

## ORIGINAL ARTICLE

# Two new brackish-water species of centrohelid heliozoans (Haptista: Centroplasthelida), *Choanocystis mylnikovi* sp. n. and *C. punctata* sp. n., from Russia

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## Summary

The morphology of two new species of centrohelid heliozoans, *Choanocystis mylnikovi* and *C. punctata*, from the brackish-water Tuzlukkol' River, South-Eastern Russia, were studied using scanning electron microscopy. The new species are covered with two types of scales that are plate scales and spine scales with heart-shaped basal plates and proximally curved shafts. *C. mylnikovi* has spine scales that are highly variable in length, which differs up to six times (2.0–12.9  $\mu\text{m}$ ). Spine scales of *C. mylnikovi* are slightly tapering, with two small teeth of the same length on the apices. This species also has oviform or oval plate scales (1.6–2.9  $\times$  1.0–1.8  $\mu\text{m}$ ) with an axial ridge and thin marginal rim. *C. punctata* has both short (3.6–9.9  $\mu\text{m}$ ) and long (10.0–18.5  $\mu\text{m}$ ) spine scales with pointed apices. Sometimes short spine scales of *C. punctata* end with a bent apex or a small bent hook. Plate scales (2.9–4.7  $\times$  1.5–3.2  $\mu\text{m}$ ) and basal plates of spine scales are ornamented with numerous granules. *C. punctata* sporadically demonstrates the presence of spine scales with the shaft in a nearly central position and membranous extensions of the basal plate along the shaft, which are the characteristics for the other morphologically defined genera of centrohelids – *Acanthocystis*, *Pterocystis* or *Raineriophrys*. Similarities and differences between the new species and other members of the genus *Choanocystis* are discussed.

**Key words:** centrohelids, *Choanocystis*, external skeleton, morphology, systematics, protists

## Introduction

Centrohelid heliozoans (Centroplasthelida Febvre-Chevalier et Febvre, 1984), or centrohelids, represent a monophyletic group of free-living ubi-

quitous predatory protists forming cysts (Cavalier-Smith et al., 2015; Zlatogursky, 2013). Centrohelids are characterized by the worldwide distribution; they inhabit benthos and periphyton in both marine and freshwater ecosystems, being a component of

microbial loops that are responsible for a substantial part of transformation of matter and effective energy flows in aquatic ecosystems (Mikrjukov, 2002). Centrohelids along with haptophytes constitute the phylum Haptista (Burki et al., 2016). According to the recent taxonomic revision, centrohelids are divided into Panacanthocystida Shishkin et Zlatogursky 2018 characterized by the presence of 7–13 insertions in 18S rRNA gene and Pterocystida Cavalier-Smith and Heyden 2007 without insertions (Cavalier-Smith and Heyden, 2007; Shishkin et al., 2018). Centrohelids are characterized by a conservative internal structure (slender axopodia radiating from the cell surface, axopodial axonemes radiating from the microtubules-organizing center, kinetocysts, flat mitochondrial cristae), whereas morphology of cell coverings (organic spicules, siliceous spine and plate scales) is highly variable within the group (Siemensma, 1991; Mikrjukov, 2002;). Morphology of cell coverings is one of the main diagnostic features for species identification and classification in this group (Siemensma, 1991; Mikrjukov, 2002; Cavalier-Smith and Heyden, 2007; Tikhonenkov and Myl'nikov, 2010; Zlatogursky, 2010, 2013, 2016; Plotnikov and Ermolenko, 2015; Gerasimova and Plotnikov, 2016; Zlatogursky et al., 2017, 2019; Shishkin et al., 2018; Zagumyonnyi et al., 2020). In addition, the structure of the scales reflects the evident morphological and taxonomic diversity of centrohelids (Siemensma, 1991; Mikrjukov, 2002).

The genus *Choanocystis* Penard 1904 is characterized by two contrasting types of siliceous scales, oval or bilobed tangential plate scales (margin not hollow and enrolled) forming the inner layer, and outer bipartite spine scales consisting of a shaft, sometimes curved or branched but lacking lateral wings, emanating from near a strong indentation on one side of a flat horizontal basal plate (Adl et al., 2019).

Here we describe two new species of *Choanocystis* from brackish waters and discuss the similarities and differences between the new species and other members of the genus, as well as brackish-water distribution of the representatives of *Choanocystis*.

## Material and methods

Samples were collected from the upper layer of bottom sediment with near-bottom water from 5–10 cm depth, using 500-ml sterile containers, at three sampling sites of the Tuzlukkol' River (South-

East of the European part of Russia) with different water salinity (N 51°17'3", E 56°36'21", salinity 0.6 ppt; N 51°17'8", E 56°36'16", 20 ppt; and N 51°17'9", E 56°36'16", 22 ppt). Enriched cultures of centrohelids were obtained by inoculation of 10 ml of the initial environmental samples into 60-mm plastic Petri dishes with the addition of bacteria *Pseudomonas fluorescens*. The enriched cultures were incubated at +10–14 °C under room light during one month and heliozoans grew without isolation in pure culture. Observation of the living cells was carried out using an inverted Nikon Eclipse TS2 microscope equipped with phase contrast (total magnification ×800). Total preparation for scanning electron microscopy (SEM) was conducted according to Gerasimova and Plotnikov (2016). Examination of scales was carried out with an electron microscope (EM) Jeol JSM 6510 (Jeol, Japan). Scales were measured in EM images. Scales sizes are given in Table 1. The Venice System (1958) for the classification of waters according to salinity was used.

## Results

### CELL MORPHOLOGY

#### *Choanocystis mylnikovi* sp. n. (Fig. 1, A–E).

**Material.** 13 cells from the sampling site with salinity 20 ppt, 3 cells from the sampling site with salinity 22 ppt.

**Description.** The cell coat consists of plate scales and spine scales. Spine scales are highly variable in length and differ up to six times. Spine scale slightly tapers to their apexes possessing two small teeth of the same length (Fig. 1, D). Shafts of spine scales are proximally (or, rarely more distally) curved (Fig. 1, A) and seat eccentrically in the base of the heart-shaped basal plates, surrounded with a marginal rim (Fig. 1, B). Spine scales are 2.0–12.9 μm long, 0.1–0.18 μm thick, basal plates are 0.6–1.4 μm in diameter. Plate scales are oviform or oval (Fig. 1, E), 1.6–2.9 μm long, 1.0–1.8 μm wide, with a clearly expressed axial ridge and a thin marginal rim. Axial ridge is located on the convex part in the center of the plate scales.

**Comparative description.** *C. mylnikovi* has spine scales with two small teeth. Among the described species of *Choanocystis*, there are only two ones with two small teeth on the scale apexes – *C. perpusilla* Petersen et Hansen 1960 and *C. rhytidus* Dürschmidt 1987. Similarly, in *C. rhytidus* from two

**Table 1.** Morphometry of *Choanocystis mylnikovi* and *C. punctata*.

Species	Parameter	Min (µm)	Max (µm)	Mean unweighted, ±SE (µm)	n (i)	n
<i>C. mylnikovi</i>	Spine scale length	2.0	12.9	6.46±0.27	16	90
	Scale thick	0.1	0.18	0.15	16	36
	Basal plates diameter	0.6	1.4	1.02±0.02	16	72
	Plate scales length	1.6	2.9	2.2±0.05	16	29
	Plate scales width	1.0	1.8	1.3±0.03	16	28
<i>C. punctata</i>	Long spine scale length	10.0	18.5	12.8±0.2	10	57
	Scale thick	0.18	0.3	0.23±0.01	10	24
	Basal plates diameter	1.0	2.2	1.8±0.04	10	54
	Short spine scale length	3.67	9.9	7.6±0.2	10	47
	Basal plates diameter	1.0	2.3	1.6±0.04	10	48
	Scale thick	0.1	0.2	0.17±0.01	10	23
	Oval plate scales length	2.9	4.7	3.7±0.07	10	36
	Oval plate scales width	2.5	3.2	2.3±0.06	10	33
	Roundish plate scales diameter	2.7	4.0	3.3±0.13	10	9

Notes: Min – minimum, Max – maximum, Mean – arithmetic mean, SE – standard error of the mean, n (i) – number of individuals, n – number of measurements.

to four teeth were observed (Dürschmidt, 1987). In contrast, *C. mylnikovi* always bears only two teeth. The number of teeth described in *C. perpusilla* is two. By contrast with *C. mylnikovi*, radial scales of *C. perpusilla* are short in relation to the basal plates (Petersen and Hansen, 1960; Siemensma, 1981), whereas the *C. mylnikovi* radial scales are up to nine times longer than basal plates. In addition, length of radial scales of *C. perpusilla* differ approximately by two times – 2.0–4.5 µm (Petersen and Hansen, 1960), 3.0–5.0 µm (Dürschmidt, 1985) and 4.8–8.2 µm (Siemensma, 1981), whereas radial scales of *C. mylnikovi* can differ by more than six times (2.0–12.9 µm). In addition, in comparison with *C. mylnikovi*, *C. perpusilla* has a more expressed tapering of apices (Figs. 35 and 36, in Petersen and Hansen, 1960; Fig. 38 in Dürschmidt, 1985). Unlike *C. mylnikovi*, plate scales of *C. rhytidus* are elliptical to pear-shaped and ornamented with radially located and branched grooves. Meanwhile, the plate scales of *C. mylnikovi* are oviform or oval, with axial ridge and a thin marginal rim. By contrast with *C. mylnikovi*, plate scales of *C. perpusilla* are oval, without particular texture, but with a more or less pronounced structure in the center, mentioned in the original description as a central thicker part (Petersen and Hansen, 1960, p. 552).

Interestingly, findings of *C. perpusilla* with an axial ridge on the plate scales have been reported (Leonov and Plotnikov, 2009; Prokina et al., 2020). However, the isolate from Vietnam identified as *C. perpusilla* (Prokina et al., 2020) differs from the

original description (Petersen and Hansen, 1960) in having longer spine scales (3.29–8.73 µm) and the plate scales with a “concave central part and axial ridge” (Prokina et al., 2020, p. 736). Although the isolate of *C. perpusilla* from another source described by the same authors (Prokina and Mylnikov, 2019) differs from the “Vietnam isolate” (Prokina et al., 2020) by the presence of basal plates “with straight edges” and plate scales “without texture” (Prokina and Mylnikov, 2019, p. 13–14). The isolate of *C. perpusilla* described by Leonov and Plotnikov (2009) also differs from the original description by having larger spine scales (6.0–10.0 µm), larger diameter of basal plates (2.6–3.0 µm) and larger plate scales (3.5–4.5 × 2.5–3.1 µm), as well as by the presence of an axial ridge on the plate scales, as shown in Fig. 2 (Leonov and Plotnikov, 2009, p. 648), but missing in the description.

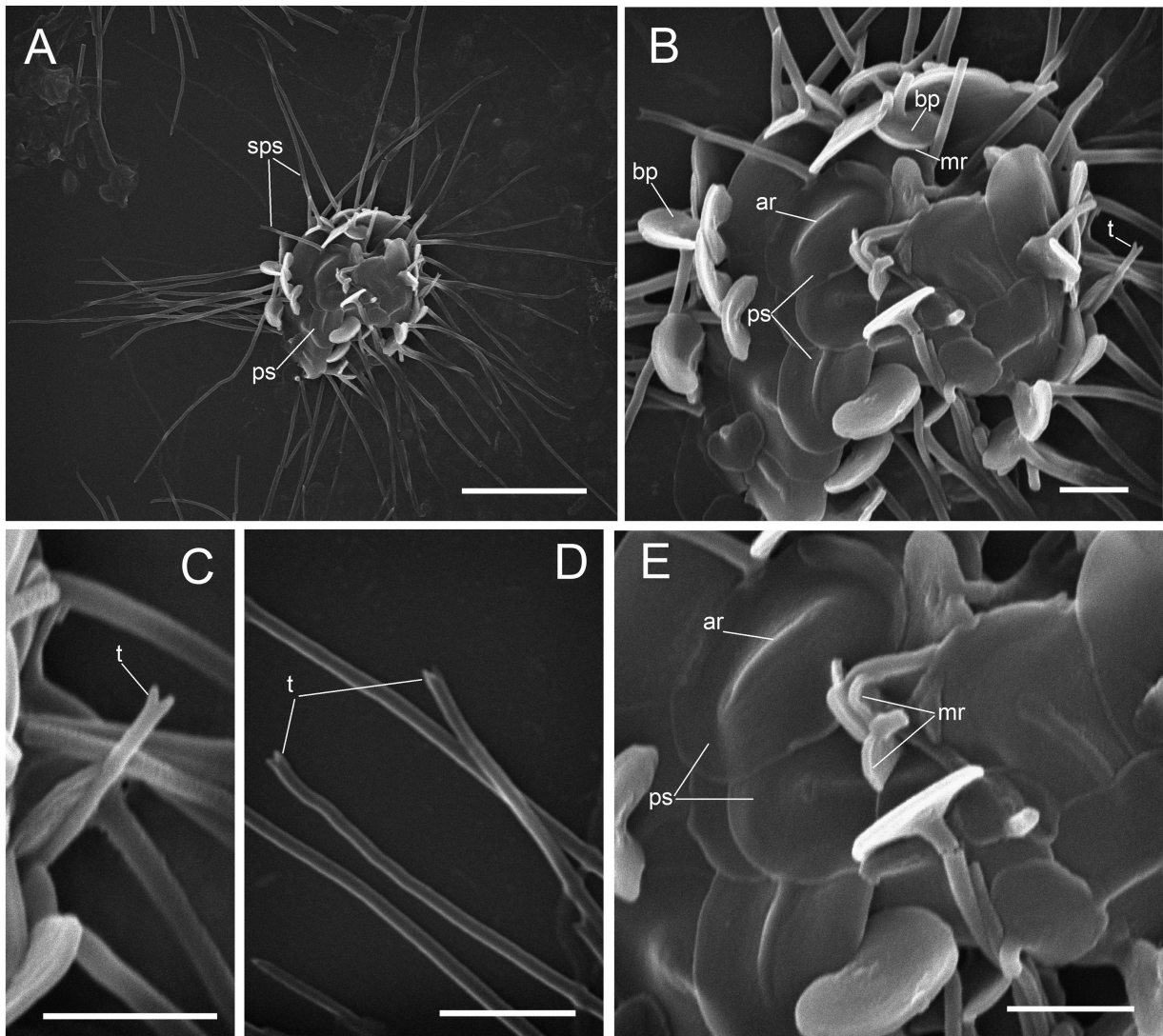
**Remarks.** This species was previously described as *Choanocystis perpusilla* (Gerasimova and Plotnikov, 2015; Gerasimova, 2021).

#### *Choanocystis punctata* sp. n. (Fig. 2, A–P).

**Material.** 10 cells from the sampling site with salinity 0.6 ppt.

**Description.** The cell coat consists of short and long spine scales, as well as of plate scales. Both types of spine scales have pointed apices. Sometimes short spine scales end with a small bent hook or a bent apex (Fig. 2, M). Shafts of spine scales are hollow, cylindrical, straight or curved. Some of them have the heart-shaped basal plates, and in this case,



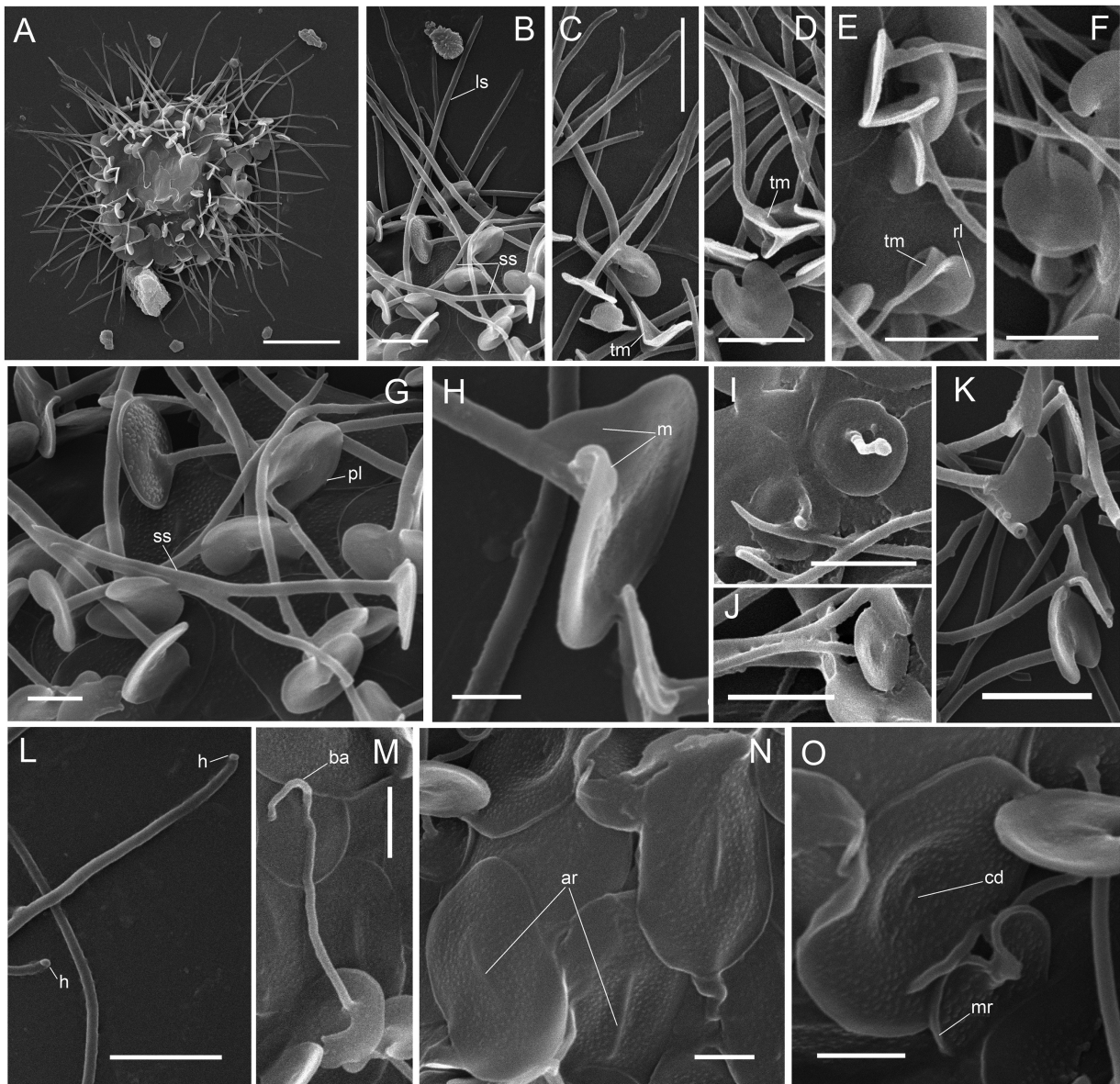


**Fig. 1.** Surface morphology of *Choanocystis mylnikovi* (SEM). A, B – General view; C, D – apices of a spine scales with teeth; E – plate scales. *Abbreviations:* ar – axial ridge; bp – basal plate; mr – marginal rim; ps – plate scale; sps – spine scale; t – teeth. Scale bars: A – 5  $\mu\text{m}$ ; B–E – 1  $\mu\text{m}$ .

shafts are located on the basal plates eccentrically (Fig. 2, H, I). In other spine scales, the basal plate is rounded and the shafts are located near the center of the basal plates, but not centrally (Fig. 2, J, K, M). Basal plates are flat, ornamented with numerous granules and surrounded with a marginal rim (Fig. 2, H, P). Most often, the marginal rim of basal plates ends near the base of the shaft at the invagination of the basal plates (Fig. 2, P). In the other cases, basal plates with rim have upward bent edges at the base of the invagination of the basal plates and form two membranes (occasionally of different length and asymmetric) extending along the shaft for a short distance (0.3–0.5  $\mu\text{m}$ ) (Fig. 2, H, I). Sometimes, edges of basal plates

form almost triangular membranes (Fig. 2, C–E) extending at the distance not exceeding 1.2  $\mu\text{m}$  from basal plates. Short spine scales are 3.6–9.9  $\mu\text{m}$  in length, 0.1–0.2  $\mu\text{m}$  thick, with heart-shaped (1.0–2.3  $\mu\text{m}$  in diameter) or rounded basal plates (1.5–2.0  $\mu\text{m}$  in diameter). Shafts of the short spine scales can be curved proximally, distally, along the entire length, as well as curved on apex and form a curl or a small bent hook (Fig. 2, M). Long spine scales are 10.0–18.5  $\mu\text{m}$  in length, 0.18–0.3  $\mu\text{m}$  thick, eccentrically located at the base of heart-shaped basal plates, 1.0–2.2  $\mu\text{m}$  in diameter (Fig. 2, B). Straight and proximally curved shafts with pointed apices were found. Plate scales from oval to roundish were found, both types are ornamented





**Fig. 2.** Surface morphology of *Choanocystis punctata* (SEM). A – General view; B – short and long spine scales; C – long spine scales with triangular membrane; D–G – short spine scales with unusual form of basal plates; H, I – membrane at the base of basal plates; J, K – spine scales with rounded basal plates; L – hollow shaft of spine scales; M – short spine scale with bent apex and rounded basal plate; N – apices of long spine scales end with small hook; O – oval plate scales; P – rounded plate scales. *Abbreviations:* ba – bent apex; cd – central depression; h – hook; ls – long scale; m – membrane; pl – *Pterocystis*-like basal plates; rl – *Raineriophrys*-like basal plates; ss – short scale; tm – triangular membrane; for the explanation of other symbols see Fig. 1. Scale bars: A – 10  $\mu\text{m}$ ; B–F, H, J–L – 2  $\mu\text{m}$ ; G, M–P – 1  $\mu\text{m}$ ; I – 0.5  $\mu\text{m}$ .

with numerous granules and a thin marginal rim. The oval plate scales (Fig. 2, O) are 2.9–4.7  $\mu\text{m}$  long and 1.5–3.2  $\mu\text{m}$  wide, with axial ridge. The rounded plate scales (Fig. 2, P) are not numerous, 2.7–4.0  $\mu\text{m}$  in diameter, with well-expressed central depression.

**Comparative description.** The morphology of

*C. punctata* is similar to *C. lepidula* Penard 1904. The most similar diagnostic features are plate scales ornamented with granules and axial ridge as well as proximally curved spine scales with upward bent edges that slightly extend along the shaft. Nevertheless, unlike *C. lepidula* having bluntly

cut spine scales of just one type (7.1–9.5  $\mu\text{m}$ ), *C. punctata* has both long (10.0–18.5  $\mu\text{m}$ ) and short (3.6–9.9  $\mu\text{m}$ ) spine scales with tapering apices that are sometimes bent or even form a small hook. By contrast with *C. lepidula*, basal plates of spine scales in *C. punctata* are ornamented with numerous granules. In *C. lepidula*, plate scales vary from oval to oblong shape, all with a central depression (Patterson and Dürschmidt, 1988). In *C. punctata*, plate scales can also be oval; however, unlike *C. lepidula*, oval plate scales of *C. punctata* have an axial ridge. Additionally, rounded plate scales with central depression can be observed in *C. punctata* but they are absent in *C. lepidula*. Oval plate scales in *C. punctata* are shorter (2.9  $\mu\text{m}$  compared to 4.2  $\mu\text{m}$  in *C. lepidula*) and wider (3.2  $\mu\text{m}$  compared to 2.3  $\mu\text{m}$  in *C. lepidula*).

The isolate, identified as *C. pelagica* Ostenfeld 1904 (Mikrjukov, 1994a), but presumably representing a separate species (see Cavalier-Smith and Heyden 2007, p. 1198, for discussion), is similar to the new species in having pointed spine scales with proximally curved shafts and ornamented heart-shaped plate scales. But this isolate has only one type of spine scales, 6.0–12.6  $\mu\text{m}$  (often 6–7  $\mu\text{m}$ ) long, while *C. punctata* has both long (10.0–18.5  $\mu\text{m}$ ) and short (3.6–9.9  $\mu\text{m}$ ) spine scales. In addition, this isolate has no granules on the basal plates. By contrast with *C. punctata*, plate scales of “*C. pelagica*” (Mikrjukov, 1994a), are ornamented with granules only in the central part.

Another isolate, identified as *C. pelagica* (Mikrjukov, 1994b) and corresponding to the original description by Ostenfeld (Ostenfeld, 1904), is similar to *C. punctata* in having taper spine scales and basal plates with many small granules. At the same time, *C. pelagica* (Mikrjukov, 1994b) has one type of spine scales of a similar length (7  $\mu\text{m}$ ) and patternless plate scales with a clear medial constriction.

**Remarks.** This species was previously described as *Choanocystis pelagica* (Gerasimova and Plotnikov, 2015) and as *Choanocystis* aff. *pelagica* (Gerasimova, 2021).

#### TAXONOMIC SUMMARY

**Assignment.** Eukaryota; Diaphoretickes; Haptista; Centroplasthelida; Choanocystidae.

#### *Choanocystis mylnikovi* sp. n.

**Diagnosis.** Centrohelids with plate scales and spine scales. Spine scales are highly variable in length. Spine scales are slightly tapering to apex and

possessing two small teeth of the same length. Shafts of spine scales seat eccentrically in heart-shaped basal plates surrounded by a marginal rim. Spine scales are 2.0–12.9  $\mu\text{m}$  long, 0.1–0.18  $\mu\text{m}$  thick, basal plates are 0.6–1.4  $\mu\text{m}$  in diameter. Plate scales are oviform or oval, 1.6–2.9  $\times$  1.0–1.8  $\mu\text{m}$ , with a clearly expressed axial ridge and a thin marginal rim.

**Etymology.** The species group name “*mylnikovi*” is given in honor of Alexander Mylnikov, in the memory and with gratitude to him as a teacher.

**Type Figure.** Figure 1, A–E.

**Zoobank LSID:** urn:lsid:zoobank.org:act:A7940B96-F88A-4AC9-B3BB-4EAAC5955E4F.

**Type locality.** Bottom sediment from the Tuzlukkol’ River, salinity 20–22 ppt, South-East of the European part of Russia, N 51°17’8”, E 56°36’16”. Collected on 02.07.2015.

#### *Choanocystis punctata* sp. n.

**Diagnosis.** Centrohelids with plate scales and short and long spine scales. Spine scales with hollow and cylindrical shaft tapering to the pointed apex. Short spine scales are 3.6–9.9  $\mu\text{m}$  long, 0.1–0.2  $\mu\text{m}$  thick, eccentrically located at the heart-shaped basal plates (1.0–2.3  $\mu\text{m}$  in diameter), or located almost centrally at the rounded (1.5–2.0  $\mu\text{m}$  in diameter) basal plates. Shafts of the short spine scales can form a curl or small bent hook. Long spine scales are 10.0–18.5  $\mu\text{m}$  long, 0.18–0.3  $\mu\text{m}$  thick, eccentrically located at the heart-shaped basal plates that are 1.0–2.2  $\mu\text{m}$  in diameter. Basal plates of both long and short spine scales are flat, ornamented with numerous granules and marginal rim. In some cases, marginal rim forms two short membranous extensions along the shaft and can form almost triangular membranes. Plate scales are from oval (2.9–4.7  $\times$  1.5–3.2  $\mu\text{m}$ , with axial ridge) to roundish (2.7–4.0  $\mu\text{m}$  in diameter, with well-expressed central depression), ornamented with numerous granules and thin marginal rim.

**Etymology.** The species group name “*punctata*” (from the Latin *punctum* – a dot) refers to dot-like granules ornamenting the plate scales and basal plates of the spine scales.

**Type Figure.** Figure 2, A–P.

**Zoobank LSID:** urn:lsid:zoobank.org:act:E7FA5E08-163A-4257-A527-AD76A0F64C52.

**Type locality.** Bottom sediment from the Tuzlukkol’ River, salinity 0.6 ppt, South-East of the European part of Russia, N 51°17’3” E 56°36’21”. Collected 02.07.2015.



## Discussion

### JUSTIFICATION OF THE NEW SPECIES

The genus *Choanocystis* was established by Penard (1904) and for a long time remained monotypic. Only in 1988, the genus was re-defined based on ultrastructural data, and many of the former *Acanthocystis* species, including the classical *A. aculeata*, were transferred to the genus *Choanocystis* (Siemensma, 1988). According to an emended diagnosis, *Choanocystis* was characterized by two contrasting types of siliceous scales: spine scales with heart-shaped basal plates, as well as oval or bilobed tangential plate scales (Adl et al., 2019). It was established that the main taxonomic features suggested for *Choanocystis* are: (1) structure of the spine scale apexes and presence of marginal teeth on them, (2) structure of the spine scale basal plate margin, (3) shape of the spine scales shafts, and (4) the shape and ornamentation of the plate scales (Mikrjukov, 1995). According to the morphological features of the spine scales' apexes, *Choanocystis* was divided into four groups: *C. pelagica*-group with pointed apexes; *C. lepidula*-group with truncated apexes bearing marginal teeth; *C. bicornioides*-group with dichotomously branched apexes; *C. malyutini*-group with three furcae (Mikrjukov, 1995, 1997). *C. mylnikovi* has slightly tapering spine scales with two small teeth of the same length on the apex and can be attributed to *C. lepidula*-group. *C. punctata* has spine scales with pointed apexes and can be attributed to *C. pelagica*-group.

*C. punctata* clearly belongs to a traditional *Choanocystis* morphospecies sensu Siemensma and Roijackers (1988), due to the eccentric shaft position in the majority of their spine scales – the main diagnostic feature of *Choanocystis* (Siemensma and Roijackers, 1988; Mikrjukov, 1995, 1997). At the same time, noteworthy is the occasional presence of some features that are characteristic for other morphologically defined genera of centrohelids. Sporadically present spine scales with almost central position of the shaft and ovoid basal plate (Fig. 2, I, J) resemble the typical spine scales of the genus *Acanthocystis* (Patterson and Dürrschmidt, 1988). Similarly, membranous extensions of the basal plate along the shaft, observed in some of *C. punctata* scales, are somewhat similar to the membranes (Fig. 2, F) in spine scales of *Pterocystis* (e.g., *P. pyriformis* Dürrschmidt, 1987) or *Raineriophrys* (e.g., *R. echinata* Rainer, 1968 and *R. scaposa* Dürrschmidt, 1987)

(Fig. 2, C–E) (Dürrschmidt, 1987; Siemensma and Roijackers, 1988). Interestingly, *R. scaposa* is similar to *Choanocystis* spp. by the heart-shaped basal plates of its spine scales. All these observations indicate that clear morphological diagnoses of the centrohelid genera become less distinct as the more complete picture of centrohelid diversity is revealed. It is becoming more and more difficult to establish a taxonomy basing solely on skeleton ultrastructure, and the application of molecular phylogenetics as well as the search for additional characters unifying the genus-level taxa are necessary.

Dealing with *C. mylnikovi*, we had difficulties with the correct naming and identification of the similar species, *C. perpusilla*, due to the variable scale morphology in the descriptions of different authors. *C. perpusilla* was originally described by Petersen and Hansen (1960) as *Acanthocystis perpusilla*, using light and electron microscopy. The authors (Petersen and Hansen, 1960) described straight or slightly curved spine scales (2.0–4.5 µm) that seat eccentrically on the nodular-shaped basal plates; oval plate scales (2.5 × 1.7 µm) without particular texture, but with a more or less pronounced structure in the center. Later Siemensma (1981) re-isolated this species and described the same morphology, i.e. the straight or slightly curved spine scales (except for longer spine scales, 4.8–8.2 µm) and plate scales without any structure. In addition, Siemensma (1981) noted the short length of the spine scales in relation to discoid or nodular-shaped basal plates and the characteristic tapering of the spine scales apexes observed in a light microscope. Dürrschmidt (1985) described this species from Chilean Lake Calafquén and noted that “the fine structure of spine scales and plate scales agrees with Petersen and Hansen’s description, although have discrepancies in the dimensions”, 3.0–5.0 µm for spine scales and 1.8–2.5 × 1.6–2.0 µm for plate scales (Dürrschmidt, 1985, p. 69, 71). However, many divergent forms of the spine scales were observed, but it remains unclear if it is a distinctive feature to separate different species or it should be considered as intraspecific variation. Later Dürrschmidt (1987) described the species *Acanthocystis cordiformis* ssp. *parvula* closely related to *A. perpusilla* in the construction of the spine scales (Dürrschmidt, 1987, p. 41). Nevertheless, it was noted that the spine scales of *A. perpusilla* are only half of the size of *A. cordiformis* ssp. *parvula*, also possessing from 2 to 4 teeth (Dürrschmidt, 1987, p. 40, Table 2). Therefore, *A. cordiformis* ssp. *parvula* was established as a separate taxon (Dür-



rschmidt, 1987). Despite that, Mikrjukov (2002) in his identification guide mistakenly recognized *A. cordiformis* ssp. *parvula* as a junior synonym of *C. perpusilla*. It caused the confusion in the subsequent identification of *C. perpusilla*. For example, the isolates from Vietnam described as *C. perpusilla* (Prokina et al., 2020) possess plate scales with an axial ridge and longer spine scales (3.29–8.73 µm). The author described the “Vietnam isolate” as *C. perpusilla* and compared its dimensional characteristics with *C. cordiformis* ssp. *parvula*, confusingly recognized as a junior synonym by Mikrjukov (2002). *C. perpusilla* from another source, described by the same authors (Prokina and Myl’nikov, 2019), differs from the “Vietnam isolate” (Prokina et al., 2020) by the presence of basal plates “with straight edges” and plate scales “without texture” (Prokina and Myl’nikov, 2019, p. 13–14).

One more finding of *C. perpusilla* was described by Leonov and Plotnikov (2009) and Leonov and Myl’nikov (2012). However, *C. perpusilla* (Leonov and Plotnikov, 2009) differs from the original description by having larger spine scales (6.0–10.0 µm), larger diameter of basal plates (2.6–3.0 µm), and larger plate scales (3.5–4.5 × 2.5–3.1 µm), as well as by the presence of an axial ridge on the plate scales shown in Fig. 2 (Leonov and Plotnikov, 2009, p. 648) but missing in the description. Other cells from the different source (Leonov and Myl’nikov, 2012) were called *C. perpusilla*, despite the lack of morphological description of the plate scales and larger spine scales (8–10 µm). Additionally, the authors wrongly estimated the dimensions of the basal plates (2.6–3.0 µm) that in their illustrations were smaller than the scale bar (= 2 µm) and did not exceed one half of it (Leonov and Plotnikov, 2009, Fig. 2).

The isolates named “*C. perpusilla*” (Mikrjukov, 2002; Leonov and Plotnikov, 2009; Leonov and Myl’nikov, 2012; Prokina and Myl’nikov, 2019; Prokina et al., 2020) differ by morphology from the original description of this species (Petersen and Hansen, 1960) presumably representing a separate species of *Choanocystis*.

#### BRACKISH-WATER DISTRIBUTION OF CHOANOCYSTIDS

Mikrjukov (1995) described *C. pelagica*-group as a complex of species from marine habitats. However, *C. ebelii* Wujek et Elsner, 1992, also included in this group, was described from fresh waters (Wujek, 1992 (as *Pterocystis ebelii*)) and from brackish-wa-

ter habitats (10 ppt, Plotnikov and Gerasimova, 2017). Therefore, the group cannot be considered truly marine. Two other members of this group, *C. pelagica* and *C. kareliensis*, were isolated from marine habitats (Ostenfeld, 1904; Mikrjukov, 1994a, 1994b; Zagumyonnyi et al., 2020); however, *C. kareliensis* was also noted in a freshwater habitat (Leonov, 2012). *C. mylnikovi* was isolated from continental brackish waters with salinity 20–22 ppt, which also contradicts purely marine nature of *C. pelagica*-group.

The species list of centrohelids in brackish waters worldwide includes 29 species, with only 9 species of *Choanocystis* (see Table 1 in Gerasimova, 2021):

1. *C. aculeata* was found in the Kuchuk-Adjigol Lake (3–4 ppt; Mikrjukov, 1999), the Baltic Sea (10–12 ppt; Vørs, 1992a), the Coast of Alabama (20 ppt; Jones, 1974), and the Kattegat (22 ppt; Vørs, 1992b).

2. *C. bicornioides* was noted in the Black Sea (18 ppt; Mikrjukov, 1999).

3. *C. cordiformis* ssp. *parvula* was noted in the Sea of Japan (29 ppt; Zagumyonnyi et al., 2020).

4. *C. ebelii* was noted in the Bol’shaya Samoroda River (10 ppt; Plotnikov and Gerasimova, 2017).

5. *C. kareliensis* was noted in the unnamed brackish lake on the Cape Zelenyi (23 ppt; Mikrjukov, 1994a).

6. *C. aff. pelagica* was noted in the Tuzlukkol’ River (0.6 ppt; Gerasimova, 2021).

7. *C. perpusilla* was noted in the Maloe Gorodskoe Lake (1.6–1.7 ppt; Leonov and Plotnikov, 2009), and the Gulf of Finland of the Baltic Sea (6 ppt; Vørs, 1992a).

8. *C. rotoairense* was noted in the Baltic Sea (12 ppt; Vørs, 1992a).

9. *C. rotundata* was noted in the Baltic Sea (18 ppt; Mikrjukov, 1999).

In the continental brackish and hyperhaline waters of Russia (2–42.2 ppt) twenty centrohelids species have been described (Mikrjukov, 1994a, 1994b, 1999, 2001; Leonov and Plotnikov, 2009; Plotnikov and Ermolenko, 2015; Plotnikov and Gerasimova, 2017; Gerasimova, 2021), including 5 species of *Choanocystis*. This study adds two new species to the list of centrohelids from the continental brackish waters of Russia.

Potentially vast diversity of brackish-water centrohelids was supported by the recent description of the new genus and species *Pinjata ruminata* (Zlatogursky et al., 2019) and by the high-throughput sequencing survey focused on environmental

samples (Gerasimova et al., 2020). That survey revealed six potentially novel phylotypes without morphological equivalents, with no obvious homology with known centrohelids' species, and represented first sequenced members of understudied centrohelids' lineages (Gerasimova et al., 2020). The phylogenetic 18S rDNA tree of centrohelid heliozoans contained at least eleven clades with members representing environmental sequences of marine/saline/brackish origin, which remain undescribed in terms of scale morphology (Cavalier-Smith and Heyden, 2007; Shishkin et al., 2018; Gerasimova et al., 2020). Potentially vast diversity of undescribed species of centrohelid heliozoans in brackish waters indicates the necessity of further morphological and molecular surveys.

## Conclusions

Based on morphological differences with the described species of centrohelid heliozoans, *C. mylnikovi* and *C. punctata* were described as new species of the genus *Choanocystis*. Potentially vast diversity of undescribed species of centrohelid heliozoans in brackish waters indicates the necessity of their further morphological and molecular surveys.

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