 1996] (Figure 4a–4c)

Description: Oval plate scales are 4.64–8.48 × 2.08–3.09 µm. Length to width ratio is 1.84–4.05. Hollow dense marginal rim is 0.20–0.34 µm in diameter. Inner surface with reticular structure, divided into two halves by longitudinal rib (0.08–0.12 µm in length).

Distribution: Freshwaters of the European part of Russia (Leonov 2010a, Prokina *et al.* 2017c); Sweden, Netherlands (Siemensma & Roijackers 1988); Canada (Nicholls & Dürschmidt 1985); Greenland (Ikävalko *et al.* 1996).

***Pterocystida Cavalier-Smith & von der Heyden, 2007

****Raphidista Shishkin & Zlatogursky, 2018

*****Choanocystidae Cavalier-Smith & von der Heyden, 2007

Choanocystis aculeata (Hertwig & Lesser, 1874) Siemensma & Roijackers, 1988 [syn.: *Acanthocystis aculeata* Hertwig & Lesser, 1874; *A. serrata* Nicholls, 1983] (Figure 4d–4f)

Description: Spine scales consist of hollow cylindrical shaft and a circular flattened base. Shaft is 4.50–7.70 μm in length. Proximal part of shaft is 0.45–0.61 μm in diameter; consists of round finger-shaped outgrowths (0.16–0.24 μm in length, 0.05–

0.31 μm in diameter). Distal part of shaft is 0.22–0.31 μm in diameter, with 4–7 terminal teeth. Heart-shaped base of shaft is 1.69–2.47 μm in diameter. Dumbbell-shaped plate scales are 4.39–5.68 μm in length, broadest parts are 2.59–3.11 μm in width, narrow central part is 0.89–1.70 μm in length.

Distribution: Freshwaters of the European part of Russia (Mikrjukov 1993a, 1995, 1999, Prokina *et al.* 2018); Estonia (Mikrjukov 1993b); Sweden, Netherlands (Siemensma & Roijackers 1988); India (Wujek & Saha 2006); China (Biyu 2000); Japan (Takamura *et al.* 2000); Australia (Croome 1986); USA (Wujek 2005); Canada (Nicholls 1983); Chile (Dürschmidt 1985). Brackish inland waters of the European part of Russia, 3–4 ‰ (Mikrjukov 1999).

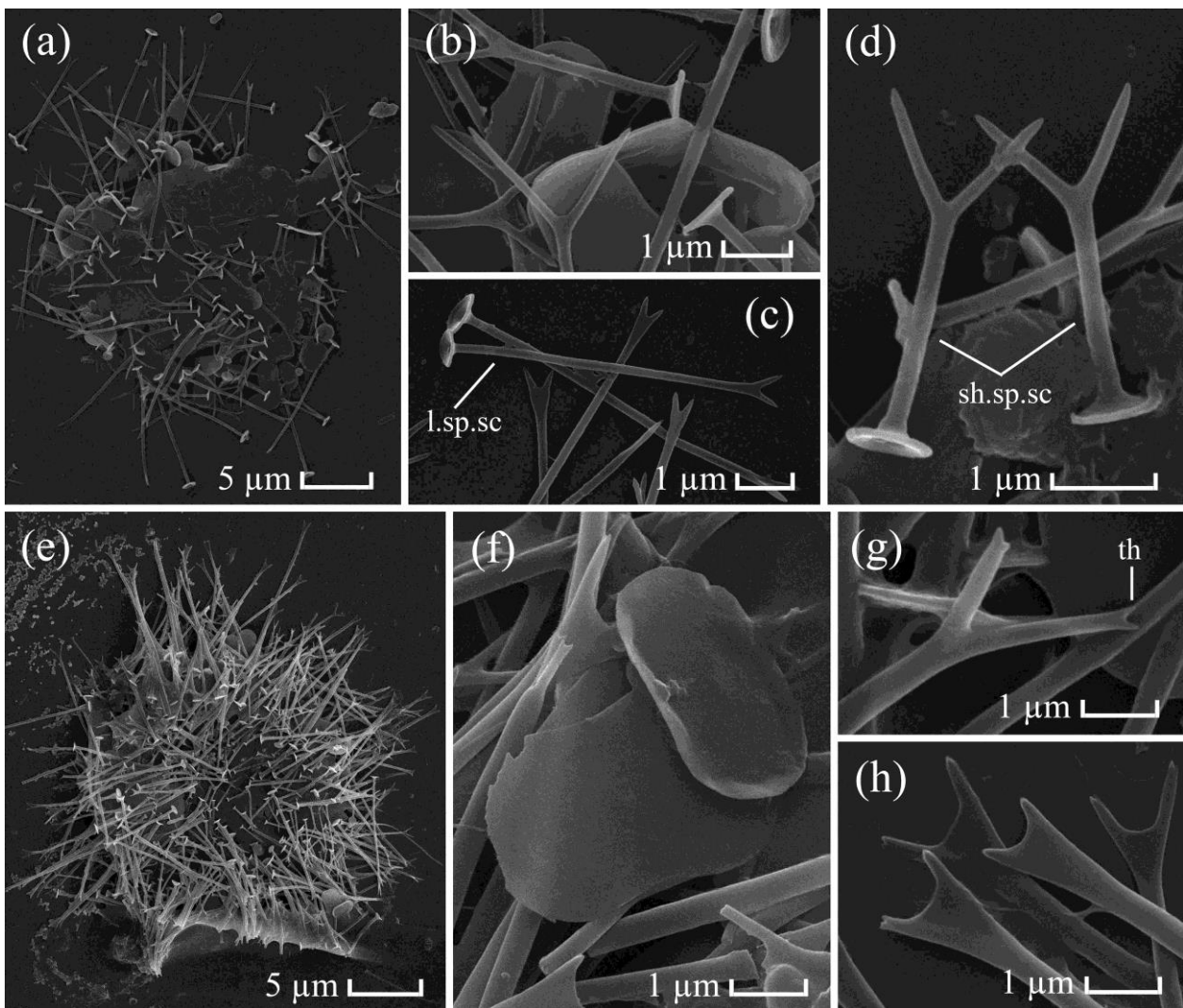


Figure 3. Morphology of observed scales of centrohelic species *Acanthocystis turfacea*, SEM. (a)–(d) *A. turfacea* “*viridis*”: (a) scales of single cell; (b) short spine scales and plate scales; (c) long spine scales; (d) short spine scales. (e)–(h) *A. turfacea* “*gigas*”: (e) scales of single cell; (f) plate scales; (g) tip of short spine scale; (h) tips of long spine scales. Abbreviations: l.sp.sc = long spine scales; sh.sp.sc = short spine scales; th = teeth.

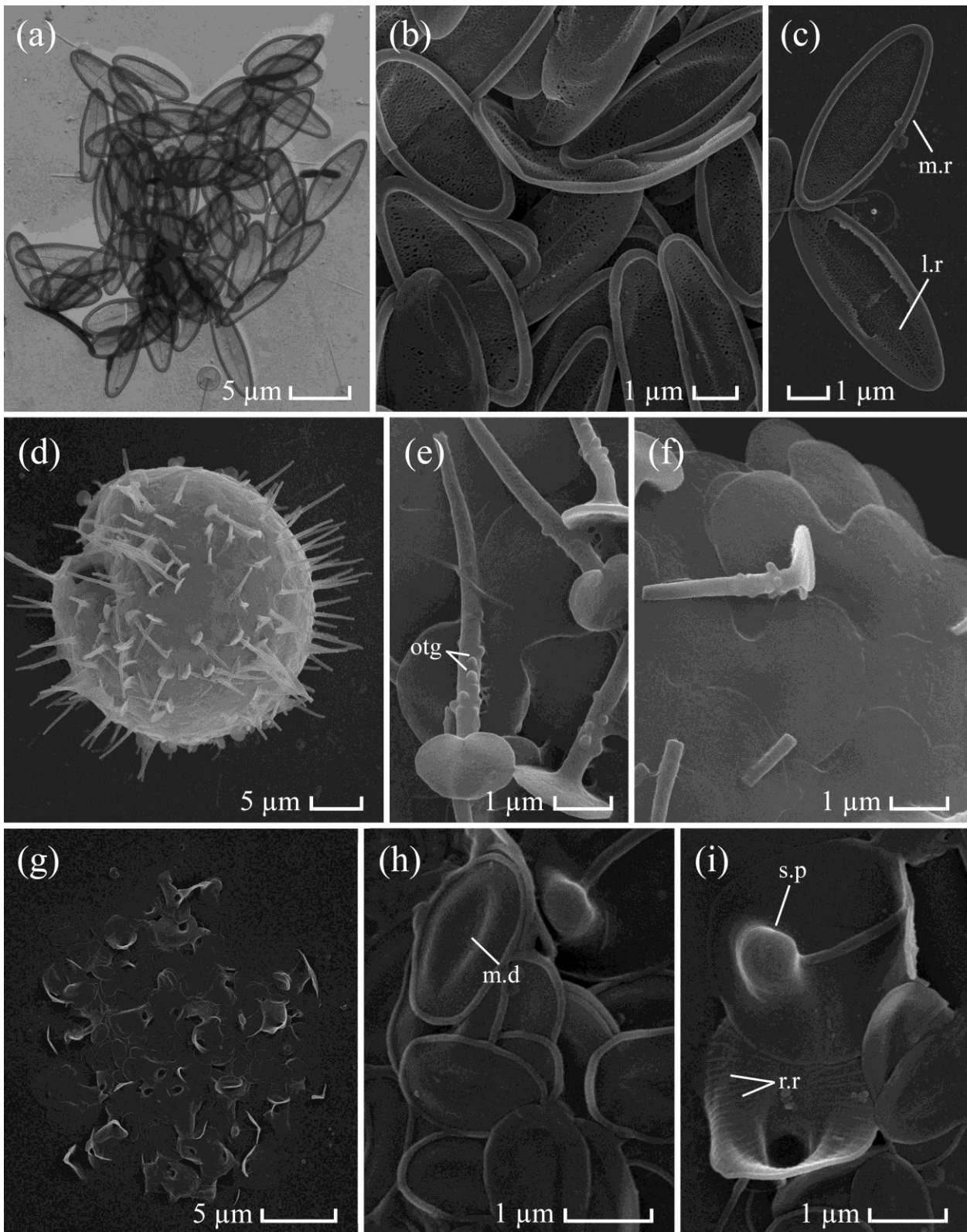


Figure 4. Morphology of observed scales of centrohelid genera *Raphidocystis*, *Choanocystis*, *Pseudoraphidiophrys*, (a) TEM, (b)–(i) SEM. (a)–(c) *R. symmetrica*: (a) scales of single cell; (b)–(c) plate scales. (d)–(f) *C. aculeata*: (d) scales of single cell; (e) spine scales; (f) spine and plate scales. (g)–(i) *P. veliformis*: (g) scales of single cell; (h) plate scales; (i) spine scales. Abbreviations: l.r = longitudinal rim; m.d = medial depression; m.r = marginal rim; otr = outgrowths; r.r = radial ribs; s.p = subapical protrusion.

****Pterista Shishkin & Zlatogursky, 2018

*****Pterocystidae Cavalier-Smith & von der Heyden, 2007

Pseudoraphidiophrys veliformis (Dürschmidt, 1985) Mikrjukov, 1997 [bas.: *Acanthocystis veliformis* Dürschmidt, 1985; syn.: *Pterocystis veliformis* (Dürschmidt, 1985) Siemensma, 1991] (Figure 4g–4i)

Description: Spine scales sail-shaped, large, almost circular in outline, 2.06–2.70 µm. Shaft is 1.59–2.07 µm in length, 0.13–0.16 µm in diameter. Basal part of shaft departs from rounded subapical protrusion 0.38–0.69 µm in diameter. From shaft to dense margins departs 56–68 radial ribs. Oval-ovoid plate scales are 1.0–2.23 × 0.50–1.40 µm, with oval medial depression (0.82–1.33 × 0.29–0.55 µm), weakly-expressed axial ridge and dense margins.

Distribution: Freshwaters of Chile (Dürschmidt 1987b, Prokina & Mylnikov 2019); USA (Wujek 2005).

Pterocystis anapoda Siemensma et Roijackers, 1988 (Figure 5a–5d)

Description: Spine scales consist of hollow cylindrical shaft and basal membrane, presented by two lateral wings. Shaft is 9.3–14.07 µm in length, 0.21–0.27 µm in diameter, with truncated apex and curved proximal part. Basal membrane is trapezium in outline, 2.81–3.37 µm in length, width is 2.32–2.66 µm on broader proximal side and 1.20–1.64 µm on narrowed distal part. Margins of wings are bent. Oval plate scales are 2.79–3.82 × 1.80–2.53 µm, with weakly-expressed medial depression, axial ridge (1.23–2.06 µm in length) and dense margins.

Remarks: In original description, Siemensma & Roijackers (1988) pointed out the presence of radial ribs on plate scales and on the bases of spine scales; however, these morphological features are not visible in the type Figures (30–33, page 206).

Distribution: Freshwaters of Netherlands (Siemensma & Roijackers 1988).

Pterocystis pinnata (Nicholls, 1983) Siemensma et Roijackers, 1988 [bas.: *Acanthocystis pinnata* Nicholls, 1983] (Figure 5e–5g)

Description: Spine scales consist of hollow cylindrical shaft and basal membrane, including lateral and basal wings. Shaft is 3.44–5.67 µm in length, 0.13–0.19 µm in diameter, basal part slightly curved, tip of shaft sharpened. Lateral wings stretched along entire length of shaft, distal part is

0.23–0.48 µm in width, proximal part is 1.40–2.22 µm in width, with shoulders and small outgrowths ("feathers"). Basal wing connected with lateral wings at 90° angles, forming subapical protrusion. Oval plate scales are 1.70–2.33 × 1.19–1.65 µm, with small axial ridge 0.57–0.62 µm in length.

Remarks: This species is similar to *P. tropica* (see below), and differs by absence of radial ribs on spine.

Distribution: Freshwaters of the European part of Russia (Leonov 2009, 2012; Leonov & Plotnikov 2009, Prokina *et al.* 2017c); Sweden (Siemensma & Roijackers 1988); USA (Wujek 2003); Canada (Nicholls 1983); Chile, Malaysia, Sri Lanka, New Zealand (Dürschmidt 1987b). Brackish inland waters of the European part of Russia, 2% (Gerasimova & Plotnikov 2015).

Pterocystis tropica (Dürschmidt, 1987) Siemensma, 1991 [bas.: *Acanthocystis tropica* Dürschmidt, 1987] (Figure 5h–5j)

Description: Spine scales consist of hollow cylindrical shaft and basal membrane, including lateral and basal wings. Shaft is 3.18–4.32 µm in length, 0.16–0.22 µm in diameter, basal part slightly curved, tip of shaft sharpened. Lateral wings stretched along shaft on 1.52–2.47 µm, basal part is almost rectangular in outline, 0.69–1.78 µm in length. Basal wing connected with lateral wings at 90° angles, forming subapical protrusion. Both wings ornamented by radial ribs. Oval plate scales are 2.20–2.42 × 1.42–1.76 µm, with axial ridge (0.79–0.96 µm in length).

Distribution: Freshwaters of the European part of Russia (Prokina *et al.* 2017c); Germany, Chile, Sri Lanka (Dürschmidt 1987a).

Pterocystis anglocollegarum sp. nov. (Figure 6)

Material: Type material (preparation for SEM) stored in the Laboratory of Microbiology of IBIW RAS, Borok, Russia. Type: Figure 6. Scales of two cells were observed in an extract of moss *Hamatocaulis vernicosus* (Mitt.) Hedenäs from a carpet located around a mire pool in Ushtulu Mire, Kabardino-Balkaria. The sample was collected on 21 September 2018 by Dmitriy A. Philippov (IBIW RAS).

Diagnosis: Protoplast is unknown. Cells covered by spine and plate scales. Spine scales leaf-shaped, with circular base, surrounded by lateral wings forming a short cylindrical cup. Shaft is completely reduced. Distal part of spine scales sharpened. Spine scales are 2.38–3.16 µm in length, base is 1.05–1.34 µm in diameter, cup-shaped structure is 0.29–0.37 µm in

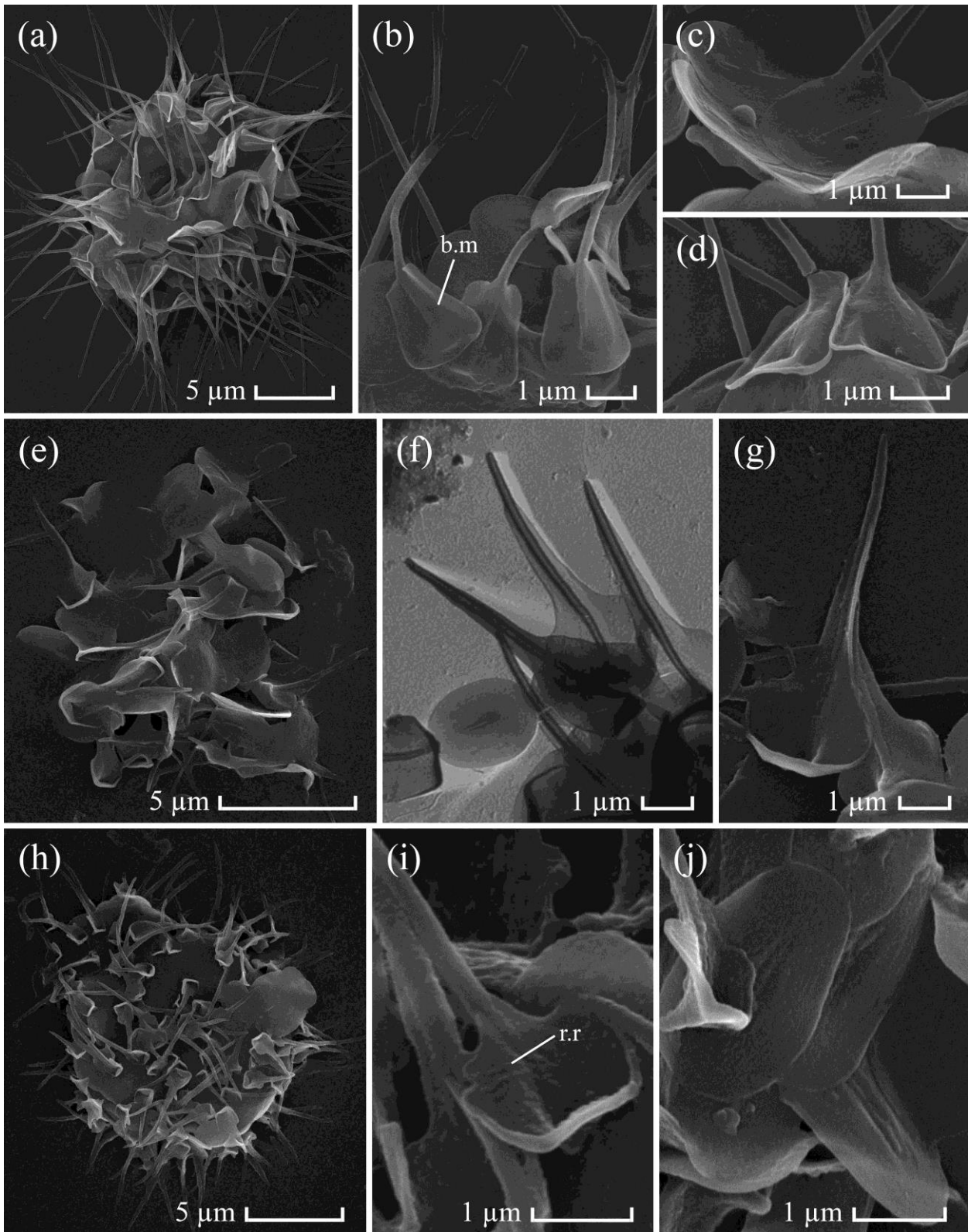


Figure 5. Morphology of observed scales of centrohelid genus *Pterocystis*, (a)–(e) and (g)–(j) SEM, (f) TEM. (a)–(d) *P. anapoda*: (a) scales of a single cell; (b) spine scales; (c) plate scales; (d) basal membrane of spine scales. (e)–(g) *P. pinnata*: (e) scales of single cell; (f) spine and plate scales; (g) spine scales. (h)–(j) *P. tropica*: (h) scales of a single cell; (i) basal membrane of spine scale; (j) plate scales and basal membrane of spine scale. Abbreviations: b.m = basal membrane. Other abbreviations as in Figure 4.

height. Margins of lateral wings and base consist of hollow marginal rim 0.10–0.12 μm in diameter. Marginal rim of lateral wings in the distal part of scale can be joined and extended out into a small rod 0.14–0.16 μm in diameter. Oval plate scales are 0.61–0.75 \times 0.41–0.45 μm , with well-marked hollow marginal rim (0.08–0.09 μm in diameter).

Differential diagnosis: Observed cells have a unique morphology of scales; shaft of spine scales completely reduced. Cup-shaped structure formed by lateral wings is characteristic of the following species: *Pterocystis plumosa* (Dürschmidt, 1987) Siemensma, 1991, *P. pteracantha* (Siemensma, 1981) Siemensma & Roijackers, 1988, *P. pulchra* (Dürschmidt, 1985) Siemensma, 1991 and *Pseudoraphidocystis umbraculiformis* (Dürschmidt, 1987) Mikrjukov, 1997. However, these species differ by the presence of a long and hollow stalk, cup-

shaped structure is conical, not cylindrical. Also, these species have a shaft and radial ribs on lateral wings. Leaf-shaped spine scales are present on the following species: *Pterocystis foliacea* (Dürschmidt, 1985) Siemensma, 1991, *P. contorta* Cavalier-Smith & von der Heyden, 2007, *P. cuspidata* Cavalier-Smith & von der Heyden, 2007, *P. ovata*, *Pseudoraphidiophrys ovata* (Dürschmidt, 1985) Mikrjukov, 1997, *P. pyriformis* (Dürschmidt, 1987) Siemensma, 1991, *P. veliformis*. However, scales of these species do not have a cup-shaped structure; instead, some species have a subapical protrusion. The observed plate scales of this new species are smaller than plate scales of most known species of centrohelids.

Remarks: Two spine scales and one plate scale of this species without a description of morphology were observed by G.F. Esteban and coauthors (Esteban *et*

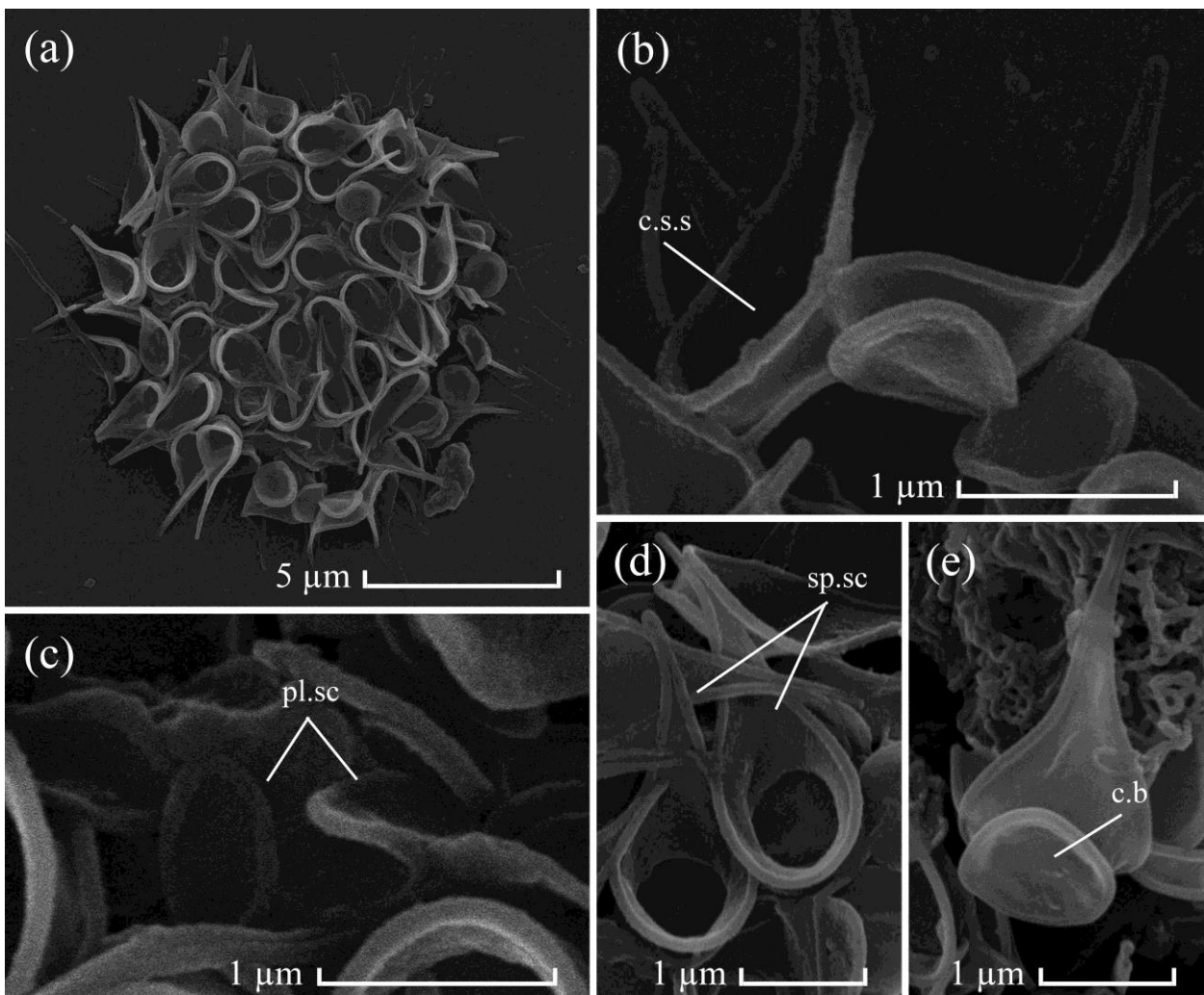


Figure 6. Morphology of observed scales of new centrohelid species *Pterocystis anglocollegarum* sp. n., SEM: (a) scales of a single cell; (b) spine scales, side view; (c) plate scales; (d) spine scales, view from above; (e) spine scale, bottom view. Abbreviations: c.b = circular base of spine scale; c.s.s = cup-shaped structure. Other abbreviations as in Figures 1 and 4.

al. 2012) from freshwaters of England. Those researchers did not describe a new species, possibly due to insufficient material. The indicated size of spine scales (2.7 μm in length) and calculated size of plate scales (about 0.64 μm) corresponds to the sizes of scales we found.

Zoobank Registration:

urn:lsid:zoobank.org:act:BA78B5FB-09D1-4CAF-BEBF-97DBC6F3E62E.

Etymology: The species is named after the English colleagues G.F. Esteban, B.J. Finlay and K.J. Clarke who first discovered scales of this species.

Raineriophrys erinaceoides (Petersen et Hansen, 1960) Mikrjukov, 2001 [bas.: *Acanthocystis erinaceoides* Petersen et Hansen, 1960; syn.: *Echinocystis erinaceoides* (Petersen et Hansen, 1960) Mikrjukov, 1997; *Pterocystis erinaceoides* (Petersen

et Hansen, 1960) Siemensma, 1991; *Raineria erinaceoides* (Petersen et Hansen, 1960) Mikrjukov, 1999] (Figure 7a–7d)

Description: Spine scales consist of hollow cylindrical shaft and basal membrane, including lateral and basal wings. Shaft is 3.69–10.54 μm in length, 0.15–0.38 μm in diameter, distal part conically tapers to a pointed tip. Lateral wings are 1.90–4.71 μm in length. Basal wing connected with lateral wings at 90° angles, forming subapical protrusion (1.38–1.56 μm in diameter). Ovoid plate scales are 4.43–4.73 \times 2.09–2.26 μm , with axial ridge (3.36–3.77 μm in length) and dense margins.

Remarks: This species is very similar to *R. kilianii* (see below), differing by the absence of radial ribs on lateral wings, presence of pointed shaft on spine scales and well-marked axial ridge on ovoid plate scales.

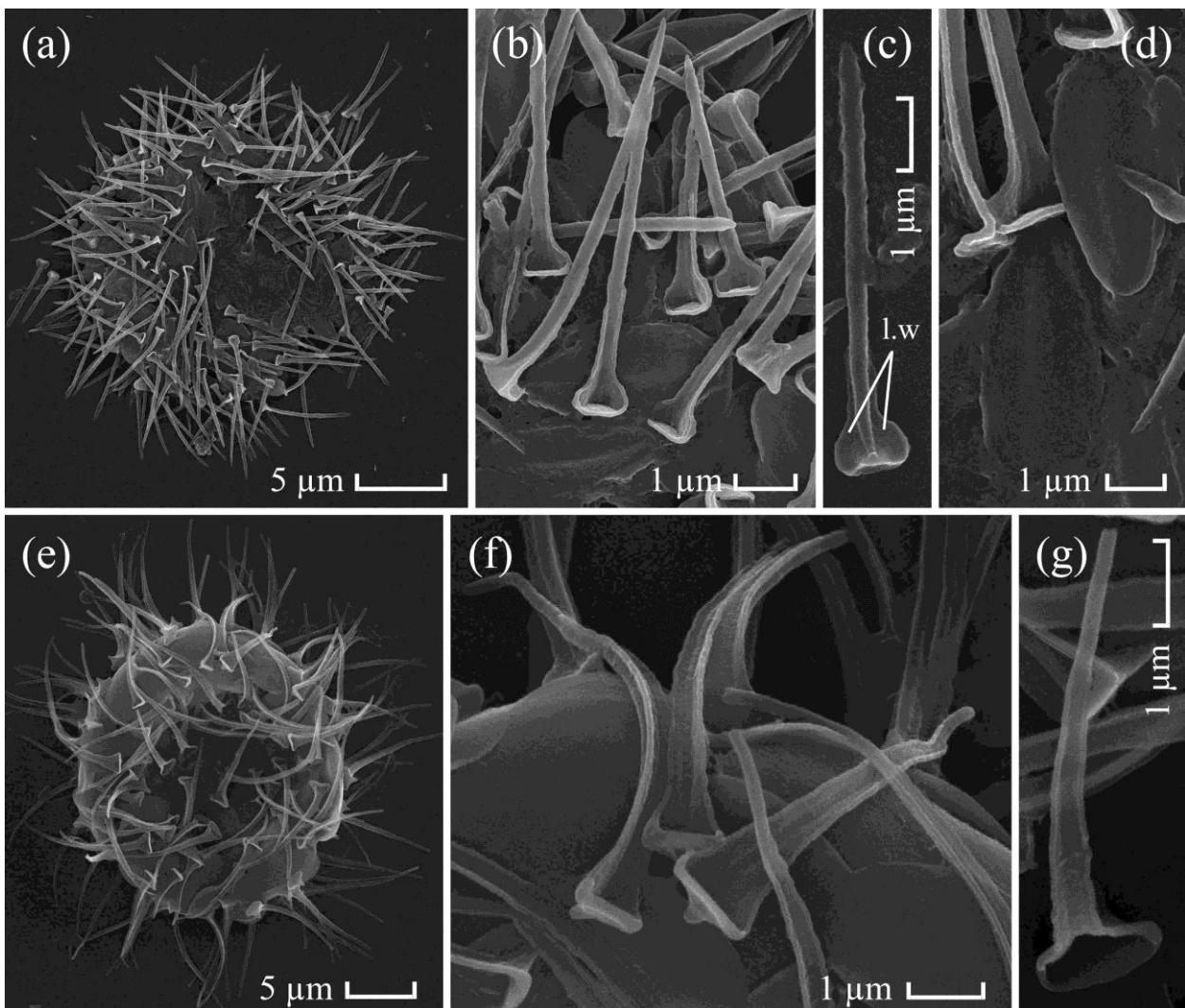


Figure 7. Morphology of observed scales of centrophelid genus *Raineriophrys*, SEM. (a)–(d) *R. erinaceoides*: (a) scales of single cell; (b) spine scales; (c) spine scale, dorsal side; (d) plate scales. (e)–(g) *R. kilianii*: (e) scales of single cell; (f) spine and plate scales; (g) spine scale. Abbreviations: l.w = lateral wings.

Distribution: Freshwaters of the European part of Russia (Mikrjukov 1993a, Leonov 2010a, 2012 [as *R. kilianii*], Leonov & Mylnikov 2012, Zlatogursky 2014, Prokina *et al.* 2018); Ukraine (Gaponova 2008); Estonia (Mikrjukov 1993b); Mongolia (Kosolapova & Mylnikov 2015); Canada (Nicholls 1983); Chile (Dürschmidt 1985, 1987a, Prokina & Mylnikov 2019); Sri Lanka (Dürschmidt 1987a); Australia (Croome 1986); brackish inland waters of the European part of Russia, 2‰ (Plotnikov & Gerasimova 2017), ~ 20‰ (Mikrjukov 2001).

Raineriophrys kilianii (Dürschmidt, 1987) Mikrjukov, 2001 [bas.: *Acanthocystis kilianii* Dürschmidt, 1987; syn.: *Pterocystis kilianii* (Dürschmidt, 1987) Siemensma et Roijackers, 1991; *Echinocystis kilianii* (Dürschmidt, 1987) Mikrjukov, 1997] (Figure 7e–7g)

Description: Spine scales consist of hollow cylindrical shaft and basal membrane, including lateral and basal wings. Shaft is 6.29–8.88 µm in length, 0.18–0.21 µm in diameter, basal part slightly curved, tip of shaft truncated. Lateral wings narrowed, stretched along 2/3 of length of shaft, at the base sharply expanded and connected with the basal wing, forming a subapical protrusion 1.24–1.56 µm in width. Lateral wings with jagged margins and poorly distinguishable radial ribs. Oval plate scales are 3.10–3.37 × 2.11–2.25 µm, without any texture.

Remarks: In the original description M. Dürschmidt (1987b) noticed the presence of a poorly visible axial ridge on plate scales; however, on type Figures (15–19, page 54) the axial ridge is not visible at all, plate scales are without any texture and very similar to the scales we studied.

Distribution: Freshwaters of the European part of Russia (Prokina *et al.* 2017c); Netherlands (Siemensma & Roijackers 1988); Germany, Chile (Dürschmidt 1987b); Australia (Croome 1987).

DISCUSSION

A total of 13 species of centrohelic heliozoans from six genera and four families were found (Table 2). All of them are new records for the freshwater ecosystems of the Caucasus (due to lack of any relevant research in this region to date). Four species are new records for Russia: *Acanthocystis nigeriensis*, *Pseudoraphidiophrys veliformis*, *Pterocystis anapoda* and *Pterocystis anglocollegarum* sp. n. Most of the species observed belong to the families Pterocystidae (*Pterocystis* - four species, *Raineriophrys* - two species, *Pseudoraphidiocystis* - one species) and

Acanthocystidae (*Acanthocystis* - four species). Families Raphidocystidae (*Raphidocystis* - one species) and Choanocystidae (*Choanocystis* - one species) were characterised by the lowest species richness. The most common species (observed in four of the sixteen samples) were *Acanthocystis nigeriensis*, *A. trifurca*, *A. turfacea* and *Pterocystis pinnata*. *Raphidocystis symmetrica* was observed in three samples. *Acanthocystis penardi*, *Choanocystis aculeata*, *Pseudoraphidiophrys veliformis*, *Pterocystis anapoda*, *P. anglocollegarum*, *P. tropica*, *Raineriophrys erinaceoides* and *R. kilianii* were found in only one sample.

The morphology of observed scales mainly corresponded to previous descriptions, but some deviations in shape and size were present. The majority of identified species are common on multiple continents in both the Northern and Southern Hemispheres (*Acanthocystis penardi*, *A. trifurca*, *A. turfacea*, *Choanocystis aculeata*, *Pseudoraphidiophrys veliformis*, *Pterocystis pinnata*, *P. tropica*, *Raineriophrys erinaceoides*, *R. kilianii*) (Nicholls 1983, Dürschmidt 1985, 1987b; Croome 1986, Croome *et al.* 1987, Siemensma & Roijackers 1988, Mikrjukov 1993a, 1993b). Two species (*Pterocystis anapoda* and *P. anglocollegarum*) have previously been found only in Europe (Siemensma & Roijackers 1988, Esteban *et al.* 2012) and one species (*Acanthocystis nigeriensis*) only in Africa (Wujek *et al.* 2007). Most (eight) species were previously observed only in freshwater habitats: *Acanthocystis penardi*, *A. nigeriensis*, *Raphidocystis symmetrica*, *Pseudoraphidiophrys veliformis*, *Pterocystis anapoda*, *P. anglocollegarum*, *P. tropica* and *Raineriophrys kilianii*. Two species have been found also in brackish waters (2–4‰): *Choanocystis aculeata* and *Pterocystis pinnata* (Mikrjukov 1999, Gerasimova & Plotnikov 2015). Three species have been found also in marine and saline inland waters: *Acanthocystis trifurca*, *A. turfacea* and *Raineriophrys erinaceoides* (Croome *et al.* 1987, Vørs 1992, Mikrjukov 2001, Leonov 2010a, Plotnikov & Gerasimova 2017, Prokina *et al.* 2019).

The distribution of centrohelic species among sampling plots was even, and the average number of species per sample was 1.7. The maximum species richness was observed in water samples from a hollow-pool in Kubus mire (three species) and in an extract of the moss *Hamatocaulis vernicosus* from a carpet in Ushtulu Mire (four species). In other sampling plots, the species richness was 1–2 species.

Most species were observed in Kabardino-Balkaria (average number of species per sample was 1.8); seven of eleven species were found only here. In North Ossetia, only six species were found

(average number of species per sample was 1.6) and two of them were found only here. *Acanthocystis nigeriensis*, *A. trifurca*, *A. turfacea* and *Pterocystis pinnata* were common to both studied regions.

In the eutrophic mires (Chifandzar and Ushtulu), twelve centrohelicid species were recorded in total (average number of species per sample was 1.8). Among the eutrophic mires, the highest species richness was recorded in Ushtulu Mire, namely: *Acanthocystis penardi*, *A. trifurca*, *A. turfacea*, *Raphidocystis symmetrica*, *Pterocystis anapoda*, *P. pinnata*, *P. tropica*, *P. anglocollegarum*, *Raineriophrys erinaceoides* and *R. kiliani*. Of these, seven (bold type here and below) were found only in this mire. In Chifandzar Mire, five species were recorded, namely: *Acanthocystis nigeriensis*, *A. trifurca*, *A. turfacea*, *Pseudoraphidiocystis veliformis* and *Pterocystis pinnata*. Only three species were observed in the oligotrophic mires (average number of species per sample was 1.5). Among the oligotrophic mires, the greatest species richness was recorded in Kubus Mire: *Acanthocystis nigeriensis*, *A. turfacea* and *Choanocystis aculeata*. *Acanthocystis nigeriensis* was found only in the oligotrophic Konskoe and Zayachye Mires.

Acanthocystis turfacea and *A. nigeriensis* were common to more than half of the studied mires, and we can assume that they are typical for the area. *A. turfacea* is widespread all over the world, while *A. nigeriensis* has been found only once previously, in freshwaters of Nigeria, and is probably a rare species. On the other hand, scales of this species are similar to scales of the more common *A. trifurca*, so we cannot draw firm conclusions about the rarity of *A. nigeriensis*.

Above-water (hummock and carpet) levels in the microtopography were characterised by significantly higher species diversity, as compared with mire water bodies (in-mire lakes, inter-hummock spaces and hollow-pools). This may be due to their greater variety of microhabitats with precise conditions required by centrohelicids. A total of 12 species were observed at levels above the water table (average number of species per sample was 1.9). Eight species were found in carpets, namely: *Acanthocystis nigeriensis*, *A. trifurca*, *Raphidocystis symmetrica*, *Pterocystis anapoda*, *P. tropica*, *P. anglocollegarum*, *Raineriophrys erinaceoides*, *R. kiliani*; five of which were observed only at this microtopographical level. Five species were recorded in hummocks, namely: *Acanthocystis penardi*, *A. trifurca*, *A. turfacea*, *Pseudoraphidiophrys veliformis*, *Pterocystis pinnata*. Five species were recorded in mire water bodies (average number of species per sample was 1.5). Three species, *Acanthocystis nigeriensis*, *A. turfacea*,

and *Choanocystis aculeata*, were observed in hollow-pools, the last one being found only at this microtopographical level. Four species were found in inter-hummock spaces, namely: *Acanthocystis nigeriensis*, *A. turfacea*, *Raphidocystis symmetrica* and *Pterocystis pinnata*. Only *Acanthocystis nigeriensis* was found in the in-mire lake. A dendrogram of the similarities between microtopographical levels in terms of species composition (Figure 8) shows that mire water bodies had the greatest similarity, while microtopographical levels above the water table diverged both from each other and from mire water bodies.

Among the observed microhabitat types, moss extracts are characterised by higher species richness. Hypnales were the most populated by centrohelicids. In total, eight centrohelicid species were found in Hypnales (average number of species per sample was 2.5): *Acanthocystis trifurca*, *Raphidocystis symmetrica*, *Pterocystis anapoda*, *P. pinnata*, *P. tropica*, *P. anglocollegarum*, *Raineriophrys erinaceoides*, *R. kiliani*, five of which were found only here. In *Sphagnum* mosses, six species were observed (average number of species per sample was 1.4): *Acanthocystis nigeriensis*, *A. penardi*, *A. trifurca*, *A. turfacea*, *Pseudoraphidiophrys veliformis*, *Pterocystis pinnata*. Only *Sphagnum teres* (Schimp.) Ångstr. had high species diversity of associated heliozoans (four species). Low similarity of centrohelicid species composition was recorded in the observed *Sphagnum* mosses. Only *Acanthocystis trifurca* was associated with two *Sphagnum* species at the same time.

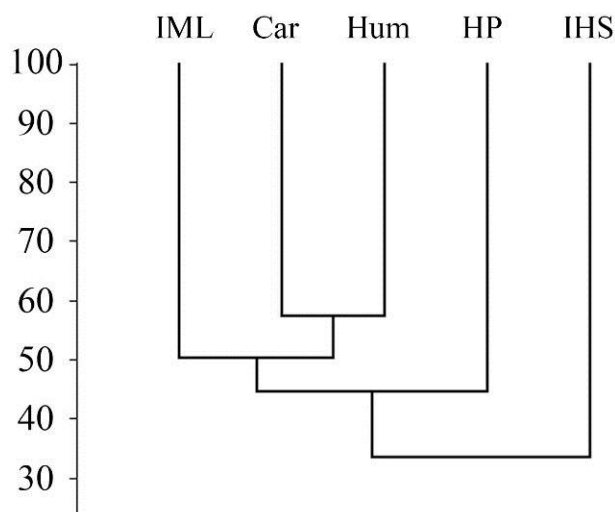


Figure 8. Dendrogram showing the Bray-Curtis similarity (%) of biotope types by species diversity of centrohelicids. Abbreviations: IML = in-mire lake; Car = carpets; Hum = hummocks; HP = hollow-pools; IHS = inter-hummock space.

The smallest numbers of centrohelid species were found in samples of bottom sediments and surface water (four species in each microhabitat, average numbers of species per sample were 1.7 and 1.5, respectively). *Acanthocystis nigeriensis*, *A. turfacea*, *Raphidocystis symmetrica* and *Pterocystis pinnata* were associated with bottom sediments; *Acanthocystis nigeriensis*, *A. turfacea*, *Raphidocystis symmetrica* and *Choanocystis aculeata* were associated with water samples, the latter being found only here.

More than half (eight) of the centrohelid species were observed only in one type of microhabitat, and we assume that this indicates their high adaptation to specific micro-environmental conditions. However, more research is needed. Only *Acanthocystis trifurca* was found in two types of microhabitat, and four species (*Acanthocystis nigeriensis*, *A. turfacea*, *Raphidocystis symmetrica* and *Pterocystis pinnata*) were recorded in three types of microhabitat. Thus, all of these species may be regarded as being poorly adapted to a range of conditions. The bottom sediments and water surface communities showed greater similarity (75 %) to each other than to moss communities (Figure 9). *Sphagnum* cenoses showed the greatest uniqueness.

It has been shown previously that hydrochemical and hydrophysical factors play an important role in determining the distribution of protists (Mazei *et al.* 2005, Lamentowicz *et al.* 2007, Mieczan 2009, Prokina *et al.* 2017a). Low pH values of water probably reflect extreme habitat conditions for centrohelids. Figure 10 shows the dependence of community species richness (directly proportional to the diameter of the circle) on water properties, pH on the X-axis and temperature on the Y-axis. Biotopes with high temperature and low pH (Samples 8 and 16), as well as biotopes with low temperature and high pH (Sample 1) are associated with the lowest species diversity, whereas high water temperature and pH associate with higher species richness in biotopes.

CONCLUSIONS

The species diversity of centrohelid heliozoans in the studied mountain mires is relatively high; 13 species were recorded. *Acanthocystis nigeriensis* was found for the first time since its original description. The new species *Pterocystis anglocollegarum* sp. n. has a unique morphology of cell coverings. The data obtained on the morphology of scales further our knowledge of the intraspecific variability of centrohelid heliozoans. New records for Russia were the following four species: *Acanthocystis nigeriensis*,

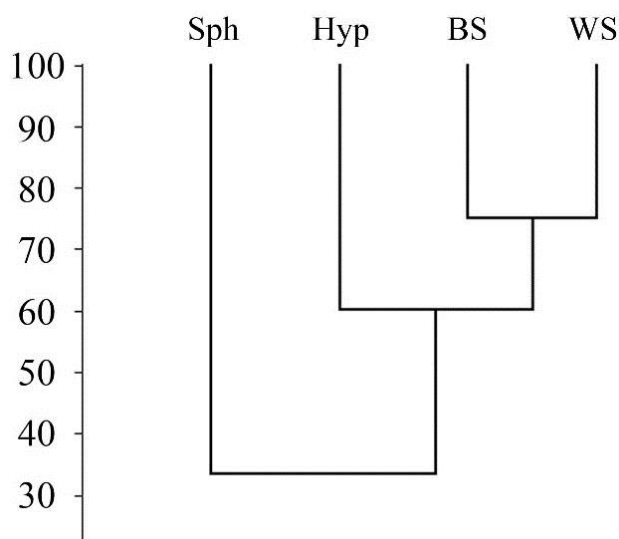


Figure 9. Dendrogram showing the Bray-Curtis similarity (%) of microhabitat types by species diversity of centrohelids. Abbreviations: Sph = extracts of *Sphagnum* mosses; Hyp = extracts of Hypnales mosses; BS = bottom sediment; WS = water surface.

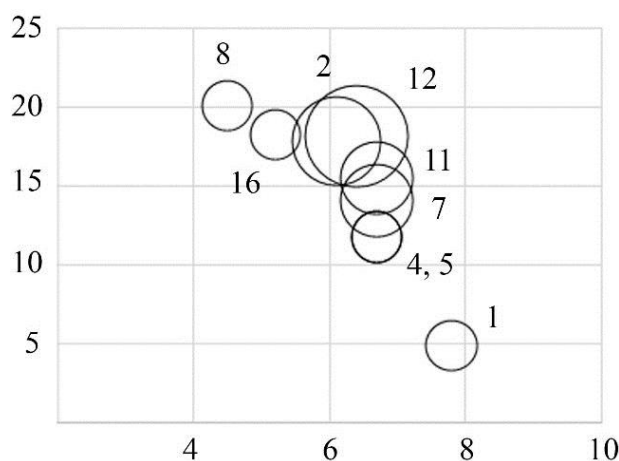


Figure 10. Dependence of species richness of centrohelids per biotope on the pH (X-axis) and temperature (Y-axis) of water. The diameter of the circle is directly proportional to the species richness of the biotope. The numbers of biotopes are given in accordance with Table 1.

Pseudoraphidiophrys veliformis, *Pterocystis anapoda* and *P. anglocollegarum* sp. n. The most common species were *Acanthocystis nigeriensis*, *A. trifurca*, *A. turfacea* and *Pterocystis pinnata*. Above-water microtopographical levels (hummock and carpet) were characterised by significantly higher species richness in comparison with mire water bodies (in-mire lakes, hollow-pools and inter-hummock

spaces). Among the types of microhabitats observed, moss extracts were characterised by higher species richness. The bottom sediments and water surface communities showed greater similarity amongst themselves than with communities associated with mosses. It was shown that water temperature and pH significantly influence the species richness of the studied biotopes. The distribution of the observed morphospecies confirms the cosmopolitanism of many centrohelids and highlights the importance of the water body and microhabitat type in determining the species composition.

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