Available online at www.sciencedirect.com

ScienceDirect

European Journal of Protistology 86 (2022) 125916

European Journal of PROTISTOLOGY

www.elsevier.com/locate/ejop

Centrohelid heliozoans of Ukraine with a description of a new genus and species (Haptista: Centroplasthelida)

Dmitry G. Zagumyonnyi [a,*], Liudmila V. Radaykina [a], Patrick J. Keeling [b], Denis V. Tikhonenkov [a]

[a] *Papanin Institute for Biology of Inland Waters, Russian Academy of Sciences, 152742 Borok, Russia*
[b] *Department of Botany, University of British Columbia, 6270 University Boulevard, Vancouver, BC V6T1Z4, Canada*

Received 11 June 2022; revised 4 August 2022; accepted in revised form 22 August 2022; Available online 27 August 2022

Abstract

New data on the species diversity and morphology of centrohelid heliozoans in freshwater, marine, and soil habitats of Ukraine were obtained. Cell coverings (scales and spicules) were observed using scanning and transmission electron microscopy. Eighteen species from seven genera of centrohelids and unidentifiable *Heterophrys*-like organisms were revealed. The micrographs and detailed morphological descriptions of observed species and their comparison with previously found centrohelids are provided. A new genus and three new species *Acanthocystis tyrasiana* sp. nov., *Pterocystis borysthenica* sp. nov., and *Khitsovia mutabilis* gen. et sp. nov. were described. Molecular phylogenetic analyses based on 18S rRNA sequences, obtained for three strains, expand our knowledge on the diversity and evolution of centrohelids within the Pterista clade. The novel data on the morphology of studied scales supplement available information on the intraspecific variability of centrohelid heliozoans.

Keywords: Protists; Morphology; *Acanthocystis tyrasiana*; *Pterocystis borysthenica*; *Khitsovia mutabilis*; 18S rRNA gene

Introduction

Centrohelid heliozoans (Centroplasthelida Febvre-Chevalier and Febvre 1984) are widespread amoeboid non-photosynthetic protists with a spherical cell body and radially oriented unbranched axopodia. Axopodia usually have numerous stinging organelles called kinetocysts that serve to capture prey. Centrohelids are an important functional component of benthic and periphyton food webs of aquatic habitats of various salinities (Siemensma 1991; Mikrjukov 2002). The cells of most centrohelid heliozoans are covered with siliceous scales of various shapes and/or needle-like organic spicules (Siemensma 1991, Mikrjukov 2002). The shape of siliceous scales is species-specific and correlates well with the data of molecular phylogeny (Zlatogursky and Klimov 2016).

Multigene phylogenetic studies have resolved the position of centrohelids in the eukaryotic tree by placing them with haptophyte algae, together making up two most diverse lineages of the Haptista supergroup (Burki et al.

Abbreviations: DIC, differential interference contrast; PhC, phase contrast; SEM, scanning electron microscopy; TEM, transmission electron microscopy; HLO, "Heterophrys"-like organism

*Corresponding author.
E-mail address: zdmitryg@gmail.com (D.G. Zagumyonnyi).

https://doi.org/10.1016/j.ejop.2022.125916
0932-4739/

2016). However, phylogenetic relationships between different groups within centrohelid heliozoans are largely unresolved (Cavalier-Smith and von der Heyden 2007; Cavalier-Smith, T., Chao 2012; Zagumyonnyi et al. 2020b). Centrohelids generally are divided into two major groups: Panacanthocystida and Pterocystida. Panacanthocystida are characterized by the presence of 5–13 insertions in the 18S rRNA gene (Yabuki et al. 2012) and contain the taxa Chthonida (with the family Yogsothothidae) and Acanthocystida (with the families Marophryidae, Acanthocystidae, and Raphidocystidae). Pterocystida possesses no insertions in the 18S rRNA gene and consists of Raphidista (with the families Choanocystidae and Raphidiophryidae) and Pterista (with the families Oxnerellidae, Pterocystidae, Heterophryidae, and Clypiferidae) (Shishkin et al 2018; Shishkin et al 2021). The family Spiculophryidae is of an uncertain systematic position within Centroplasthelida. Molecular data for the genera *Choanocystis*, *Raineriophrys*, and *Raphidiophrys* are very limited, and there are no 18S rRNA gene sequences available for *Parasphaerastrum, Pseudoraphidiophrys*, and *Pseudoraphidocystis*.

Currently, most of the known diversity of centrohelids is made up of groups known only from environmental 18S rRNA sequences, and without any morphological data. Pterocystida contains the greatest number of such environmental lineages (Cavalier-Smith and von der Heyden 2007; Cavalier-Smith and Chao 2012; Shishkin et al. 2018), and the deep branching relationships within this group remain poorly resolved (Cavalier-Smith and Chao 2012).

Centrohelid heliozoans have been studied poorly in general, which is made worse by their uneven sampling across the globe. For a large number of regions of the world, there is almost no data on centrohelids (Cavalier-Smith and von der Heyden 2007; Plotnikov and Ermolenko 2015; Prokina et al. 2020). Studies concerning the species diversity of centrohelids in Ukraine have been undertaken since the 1880s (Gaponova 2006), however, these are largely restricted to faunistic lists of aquatic organisms (Pereyaslavceva 1886; Vysockij 1888; Jaworowski 1893; Buchinskij1895; Faszynski 1910; Dobrovlyanskij 1914; Beling 1923; Krasheninnikiv 1925; Fadeev 1929). Only one paper was devoted to a special study and description of the new species *Acanthocystis wiasemskii* (Ostroumoff 1917). The investigation of species morphology in these studies was done solely by light microscopy, which does not allow correct identification of species in most cases (Roijackers 1988). Thus, published lists of species are doubtful, probably with the exception of *Acanthocystis turfacea* Carter, 1863, a large heliozoan with silica scales that can generally be resolved with high magnification light microscopy. This species was reliably noted by Faszynski (1910) in the pond at Ivano-Frankovo (formerly Janow city). Studies of centrohelid heliozoans using electron microscopy include Ukrainian Polesie (Kyiv and Chernihiv regions) and Crimean Peninsula (Mikrjukov 1995, 1997, 1999; Gaponova 2009; Prokina et al. 2017c; Prokina et al. 2019). This study aimed to examine the diversity and morphology of centrohelid heliozoans from freshwater, brackishwater and soil habitats of Ukraine using microscopy and molecular phylogeny methods.

Material and methods

Cultures and samples

Samples were taken from various microbiotopes of freshwater, marine and soil habitats of Ukraine during 2013–2018 (see Table 1) at three main sites: I) the Dniester river basin (Chernivtsi, Khmelnytsky and Ternopil regions); II) the Dnieper river basin (Kyiv and Cherkasy regions); III) the Northern Azov river basins, and the Azov Sea proper (Zaporizhzhya region). The first two sites are located in forest-steppe zone, whereas the third is in the steppe zone. Samples were placed into plastic tubes and transported to the laboratory at 4 °C. Soil samples were diluted with sterile mineral water (Aqua Minerale, PepsiCo, Inc., Moscow region, Russia).

Each sample was inoculated into a 60 mm Petri dish. One rice grain was added to each dish to stimulate bacteria growth. Samples were incubated at 20–22 °C in the dark, and periodically examined with a light microscope. To obtain clonal cultures, single cells were picked using a glass micropipette, and transferred into Petri dishes with inorganic medium and a food source.

For freshwater centrohelids, the cell culture of flagellate *Parabodo caudatus* (Dujardin, 1841) Moreira et al., 2004 (BAS-1 strain, IBIW RAS), grown in the autoclaved spring water (Aqua Minerale, PepsiCo, Inc., Moscow region, Russia) was used as prey. Prey cultures were enriched with *Pseudomonas fluorescens* Migula, 1895 bacteria.

For marine centrohelids, the flagellate *Procryptobia sorokini* (Zhukov, 1975) Frolov et al., 2001 (strain B-69, IBIW RAS) was used as prey, grown in an artificial marine medium (RS-R11040, Red Sea).

MICROSCOPY: An AxioScope A1 upright light microscope (Carl Zeiss, Jena, Germany) with DIC and phase contrast, and water and oil immersion objectives (63×) was used for observation of living cells. An inverted light microscope (CKX41 Olympus, Tokyo, Japan) with phase contrast objectives (20×, 40×) and inverted microscope Axio Observer 5 (Carl Zeiss, Jena, Germany) with phase contrast (objectives 20×) was used for isolation of cell cultures. Light microscopic images were taken with a MC-20 camera (Lomo-Microsystems, Saint Petersburg, Russia) and MC-1009/S video camera (AVT Horn; Aalen, Germany).

Preparations for studying skeletal elements were air dried and carried out according to the described methods (Zagumyonnyi et al. 2021), and observed in transmission JEM-1011 (Jeol, Tokyo, Japan) and scanning

Table 1. Characteristics of sampling sites.

	Habitat	Microbiotope	Coordinates. N. E.	Date	Species
Ternopil region					
1	Dniester River	coastal bottom sediments	48°46′13.06″ 25°35′45.45″	September 12, 2014	HLO
2	The tributary of the Djurin River, Dniester basin	water from a stream, a tributary of the Djurin River	48°48′26.8″ 25°34′51.6″	August 15, 2015	*Acanthocystis* aff. *turfacea*
Khmelnitsky region					
3	Zhvanchik River, Dniester basin	water column	49°00′36.2″ 26°21′08.8″	August 11, 2015	*Pterocystis* aff. *tropica*
Chernivtsi region					
4	Nameless forest spring, Dniester basin	spring water in lime rubble in an oak and hornbeam forest	48°28′12.06″ 26°46′13.91″	June 18, 2014	*Acanthocystis tyrasiana* sp. nov.
Kiev region					
5	Soil of Kam'yaniy island, Dnepr River	soil within the grasses and roots of *Amorpha* sp.	50°34′48.41″ 30°31′18.56″	October 14, 2014	*Raineriophrys* aff. *fortesca*
6	Soil of Ptashynyi island, Dnepr River	loamy sand soil within grasses and roots of *Salix* sp.	50°34′33.23″ 30°31′14.16″	October 5, 2014	*Raineriophrys* aff. *fortesca*
7	Soil of nameless island, Dnepr River	sand within the grasses	50°34′28.44″ 30°30′55.32″	October 5, 2014	*Acanthocystis* aff. *turfacea*; *Raineriophrys* aff. *fortesca*
8	Dnepr River	coastal bottom detritus	50°33′39.14″ 30°30′25.17″	July 3, 2014	*Acanthocystis pectinata*
9	Dnepr River	water column	50°33′37.26″ 30°30′24.00″	July 3, 2014	*Acanthocystis nichollsi*; *Raineriophrys erinaceoides*
10	Dnepr River	coastal bottom detritus	50°33′37.26″ 30°30′24.00″	July 3, 2014	*Acanthocystis pectinata*
11	Desna River	water column	50°33′43.65″ 30°33′38.51″	September 7, 2014	*Acanthocystis pectinata*
12	Dnepr River	coastal bottom detritus	50°33′27.90″ 30°31′04.23″	July 3, 2014	*Acanthocystis aff. nichollsi*; *Acanthocystis penardi*
13	Dnepr River	water column	50°33′27.90″ 30°31′04.23″	July 3, 2014	*Raineriophrys erinaceoides*
14	Dnepr river	coastal bottom detritus	50°33′02.57″ 30°30′54.72″	July 3, 2014	*Raphidocystis ambigua*
15	Dnepr river	coastal bottom detritus	50°32′11.99″ 30°32′14.72″	June 19, 2014	*Raineriophrys erinaceoides*
16	Soil of Pesiv island, Dnepr river	loamy sand soil within the roots of *Carex* sp. and *Salix* sp.	50°30′13.95″ 30°31′52.90″	October 11, 201	*Raineriophrys* aff. *fortesca*
17	Soil of Kachinij island, Dnepr river	soil within the grasses and roots of *Salix* sp.	50°30′14.68″ 30°31′57.16″	October 11, 2014	*Pterocystis* aff. *pinnata; Triangulopteris lacunata*
18	Hollow in the tree in Feofaniya Park, Dnepr basin	water inside the tree hollow	50°20′22.6″ 30°29′05.9″	October 27, 2017	*Raineriophrys* aff. *fortesca*
19	Hollow in the tree in Feofaniya Park, Dnepr basin	water inside the tree hollow	50°20′21.6″ 30°29′05.2″	October 27, 2017	*Choanocystis* aff. *perpusilla, Raineriophrys* aff. *fortesca*
20	Pond Palladinsky, Dnepr basin	coastal bottom detritus	50°20′21.1″ 30°29′26.6″	October 27, 2017	HLO; *Acanthocystis* aff. *turfacea*; *Raineriophrys scaposa*
21	Nameless pond on Vita Stream, Dnepr basin	coastal bottom detritus	50°20′22.6″ 30°29′32.1″	October 27, 2017	*Pterocystis borysthenica* sp. nov.
Chernihiv region					
22	Trubin (Bekhova) river, Desna basin	periphyton on *Ceratophyllum* sp.	51°23′30.1″ 32°21′41.6″	August 9, 2016	*Acanthocystis* sp.1

23	Trubin (Bekhova) River floodplain, Desna basin	soil of a wet floodplain meadow among *Carex* sp. and *Elytrigia* sp.	51°23′28.9″ 32°21′41.5″	August 9, 2016	*Khitsovia mutabilis* gen. et sp. nov.
Zaporozhye region					
24	Obitochnaya River	periphyton on *Phragmites australis*	46°40′11.31″ 36°12′47.07″	August 28, 2013	*Raphidocystis symmetrica*
25	Berdyansk Bay, Sea of Azov	coastal bottom detritus	46°41′10.62″ 36°49′12.70″	August 28, 2013	HLO

JSM-6510LV (Jeol, Tokyo, Japan) electron microscopes. Acceleration voltage was 80 kV for TEM and 15–30 kV for SEM.

Cells and skeletal elements were measured using ImageJ 1.52a (Schneider et al. 2012). Detailed morphometric parameters are given for new taxa and species whose 18S rRNA gene were sequenced.

Molecular phylogeny

Cells of centrohelid heliozoans (strains HF-24, HF-51, HF-55) were grown in clonal cultures and collected by centrifugation (1000× g, room temperature) onto the 0.8 μm membrane of a Vivaclear mini columns (Sartorius Stedim Biotech Gmng, Cat. No. VK01P042). Genomic DNA was isolated using the MasterPure™ Complete DNA and RNA Purification Kit (Epicentre, Madison, WI, USA, Cat. No. MC85200). The 18S rRNA genes (GenBank accession numbers: OP101620, OP101622, and OP104322) were amplified by polymerase chain reaction (PCR) using EconoTaq Plus Green 2 Master Mix (Lucigen, Middleton, WI, USA, Cat. No. 30033-1) and eukaryotic primers PF1-FAD4 (for strains HF-51 and HF-55) and 18SFU-18SRU (HF-24) (Keeling, 2002; Medlin et al., 1988; Tikhonenkov et al., 2016). The PCR-program for amplification was as follows: initial denaturation 95 °C for 3 min, 35 cycles of 95 °C for 30 s, 52 °C for 30 s, 72 °C for 1.5 min, and a final extension 72 °C for 5 min. Amplified DNA fragments were purified with QIAquick PCR Purification Kit (Quagen, Hilden, Germany, Cat. No. 433160764). PCR products were subsequently cloned (HF-55) using the StrataClone PCRCloning Kit (Agilent, Santa Clara, USA, Cat. No. 240205) or sequenced directly (HF-24, HF-51) using Sanger dideoxy sequencing. Sequencing was conducted using the Applied Biosystems 3730 DNA analyzer (Applied Biosystems, USA; Hitachi, Japan) at the NAPS Unit at the University of British Columbia, Vancouver, BC. The resulting sequences were assembled using Geneous v. 6.0.6.

All centrohelid sequences were aligned using L-INS-i algorithm in MAFFT version 7.475 (Katoh and Standley 2013) and trimmed using '-gappyout' method in TrimAl version 1.2 (Capella-Gutierrez et al. 2009). The resulting alignment consisted of 1489 sites and was used to build the phylogenetic trees.

To infer Bayesian phylogenetic tree, MrBayes version 3.2.7a (Ronquist et al. 2012) was used with four categories of Gamma-distributed among-site rate variation under GTR +I+GAMMA4 substitution model, calculating proportion of invariable sites. To calculate posterior probability, four independent Metropolis-coupled Markov chains were run for 20 million generations, and summarized with a 50 % burn-in.

Additionally, maximum likelihood phylogeny was inferred using IQ-TREE v1.6.12. (Nguyen et al. 2015) with 1000 nonparametric bootstraps under the best fit model (TN +F+I+G4) determined by the in-built ModelFinder.

Results and discussion

Morphology

Eighteen species of centrohelids and non-identifiable *Heterophrys*-like organisms were found and listed systematically according to the system of eukaryotes by Adl et al. (2019). Black circles "•" indicate the ranks of taxa from greater to lesser. The more symbols that are used, the lower the rank. Morphological descriptions of species and taxonomic diagnoses of new centrohelids *Acanthocystis tyrasiana* sp. nov., *Pterocystis borysthenica* sp. nov., and *Khitsovia mutabilis* gen. et sp. nov. are listed below. Information on the distribution of the observed species is provided in Supplementary Table S1.

DIAPHORETICKES Adl et al., 2012.

• Haptista Cavalier-Smith, 2003.

•• Order Centroplasthelida Febvre-Chevalier et Febvre, 1984.

••• Panacanthocystida Shishkin et Zlatogursky, 2018.

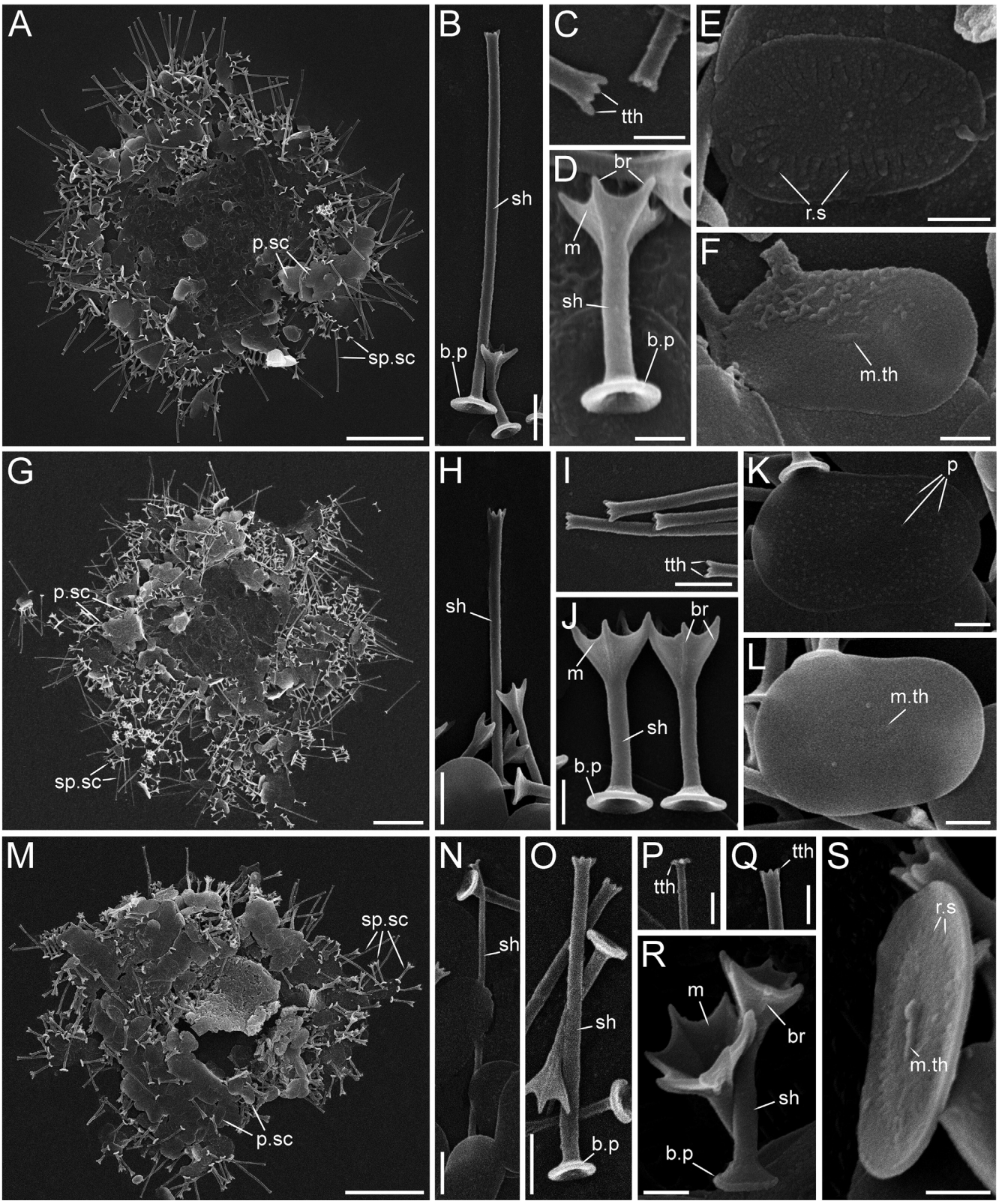
•••• Acanthocystida Cavalier-Smith et von der Heyden, 2007 emend. Shishkin et Zlatogursky, 2018

••••• Chalarothoracina Hertwig et Lesser, 1874 sensu Cavalier-Smith in Yabuki et al., 2012 emend. Shishkin et Zlatogursky, 2018

•••••• Family Acanthocystidae Claus, 1874 emend. Shishkin et Zlatogursky, 2018

••••••• *Acanthocystis* Carter, 1863

Acanthocystis nichollsi Siemensma et Roijackers, 1988 (Fig. 1A–F)


A
p.sc
sp.sc
B
sh
b.p
C
tth
D
br
m
sh
b.p
E
r.s
F
m.th
G
p.sc
sp.sc
H
sh
I
tth
J
br
m
sh
b.p
K
p
L
m.th
M
sp.sc
p.sc
N
sh
O
sh
b.p
P
tth
Q
tth
R
m
br
sh
b.p
S
r.s
m.th

Material: 23 cells from the sampling site No. 10 (see Table 1).

Description: Skeletal elements of cells are represented by plate-scales and spine-scales of two types (Fig. 1A–F). The length of long spine-scales is 7.5–9.4 μm (Fig. 1B). The basal plate is circular 0.87–1.06 μm in diameter, the shaft diameter is 0.24–0.28 μm (Fig. 1B). The apical part of the scale ends with six to nine short teeth, 0.26–0.41 μm wide (Fig. 1C). The length of short spine-scales is 0.8–2.9 μm (Fig. 1D). The basal plate is circular, 0.70–0.85 μm in diameter, the shaft is hollow, 0.22–0.26 μm in diameter. The top of short spine-scales is in the form of a bowl, 0.65–0.95 μm wide, with four to six branches connected by a membrane (Fig. 1D). Plate-scales are elliptical, sometimes with a medial constriction, 2.3–3.1 × 1.4–1.8 μm (Fig. 1E–F). Medial thickening 0.45–0.49 μm in length, marginal rim 0.02–0.05 μm wide. Radial slits are visible on the surface of the scales (Fig. 1E).

Remarks: Some authors noted long spine-scales of larger or smaller size: 9–12 μm (Leonov 2009; Leonov and Plotnikov 2009; Leonov and Mylnikov 2012), 12–15 μm (Leonov 2010), 3.8–7.5 μm (Zagumyonnyi et al. 2020a), and 4.13–10.95 μm (Prokina et al. 2018). Short spine-scales of a longer size up to 3.2–3.5 μm were also noted (Leonov 2009; Prokina et al. 2018; Prokina and Mylnikov 2019; Zagumyonnyi et al. 2020a). Larger plate-scales 3.8 × 2.1 μm (Leonov and Plotnikov 2009; Leonov and Mylnikov 2012; Kosolapova and Mylnikov 2015), and 3.2–3.6 × 2.0–2.3 μm (Leonov 2010) were described previously. Radial slits are often not visible, probably due to poor washing of the electron microscopy preparations. This species was found in Ukraine for the first time.

Acanthocystis pectinata Penard, 1889 emend. Siemensma et Roijackers, 1988 (Fig. 1G–L)

Material: 21 cells from the sampling site No. 8; 13 cells from the site No. 9; 17 cells from the site No. 11 (see Table 1).

Description: Skeletal elements of cells are represented by plate-scales and spine-scales of two types (Fig. 1G–L). The long spine-scales have a length of 3.9–13.8 μm (Fig. 1H, I). The circular basal plate is 0.8–1.2 μm in diameter. The shaft is hollow and 0.22–0.30 μm in diameter. The apical part of the scale ends with six to nine short teeth 0.20–0.60 μm wide (Fig. 1I). The length of short spine-scales is 1.4–3.3 μm (Fig. 1J). The basal plate is circular and 0.61–1.06 μm in diameter. The shaft is hollow and 0.20–0.28 μm in diameter. The top of short spine-scales is in the form of a bowl, 0.5–1.4 μm wide, with four to six branches connected by a membrane. Plate-scales are elliptical or elliptical with a medial constriction, 2.2–3.1 × 1.4–2.1 μm (Fig. 1K, L), sometimes covered with papillae. Medial thickening 0.42–0.65 μm in length, marginal rim 0.02–0.05 μm wide. Radial slits are absent on plate-scales.

Remarks: The morphology and size of scales of the investigated cells are similar to the earlier descriptions. The most morphologically similar cells have been described from Usman' River of Don Basin (Prokina et al. 2018). In other works, the lower values of the length of long spine-scales were much higher, e.g., 8–12 μm in Dürrschmidt (1987a), 6–10 μm in Siemensma and Roijackers (1988a), and 7–10 μm in Prokina and Mylnikov (2019).

Acanthocystis* aff. *nichollsi Siemensma et Roijackers, 1988 (Fig. 1M–S)

Material: 6 cells from the sampling site No. 12 (see Table 1).

Description: Live cells are ~20 μm in diameter. Skeletal elements are represented by plate-scales and spine-scales of three types (Fig. 1M–S). The length of spine-scales of the first type is 6.1–8.2 μm (Fig. 1N). The circular basal plate is 0.53–0.74 μm in diameter. The shaft is hollow, 0.15–0.21 μm in diameter. The apical part of the scales is 0.26–0.36 μm wide and ends with four short curved teeth (Fig. 1N, P). The length of spine-scales of the second type is 5.3–6.9 μm (Fig. 1O). The circular basal plate is 0.81–0.92 μm in diameter. The shaft is hollow, 0.24–0.31 μm in diameter. The apical part of the scale is 0.32–0.45 μm wide and ends six short straight teeth (Fig. 1O, Q). The length of spine-scales of the third type is 2.4–3.4 μm (Fig. 1R). The circular basal plate is 0.52–0.87 μm in diameter. The shaft is hollow, 0.22–0.27 μm in diameter. The top of the scale is in form of a bowl, 0.71–0.95 μm wide, with four to six branches connected by a membrane. Plate-scales are elliptical or elliptical with a medial constriction, 2.2–2.9 × 1.5–1.8 μm with medial thickening and short radial slits between the outer edge and the middle part (Fig. 1S).

Remarks: This species differs from *A. nichollsi* by the presence of the second type of long spine-scales resembling the spine-scales of *A. amura* Zlatogursky, Gerasimova et Plotnikov, 2017. This type of scales has a smaller diameter of the shaft, a smaller diameter of the circular basal plate,

Fig. 1. Morphology of observed scales of the genus *Acanthocystis* (SEM). A–F – *A. nichollsi*: A – general view of the dried cell; B – long spine-scale; C – apical part of long spine-scales; D – short spine-scale; E–F – plate-scales; G–L – *A. pectinata*: G – general view of the dried cell; H – long spine-scales; I – apical part of long spine-scales; J – short spine-scales; K–L – plate-scales; M–S – *A.* aff. *nichollsi*: M – general view of the dried cell; N – first type of long spine-scale; O – second type of long spine-scale; P – apical part of first type of long spine-scale; Q – apical part of second type of long spine-scale; R – short spine-scales; S – plate-scale. Abbreviations: b.p – basal plate; br – branches; m – membrane; m.th – medial thickening; p – papillae; r.s – radial slits; sh – shaft; p.sc – spine-scale; tth – teeth. Scale bar: A, G, M – 10 μm; B, H, I, N–Q – 1 μm; C–F, J–L, R, S – 0.5 μm.

and carries four proximally curved teeth at the apex. Cells with the second type of long spine-scales were previously noted in South Vietnam as *A.* aff. *nichollsi* (Zagumyonnyi et al. 2020a), in South Russia as *A. pectinata* (Leonov and Plotnikov 2009), and in Northern Vietnam and European Russia (unpublished data). The taxonomic status of this organism will be clarified after a detailed study using 18S rRNA sequencing.

Acanthocystis penardi Wailes, 1925 (Fig. 2A–D)

Material: 7 cells from the sampling site No. 12 (see Table 1).

Description: Live cells with a diameter of ~20 μm. The surface of the cells is covered with plate and spine-scales (Fig. 2A–D). Spine-scales are 3.2–14.0 μm in length. They consist of a circular basal plate with a diameter of 1.0–2.0 μm, and a cylindrical hollow shaft with a diameter of 0.25–0.46 μm (Fig. 2B–C). There are 4 to 8 marginal teeth at the top of the scale, but more often 5–6 teeth. (Fig. 2C, D). Plate-scales are granular, elliptical, 2.9–4.1 × 1.7–2.7 μm (Fig. 2D).

Remarks: The morphology and size of scales of the investigated cells are similar to the earlier descriptions. The greatest similarity is observed with cells from the European part of Russia (Prokina et al. 2017a; Prokina et al. 2018). Larger and smaller spine-scales were observed in several studies: 9–30 μm (Leonov 2009), 8–26 μm (Leonov 2010), 8–26 μm (Leonov and Mylnikov 2012), 9–25 μm (Leonov and Plotnikov 2009), 8–33 μm

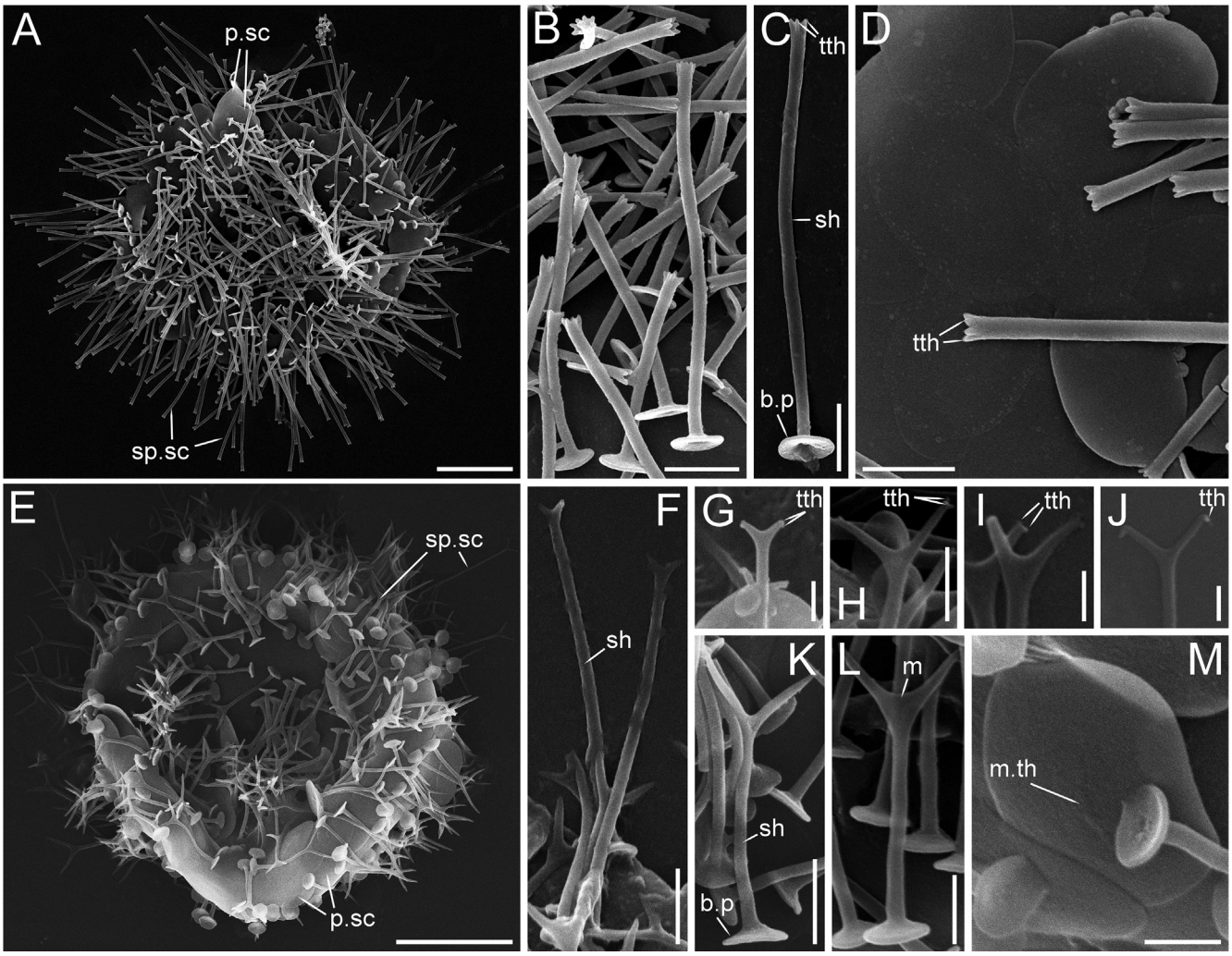


Fig. 2. Morphology of observed scales of the genus *Acanthocystis* (SEM). A–D – *A. penardi*: A – general view of the dried cell; B, C – spine-scales; D – plate-scales and distal tips of the spine-scales; E–M – *Acanthocystis* aff. *turfacea*; E – general view of the dried cell (from the sampling site No. 20); F – long and short spine-scales (from the sampling site No. 7); G–J – distal tips of the long spine-scales: (G – from the sampling site No.7; H, I – from the sampling site No. 2; J – from the sampling site No. 20); K–L – short spine-scales (K – from the sampling site No. 20; L – from the sampling site No. 2), M – plate-scale (from the sampling site No. 2). Abbreviations: b.p – basal plate; m – membrane; m.th – medial thickening; p.sc – plate-scale; sh – shaft; sp.sc – spine-scale; tth – teeth. Scale bar: A – 10 μm; E – 5 μm; B–D – 2 μm; F–K – 1 μm; G, H, L, M – 0.5 μm; I, J – 0.25 μm.

(Mikrjukov 1993b), 3.9–6.6 μm (Dürrschmidt 1987a), and 3.2–6.7 μm (Kosolapova and Mylnikov 2015).

Acanthocystis* aff. *turfacea Carter, 1863 (Fig. 2E–M)

Material: 3 cells from the sampling site No. 2; 11 cells from the site No. 7; 2 cells from the site No. 20 (see Table 1).

Description: The skeletal elements of all studied organisms had a similar type of structure. The surface of the cells is covered with plate- and spine-scales of two types. Both types of spine-scales consist of a hollow cylindrical shaft and a circular basal plate. The apex of the spine-scale is furcate with each of the resulting two branches tapering to the top. Short scales have longer and wider apical branches with a small membrane between them (see Supplementary Table S2 for detailed morphometric information).

The diameter of the cells of specimens from the sampling site No. 2 is about 10 μm. Long spine-scales 3.8–4.2 μm in length have 2 or 3 small teeth (0.41–0.57 μm in length) at the tops of each branches (Fig. 2H, I). Short spine-scales 2.5–2.6 μm in length have sharpened 0.60–0.70 μm long apical branches (Fig. 2L). Elliptical plate-scales 1.7–2.5 × 1.2–1.3 μm, with a medial thickening of 0.60–0.74 × 0.06–0.08 μm (Fig. 2M).

The diameter of the cells of specimens from the site No. 7 is about 12 μm. Long spine-scales are 3.7–6.5 μm in length (Fig. 2F). Apical branches are 0.21–0.39 μm in length bent outward in the distal part, terminating in two small teeth (Fig. 2G). Short spine-scales 2.3–2.7 μm in length bear pointed 0.48–0.72 μm apical branches. Elliptical plate-scales are 2.2–3.0 × 1.5–1.8 μm, with a medial thickening of ~ 0.80× 0.09 μm.

The diameter of the cells of specimens from the site No. 20 is about 12 μm (Fig. 2E). Long spine-scales 3.8–4.2 μm in length are rare. Apical branches 0.40–0.53 in length end with two teeth bent outwards (Fig. 2J). Short spine-scales 2.6–3.7 in length bear pointed apical branches 0.59–1.04 μm (Fig. 2K). Elliptical plate-scales are 2.3–2.9 × 1.4–1.7 μm with a medial thickening of 1.09 × 0.06 μm.

Remarks: Cells from different habitats have similar morphometric parameters, but there are some variations in the structure of the distal parts of the spine-scales. In previous works, significant differences were noted in the size and structure of the cover elements (see Prokina et al. 2020). Leonov (2012) noted two variations of *A. turfacea* in his Ph.D. thesis, calling them "*gigas*" and "*viridis*" but not formally describing them. Two different morphological forms tentatively designated as "*gigas*" and "*viridis*" were also noted in subsequent papers (Prokina and Philippov 2019; Prokina et al. 2017a). Prokina et al. (2019) found *A. turfacea*-like organisms in marine waters with two rounded apical branches on spine-scales, with the weakly expressed membrane. Prokina et al. (2020) noted narrower plate-scales and longer apical branches on long spine-scales. The cells described in this article are also different from previous descriptions. Thus, the observed cell covers are more similar to the so-called "viridis" form having similar dimensions. But the presence of long spine-scales with proximally bent apical branches and long spine-scales with denticles on the apical branches of so-called "*viridis*" cells have not been previously noted. There are likely several different species among the listed morphological forms described as *A. turfacea*.

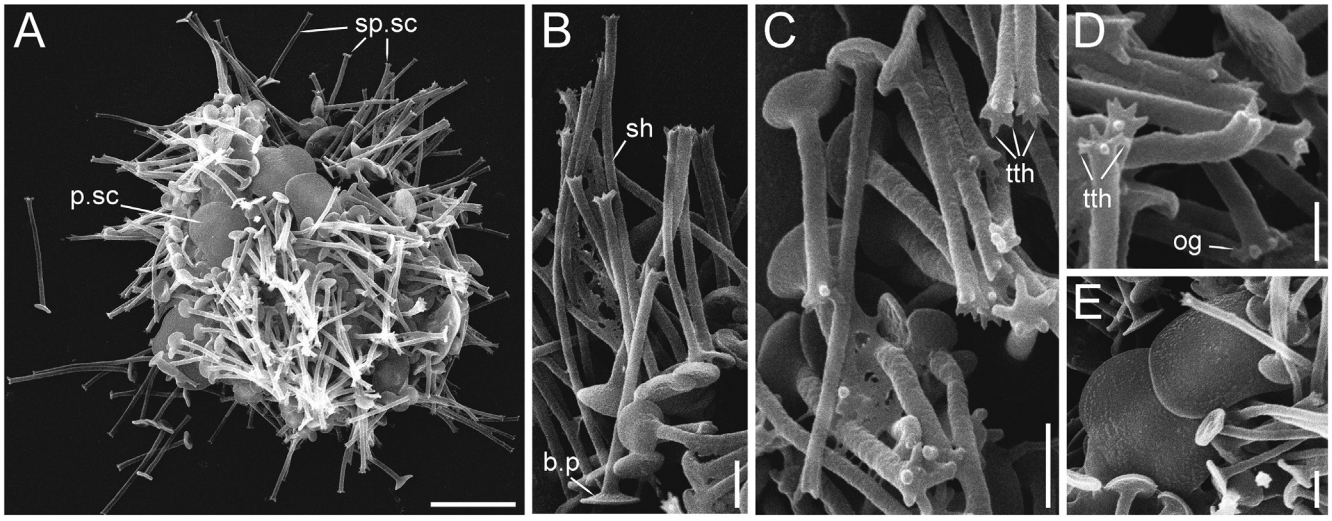


Fig. 3. Morphology of observed scales of *Acanthocystis* sp. 1 (SEM). A – general view of the dried cell; B–C – spine-scales; D – distal tips of the spine-scales; E – plate-scale; Abbreviations: b.p – basal plate; og – outgrowths; p.sc – plate-scale; sh – shaft; sp.sc – spine-scale; tth – teeth. Scale bar: A – 5 μm; B, C, E, – 1 μm; D– 0.5 μm.

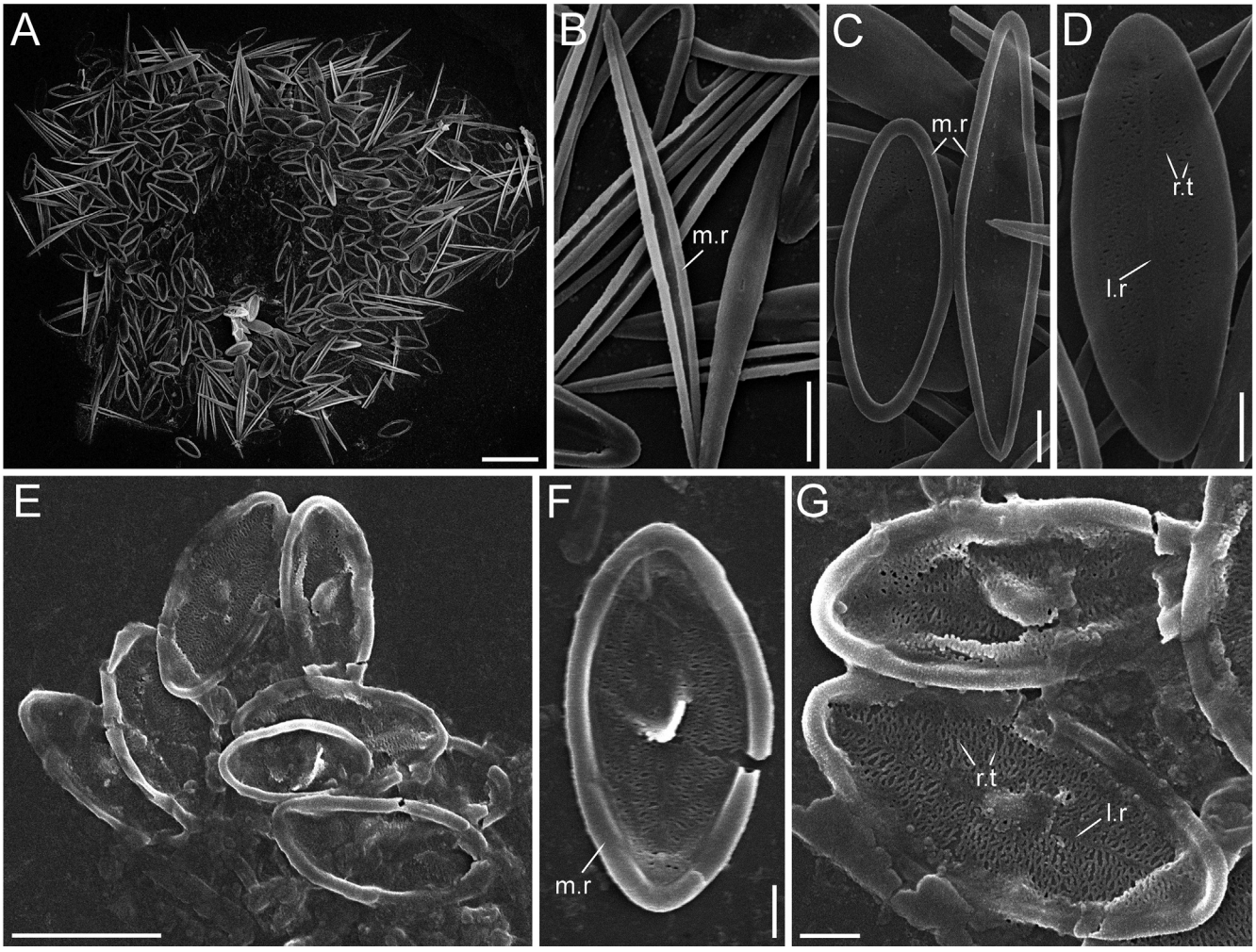


Fig. 4. Morphology of cells and scales of the genus *Raphidocystis* (SEM). A–D – *R. ambigua*: A – general view of the dried cell; B – plate-scales of the first type; C – plate-scales of the second and third types; D – back view of the plate-scale; E–G – *R. symmetrica*: E – general view of the dried cell; F–G – plate-scales. Abbreviations: l.r – longitudinal rib; m.r – marginal rim; r.t – reticular texture. Scale bar: A – 10 μm; E – 5 μm; B–D, F, G – 1 μm.

Further molecular phylogenetic studies and observations in clonal cultures are required to understand the taxonomic status of these organisms.

***Acanthocystis* sp. 1** (Fig. 3A–E)

Material: 1 cell from the sampling site No. 22 (see Table 1).

Description: The diameter of the live cell is about 18 μm. The surface of the cell is covered with spine- and plate-scales (Fig. 3A). Spine-scales are 3.4–9.5 μm long, consist of a basal plate, a shaft, and a denticulate apex. A circular basal plate is 1.1–1.3 μm in diameter (Fig. 3B, C). The shaft is 0.23–0.30 μm in diameter, straight or slightly curved (Fig. 3B, C). There are 4–7 uneven teeth of different lengths from 0.12 to 0.33 μm on the apical part of the spine-scales (Fig. 3C–D). Some of the teeth extend perpendicularly from the shaft. Some teeth have proximally curved outgrowths (Fig. 3D). The apical part of the spine-scales is 0.43–0.87 μm wide. Plate-scales are elliptical 3.5–4.5 × 2.8–3.2 μm with a small medial constriction (Fig. 3E). The surface of the plate-scales is granular, without pronounced medial thickening.

Remarks: Similar to *A. taurica* Mikrjukov, 1997 by the presence of 5–7 apical teeth. However, in *A. taurica* the teeth are of equal size, smooth, and not bifurcated. Also, the plate-scales of *A. taurica* are elliptical without constriction, whereas in the species examined, the plate-scales have medial constrictions.

●●●●●● Raphidocystidae Zlatogursky, 2018.

●●●●●●● *Raphidocystis* (Penard, 1904) Zlatogursky, 2018.

Raphidocystis ambigua (Penard, 1904) Zlatogursky, 2018 (Fig. 4A–D)

Material: 11 cells from the sampling site No. 14 (see Table 1).

Description: The diameter of the live cells is about 30–40 µm. The surface of the cells is covered with three types of plate-scales (Fig. 4A). Scales of the first type are fusiform 10.5–13.9 × 0.6–1.1 µm with sharp ends (Fig. 4B). Scales of the second type are elongated-oval, 5.6–10.8 × 1.2–2.0 µm (Fig. 4C). Scales of the third type are oval, 4.6–8.1 × 1.7–2.9 µm (Fig. 4C, D). All types of scales have a hollow cylindrical marginal rim 0.22–0.42 µm wide (Fig. 4C). The inner surface of the scales has a reticular texture (Fig. 4D). There is a faint longitudinal rib on the outer side of the scales. The scales of the first type were predominantly localized around the basal portions of the axopodia and located in a longitudinal direction. The scales of other types were located tangentially on the cell surface.

Remarks: The morphology and size of scales of the investigated cells are similar to the earlier descriptions.

Raphidocystis symmetrica (Penard, 1904) Zlatogursky, 2018 (Fig. 4E–G)

Material: 3 broken cells from the sampling site No. 24 (see Table 1).

Description: The surface of the cells is covered with one type of elliptical scales with a reticular texture of the surface (Fig. 4E–G). The scales are oval or elongated-oval 5.1–7.7 × 2.7–4.2 µm in size (Fig. 4E). Marginal rim is 0.27–0.52 µm in width (Fig. 4F). Scale length to width ratio is 1.8–2.5. The longitudinal rib is visible in the central part of the scale and divides the scale into two halves (Fig. 4G).

Remarks: Similar scales are present in *Raphidocystis tubifera* Penard, 1904. However, the funnel-shaped scales characteristic of *R. tubifera* were not recorded in the light and electron microscope. The morphology and sizes of skeletal elements are consistent with the descriptions in other studies. Scales of slightly shorter length, up to 3.9 µm (Ikävalko et al. 1996; Zagumyonnyi et al. 2020a) or slightly longer length, up to 9.6 (Prokina et al. 2017a), as well as scales of smaller width of 1.8 µm (Prokina et al. 2019) and 2.0 µm (Prokina et al. 2017a; Prokina et al. 2018) were found in previous investigations. The widest known scales (up to 4.17 µm) were revealed in our study. *R. symmetrica* has not previously been recorded in brackish waters.

This is the first validated by electron microscopy finding of *R. symmetrica* in Ukraine. It is not possible to distinguish species of the genus *Raphidocystis* in a light microscope, however, *R. symmetrica* was noted from freshwater biotopes in the vicinity of Kiev in a light microscope investigation by Dobrovlyanskij (1914).

●●● Pterocystida Cavalier-Smith et von der Heyden, 2007 emend. Shishkin et Zlatogursky, 2018

●●●● Raphidista Shishkin et Zlatogursky, 2018

●●●●● Choanocystidae Cavalier-Smith et von der Heyden, 2007

●●●●●● *Choanocystis* Penard, 1904

Choanocystis aff. *perpusilla* (Petersen et Hansen 1960) Siemensma, 1991 (Fig. 5A–E)

Material: 15 cells from the sampling site No. 19 (see Table 1).

Description: The diameter of the live cells is about 8 µm. Surface of cells is covered with spine- and plate-scales (Fig. 5A). The length of the spine-scales is 1.4–8.1 µm (Fig. 5B). Spine-scales consist of a hollow, non-narrowing cylindrical shaft 0.13–0.23 µm in diameter, which asymmetrically sitting on a heart-shaped flattened 0.8–1.6 µm wide base with a well-defined marginal rim (Fig. 5B, C).

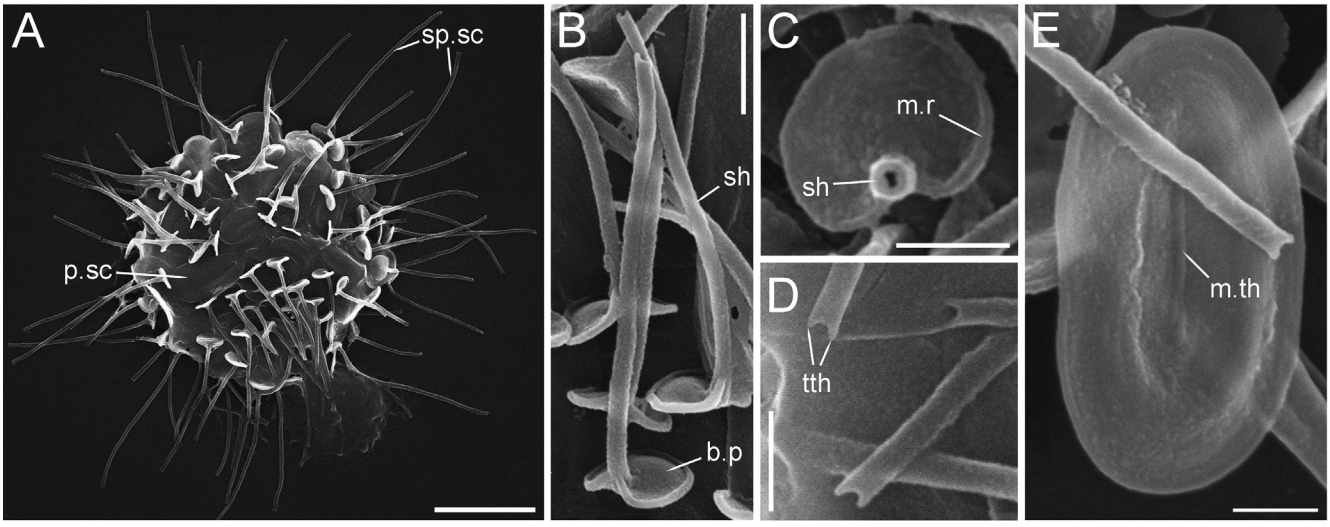


Fig. 5. Morphology of observed scales of *Choanocystis* aff. *perpusilla* (SEM). A – general view of the dried cell; B – spine-scales; C – basal plate of the spine-scale; D – distal tips of the spine-scales; E – plate-scale. Abbreviations: m.r – marginal rim; m.th – medial thickening; p.sc – plate-scale; sh – shaft; sp.sc – spine-scale; tth – teeth. Scale bar: A – 5 µm; B – 1 µm; C–E – 0.5 µm.

The shaft of the scales is not straight, and ends with two small teeth on the distal part (Fig. 5D). Plate-scales are oval or ovoid, 1.4–3.7 × 0.9–2.1 μm with a large noticeably depressed central area. Medial thickening is 0.53–1.51 × 0.05–0.09 μm (Fig. 5E).

Remarks: Since the original description of the species now called *Ch. perpusilla*, many morphologically similar organisms have been found. Their main common morphological feature was the presence of two teeth on the apex of the spine-scales. However, such features as the shape of plate-scales, the presence of medial thickening (or axial ridges), the length of spine-scales, and the degree of constriction of spine-scales vary greatly (Gerasimova 2022). Gerasimova (2022) described *Choanocystis mylnikovi* which is similar to the specimens we found by a medial thickening (axial ridge) on the plate-scales and longer spine-scales compare to the original description (2.0–12.9 μm *vs* 2.0–4.5 μm). However, *Ch. mylnikovi* differs from our isolate in the greater length of spine-scales (12.9 μm *vs* 8.1 μm), a more pronounced medial thickening (axial ridge), and the presence of a tapering at apex of spine-scales. Also, *Ch. mylnikovi* was isolated from the saline (20–22 ‰) waters of the Tuzlukkol' River, whereas our isolate is freshwater. Due to the lack of molecular data and information on the variability of skeletal elements of *Ch. perpusilla* and *Ch. mylnikovi*, accurate identification of studied isolate is difficult. Morphology and sizes of the observed scales are closest to the descriptions of *Choanocystis perpusilla* in Leonov (2010) and Prokina et al. (2020). This species was found in Ukraine for the first time.

●●●● Pterista Shishkin et Zlatogursky, 2018

●●●●● Pterocystidae Cavalier-Smith et von der Heyden, 2007

●●●●●● *Pterocystis* Siemensma et Roijackers, 1988

Pterocystis* aff. *pinnata (Nicholls, 1983) Siemensma et Roijackers, 1988 (Fig. 6A–D)

Material: 3 cells from the sampling site No. 17 (see Table 1).

Description: The diameter of the live cells is about 8 μm. The surface of the cells is covered with plate- and spine-scales (Fig. 6A). Spine-scales are 3.7–5.1 μm in length. They consist of a shaft, a basal wing, and lateral wings (Fig. 6B, C). The shaft is hollow, 0.14–0.17 μm in diameter, curved, tapering to a rounded tip. The proximal part is represented by a basal wing, located at the right angles to the shaft and connected with two lateral wings stretching on both sides of the shaft. The lateral wings in the widest part are of 1.3–2.0 μm. The lateral wings taper towards the apex of the scale and cover 2/3 of its entire length. The inner side of the lateral wings is smooth and without radial ribs. The basal wing and lateral wings of scales form a bucket-like structure of various shapes, from leaf-shaped to triangular and square. Plate-scales are oval, 1.9–2.2 × 1.4–1.6 μm, and textureless (Fig. 6D).

Remarks: The found specimens differs from the majority of previously described cells by the less distinct shoulders of lateral wings on spine-scales. The dimensional characteristics are similar to those described by other authors. This species was found in Ukraine for the first time.

Pterocystis tropica (Dürrschmidt, 1987) Siemensma et Roijackers, 1988 (Fig. 6E–H)

Material: 6 cells from the sampling site No. 3 (see Table 1).

Description: The diameter of the cells is about 8–10 μm. Skeletal elements are represented by spine- and plate-scales (Fig. 6E). Spine-scales are 2.6–6.7 μm in length, consist of a shaft, a basal wing, and lateral wings (Fig. 6F, G). The shaft is hollow, 0.14–0.20 μm in diameter, slightly curved, tapering to a sharp tip. A basal wing is located at right angle to the shaft and connected with two lateral wings stretching on both sides of the shaft. Lateral wings in the widest part are of 1.3–2.0 μm. The edges of the wings are uneven, sometimes asymmetric. Rare ribs extend from the shaft on the inner side of the lateral wings of some scales. Plate-scales are oval, 2.1–3.5 × 1.3–2.0 μm. There are radial slits and a small medial thickening 0.74–0.95 μm in length (Fig. 6H).

Remarks: Morphology and sizes of the observed scales are closest to the descriptions of *P. tropica* by Dürrschmidt (1987b), Prokina et al. (2017a), and Zagumyonnyi et al. (2020a). The description of *Acanthocystis tropica* ssp. *paucistriata* (Dürrschmidt 1987b) and *P. tropica* (Prokina et al. 2017b) indicates the presence of a small number of radial ribs on the lateral wings of the spine-scales and the absence of radial slits on the plate-scales. The description of *A. tropica* ssp. *tropica* (Dürrschmidt 1987b) and *P. tropica* (Zagumyonnyi et al. 2020a) instead, shows a large number of radial ribs on the lateral wings of the spine-scales and the presence of radial slits on the plate-scales. The cells we studied, however, possess common features: the lateral wings have a small number of radial ribs and, at the same time, the plate-scales have radial slits. In contrast to *P. quadrata* Cavalier-Smith and von der Heyden, 2007, the studied cells have the ribs on the lateral wings and slits on the plate-scales. This species was found in Ukraine for the first time.

●●●●●● *Raineriophrys* Mikrjukov, 2000

Raineriophrys erinaceoides (Petersen et Hansen, 1960) Mikrjukov, 2001 (Fig. 7A–J)

Material: 9 cells from the sampling site No. 9; 88 cells from the sampling site No. 13; 34 cells from the site No. 15 (see Table 1).

Description: Skeletal elements were represented by plate- and spine-scales. Specimens from different sampling sites were characterized by slightly morphologically different scales of two types. Spine-scales of the first type (sampling site No. 9; Fig. 7A–E) consist of a hollow cylindrical

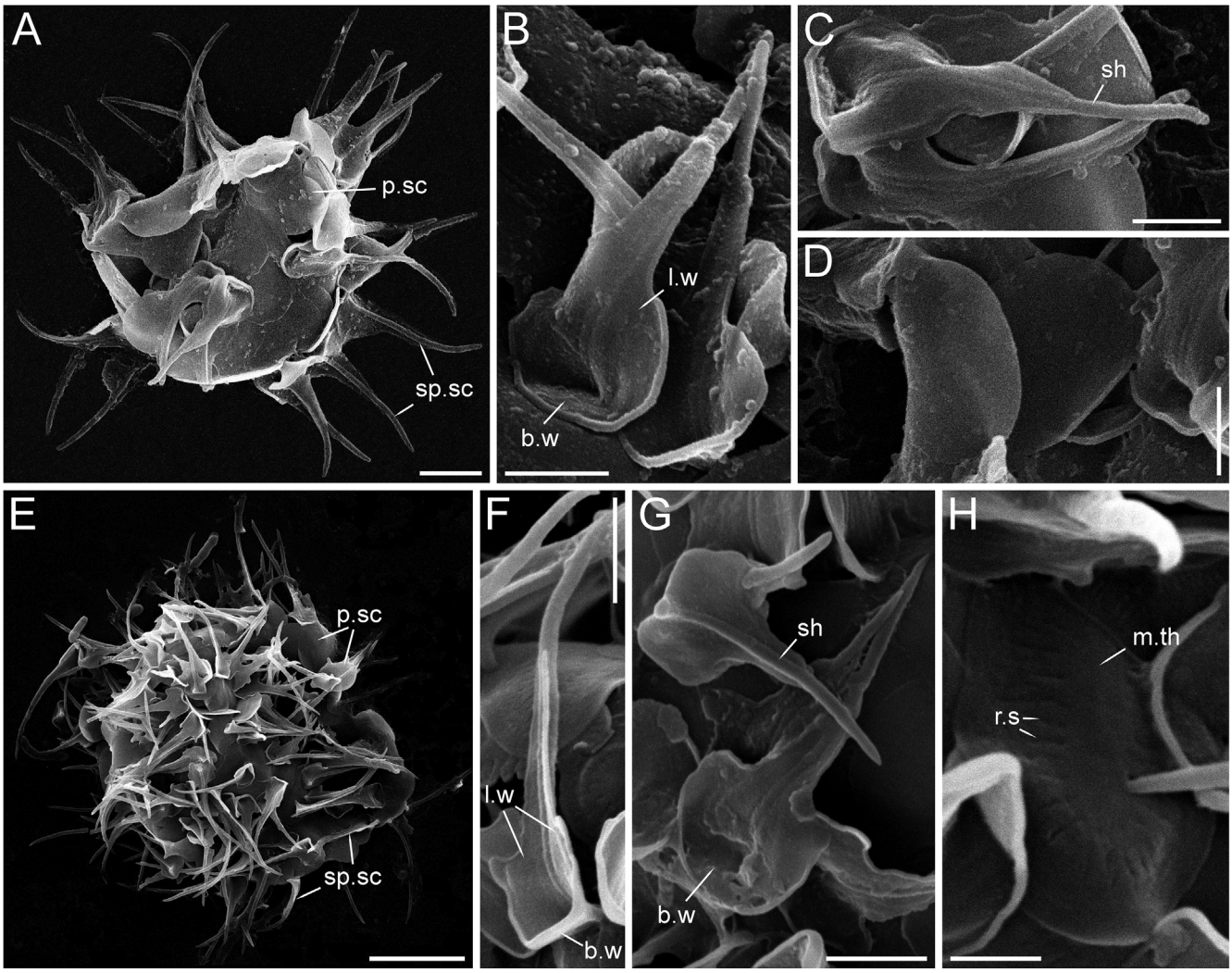


Fig. 6. Morphology of observed scales of the genus *Pterocystis* (SEM). A–D – *P.* aff. *pinnata*: A – general view of the dried cell; B, C – spine-scales; D – plate-scales; E–H – *P. tropica*: E – general view of the dried cell; F, G – spine-scales; H – plate-scale. Abbreviations: b.w – basal wing; l.w – lateral wing; m.th – medial thickening; p.sc – plate-scale; r.s – radial slits; sh – shaft; sp.sc – spine-scale. Scale bar: E – 5 μm; A – 2 μm; B–D, F, G – 1 μm; H – 0.5 μm.

shaft, lateral and basal wings (Fig. 7B–D). The shaft is 7.2–11.2 μm in length and 0.28–0.35 μm in diameter (in the widest part), conically tapers to the pointed tip. Lateral wings are 3.7–5.3 μm in length, textureless, have smooth edges, tapering from the base to distal end. They cover ⅓ to ⅔ of the shaft length. Some of the scales have asymmetric lateral wings. The basal and lateral wings together form a scoop-like structure 1.3–2.8 μm in width. Oviform plate-scales are 4.1–5.8 × 2.2–3.1 μm, with medial ridge 2.3–4.1 × 0.35–0.50 μm (Fig. 7E), and without radial slits.

The shaft of spine-scales of the second type (sampling site No. 13; No. 15; No. 27; Fig. 7F–J) is 4.3–17.6 μm in length and 0.21–0.39 μm in diameter (in the widest part), conically tapers to pointed tip (Fig. 7G). The shaft is often curved in the basal part of the scale (Fig. 7H). Narrow lateral wings are 2.7–10.1 μm in length with jagged edges, tapering from the base to distal end. They cover ½ to ¾ of the shaft length. The basal and lateral wings together form a scoop-like structure 1.4–2.3 μm in width (Fig. 7G–I). Oviform plate-scales are 2.7–5.9 × 1.7–3.0 μm with axial ridge (2.0–4.5 × 0.21–0.52 μm) and narrow radial slits (Fig. 7J).

Remarks: The species was originally described as *Acanthocystis erinaceoides* Petersen et Hansen, 1960 from a freshwater biotope near Vassingerød, Denmark. Dürrschmidt (1987b) described two subspecies *A. erinaceoides sculpta* and *A. erinaceoides undulata*. *A. erinaceoides sculpta* differs from *A. erinaceoides erinaceoides* by plate-scales with numerous 0.4 μm long cross-slits extending from the central swelling and ending at some distance from the peripheral rim. Also, longitudinally elongated lateral wings on the spine-scales of *A.*

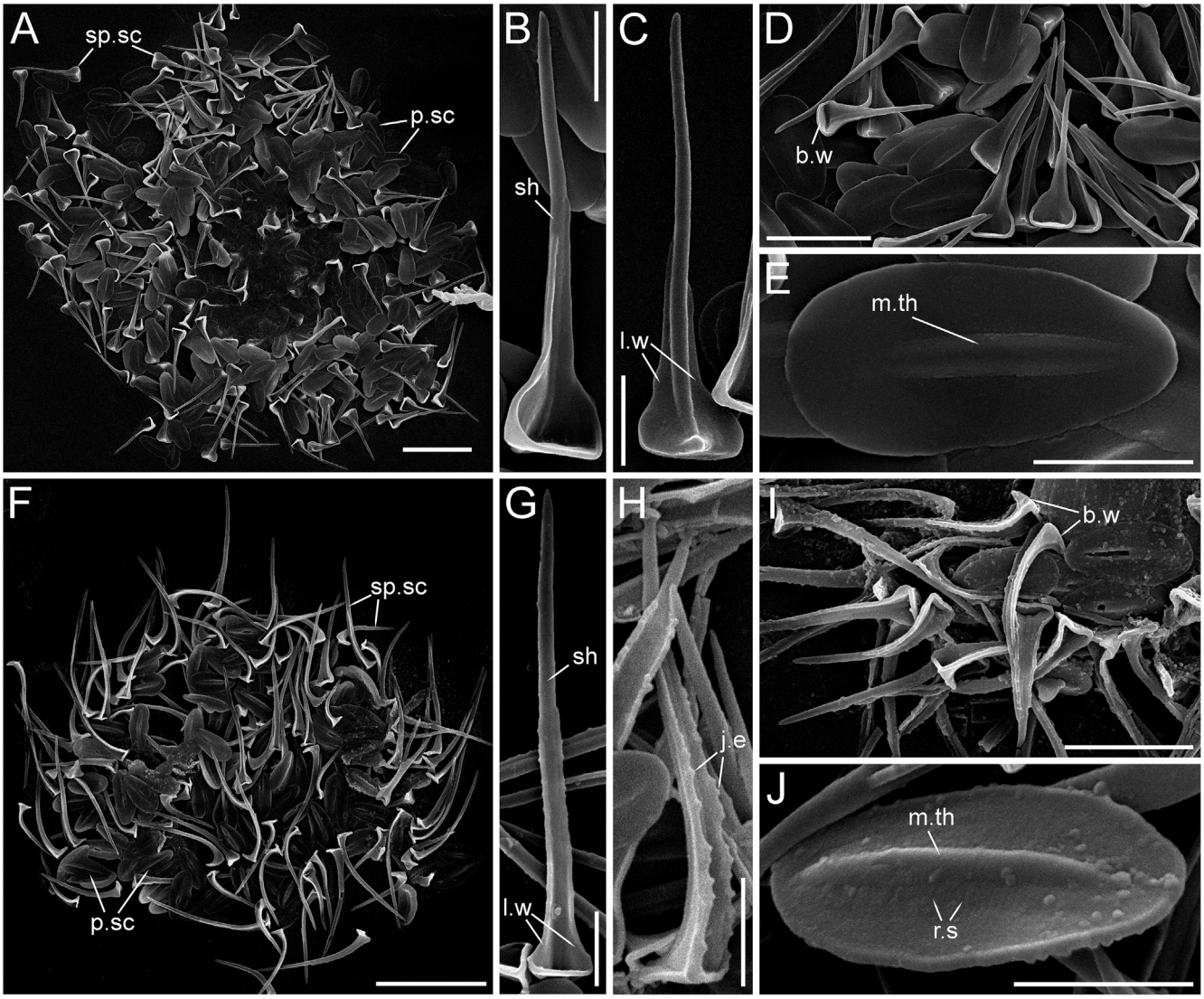


Fig. 7. Morphology of observed scales of *Raineriophrys erinaceoides* (SEM). A–E – *R. erinaceoides* with spine-scales of the first type: A – general view of the dried cell; B, C – spine-scales; D – spine- and plate-scales; E – plate-scale; F–J – *R. erinaceoides* with spine-scales of the second type: F – general view of the dried cell; G–H – spine-scales; I – spine- and plate-scales; J – plate-scale. Abbreviations: b.w – basal wing; j.e – jagged edges; l.w – lateral wing; m.th – medial thickening; p.sc – plate-scale; r.s – radial slits; sh – shaft; sp.sc – spine-scale. Scale bar: A, F – 10 μm; D, I – 5 μm; B, C, E, G, H, J – 2 μm.

erinaceoides sculpta often reach the distal third of the shaft (Dürrschmidt 1987b). Later, *Acanthocystis erinaceoides* was transferred to the genus *Pterocystis* (Siemensma 1991), then moved to *Echinocystis* (Mikrjukov 1997) and *Raineria* (Mikrjukov 1999), and finally to *Raineriophrys* (Mikrjukov and Milyutina 2001).

R. erinaceoides has been observed quite frequently. Three types of spine-scales can be conditionally distinguished: scales with smooth lateral wings not exceeding half of the length of the shaft (Leonov and Plotnikov 2009; Plotnikov and Ermolenko 2015; Nicholls 1983; Mikrjukov 1993a; 1993b; Croome 1986; Mikrjukov and Milyutina 2001; Prokina et al. 2018); scales with jagged margins of lateral wings whose length exceeds 2/3 of the shaft length (Prokina et al. 2020; Zlatogursky 2013a, 2013b; Mikrjukov 1999; Bessudova et al. 2022); and scales with jagged margins of lateral wings whose length does not exceed half the length of the shaft (Plotnikov and Gerasimova 2017). As for plate-scales, most papers lack detailed descriptions and close-up photographs, therefore, it is difficult to judge about the presence or absence of radial slits.

Such a contrasting morphology of the different findings of *R. erinaceoides* seems to indicate that these are different species. Molecular phylogenetic studies and careful investigations of the variability of the scales in monoclonal

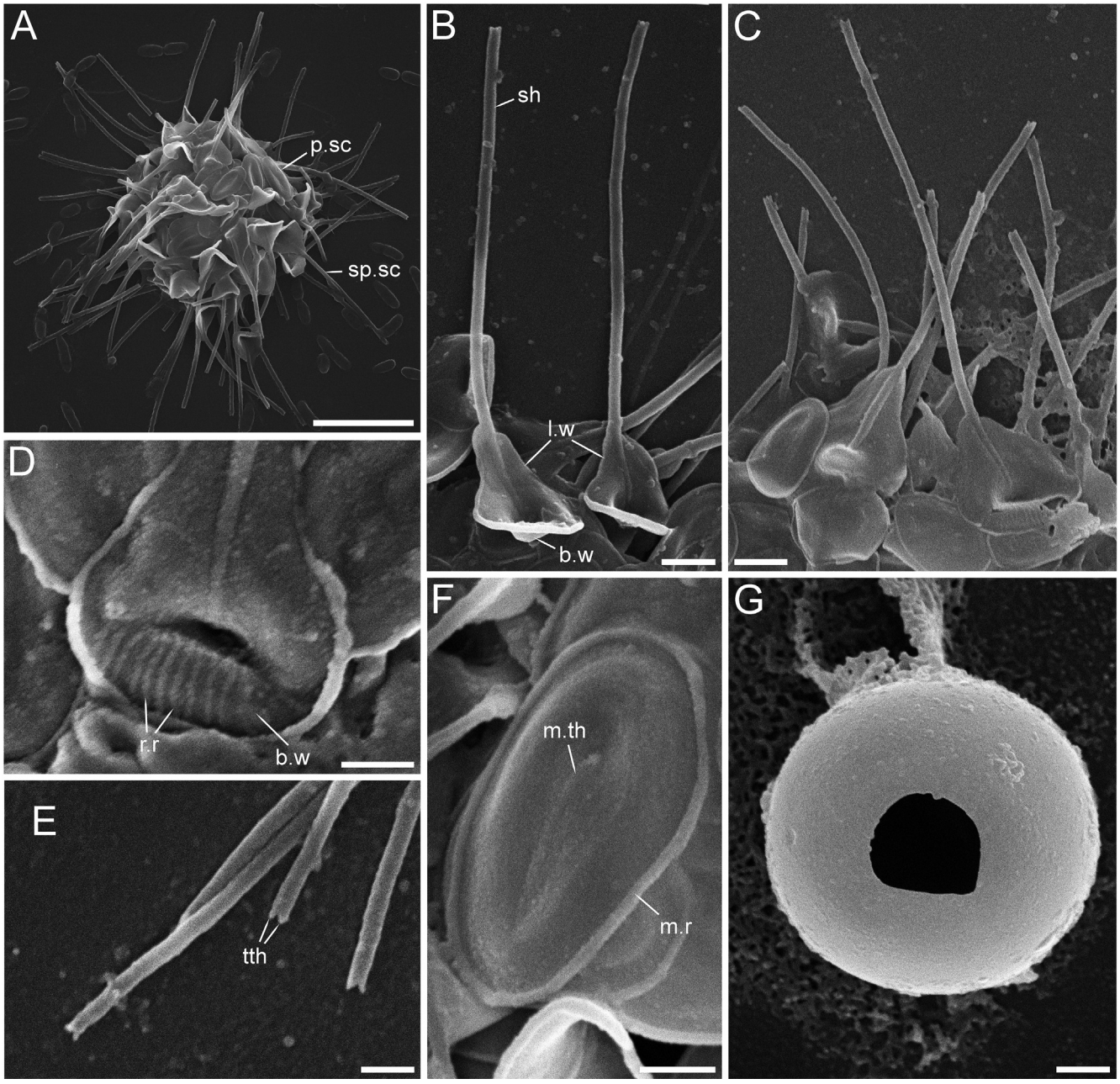


Fig. 8. Morphology of *Raineriophrys fortesca* with the spine-scales of the first type (SEM). A – general view of the dried cell; B, C – spine-scales; D – basal part of spine-scale; E – distal part of spine-scales; F – plate-scales; G – cyst. Abbreviations: b.w – basal wing; l.w – lateral wing; m.th – medial thickening; m.r – marginal rim; p.sc – plate-scale; r.r – radial ribs; sh – shaft; sp.sc – spine-scale; tth – teeth. Scale bar: A – 5 μm; B, C, G – 1 μm; D–F – 0.5 μm.

cultures is necessary for understanding the taxonomic status of the different morphological forms.

Raineriophrys* aff. *fortesca (Nicholls, 1983) Mikrjukov, 1999 (Fig. 8)

Material: 10 cells from the sampling site No. 5; 11 cells from the site No. 6; 1 cell from the sampling site No. 7; 9 cells from the site No. 16; 4 cells from the site No. 18; 3 cells from the site No. 19 (see Table 1).

Description: The cell surface is covered with plate- and spine-scales. Cells with two different types of spine-scales were observed. The diameter of the cells with the spine-scales of the first type is about 5 μm (sampling sites No. 5, No. 6, No. 16, No. 18; Fig. 8). The spine-scales are 3.4–11.4 μm in length. They consist of a shaft, a basal wing, and lateral wings (Fig. 8B, C). The shaft is hollow, 0.15–0.24 μm in diameter, straight or slightly curved.

About 4 teeth, 0.05–0.13 μm long, are located at the distal end of the shaft (Fig. 8E). The proximal part of the spine-scale is in the shape of a pear-shaped or triangular bucket, formed by the lateral and basal wings (Fig. 8D). The lateral wings in the widest part are of 1.1–2.3 μm. The length of the lateral wings is 1.3–2.4 μm. The inner side of the basal wings have 13–18 parallel ribs. The inner part of the lateral wings also has 2–7 ribs perpendicular to the shaft. (Fig. 8D). Plate-scales are oval, 1.6–3.0 × 1.0–1.9 μm, with medial depression, medial thickening of 0.8–1.4 × 0.038–0.072 μm, and 0.04–0.11 μm thick marginal rim (Fig. 8F).

Besides the cells on the trophic stage, a spherical cyst 6.2 μm in diameter was observed. It was composed of presumably organic material and possess an almost circular aperture about 2 μm in diameter (Fig. 8G).

The diameter of the cells with the spine-scales of the second type is about 5 μm (sampling sites No. 7 and No. 19; Fig. 9). Spine-scales are 4.1–11.6 μm long, consist of a shaft, a basal, and lateral wings. Hollow shaft is 0.15–0.25 μm in diameter, with small denticles (ca. 3–5) on an apical part. The basal wing and lateral wings form a subcircular plate of 1.3–2.8 × 1.7–3.0 μm with depression in the center in the proximal part of the scale (Fig. 9A–C). Scales from the sampling site No. 7 have radially diverging ribs (Fig. 9D) on the inner surface of the subcircular plate. The inner surface of the subcircular plates of the specimens from sampling site No. 19 is smooth. Plate-scales are elliptic 1.9–2.7 × 1.1–1.8 μm with medial depression, weak medial thickening (0.89–0.98 × 0.04–0.08 μm), and marginal rim 0.06–0.11 μm wide (Fig. 9E).

Remarks: Two morphologically similar species *Acanthocystis fortesca* and *A. pantopodeoides* were described by Nicholls (1983). He noted two main differences between these species: "The ratio of spine length to cell diameter and the spine shaft diameter is about two times greater in *A. pantopodeoides*. The shape of the membrane on the spine base of *A. fortesca* is obovoid in outline compared to the truncate-spatulate shape of the corresponding structure in *A. pantopodeoides*". Later, Dürrschmidt described *A. cuneiformis* (Dürrschmidt 1985), which is morphologically coincide with *A. pantopodeoides*. Croome (1986) then listed *A. pantopodeoides* as a junior synonym of *A. cuneiformis*. Siemensma and Roijackers (1988a) described a new genus *Pterocystis* and transferred *A. fortesca* to *Pterocystis*. They also synonymized *A. fortesca* and *A. pantopodeoides* based on findings of cells having both obovoid and truncate-spatulate shape of the basal part of scales, as well as presence of intermediate shapes within one cell. Mikrjukov later transferred *P. fortesca* to the genus *Raineriophrys* (Mikrjukov 2002).

We have noted cells with pear-shaped and triangular basal parts of spine-scales from four sampling sites, and cell with subcircular basal parts of spine-scales from two other sampling sites. No intermediate shapes of scales were found within a cell, as noted by Siemensma and Roijackers (1988a). The described above cells with a spine-scales of the first type are more similar to *A. pantopodeoides* described by Nichols, whereas the second type is more similar to *A. fortesca*.

Previous study reported spine-scales with triangular bases without ribs (Prokina and Mylnikov 2019). In contrast, scales of the cells from Vietnam had radial ribs not only on the basal wing but also on the inner surface of the lateral wings (Prokina et al. 2020). Cells of *R. fortesca*

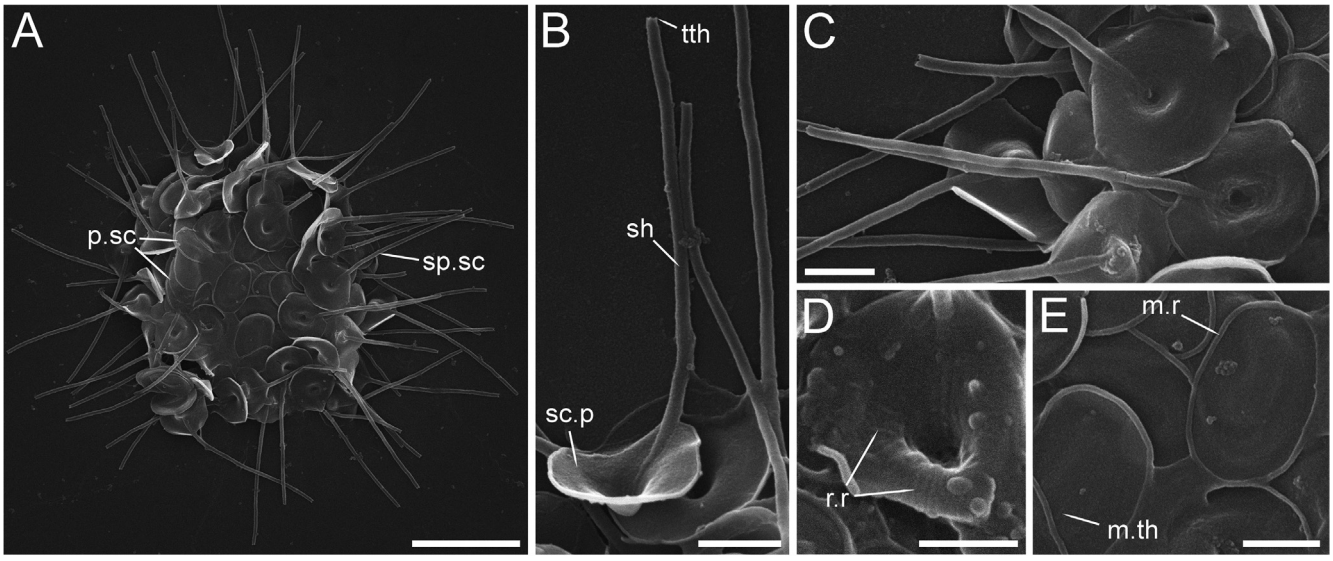


Fig. 9. Morphology of *Raineriophrys* aff. *fortesca* with the spine-scales of the second type (SEM). A – general view of the dried cell; B, C – spine-scales; D – subcircular plate of spine-scales of cells from the sampling site No. 7; E – plate-scales. Abbreviations: sc.p – subcircular plate; l.w – lateral wing; m.th – medial thickening; m.r – marginal rim; p.sc – plate-scale; r.r –radial ribs; sh – shaft; sp.sc – spine-scale; tth – teeth. Scale bar: A – 5 μm; C – 2 μm; B, D, E – 1 μm.

with much smaller spine-scales (2.9–4.1 μm) and without ribs were found in marine samples from the Black Sea (Prokina et al. 2019). Also, much longer radial scales (15–24 μm and 19–23 μm) have been reported (Mikrjukov 1993a; Nicholls 1983). As can be seen from the above, either there are a large number of related species with similar morphological features, or *R. fortesca* has a significant variability in the shape of the scales. Molecular phylogenetic studies as well as a careful investigation of the variability of cover structures in monoclonal cultures are necessary for understanding the taxonomic status of the described above forms.

Raineriophrys scaposa (Dürrschmidt, 1987) Mikrjukov, 2001 (Fig. 10A–D)

Material: 1 cell from the sampling site No. 20 (see Table 1).

Description: The diameter of the live cells is about 5 μm. The surface of the cells is covered with plate- and spine-scales (Fig. 10A). Spine-scales are 1.6–2.3 μm long and consist of a shaft, a basal plate and lateral wings (Fig. 10B, C). Shaft is 0.07–0.08 μm wide, not tubular, tapering to a rounded apex. Basal plate is 0.54–0.78 μm in diameter, heart-shaped, and surrounded by a marginal rim. Lateral wings stretch to the apex of the shaft from the basal wing tapering. Plate-scales are oval, 1.2–1.8 × 0.8–1.1 μm, and possess a medial depression with a medial thickening of 0.62–0.83 × 0.06–0.08 μm at the bottom (Fig. 10D).

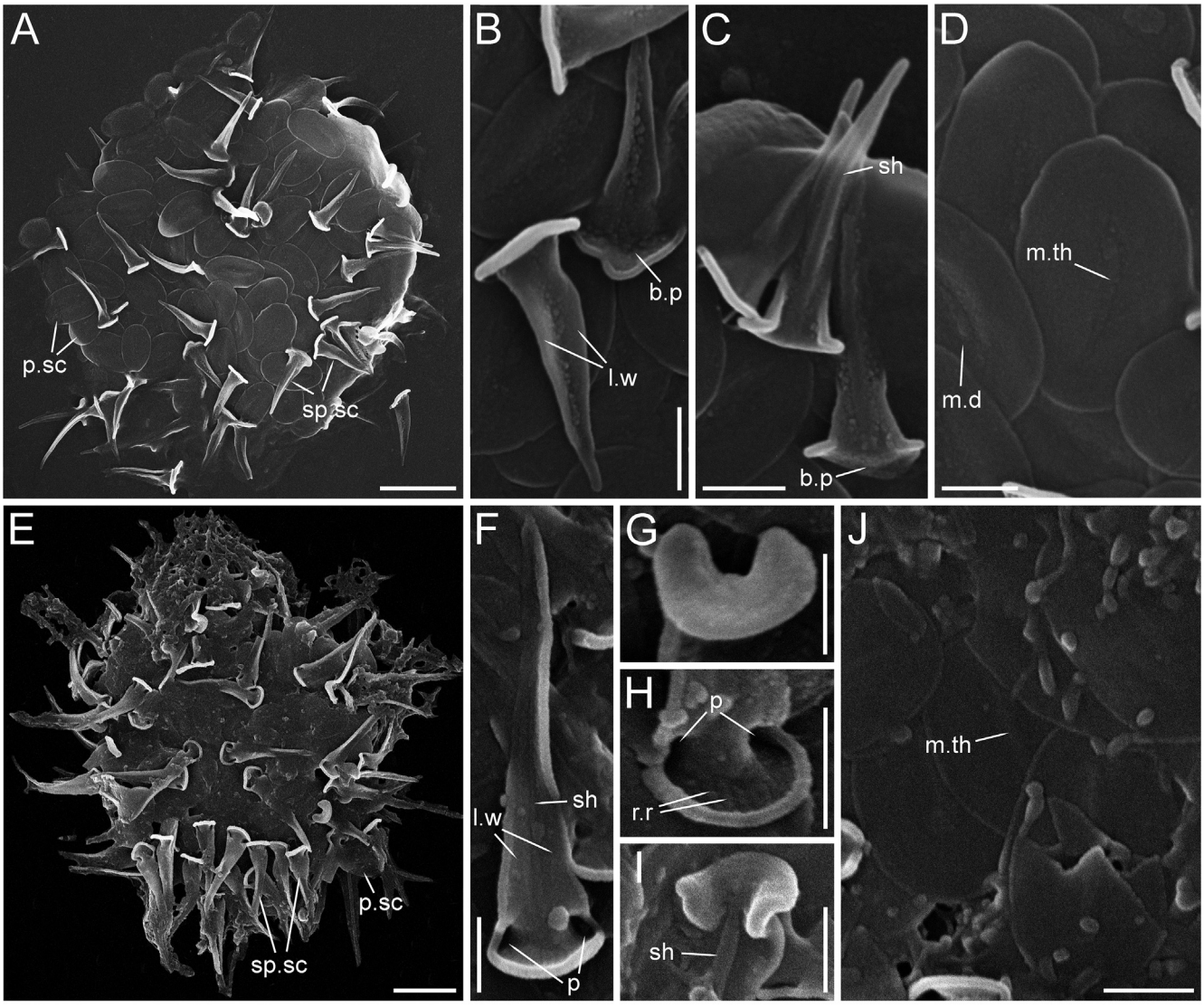


Fig. 10. Morphology of observed scales of *Raineriophrys scaposa* and *Triangulopteris lacunata* (SEM). A–D – *R. scaposa*: A – general view of the dried cell; B. C – spine-scales; D – plate-scales; E–J – *T. lacunata*: E – general view of the dried cell; F – spine-scale; G–I – basal plates of the spine-scales; J – plate-scales. Abbreviations: b.p – basal plate; l.w – lateral wing; m.d – medial depression; m.th – medial thickening; p – pockets; p.sc – plate-scale; r.r – radial ribs; sh – shaft; sp.sc – spine-scale. Scale bar: A. E – 2 μm; B–D, F–J – 0.5 μm.

Remarks: The morphology and sizes of the skeletal elements correspond to the original description (Dürrschmidt 1987a). The sizes of the plate-scales of the cells studied here are somewhat larger than in the original description: 1.2–1.8 × 0.8–1.1 μm *vs* 1.2–1.5 × 0.6–0.8 μm (Dürrschmidt 1987a).

●●●●●● *Triangulopteris* Zagumyonnyi, Radaykina et Tikhonenkov, 2021

Triangulopteris lacunata Zagumyonnyi, Radaykina et Tikhonenkov, 2021 (Fig. 10E–J)

Material: 11 cells from the sampling site No. 17 (see Table 1).

Description: Diameter of the cells is the about 5 μm. The surface of the cells is covered with plate- and spine-scales (Fig. 10E). Spine-scales are 2.1–4.7 μm long and consist of a shaft, a basal plate, and lateral wings (Fig. 10F). Shaft is 0.10–0.11 μm wide, tapering to a more or less sharp apex. Basal plate is in the form of a horseshoe, 0.70–0.90 μm in diameter, surrounded by a thick marginal rim 0.06–0.09 μm in wide. Two so-called "pockets" are formed between the shaft, lateral wings and the "horseshoe" basal plate (Fig. 10F, H). Lateral wings stretch to the apex of the shaft from the basal plate. Radial ribs are located on the inner side of the basal plate and lateral wings. Plate-scales are oval, 1.2–1.6 × 0.7–1.0 μm. The medial thickening of 0.72–0.86 × 0.072–0.085 μm (Fig. 10J) is not clearly pronounced.

Remarks: Partial information about this specimen is given in Zagumyonnyi et al. (2021).

Centroplasthelida *incertae sedis*

***Heterophrys*-like organisms** (Fig. 11)

Material: 5 cells from the sampling site No. 1; 98 cells from the sampling site No. 20; 34 cells from the sampling site No. 25 (see Table 1).

Description: Strain HF-55 from the sampling site No. 20 (Fig. 11A–D).

Live cells are 5–16 μm in diameter (Fig. 11A, B). Axopodia 8–41 μm long. The ratio of axopodia length to cell diameter is 1.47–6.30. Cell diameter and the ratio of axopodia length to cell diameter depends on feeding conditions. The surface of cell is covered with spirally twisted spicules 1.2–6.2 μm in length and 0.02–0.04 μm in width (Fig. 11C, D). The spicules are arranged both radially and irregularly relative to the cell surface.

Specimens from the sampling site No. 25 (Fig. 11E, F)

The diameter of the live cells is about 8 μm. The surface of cells is covered with radially arranged spicules. Needle-shape spicules are 1.9–4.6 μm in length and 0.05–0.07 μm in width, tapering towards pointed ends.

Specimens from the sampling site No. 1 (Fig. 11G, H)

The diameter of the live cells is about 20 μm. The surface of the cells is covered with spicules. Flattened and slightly spirally twisted spicules are 4.0–18.3 μm in length and 0.09–0.14 μm in width, tapering towards pointed ends. The spicules are arranged predominantly tangentially relative to the cell surface.

Remarks: The cells from the sampling site No. 1 have significantly larger spicule sizes than cells from other sampling sites. Also, the location of the spicules from the sampling site No. 1 is more tangential, whereas the cells from the other sampling sites have more radially oriented spicules. Centrohelids with organic spindle-shaped spicules, such as *Heterophrys*, *Marophrys*, *Sphaerastrum*, and *Spiculophrys*, are morphologically indistinguishable. Phylogenetic analysis of 18S rRNA genes showed the presence of at least six unrelated lineages of *Heterophrys*-like organisms in different parts of the centrohelid tree (Zlatogursky 2016). The presence of a spicule-bearing stage in the life cycle of the *Raphidocystis glabra* Dürrschmidt 1985 (Zlatogursky et al. 2018), *Raphidiophrys heterophryoidea* Zlatogursky, 2012 (Zlatogursky 2012; Drachko et al. 2020) and *Triangulopteris lacunata* (Zagumyonnyi et al. 2021) is also shown.

Novel species and genus of centrohelids

DIAPHORETICKES Adl et al., 2012

● Haptista Cavalier-Smith, 2003

●● Order Centroplasthelida Febvre-Chevalier et Febvre, 1984

●●● Panacanthocystida Shishkin et Zlatogursky, 2018

●●●● Acanthocystida Cavalier-Smith et von der Heyden, 2007 emend. Shishkin et Zlatogursky, 2018

●●●●● Chalarothoracina Hertwig et Lesser, 1874 sensu Cavalier-Smith in Yabuki et al., 2012 emend. Shishkin et Zlatogursky, 2018

●●●●●● Family Acanthocystidae Claus, 1874 emend. Shishkin et Zlatogursky, 2018

●●●●●●● *Acanthocystis* Carter, 1863

***Acanthocystis tyrasiana* Zagumyonnyi et Tikhonenkov sp. nov.** (Fig. 12A–G; Supplementary Table S3)

Material: 28 cells from the sampling site No. 4 (see Table 1).

Diagnosis: The diameter of the cell is about 10 μm. Spine-scales 2.9–11.2 μm long, consist of a small circular basal plate 0.61–1.08 μm wide and a hollow non-narrowing cylindrical shaft 0.21–0.31 μm in diameter with 4, less frequently 3, short straight teeth at the top. Plate-scales are oval, textureless, 2.0–3.3 × 1.1–2.4 μm. A short medial thickening is visible on few scales.

Description: The diameter of the live cell is about 10 μm. The surface of the cells is covered with plate-scales and spine-scales (Fig. 12A). Spine-scales 2.9–11.2 μm long, have a small circular basal plate, from which a hollow cylindrical shaft extends. There are 4 or (less frequently) 3 short teeth at the top of the scale (Fig. 12C–E), which never bent to the sides. The diameter of the shaft is the same along the

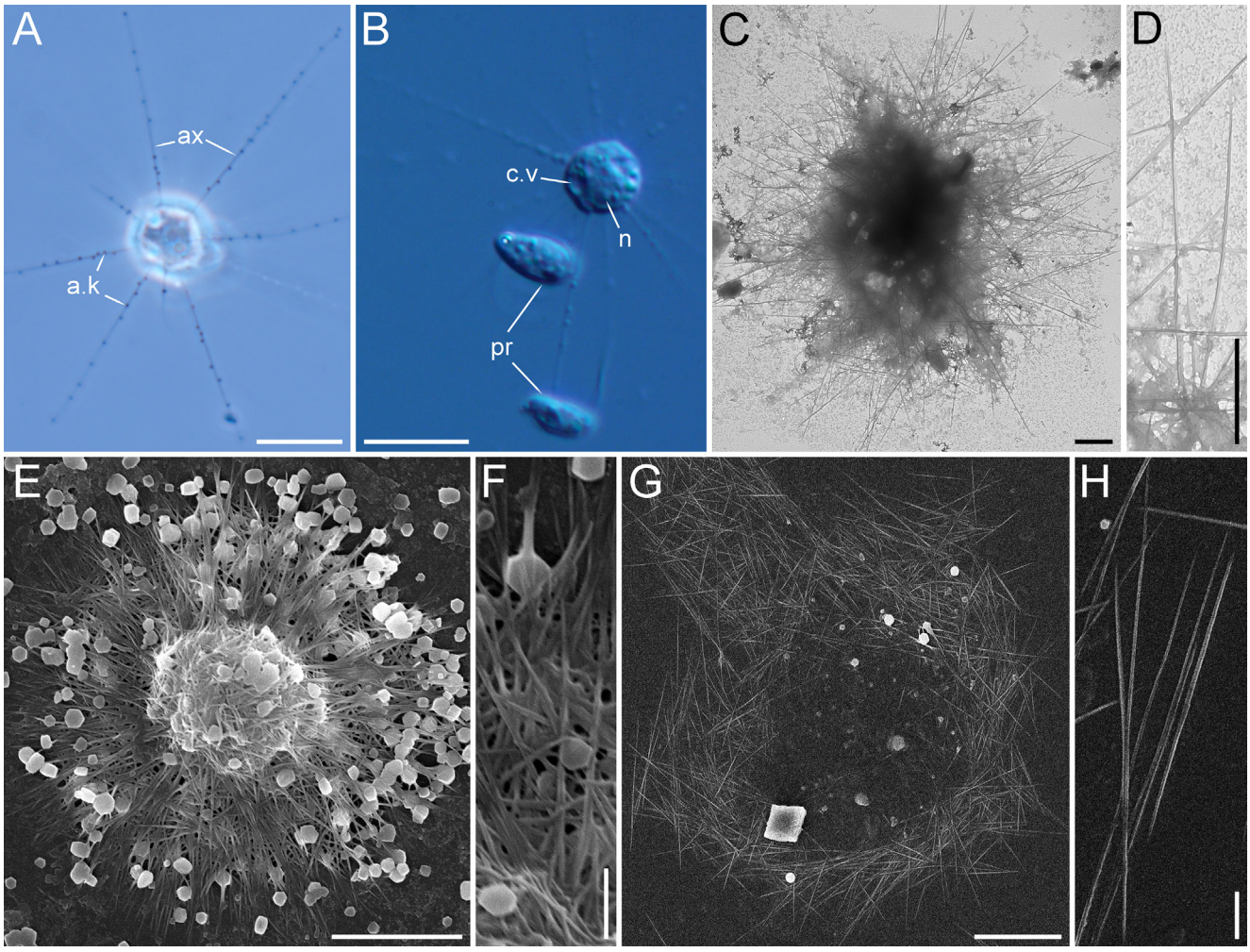


Fig. 11. Morphology of *Heterophrys*-like organisms. AD – HLO's from the sampling site No. 20 (HF-55 strain): A–B – living cells (A – PhC LM; B – DIC LM); C – general view of the dried cell (TEM); D – spicules (TEM); E,F – marine HLO's from the sampling site No. 25 (SEM); E – general view of the dried cell; F – spicules; G, H – freshwater HLO's from the sampling site No. 1 (SEM); G – general view of the dried cell; H – spicules. Abbreviations: a.k – axopodial kinetocysts; ax – axopodia; c.v – contractile vacuole; n – nucleus; pr – prey; sp – spicules. Scale bar: A, B, E, G – 10 μm; C, D, F, H – 1 μm.

entire length. Plate-scales are oval and textureless (Fig. 12F–G). A short medial thickening 0.46–0.62 μm long have been observed on a few scales (Fig. 12G). Detailed morphological parameters are shown in Supplementary Table S3.

Differences from related or similar species: This species differs from *A. taurica* Mikrjukov, 1997 in the lower number of apical teeth on the spine-scales (4 rarely 3 in *A. tyrasiana vs* 5, rarely 4, 6 or 7 in *A. taurica*), longer spine-scales (2.3–11.2 μm *vs* ~4.2 μm), and in the absence of lateral deviation of the apical teeth; differs from *A. penardi* by the lower number of apical teeth of spine-scales (4 rarely 3 *vs* 4–12); differs from *A. spinosa* Cavalier-Smith et von der Heyden, 2007 by the straight shafts and greater number of teeth on the apex (4 rarely 3 *vs* 3); differs from *Acanthocystis trifurca* Nicholls, 1983 in the greater number and smaller size of apical teeth (4, rarely 3 *vs* 3); differs from *A. quadrifurca* Nicholls, 1983 in the smaller size of the apical teeth, textureless plate-scales, and the absence of radial slits on them.

Etymology: From the Greek "Τύρας" – ancient name of the Dniester River.

Type Figure: Fig. 12A–G.

Type locality: Spring water in oak and hornbeam forest (48°28′12.06″ N 26°46′13.91″ E).

ZooBank Registration: urn:lsid:zoobank.org:act:E352DB1F-19BC-4BC3-A965-9EB3BE39C1E2.

●●● Pterocystida Cavalier-Smith et von der Heyden, 2007 emend. Shishkin et Zlatogursky, 2018

●●●● Pterista Shishkin et Zlatogursky, 2018

●●●●● Family Pterocystidae Cavalier-Smith et von der Heyden, 2007

●●●●●● *Pterocystis* Siemensma et Roijackers, 1988

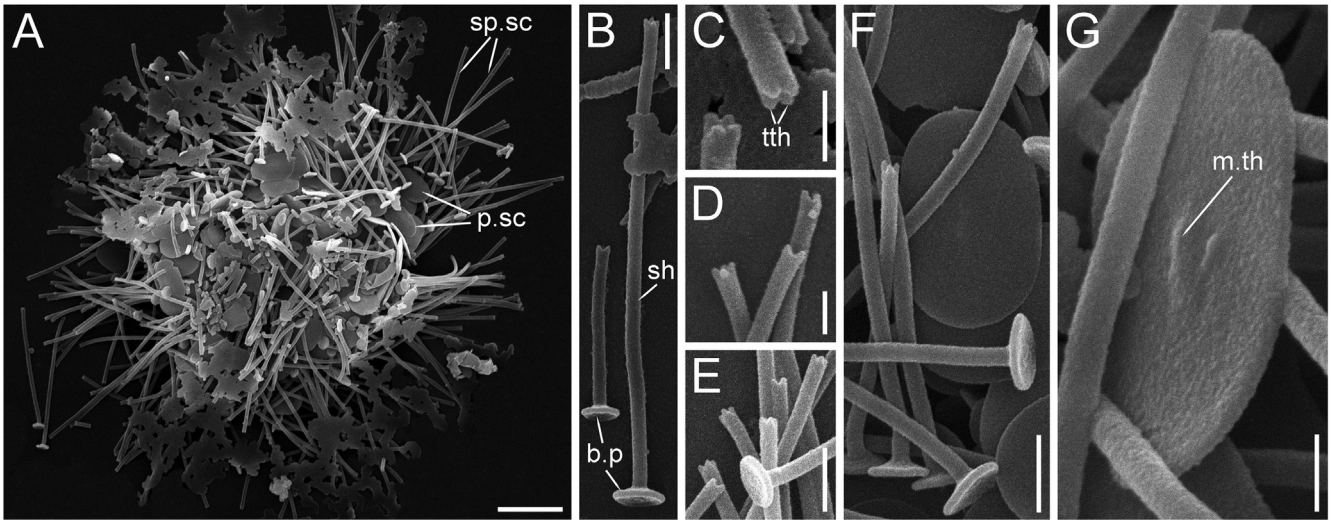


Fig. 12. Morphology of *Acanthocystis tyrasiana* sp. nov. (SEM). A – general view of the dried cell; B – spine-scales; C–E – distal tips of the spine-scales; F – spine- and plate-scales; G – plate-scale Abbreviations: b.p – basal plate; m.th – medial thickening; p.sc – plate-scale; sh – shaft; sp.sc – spine-scale; tth – teeth. Scale bar: A – 5 μm; B, E, F – 1 μm; C, D, G – 0.5 μm.

Pterocystis borysthenica Zagumyonnyi, Radaykina, Keeling et Tikhonenkov sp. nov. (Fig. 13, 14; Supplementary Table S4).

Material: Strain HF-51 from the sampling site No. 21 (40 cells were studied) (see Table 1).

Diagnosis: Cell diameter ranges between 5 and 18 μm. Spine-scales is 1.3–6.1 μm long, with a straight or curved shaft, 0.15–0.22 μm in diameter. The lateral wings are short, 0.48–1.79 μm, tapering from the basal wing to the shaft. The basal wing is triangular, 0.62–1.55 × 0.39–1.22 μm in size. There are 12–16 parallel ribs on the inner side of the basal wing. Plate-scales are elliptical, 1.6–3.1 × 1.1–2.1 μm in size with a medial thickening of 0.65–1.83 × 0.05–0.10 μm in size. Cystic scales 3.3–4.9 × 2.1–3.0 μm are dense and massive, elliptical, bean-shaped or irregular in shape with a medial thickening of 1.2–3.5 × 0.25–0.90 μm.

Description: Diameter of living cell depends on the food conditions. The axopodia are 1–5 times longer than the cell. Skeletal elements are represented by spine- and plate-scales (Fig. 13A–C). The shaft of spine-scales is hollow, straight, or curved (Fig. 13D–I). It is connected to the basal and two lateral wings in the bent proximal part, together forming a structure in the form of a ladle. The basal wings of spine-scales are triangular (Fig. 13D–I). There are 12–16 parallel ribs on the inner side of the basal wing (Fig. 13 F, J). The basal wing has a well-defined marginal border. Plate-scales are elliptical with a medial thickening (Fig. 13J, K). This strain is characterized by encystment in old cultures (Fig. 14A–B). Cystic scales were found on some cells (Fig. 14C–G). They represent massive dense plate-scales of a bean-shape or irregular shape with dimensions (Fig. 14D–G). Similar to ordinary plate-scales, cystic scales have a medial thickening. Detailed morphological parameters are shown in Supplementary Table S4.

Differences from related or similar species: The studied organism has the greatest similarity with *Pterocystis clarkii*, *P. pinnata*, *P. tropica* and *P. quadrata*. All these species have a pointed distal part of the shaft, a triangular basal wing, and lateral wings tapering to the distal part. However, *P. borysthenica* differs from other species by a different shape of lateral wings and by the absence of upturned flanges on them. Also, *P. borysthenica* differs from *P. clarkii*, *P. quadrata*, and *P. pinnata* by the presence of 12–16 parallel ribs on the inner surface of the basal wing. In *P. tropica*, ribs are present not only on the basal wing, but also on the lateral wings. The plate-scales of *P. borysthenica*, *P. clarkii*, and *P. pinnata* are generally similar. The plate-scales of *P. tropica* and *P. quadrata* are distinguished by the presence of radial slits (Dürrschmidt 1987b [as *Acanthocystis tropica* ssp. *paucistriata*]; Prokina and Philippov 2019; Prokina et al. 2019). *P. borysthenica* also has similar features in the structure of spine-scales with *P. canadensis* and *Raineriophrys erinaceoides*. In contrast to these species, *P. borysthenica* does not have widened central part of the shaft and also has radial ribs on the basal wings and possesses elliptical (but not ovoid) plate-scales with a much less pronounced central thickening. *P. borysthenica* has spine- and plate-scales of smaller sizes, and features the shorter lateral wings compared to length of the shaft than *P. canadensis* and *R. erinaceoides*.

Remark: The detailed structure of cysts and cyst scales of centrohelids is known only for *Raineriophrys erinaceoides*, *Raphidiophrys heterophryoidea* (Zlatogursky 2012; Drachko et al. 2020), *R. elongata* (Drachko et al. 2021), and *Raphidocystis ambigua* (Dürrschmidt 1987a,b). Among

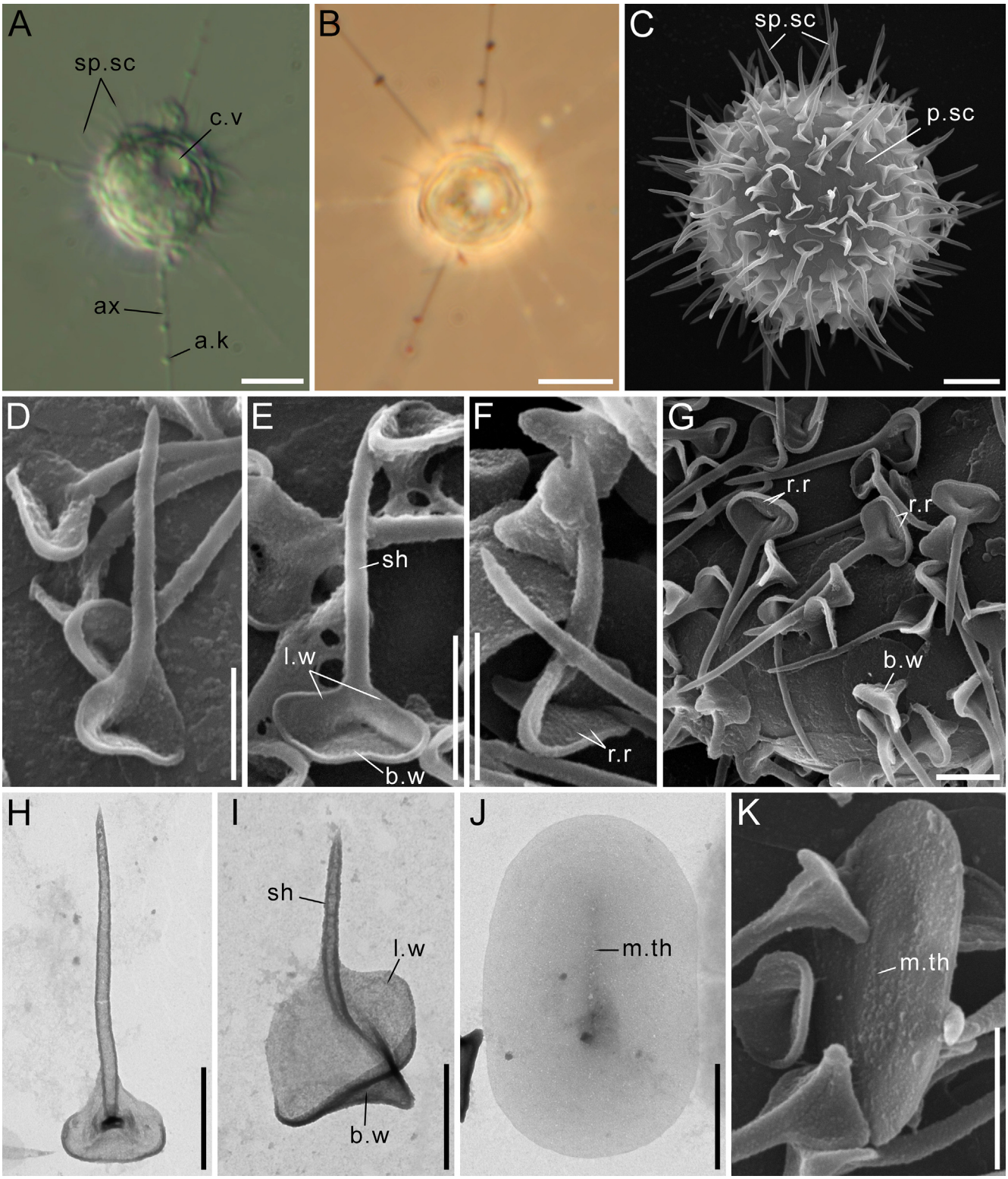


Fig. 13. Morphology of *Pterocystis borysthenica* sp. nov. (A – DIC LM; B – PhC LM; C–G, K – SEM; H–J – TEM.) A, B – general view of the living cells; C – general view of the dried cell; D–I – spine-scales; J, K – plate-scales. Abbreviations: ax – axopodia; a.k – axopodial kinetocysts; b.w – basal wing; l.w – lateral wings; m.th – medial thickening; p.sc – plate-scale; sh – shaft; sp.sc – spine-scales. Scale bar: A, B – 5 μm; C – 2 μm; D–K – 1 μm.

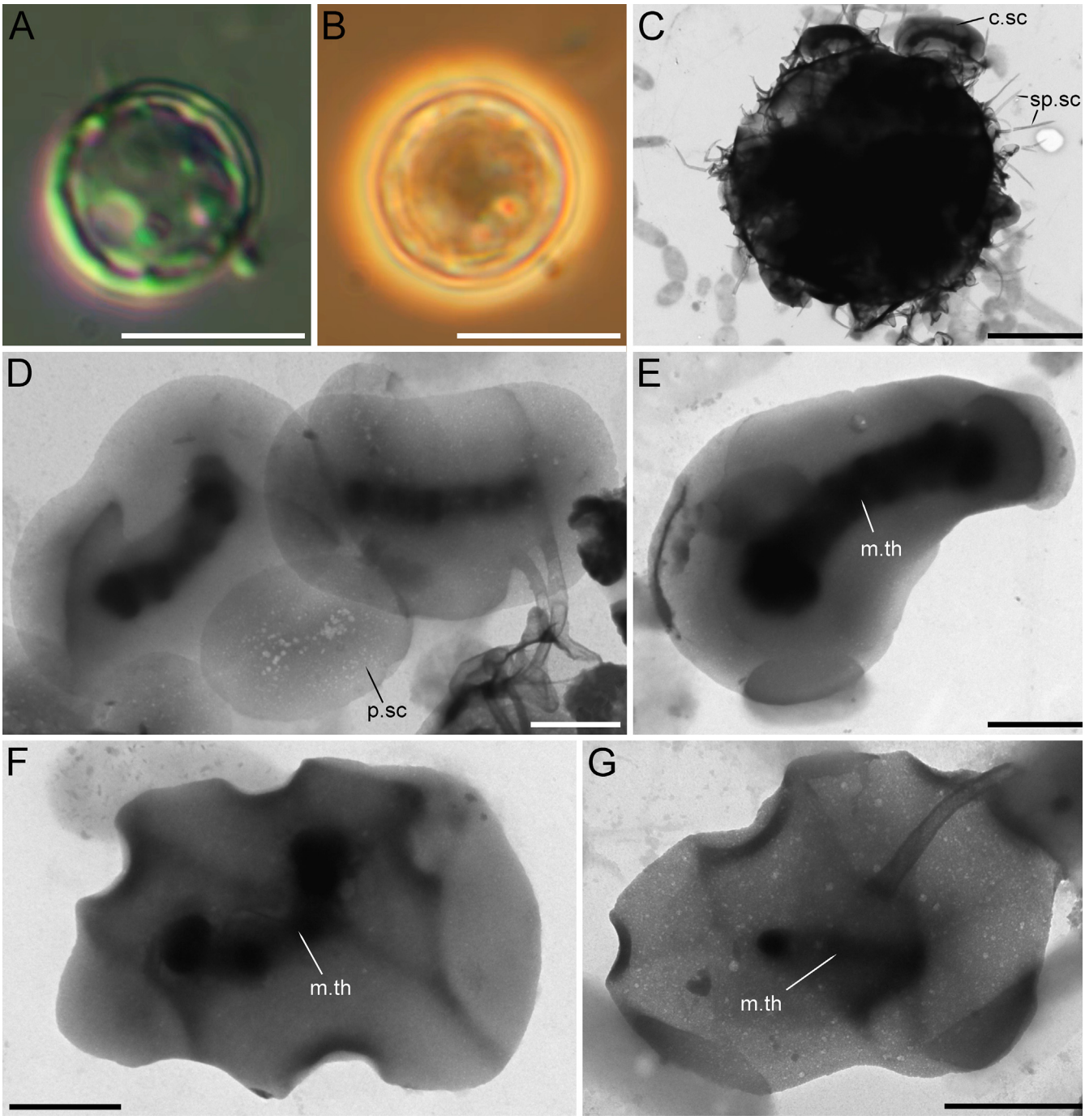


Fig. 14. Morphology of *Pterocystis borysthenica* sp. nov. (A – DIC LM; B – PhC LM; C–G – TEM). A, B – cyst with living cell; C – general view of cyst with trophic and cyst scales; D–G – cyst scales. Abbreviations: c.sc – cyst scales; m.th – medial thickening; sp.sc – spine-scales; p.sc – plate-scale. Scale bar: A–C – 5 μm; D–G – 1 μm.

the members of the genus *Pterocystis*, the details of cysts and cyst scales morphology are unknown. The closest related species with available data on the morphology of cyst scales is *Raineriophrys erinaceoides*. However, its polygonal cyst scales have little in common with the cyst scales of *P. borysthenica.* The knoll in the central part of the scales described for *R. erinaceoides* is absent in *P. borysthenica.* Instead, all cyst scales have an oblong medial thickening that resembles the medial thickening of trophic plate-scales. Bean-shaped cyst scales resembling enlarged trophic plate-scales have not been described in *R. erinaceoides.* The irregular cyst scales of *P. borysthenica* have

a few outgrowths and depressions at the edges, which somewhat resemble the structure of the cyst scales of *R. heterophryoidea.* It is likely that these scales can be attached to each other like a puzzle, as in *R. heterophryoidea.*

Etymology: From the Greek "Βορυσθένης" – ancient name of the Dnieper River.

Type strain: HF-51. Stored in the collection of live protozoan cultures at IBIW RAS.

Hapantotype: Preparation for SEM No. 2-HF-51 is kept in the laboratory of Microbiology at the Papanin Institute for Biology of Inland Waters RAS (Borok, Russia).

Type Figure: Fig. 13A–K.

Type locality: Coastal bottom sediments of unnamed pond on Vita Stream, Dnepr basin (50°20′22.6″ N 30° 29′32.1″ E).

Gene sequence: The SSU rRNA gene sequence has the GenBank Accession Number OP104322.

ZooBank Registration: urn:lsid:zoobank.org:act:A8DA6D0B-05BA-4D7D-A3F1-7A5483AE0608.

●●●●●● *Khitsovia* Zagumyonnyi, Radaykina, Keeling et Tikhonenkov gen. nov.

Diagnosis: Cell body is covered with spine- and plate-scales. Spine-scales are oriented radially. They consist of a cylindrical non-tapering shaft ending in several teeth at the apex and a proximal bucket-shaped structure formed by the basal and lateral wings. Cells are capable of encystment. There is a spicule-bearing stage.

Type species: *Khitsovia mutabilis* sp. nov.

Etymology: named after Dr., Prof. Liudmila Nikolaevna Khitsova.

ZooBank Registration: urn:lsid:zoobank.org:act:BFB00F5E-63FB-4380-B015-D26EE5E5D452.

***Khitsovia mutabilis* Zagumyonnyi, Radaykina, Keeling et Tikhonenkov sp. nov.** (Fig. 15–17; Supplementary Table S5).

Material: Strain HF-24 from the sampling site No. 23 (133 cells were studied) (see Table 1).

Diagnosis: Cell diameter ranges between 4–18 μm. The spine-scales are 1.8–9.4 in length. Their shaft is hollow 0.11–0.24 μm in diameter, straight or slightly curved, with about four 0.024–0.152 μm teeth on the apical part. Basal and lateral wings formed triangular, trapezoidal, pear-shaped, reniform, or oval-shaped bucket-like structure 0.8–2.4 μm long and 0.9–3.0 μm wide. The inner part of the lateral and basal wings is smooth, without ribs. Plate-scales are elliptical or ovoid, 1.1–4.2 × 0.7–3.0 μm, with medial depression, medial thickening 0.42–1.63 × 0.044–0.123 μm and marginal rim. Spherical cysts with a diameter of 5.3–9.6 μm and a wall thickness of 0.21–0.28 μm. An aperture of 1.9–3.3 μm in diameter is formed on one side of the cyst at excystment.

Description: Observations were made in clonal culture, where starving and well-fed cells were noted. A diameter of the starving cells without visible food vacuoles is 4–9 μm (Fig. 15A, B). A diameter of the well-fed cells is 7–18 μm. The length of the axopodia can be up to 8 times the cell diameter in starving cells and approximately equal to the cell diameter in well-fed cells. Each axopodium bears up to seven kinetocysts (Fig. 15A, B). Colony formation was not observed. The surface of the cells is covered with plate- and spine-scales (Fig. 15C). The spine-scales are consist of a shaft, a basal wing, and lateral wings (Fig. 15D–L). Teeth are located at the distal end of the shaft (Fig. 15D–J). The proximal part of the spine-scale is represented by a bucket-like structure formed by the basal and lateral wings. This "bucket" can be triangular, trapezoidal, pear-shaped, reniform, or oval-shaped (Fig. 15D–L). The inner part of the lateral and basal wings is smooth, without ribs or slits (Fig. 15G).

Plate-scales are with medial depression, medial thickening and marginal rim with a thickness of 0.017–0.055 μm (Fig. 15M–P). A subcircular scale 2.66 μm in diameter was revealed at one cell (Fig. 15P).

Cyst formation has been noted in the absence of food (Fig. 16A–J). The external surface of cysts may have single plate-scales or a large number of spine- and plate-scales as on typical feeding cells. An aperture is formed on one side of the cyst at excystment (Fig. 16C, E–H). The cyst wall (Fig. 16K) probably consists of organic material. Detailed morphological parameters are shown in Supplementary Table S5.

In a centrohelid culture that had been kept at room temperature for more than 14 days without prey, cells with only spicule-covered surfaces were found (Fig. 17A). Spicules are spindle-shaped, 1.8–10.0 μm long and 0.015–0.035 μm width. (Fig. 17B–D). Spicules are slightly flattened and twisted along the longitudinal axis, with both pointed tips. Some of the spicules are arranged radially and some irregularly.

Differences from related or similar species: Studied species is morphologically similar to *Raineriophrys fortesca* and, most likely, is closely related. However, *K. mutabilis* lacks radial ribs on the surface of the scales, and also differs by the smaller spine scales and bucket-like structure of triangular, trapezoidal, pear-shaped, reniform, or oval-shaped form. To date, there are a number of observations of the cells identified as *R. fortesca* with rather contrasting morphological differences between them. At the same time, molecular data on *R. fortesca* are still lacking.

Remarks: *K. mutabilis* produces cysts but special type of cystic scales has not been observed. However, it is possible that they can be detected in the organic covering layer after treatment of the cysts with acid. Sometimes, trophic cells possess large plate-scales (Fig. 15N) that likely synthesized prior to encystment, as noted for *Raphidiophrys ambigua* in Dürrschmidt and Patterson (1987).

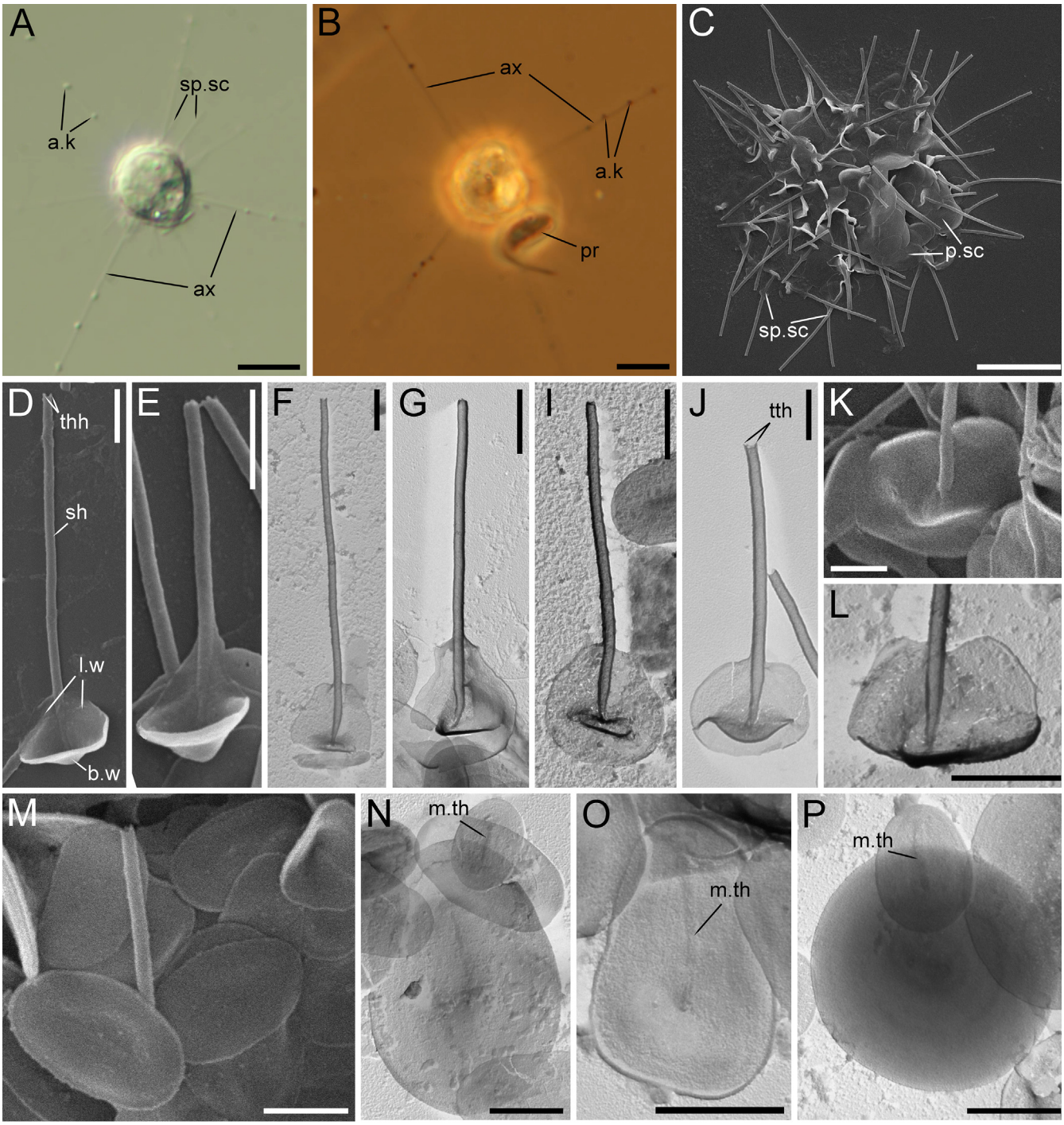


Fig. 15. Morphology of *Khitsovia mutabilis* gen. et sp. nov A – DIC LM; B – PhC LM; C–E, K, M – SEM; F–J, L, N–P – TEM; A, B – general view of the living cells; C – general view of the dried cell; D–J – spine-scales; K, L – basal part of spine-scales; M–P – plate-scales. Abbreviations: ax – axopodia; a.k – axopodial kinetocysts; b.w – basal wing; l.w – lateral wings; m.th – medial thickening; p.sc – plate-scale; pr – prey; sh – shaft; sp.sc – spine-scales; tth – teeth. Scale bar: A–C – 5 μm; D–P – 1 μm.

During excystation, cells escape through the formed aperture without destroying the cyst. Abandoned cysts often lack scales on the surface. Among the species of centrohelids with noted cysts, the formation of an aperture during excystation has not been described. Here we show that *K. mutabilis* and aforementioned *Raineriophrys fortesca* (Fig. 8G) have similar cysts with an aperture.

Etymology: From Latin "*mutabilis*" – changeable; due to the changeable shape of the proximal part of spine-scales.

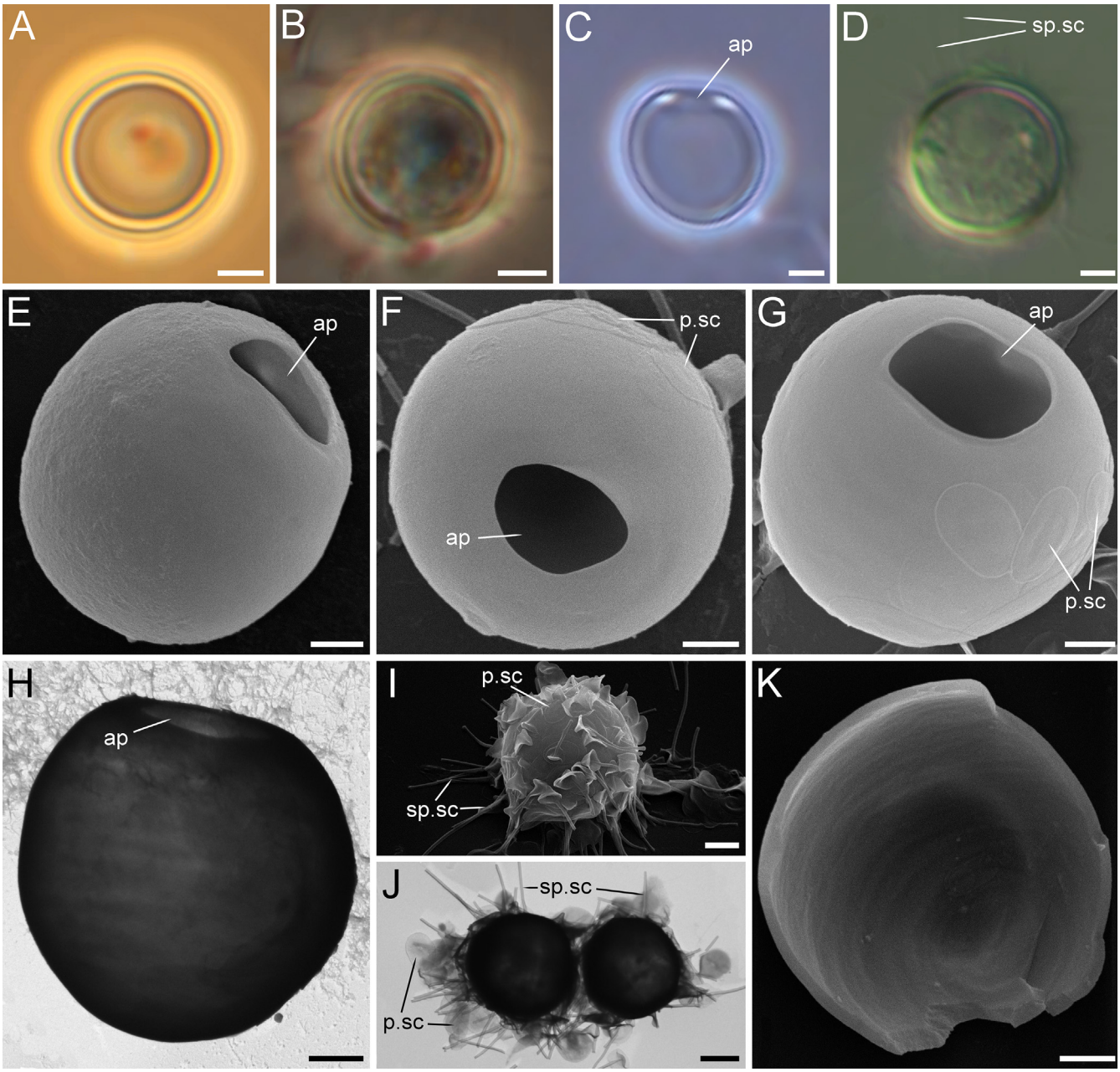


Fig. 16. Morphology of cysts of *Khitsovia mutabilis* gen. et sp. nov. A – Empty cyst (PhC LM); B – cyst with living cell (PhC LM); C – Empty cyst (PhC LM); D – cyst with living cell (DIC LM); E–H – empty cyst with aperture (E–G – SEM; H – TEM); I, J – cysts coated with scales of the trophic stage; K – inner part of cyst fragment. Abbreviations: ap – aperture; p.sc – plate-scale; sp.sc – spine-scales. Scale bar: A–D, I, J – 2 μm; E–G, H, K – 1 μm.

Type strain: HF-24. Stored in the collection of live protozoan cultures at IBIW RAS.

Hapantotype: Preparation for SEM No. 3-HF-24 is kept in the laboratory of Microbiology at the Papanin Institute for Biology of Inland Waters RAS (Borok. Russia).

Type Figure: Fig. 15

Type locality: Soil of a wet floodplain meadow among Carex sp. and *Elytrigia* sp., Trubin (Bekhova) River floodplain (51°23′28.9″N 32°21′41.5″E).

Gene sequence: The SSU rRNA gene sequence has the GenBank Accession Number OP101622.

ZooBank Registration: urn:lsid:zoobank.org:act:882E5A74-F007-4722-A6EE-D3C30623681E.

Molecular phylogeny

All of the molecularly characterized centrohelids in this study belong to the Pterista (Fig. 18). Strain HF-55

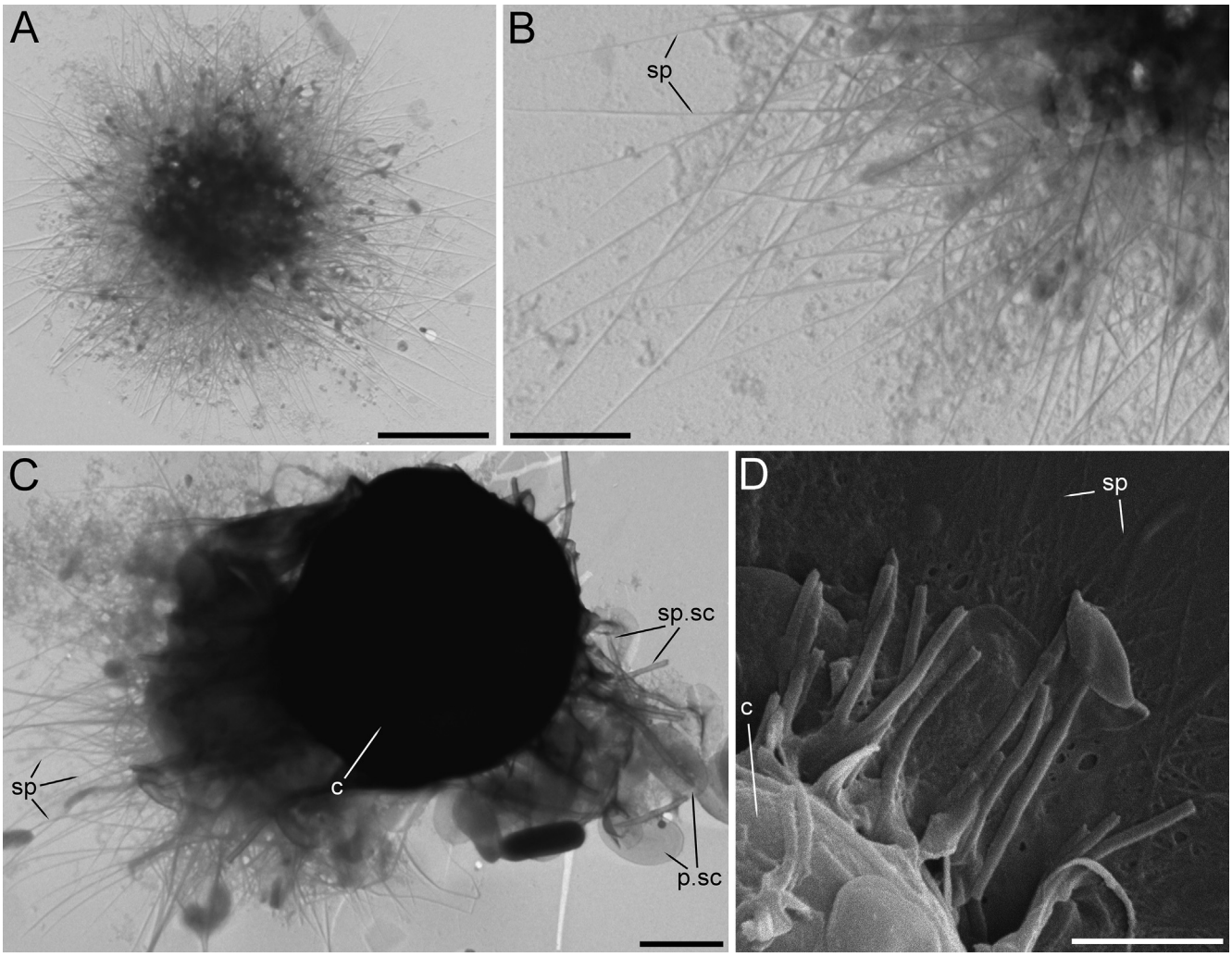


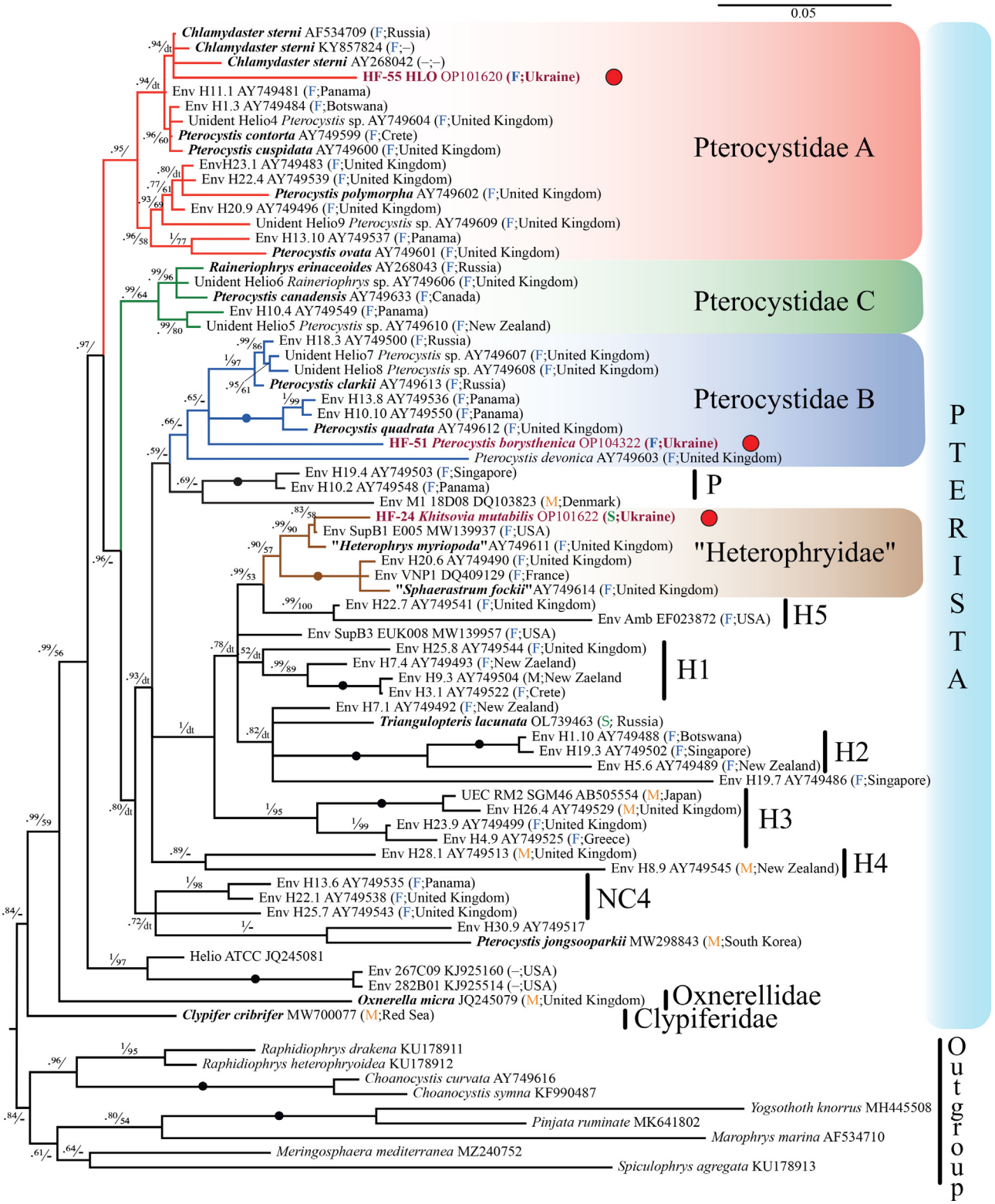
Fig. 17. Spicule-bearing stage of *Khitsovia mutabilis* gen. et sp. nov. A – general view of the dried cell; B – spicules on the peripheral part of cell; C, D – dried cell at the spicule-bearing stage near cyst. Abbreviations: c – cyst; p.sc – plate-scale; sp – spicula; sp.sc – spine-scales. Scale bar: A – 5 μm; B–D – 2 μm.

(*Heterophrys*-like organism) is located in the clade Pterocystidae A. This strain forms an unresolved polytomy (BPP 0.94) with three freshwater sequences annotated in GeneBank as *Chlamydaster sterni* (AF534709; KY857824; AY268042).

Ch. sterni is a centrohelid heliozoan covered with a mucus sheath and possessing neither siliceous covering elements nor organic spicules. Thus, clade Pterocystidae A unites heliozoans with leaf-like and siliceous plate-scales (e.g. *Pterocystis ovata*, *P. cuspidata*), mucous sheaths (*Ch. sterni*), and needle-like spicules (HF-55).

Strain HF-51 (*Pterocystis borysthenica* sp. nov.) is grouped within the Pterocystidae B clade and forms an unresolved trichotomy with two clades including *Pterocystis clarkii* (AY749613), *P. quadrata* (AY749612), unidentified *Pterocystis*, and some environmental sequences, albeit with low support. The morphology of the spine- and plate-scales of *P. borysthenica* is similar to that of the described species of the Pterocystidae B clade so its inclusion in this clade is not controversial. To date, members of this clade are represented exclusively by freshwater organisms.

Strain HF-24 (*Khitsovia mutabilis* gen. et sp. nov.) is grouped within the clade formerly named as "Heterophryidae" (Fig. 18). HF-24 is closely related to the lineage, represented by the environmental sequence SupB E005 (MW139937) from Lake Superior (USA), and HLO annotated as "*Heterophrys myriopoda*" (AY749611) from the lake sediments of Oxford University Parks. There are only two centrohelids with data on their skeletal morphology within the clade "Heterophryidae". Cavalier-Smith and von der Heyden (2007) identified them as *Heterophrys myriopoda* (AY749611) and *Sphaerastrum fockii* (AY


0.05
Chlamydaster sterni AF534709 (F;Russia)
Chlamydaster sterni KY857824 (F;–)
Chlamydaster sterni AY268042 (–;–)
HF-55 HLO OP101620 (F;Ukraine)
Env H11.1 AY749481 (F;Panama)
Env H1.3 AY749484 (F;Botswana)
Unident Helio4 Pterocystis sp. AY749604 (F;United Kingdom)
Pterocystis contorta AY749599 (F;Crete)
Pterocystis cuspidata AY749600 (F;United Kingdom)
EnvH23.1 AY749483 (F;United Kingdom)
Env H22.4 AY749539 (F;United Kingdom)
Pterocystis polymorpha AY749602 (F;United Kingdom)
Env H20.9 AY749496 (F;United Kingdom)
Unident Helio9 Pterocystis sp. AY749609 (F;United Kingdom)
Env H13.10 AY749537 (F;Panama)
Pterocystis ovata AY749601 (F;United Kingdom)
Pterocystidae A
Raineriophrys erinaceoides AY268043 (F;Russia)
Unident Helio6 Raineriophrys sp. AY749606 (F;United Kingdom)
Pterocystis canadensis AY749633 (F;Canada)
Env H10.4 AY749549 (F;Panama)
Unident Helio5 Pterocystis sp. AY749610 (F;New Zealand)
Pterocystidae C
Env H18.3 AY749500 (F;Russia)
Unident Helio7 Pterocystis sp. AY749607 (F;United Kingdom)
Unident Helio8 Pterocystis sp. AY749608 (F;United Kingdom)
Pterocystis clarkii AY749613 (F;Russia)
Env H13.8 AY749536 (F;Panama)
Env H10.10 AY749550 (F;Panama)
Pterocystis quadrata AY749612 (F;United Kingdom)
HF-51 Pterocystis borysthenica OP104322 (F;Ukraine)
Pterocystis devonica AY749603 (F;United Kingdom)
Pterocystidae B
Env H19.4 AY749503 (F;Singapore)
Env H10.2 AY749548 (F;Panama)
Env M1 18D08 DQ103823 (M;Denmark)
P
HF-24 Khitsovia mutabilis OP101622 (S;Ukraine)
Env SupB1 E005 MW139937 (F;USA)
"Heterophrys myriopoda" AY749611 (F;United Kingdom)
Env H20.6 AY749490 (F;United Kingdom)
Env VNP1 DQ409129 (F;France)
"Sphaerastrum fockii" AY749614 (F;United Kingdom)
"Heterophryidae"
Env H22.7 AY749541 (F;United Kingdom)
Env Amb EF023872 (F;USA)
H5
Env SupB3 EUK008 MW139957 (F;USA)
Env H25.8 AY749544 (F;United Kingdom)
Env H7.4 AY749493 (F;New Zaeland)
Env H9.3 AY749504 (M;New Zaeland
Env H3.1 AY749522 (F;Crete)
H1
Env H7.1 AY749492 (F;New Zealand)
Triangulopteris lacunata OL739463 (S; Russia)
Env H1.10 AY749488 (F;Botswana)
Env H19.3 AY749502 (F;Singapore)
Env H5.6 AY749489 (F;New Zealand)
H2
Env H19.7 AY749486 (F;Singapore)
UEC RM2 SGM46 AB505554 (M;Japan)
Env H26.4 AY749529 (M;United Kingdom)
Env H23.9 AY749499 (F;United Kingdom)
Env H4.9 AY749525 (F;Greece)
H3
Env H28.1 AY749513 (M;United Kingdom)
Env H8.9 AY749545 (M;New Zealand)
H4
Env H13.6 AY749535 (F;Panama)
Env H22.1 AY749538 (F;United Kingdom)
Env H25.7 AY749543 (F;United Kingdom)
NC4
Env H30.9 AY749517
Pterocystis jongsooparkii MW298843 (M;South Korea)
Helio ATCC JQ245081
Env 267C09 KJ925160 (–;USA)
Env 282B01 KJ925514 (–;USA)
Oxnerella micra JQ245079 (M;United Kingdom)
Oxnerellidae
Clypifer cribrifer MW700077 (M;Red Sea)
Clypiferidae
PTERISTA
Raphidiophrys drakena KU178911
Raphidiophrys heterophryoidea KU178912
Choanocystis curvata AY749616
Choanocystis symna KF990487
Yogsothoth knorrus MH445508
Pinjata ruminate MK641802
Marophrys marina AF534710
Meringosphaera mediterranea MZ240752
Spiculophrys agregata KU178913
Outgroup

749614). Both of these organisms carry a layer of organic needle-shaped spicules.

The position of *K. mutabilis* with spine- and plate-scales within the clade "Heterophryidae" shows that its members have not lost the ability to form siliceous scales as previously suggested (Cavalier-Smith and von der Heyden 2007). The presence of centrohelids with siliceous scales in HLO clades was suggested by Zlatogursky (2016). It is likely that Cavalier-Smith and von der Heyden (2007) analyzed only the spicules-bearing stage of "*Heterophrys myriopoda*" and "*Sphaerastrum fockii*". It is possible that these heliozoans also have a stage with spine- and plate-scales in their life cycles, similar to those in *K. mutabilis* or *R. fortesca*. We expect that sequences of *R. fortesca*-like organisms will be grouped within the "Heterophryidae" clade in future phylogenetic reconstructions.

The current phylogenetic tree contains the only available sequence from the genus *Raineriophrys* (*R. erinaceoides*), which is related to *Pterocystis canadensis* within Pterocystidae C clade. Taking into account morphological similarity of *R. fortesca* to *Khitsovia mutabilis* and *R. scaposa* to *Triangulopteris lacunata* (Zagumyonnyi et al. 2021) it is possible to suppose that genus *Raineriophrys* may eventually come to be seen as polyphyletic.

Conclusion

Eighteen species from seven genera of centrohelids and unidentifiable HLOs were found in freshwater, marine, and soil habitats of Ukraine. The most frequently observed centrohelids in this study were *Acanthocystis* aff. *turfacea*, *Raineriophrys* aff. *fortesca*, *R. erinaceoides* and *A. pectinata*. One new genus and three new species were described. Seven species (*Acanthocystis nichollsi, Acanthocystis* sp. 1, *Choanocystis* aff. *perpusilla*, *Pterocystis* aff. *pinnata*, *P. tropica*, *Raineriophrys scaposa*, *Triangulopteris lacunata*) were found in Ukraine for the first time. The resulting list of centrochelid species of Ukraine is far from being complete. It is undoubtedly possible to identify a greater species diversity by examining a larger number of diverse biotopes.

Almost all of the revealed species (except those described in this work and *Triangulopteris lacunata*, described in 2021) have been recorded in North America, South America and Eurasia. *Acanthocystis pectinata* and *Choanocystis perpusilla* have not been recorded in North America, and *Raphidocystis symmetrica* has not been occurred in South America. Only seven of the species we found are known from Australia, four from Africa, and two from Antarctica, which can be explained by the smaller number of studies conducted in these regions. It should be borne in mind, however, that a number of species have a certain degree of morphological variability. At the same time, specimens described as one species may be represented by two or more species. It is still premature to draw conclusions about the real species diversity and geographic distribution patterns of centrohelids due to the insufficient number of studied regions and lack of molecular data on many known species.

Some of the species have similar morphology with others from the works of other authors. At the same time, *Acanthocystis* aff. *nichollsi*, *A*. aff. *turfacea*, *Raphidocystis symmetrica*, *Pterocystis* aff. *pinnata*, *P. tropica* and *Raineriophrys scaposa* possessed previously undescribed or different morphological details. *Acanthocystis* aff. *turfacea*, *Raineriophrys erinaceoides* and *R.* aff. *fortesca* isolated from different biotopes were characterized by the quite different morphology of covering elements. The obtained data on the morphology and gene sequences will help to further refine the boundaries of the morphological variability of centrohelids.

Interestingly, novel species *K. mutabilis* possesses a spicule-bearing stage. Similar stages were observed in *Raphidocystis glabra*, *Raphidiophrys heterophryoidea,* and *Triangulopteris lacunata* (Zlatogursky et al. 2018; Zlatogursky 2012; Drachko et al. 2020; Zagumyonnyi et al. 2021). Described here *Pterocystis borysthenica* is the first species of *Pterocystis* with studied cyst scales whose morphology differ from all other previously described scales of the cysts. *Khitsovia mutabilis* and *Raineriophrys fortesca* have a previously undescribed type of cysts with an aperture. Molecular data on three centrohelids expand our knowledge of the Pterocistydae B and Pterocistydae C clades, and Pterista phylogeny. We have shown that "Heterophryidae" unites heliozoans capable of cyst formation, siliceous scale formation, and contain species with a spicule-bearing stage.

CRediT authorship contribution statement

Dmitry G. Zagumyonnyi: Conceptualization, Investigation, Methodology, Visualization, Writing – original draft. **Liudmila V. Radaykina:** Methodology, Investiga-

Fig. 18. Phylogenetic tree generated from Bayesian analysis of small subunit ribosomal RNA gene sequences of 77 centrohelid heliozoans, including the outgroup of 9 centrohelid species. Bayesian posterior probabilities (BPP) and Maximum Likelihood (ML, NT + R4 model) bootstrap values are indicated at branches (values greater than 50 % are shown); black, brown, and blue filled circles indicate values of BPP = 1.00 and ML bootstrap = 100 %; dt – different topology. The values were not shown if the bootstrap values are below 50 or posterior probability below 0.50. The sequences generated in this study are highlighted in bold, colored in red and marked with red circles. The information on the sequences in parentheses indicate the biotope (freshwater (F), soil (S), or marine (M)) and source (country/sea) of isolation. Clades Heterophryidae, H1–H5, NC4 are marked similarly to previous studies (Shishkin et al. 2018; Cavalier-Smith and Chao 2012).

tion. **Patrick J. Keeling:** Resources, Funding acquisition, Writing – review & editing. **Denis V. Tikhonenkov:** Conceptualization, Supervision, Funding acquisition, Resources, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors are grateful to Dr. Y.V. Dubrovsky (Institute for Evolutionary Ecology of the National Academy of Sciences of Ukraine) and Dr. A.P. Mylnikov for providing the samples and A.S. Borodina (Papanin Institute for Biology of Inland Waters) for text editing.

Funding

This work was supported by the Russian Science Foundation grant no. 18-14-00239, https://rscf.ru/project/18-14-00239/.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejop.2022.125916.

References

Adl, S.M., Bass, D., Lane, C.E., Lukeš, J., Schoch, C.L., Smirnov, A., Agatha, S., Berney, C., Brown, M.W., Burki, F., et al., 2019. Revisions to the classification, nomenclature, and diversity of eukaryotes. J. Eukaryot. Microbiol. 66, 4–119. https://doi.org/10.1111/jeu.12691.

Beling, D., 1923. Dneprovskaia biologicheskaia stantsiia i ee raboty za poslednie gody. Russkii gidrobiologicheskii zhurnal 2 (1–2), 42–44, in Russian.

Bessudova, A.Y., Firsova, A.D., Likhoshway, Y.V., 2022. Silica-Scaled Heterotrophic protists Rotosphaerida, Thaumatomonadida, and Centroplasthelida in the large continuous ecosystem connecting Lake Baikal to the Kara Sea. J. Eukaryot. Microbiol. 69 (1), e12871.

Buchinskij, P.N., 1895. Prostejshie organizmy Hadzhibejskogo i Kuyal'nickogo limanov. Zapiski Novorossijskogo obshchestva estestvoispytatelej. 20 (1), 137–148, in Russian.

Burki, F., Kaplan, M., Tikhonenkov, D.V., Zlatogursky, V., Minh, B.Q., Radaykina, L.V., Smirnov, A., Mylnikov, A.P., Keeling, P.J., 2016. Untangling the early diversification of eukaryotes: a phylogenomic study of the evolutionary origins of Centrohelida, Haptophyta and Cryptista. Proc. Royal Soc. B 283 (1823), 20152802. https://doi.org/10.1098/rspb.2015.2802.

Capella-Gutierrez, S., Silla-Martinez, J.M., Gabaldon, T., 2009. TrimAl: A tool for automated alignment trimming in large-scale phylogenetic analyses. Bioinformatics 25, 1972–1973. https://doi.org/10.1093/bioinformatics/btp348.

Cavalier-Smith, T., Chao, E.-E.-Y., 2012. *Oxnerella micra* sp. n. (Oxnerellidae fam. n.), a tiny naked centrohelid, and the diversity and evolution of heliozoa. Protist 163 (4), 574–601. https://doi.org/10.1016/j.protis.2011.12.005.

Cavalier-Smith, T., von der Heyden, S., 2007. Molecular phylogeny, scale evolution and taxonomy of centrohelid heliozoan. Mol. Phylogenet. Evol. 44 (3), 1186–1203. https://doi.org/10.1016/j.ympev.2007.04.019.

Croome, R., 1986. Observations of the heliozoan genera *Acanthocystis* and *Raphidocystis* from Australia. Arch. Protistenkd. 131, 189–199. https://doi.org/10.1016/S0003-9365(86)80040-9.

Croome, R., 1987. Observations of the genera *Acanthocystis*, *Raphidiophrys*, *Clathrulina* and *Pompholyxophrys* (Protozoa, Sarcodina) from Australian freshwaters. Arch. Protistenkd. 133, 237–243. https://doi.org/10.1016/S0003-9365(87)80055-6.

Croome, R.L., Van den Hoff, J., Burton, H.R., 1987. Observations of the heliozoean genera *Pinaciophora* and *Acanthocystis* (Heiliozoea, Sarcodina, Protozoa) from Ellis Fjord, Antarctica. Polar Biol. 8 (1), 23–28. https://doi.org/10.1007/BF00297160.

Dobrovlyanskij, V.V., 1914. Spisok presnovodnyh prostejshih okrestnostej g. Kieva. Trudy Dneprovskoj biologicheskoj stancii 1, 35–47, in Russian.

Drachko, D., Mikhailovskii, V., Shishkin, Y., Zlatogursky, V.V., 2021. Phylogenetic position and morphology of *Raphidiophrys elongata* sp. nov. (Haptista: Centroplasthelida) with notes on cyst wall structure and evolution. Eur. J. Protistol. 81, 125836. https://doi.org/10.1016/j.ejop.2021.125836.

Drachko, D., Shishkin, Y., Zlatogursky, V.V., 2020. Phenotypic masquerade: Polymorphism in the life cycle of the centrohelid heliozoan *Raphidiophrys heterophryoidea* (Haptista: Centroplasthelida). Eur. J. Protistol. 73, 125686. https://doi.org/10.1016/j.ejop.2020.125686.

Dürrschmidt, M., 1985. Electron microscopic observations on scales of species of the genus *Acanthocystis* (Centrohelidia, Heliozoa) from Chile. I. Arch. Protistenkd. 129, 55–87. https://doi.org/10.1016/S0003-9365(85)80011-7.

Dürrschmidt, M., 1987a. An electron microscopical study of freshwater Heliozoa (genus *Acanthocystis*, Centrohelidia) from Chile, New Zealand, Malaysia and Sri Lanka. II.. Arch. Protistenkd. 133 (1–2), 21–48. https://doi.org/10.1016/S0003-9365(87)80038-6.

Dürrschmidt, M., 1987b. An electron microscopical study on freshwater Heliozoa (genus *Acanthocystis*, Centrohelidia) from Chile, New Zealand, Malaysia and Sri Lanka. III.. Arch. Protistenkd. 133 (1–2), 49–80. https://doi.org/10.1016/S0003-9365(87)80039-8.

Dürrschmidt, M., Patterson, D.J., 1987. On the organization of the Heliozoa *Raphidiophrys ambigua* Penard and *R. pallida* Schulze. Ann. Sci. Nat. Zool. 8, 135–155.

Fadeev, N.N., 1929. Katalog vodnyh zhivotnyh, najdennyh v bassejne r. Donca i prilegayushchih mestnostyah za period rabot s 1917 po 1927 g. Trudi Harkivskogo tovaristva doslidnikiv prirodi. 52, 7–32, in Russian.

Faszynski, J., 1910. Badania fauny planktonowej stawa Janowskiego w roku 1909 z uwzglednieniem fauny przybrzeżnej. Kosmos 35 (10/12), 941–993, in Polish.

Gaponova, L.P., 2006. Current knowledge about Heliozoa of Ukraine. Scientific Bulletin of Natural Sciences (Biological Sciences). 8, 13–17 (in Ukrainian).

Gaponova, L.P., 2008. The centrohelid heliozoans (Protista, Centrohelida) of algal mats from the water body in Ukrainian Polissya. Vestnik Zoologii. 42 (2), e-38-e-41.

Gaponova, L.P., 2009. The centrohelid heliozoans (Centrohelea Cavalier-Smith, 1993) of Kyiv and the Chernigiv regions of Polissya. Cand. Sci. (Biol.) Dissertation, Kyiv: Inst. Zool. im. I.I. Shmal'gauzena, Nats. Akad. Nauk Ukrainy.

Gaponova, L.P., Dovgal, I.V., 2008. The variability of exoskeleton elements in *Polyplacocystis ambigua* (Protista, Centrohelida). Vestnik zoologii 42, e79–e83. https://doi.org/10.2478/v10058-008-0012-4.

Gerasimova, E.A., 2022. Two new brackish-water species of centrohelid heliozoans (Haptista: Centroplasthelida), *Choanocystis mylnikovi* sp. n. and *C. punctata* sp. n., from Russia. Protistology 16 (1), 10–20. https://doi.org/10.21685/1680-0826-2022-16-1-2.

Ikävalko, J., Thomsen, H.A., Carstens, M., 1996. A preliminary study of NE Greenland shallow meltwater ponds with particular emphasis on loricate and scale-covered forms (Choanoflagellida, Chrysophyceae sensu lato, Synurophyceae, Heliozoea), including the descriptions of *Epipyxis thamnoides* sp. nov. and *Pseudokephyrion poculiforme* sp. nov. (Chrysophyceae). Arch. Protistenkd. 147 (1), 29–42. https://doi.org/10.1016/S0003-9365(96)80006-6.

Jaworowski, A., 1893. Fauna studzienna miast Krakowa i Lwowa. Sprawozdanie Komisyi Fizyograficznej. Materyaly dla fiziografii krajowej 28 (2), 29–48, in Polish.

Katoh, K., Standley, D.M., 2013. MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. Mol. Biol. Evol. 30, 772–780. https://doi.org/10.1093/molbev/mst010.

Keeling, P.J., 2002. Molecular phylogenetic position of *Trichomitopsis termopsidis* (Parabasalia) and evidence for the Trichomitopsiinae. Eur. J. Protistol. 38 (3), 279–286. https://doi.org/10.1078/0932-4739-00874.

Kosolapova, N.G., Mylnikov, A.P., 2015. First records of centrohelid heliozoans (Centrohelida) in Mongolia. Inland Water Biol. 8 (3), 232–235. https://doi.org/10.1134/S1995082915030074.

Krasheninnikiv, S.M., 1925. Materiali do favni Protozoa oz. Zaspi ta najblizshih do cogo ozera stoyachih vodozboriv (buvsh. oz. Domaha). Zapiski Kiyivskogo veterinarno-zootehnichnogo institutu 3, 38–42, in Ukrainian.

Leonov, M.M., 2009. Heliozoan fauna of waterbodies and watercourses of the Central Russian Upland forest-steppe. Inland Water Biol. 2, 6–12. https://doi.org/10.1134/S1995082909010027.

Leonov, M.M., 2010. Heliozoans (Heliozoa, Sarcodina, Protista) of fresh and marine waters of the European part of Russia: Species composition, morphology, and distribution. Inland Water Biol. 3 (4), 344–355. https://doi.org/10.1134/S1995082910040073.

Leonov, M.M., 2012. Vidovoe raznoobrazie i morfologiya solnechnikov (Heliozoa) vodoyomov i vodotokov Evropejskoj chasti Rossii (Species Diversity and Morphology of Heliozoa of Waterbodies and Watercourses in the European Part of Russia). PhD dissertation in Biological Sciences, Papanin Institute for Biology of Inland Waters RAS, Borok, 1–191 (in Russian).

Leonov, M.M., Mylnikov, A.P., 2012. Centroheliozoa from Southern Karelia. Zool. Zhurn. 91, 515–523.

Leonov, M.M., Plotnikov, A.O., 2009. Species composition, morphology, and distribution of centrohelid heliozoa from Central Chernozem Region and the South Urals. Zool. Zhurn. 88, 643–653.

Medlin, L., Elwood, H.J., Stickel, S., Sogin, M.L., 1988. The characterization of enzymatically amplified eukaryotic 16S-like rRNA-coding regions. Gene 71 (2), 491–499. https://doi.org/10.1016/0378-1119(88)90066-2.

Mikrjukov, K.A., 1993a. Observations on Centroheliozoa of the Volga basin (Protozoa: Sarcondina). Zoosyst. Rossica 2, 201–209.

Mikrjukov, K.A., 1993b. On the centrohelid and rotosphaerid Heliozoa from the environs of the Võrtsjärv limnological station in Estonia. Proc. Estonian Acad. Sci. 42 (2), 154–160.

Mikrjukov, K.A., 1995. Revision of the species composition of the genus *Choanocystis* (Sarcodina Centroheliozoa) and its fauna in eastern Europe. Zool. Zhurn. 74 (4), 3–16, in Russian with English summary.

Mikrjukov, K.A., 1997. Revision of the genera and specific composition of the family Acanthocystidae (Centroheliozoa, Sarcodina). Russ. J. Zool. 1 (2), 119–130.

Mikrjukov, K.A., 1999. Interesting findings of Heliozoa (Protista) in Black Sea and Crimea: data on similarity on sea and freshwater fauna of these organisms. Russ. J. Zool. 3 (2), 240–249.

Mikrjukov, K.A., Milyutina, I., 2001. Heliozoa as a component of marine microbenthos: a study of Heliozoa of the White Sea. Ophelia 54, 51–73. https://doi.org/10.1080/00785326.2001.10409455.

Mikrjukov, K.A., 2002. Centrohelid Heliozoans (Centroheliozoa). KMK Sci. Press, Moscow, in Russian with English summary.

Nguyen, L.-T., Schmidt, H.A., von Haeseler, A., Minh, B.Q., 2015. IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol. Biol. Evol. 32 (1), 268–274. https://doi.org/10.1093/molbev/msu300.

Nicholls, K.H., 1983. Little-known and new heliozoeans: the centrohelid genus *Acanthocystis*, including descriptions of nine new species. Can. J. Zool. 61 (6), 1369–1386. https://doi.org/10.1139/z83-184.

Nicholls, K.H., Dürrschmidt, M., 1985. Scale structure and taxonomy of some species of *Raphidocystis*, *Raphidiophrys*, and *Pompholyxophrys* (Heliozoea) including descriptions of six new taxa. Can. J. Zool. 63 (8), 1944–1961. https://doi.org/10.1139/z85-288.

Ostroumoff, A.A., 1917. O novom vide solnechnikov v morskom planktone *Acanthocystis wiasemskii*. Trudy Karadagskoi nauchnoi stantsii imeni T.I.. Viazemskogo 1, 62–65, in Russian.

Pereyaslavceva, S.M., 1886. Protozoa Chernogo morya. Odessa: Tipografiya Odesskogo vestnika. 1–16 (in Russian).

Petersen, J.B., Hansen, J.B., 1960. Elektronenmikroskopische Untersuchunger von zvei Arten der Heliozoa Gattung *Acanthocystis*. Arch. Protistenkunde 104, 547–552.

Plotnikov, A.O., Ermolenko, E.A., 2015. Centrohelid Heliozoa (Chromista, Hacrobia) of Southern Cis-Ural region. Biol. Bull. 42 (8), 683–695. https://doi.org/10.1134/S1995082917020109.

Plotnikov, A.O., Gerasimova, E.A., 2017. Heliozoa (Centrohelea, Haptista, Hacrobia) of saline and brackish water bodies and watercourses of Russia. Inland Water Biol. 10, 121–129.

Prokina, K.I., Zagumyonnyi, D.G., Philippov, D.A., 2017a. Centrohelids in the mires of Northern Russia. Protistology 11 (1), 3–19.

Prokina, K.I., Mylnikov, A.P., Zelalem, W., 2017b. First data on heterotrophic flagellates and heliozoans of Ethiopia. Biol. Bull. 44, 896–912. https://doi.org/10.1134/S106235901708012X.

Prokina, K.I., Mylnikov, A.A., Mylnikov, A.P., 2017c. Heterotrophic flagellates and centrohelid heliozoa from littoral and supralittoral zones of the Black Sea (the Southern part of the Crimea). Protistology 11 (3), 143–169. https://doi.org/10.21685/1680-0826-2017-11-3-2.

Prokina, K.I., Zagumyonnyi, D.G., Tikhonenkov, D.V., 2018. Centrohelid heliozoans (Centroplasthelida Febvre-Chevalier et Febvre, 1984) from different types of freshwater bodies in the Middle Russian Forest-steppe. Acta Protozool. 57, 245–268. https://doi.org/10.4467/16890027AP.18.018.10094.

Prokina, K.I., Philippov, D.A., 2019. Centrohelid heliozoans (Haptista: Centroplasthelida) from mires in the North Caucasus, Russia. Mires Peat. 24 (36), 1–20. https://doi.org/10.19189/MaP.2019.OMB.StA.1806.

Prokina, K.I., Mylnikov, A.P., 2019. Centrohelid heliozoans from freshwater habitats of South Patagonia and Tierra del Fuego, Chile. Inland Water Biol. 12, 10–20. https://doi.org/10.1134/S1995082919010164.

Prokina, K.I., Zagumyonnyi, D.G., Mylnikov, A.P., 2019. Marine centrohelid heliozoans (Centroplasthelida Febvre-Chevalier et Febvre, 1984) from bays of Sevastopol (the Black Sea shore). Russ. J. Mar. Biol. 45 (5), 377–384. https://doi.org/10.1134/S1063074019050092.

Prokina, K.I., Radaykina, L.V., Mylnikov, A.P., 2020. Centrohelid heliozoans (Centroplasthelida Febvre-Chevalier et Febvre, 1984) from Vietnam. Biol. Bull. 47 (7), 29–40. https://doi.org/10.1134/S1062359020070158.

Prokina, K.I., Keeling, P.J., Tikhonenkov, D.V., 2021. Heterotrophic flagellates and centrohelid heliozoans from marine waters of Curacao, the Netherlands Antilles. Eur. J. Protistol. 77, 125758. https://doi.org/10.1016/j.ejop.2020.125758.

Roijackers, R.M.M., 1988. External morphology as taxonomic characteristic in planktonic scale-bearing Chrysophyceae and scaled Heliozoa. Hydrobiol. Bull. 22, 69–73. https://doi.org/10.1007/BF02256785.

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A., Huelsenbeck, J.P., 2012. MrBayes 3.2: Efficient bayesian phylogenetic inference and model choice across a large model space. Syst. Biol. 61 (3), 539–542. https://doi.org/10.1093/sysbio/sys029.

Schneider, C.A., Rasband, W.S., Eliceiri, K.W., 2012. NIH Image to ImageJ: 25 years of image analysis. Nat. Methods 9 (7), 671–675. https://doi.org/10.1038/nmeth.2089.

Shatilovich, A.V., Mylnikov, A.P., Stoupin, D.V., 2010. The fauna and morphology of heterotrophic flagellates and heliozoans from Late Pleistocene fossil rodent burrows (Kolyma Lowland). Zool. Zhurn. 89 (4), 387–397.

Shishkin, Y., Drachko, D., Klimov, V.I., Zlatogursky, V.V., 2018. *Yogsothoth knorrus* gen. n., sp. n. and *Y. carteri* sp. n. (Yogsothothidae fam. n., Haptista, Centroplasthelida), with notes on evolution and systematics of centrohelids. Protist. 169 (5), 682–96. https://doi.org/10.1016/j.ejop.2020.125686.

Shishkin, Y., Drachko, D., Zlatogursky, V.V., 2021. *Clypifer cribrifer* gen. nov., sp. nov. (Clypiferidae fam. nov., Pterocystida, Centroplasthelida), with notes on evolution of centrohelid siliceous coverings. Int. J. Syst. Evol. Microbiol. 71. https://doi.org/10.1099/ijsem.0.004856.

Siemensma, F.J., 1991. Klasse Heliozoa Haeckel, 1866. In: Page, F.C., Siemensma, F.J. (Eds.), Nackte Rhizopoda Und Heliozoea, Protozoenfauna. Gustav Fisher Verlag, Stuttgart - New York, pp.171–297.

Siemensma, F.J., Microworld, world of amoeboid organisms. World-wide electronic publication. https://arcella.nl/ (accessed 1 August 2022).

Siemensma, F.J., Roijackers, R.M.M., 1988a. A study of new and little-known acanthocystid heliozoeans, and a proposed division of the genus *Acanthocystis* (Actinopoda, Heliozoea). Arch. Protistenkd. 135 (1–4), 197–212. https://doi.org/10.1016/S0003-9365(88)80069-1.

Siemensma, F.J., Roijackers, R.M.M., 1988b. The genus *Raphidiophrys* (Actinopoda, Heliozoea): scale morphology and species distinctions. Arch. Protistenkd. 136 (3), 237–248. https://doi.org/10.1016/S0003-9365(88)80023-X.

Takamura, N., Shen, Y.u., Xie, P., 2000. Species richness of protozoa in Japanese lakes. Limnology 1, 91–106. https://doi.org/10.1007/s102010070015.

Tikhonenkov, D.V., Janouškovec, J., Keeling, P.J., Mylnikov, A.P., 2016. The morphology, ultrastructure and SSU rRNA gene sequence of a new freshwater flagellate, *Neobodo borokensis* n. sp. (Kinetoplastea, Excavata). J. Eukaryot. Microbiol. 63 (2), 220–232. https://doi.org/10.1111/jeu.12271.

Vørs, N., 1992. Heterotrophic amoebae, flagellates and heliozoan from the Tvarminne area, Gulf of Finland, in 1988–1990. Ophelia 36, 1–109. https://doi.org/10.1080/00785326.1992.10429930.

Vysockij, A.V., 1888. Mastigophora i Rhizopoda, najdennye v Vejsovom i Repnom ozerah. Trudy Obshchestva ispytatatelej prirody pri imperatorskom Har'kovskom universitete. 21, 119–140 (in Russian).

Wujek, D.E., 2003a. Freshwater heliozoa (Protista, Heliozoa) from Indiana. Proc. Indiana Acad. Sci. 112 (2), 169–174.

Wujek, D.E., 2003b. Freshwater scaled heterotrophic protistans from four Gulf States, including descriptions of two species. J. Alabama Acad. Sci. 74, 164–180.

Wujek, D.E., 2005. Identification, ecology, and distribution of scale bearing amoeba, ciliates, flagellates and Heliozoa from the Carolinas. J. No. Car. Acad. Sci. 121, 1–16. https://doi.org/10.2307/24336001.

Wujek, D.E., 2006. Freshwater Heliozoa from Florida. Florida Scientist 69 (3), 177–191. https://doi.org/10.2307/24321380.

Wujek, D.E., 2015. Freshwater silica-scaled heterotrophic Protista: Heliozoa, thaumatomonad flagellates, amoebae, and bicosoecids, from the Lake Itasca region. J. Minnesota Acad. Sci. 78 (2), 1–14.

Wujek, D.E., Dziedzic, R.M., Kadiri, M.O., Adesalu, T.A., Nwankwo, D.I., 2007. Identification, ecology, and distribution of Heliozoa, scaled flagellates and scaled ciliates from western Nigeria. Trop. Freshwat. Biol. 16 (1), 1–15.

Wujek, D.E., Fritz, B.A., Dziedzic, R.M., 2010. Silica-scaled chrysophytes and Heliozoa from the Caribbean Island of Dominica (Lesser Antilles). Caribb. J. Sci. 46, 120–123. https://doi.org/10.18475/cjos.v46i1.a17.

Wujek, D.E., Saha, L.C., 2006. Freshwater scaled heterotrophic flagellates and Heliozoa from India including a description of a new species of *Choanocystis*. J. Bombay Nat. Hist. Soc. 103, 71–81.

Yabuki, A., Chao, E.E., Ishida, K.H., Cavalier-Smith, T., 2012. *Microheliella maris* (Microhelida ord. n.), an ultrastructurally highly distinctive new axopodial protist species and genus, and the unity of phylum Heliozoa. Protist 163, 356–388.

Zagumyonnyi, D.G., Gusev, E.S., Mylnikov, A.A., Mylnikov, A. P., 2020a. Novel species of centrohelid heliozoans (Centroplasthelida) for freshwaters of Central and Southern Vietnam. Inland Water Biol. 13 (3), 358–371. https://doi.org/10.1134/S1995082920030165.

Zagumyonnyi, D.G., Prokina, K.I., Tikhonenkov, D.V., 2020b. First findings of centrohelid heliozoans (Haptista: Centroplasthelida) from marine and freshwater environments of South Korea. Protistology 14 (4), 227–245. https://doi.org/10.21685/1680-0826-2020-14-4-4.

Zagumyonnyi, D.G., Radaykina, L.V., Tikhonenkov, D.V., 2021. *Triangulopteris lacunata* gen. et sp. nov. (Centroplasthelida), a new centrohelid heliozoan from soil. Diversity 13 (12), 658. https://doi.org/10.3390/d13120658.

Zlatogursky, V.V., 2012. *Raphidiophrys heterophryoidea* sp. nov. (Centrohelida: Raphidiophryidae), the first heliozoan species with a combination of siliceous and organic skeletal elements. Eur. J. Protistol. 48 (1), 9–16. https://doi.org/10.1016/j.ejop.2011.09.004.

Zlatogursky, V.V., 2013a. Puzzle-like cyst wall in centrohelid heliozoans *Raphidiophrys heterophryoidea* and *Raineriophrys erinaceoides*. Acta Protozool. 52, 229–236. https://doi.org/10.4467/16890027AP.14.004.1441.

Zlatogursky, V.V., 2013b. Puzzle-like cyst wall in centrohelid heliozoans *Raphidiophrys heterophryoidea* and *Raineriophrys erinaceoides*. Acta Protozool. 52 (4), 229–236. https://doi.org/10.4467/16890027AP.14.004.1441.

Zlatogursky, V.V., 2016. There and back again: parallel evolution of cell coverings in centrohelid heliozoans. Protist 167 (1), 51–66. https://doi.org/10.1016/j.protis.2015.12.002.

Zlatogursky, V.V., Drachko, D., Klimov, V.I., Shishkin, Y., 2018. On the phylogenetic position of the genus *Raphidocystis* (Haptista: Centroplasthelida) with notes on the dimorphism in centrohelid life cycle. Eur. J. Protistol. 64, 82–90. https://doi.org/10.1016/j.ejop.2018.03.006.

Zlatogursky, V.V., Klimov, V.I., 2016. Barcoding Heliozoa: Perspectives of 18S rDNA for distinguishing between *Acanthocystis* species. Protist 167 (6), 555–567. https://doi.org/10.1016/j.protis.2016.09.004.