Protistology 17(2): 63–72 (2023) | doi:10.21685/1680-0826-2023-17-2-1

Protistology

ORIGINAL ARTICLE

Pterocystis caudata sp. n. – the new species of centrohelid heliozoans (Pterocystidae, Pterocystida, Centroplasthelida) from the Bay of Villefranche, France

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| Submitted April 8, 2023 | Accepted May 2, 2023 |

Summary

A new species of centrohelid heliozoans *Pterocystis caudata* sp. n. from the Bay of Villefranche (France) was studied with light and electron microscopy. The cell diameter is 7.6–19.0 (av. 12.4) µm. *P. caudata* has scales of two distinct types. Plate scales (2.08–3.48 (av. 2.6) × 1.07–1.65 (av. 1.3) µm) are flat, oval, with parallel edges and axial thickening. Spine scales (2.47–7.1 (av. 4.6) µm) with a funnel-shaped base, which is indistinctly continuous with a shaft. The shaft demonstrates gradual levels of reduction; in some scales it is completely absent. The surface of the spine scales is smooth, ornamented with few slits at the base of the shaft and on the surface of the funnel and has a thin marginal border. The cells are floating or attached to the substratum, sometimes in aggregates of 2–3 cells. The species diversity of *Pterocystis* spp. with leaf-shaped and scoop-shaped spine scales is discussed.

Key words: centrohelids, Centroplasthelida, external skeleton, heliozoa, Pterocystida, systematics

Introduction

Centrohelid heliozoans (Centroplasthelida Febvre-Chevalier et Febvre, 1984) are protists belonging to the Haptista supergroup (Cavalier-Smith, 2003; Cavalier-Smith et al., 2015; Burki et al., 2016; Adl et al., 2019). Their distinct position and unique ultrastructural identity were recognized after accumulation of the critical mass of general ultrastructure surveys (Febvre-Chevalier and Febvre, 1984; Dürrschmidt and Patterson, 1987), but their proper place on the eukaryotic tree of life was possible to determine only with the help of phylogenomics (Cavalier-Smith et al., 2015; Burki et al., 2016). The first ultrastructural studies of the siliceous scales, covering the cells (Nicholls, 1983; Dürrschmidt, 1985, 1987a, 1987b; Nicholls and Dürrschmidt, 1985), revealed a considerable

https://doi.org/10.21685/1680-0826-2023-17-2-1

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morphological diversity, which led to description of many genera and species based on the details of scale morphology. Initially, most of the species having a double skeletal layer (plate scales, covering the cell surface and spine scales, facing outer environment) were described under generic name *Acanthocystis* Carter, 1863, but subsequently it was split to many genera and even families and nowadays the double layer of siliceous scales is believed to be ancestral trait of centrohelids (Cavalier-Smith and Heyden, 2007). In her classical survey on centrohelid scales structure Monika Dürrschmidt split *Acanthocystis* (in a broad sense, as described above) into 10 groups designated as A_1, A_2, B, C_1, C_2, D_1, D_2, F_1, F_2 and E (Dürrschmidt, 1987b). A_1- and A2-groups get mixed with each other in 18S rDNA molecular phylogeny (Zlatogursky et al., 2017), but together form a strongly monophyletic clade now recognized as *Acanthocystis sensu stricto* (Siemensma and Roijackers, 1988). B-group more or less corresponds with the genus *Ozanamia* Shɨshkin-Skarð, 2022, but a similar scale morphotype is also known in *Meringosphaera* Lohmann, 1902 and *Marophrys* Cavalier-Smith et von der Heyden, 2017 (Shɨshkin-Skarð et al., 2022). The taxonomic history of the groups designated by letters C-E was the most complicated and relationships between the species belonging to them are still far from being fully resolved. Siemensma and Roijackers separated C, D and E as *Pterocystis* Siemensma et Roijackers, 1988 and transferred F to *Raphidocystis* Penard, 1904 (Siemensma and Roijackers, 1988; Siemensma, 1991). Later Mikrjukov formally described E as *Pseudoraphidiophrys* Mikrjukov, 1997 and F as *Pseudoraphidocystis* Mikrjukov, 1997(Mikrjukov, 1997). He also separated part of C (not corresponding to either C_1 or C_2 of Dürrschmidt) as *Raineriophrys* Mikrjukov, 2001 (initially *Echinocystis* Mikrjukov, 1997 and *Raineria* Mikrjukov, 1999, both found to be preoccupied). None of these clades has a molecular phylogenetic confirmation. The genus *Pterocystis* in its current sense unifies the centrohelids with wing-like extensions on the spine scales that are indistinctly continuous with the spine scale base (corresponds to D and partly C of Dürrschmidt). The genus includes 29 described species. Here, we describe a new marine species *Pterocystis caudata* sp. nov. with a unique fine structure of the scales.

Material and methods

Cultures and samples

The sample was collected from the Bay of Villefranche, France (N43.683; E7.317, a monitoring point “B”) on 13 September 2019 using a 50-µm plankton net at the surface water layer. The details of the sampling procedure were described previously (Volkova and Kudryavtsev, 2021; Kudryavtsev et al., 2022). The collected material contained a colony of *Collodaria* radiolarians, which was isolated from the sample using sterile needles and inoculated into artificial seawater (40‰) with addition of a wheat grain as nutrient source. The clonal cultures were obtained by isolation of single cells in the artificial seawater with addition of 0.025% cerophyl (Weizengras, Sanatur GmbH, Germany) with bodonid flagellates, isolated from tidal salt marsh, as a prey and an unregulated bacterial community.

Microscopy and morphometry

Light micrographs were taken using the temporary preparation on an object slide with Leica DM2500 (100× planapochromate objective) microscope, equipped with differential interference contrast (DIC) and phase contrast and a Nikon DS-Fi1 camera with accompanying software (Nis-Elements, Nikon Corporation, Japan). The morphometry was studied using the photos of 109 cells taken with Leica DMI3000 inverted microscope (63× objective) equipped with phase contrast optics and with the same camera. Preparation of the scales for electron microscopy was conducted according to Zlatogursky (2014). The cells were air-dried on the surface of a coverslip (for scanning electron microscopy, SEM) or formvar-coated copper grids (for transmission electron microscopy, TEM). The coverslips/grids were washed with distilled water. Coverslips were attached to the specimen stubs, gold-coated and observed with a Tescan MIRA3 LMU scanning electron microscope at 10 kV. Grids were directly observed with the Jeol JEM-1400 transmission electron microscope at 80 kV without any shadowing or coating. All measurements of living cells and scales were made using ImageJ Ver. 1.46r (Abràmoff et al., 2004). Scatterplots were built using R v. 4.2.0 (R Core Team, 2021).

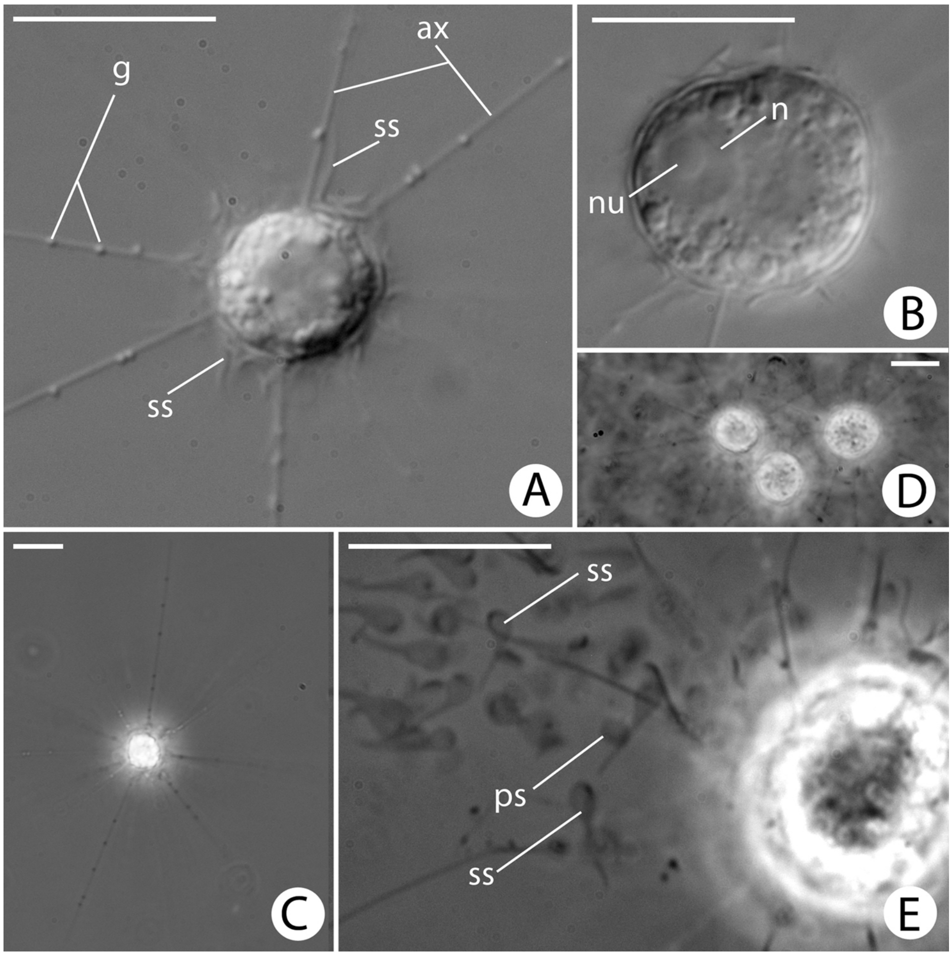

Fig. 1. Light micrographs of *Pterocystis caudata* sp. n. A, B – DIC, C–E – phase contrast. A – General view of the cell under the cover slip, optical section through the centre; B – same view showing nucleus; C – solitary cell; D – cells aggregation in a Petri dish; E – general view of the cell with scales detached from the cell surface. *Abbreviations*: ax – axopodium, g – granules, n – nucleus, nu – nucleolus, ps – plate scale, ss – spine scale. Scale bars: 10 μm.

Results

Light microscopy

The cells were spherical (Fig. 1). Most of the cells were floating and single, although aggregates of 2-3 cells have sometimes been observed (Fig. 1, D). The cell diameter was 7.6–19.0 (av. 12.4) μm [N = 109]. Axopodia were predominately 2-3 cell diameters long (Fig. 3) and were distinctively granulated. The scales formed a well visible layer surrounding the cells, their distribution was irregular.

Electron microscopy

Most of the plate scales were oval, often with parallel sides (Fig. 2, A-C, E) with a well-developed axial thickening and thin marginal border (Fig. 2, B, C, E). The length of plate scales was 2.08–3.48 (av. 2.6) μm [N = 45] and the width was 1.07–1.65 (av. 1.3) μm [N = 45] (Fig. 3). Spine scales were elongated with a well-developed shaft, which was indistinctly continuous with the funnel-shaped scale base (Fig. 2, A, B, D). The funnel-shaped part of the spine scales formed a short stalk with a

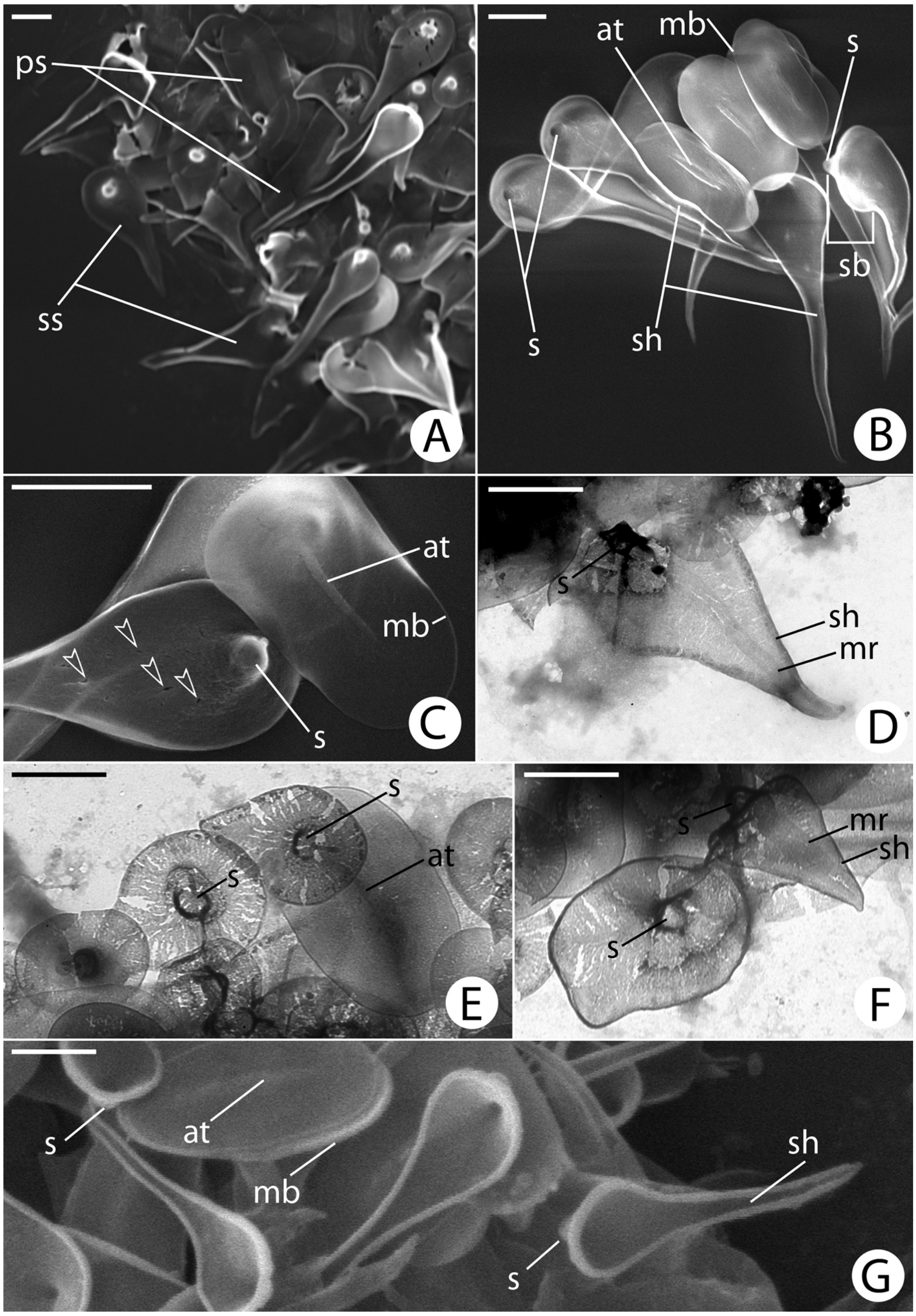

Fig. 2. *Pterocystis caudata* sp. n. scales observed with SEM (A–C) and TEM (D–F). *Pterocystis* sp. from marine aquarium, scales observed with SEM (G). A – Spine scales and plate scales under low magnification; B – spine scales showing different shapes of the shaft and plate scales; C – close up of plate scale and spine scale's base showing small slits parallel to the shaft axis (transparent arrowheads); D – close up of the spine scale with the shaft of middle length; E – close up of the spine scales with reduced shafts and plate scale; F – close up of the spine scales with more or less reduced shafts; G – close up of the spine and plate scales. *Abbreviations*: at – axial thickening, mb – marginal border, mr – midrib, ps – plate scales, s – stalk, sb – scale base, sh – shaft, ss – spine scales. Scale bars: 1 μm.

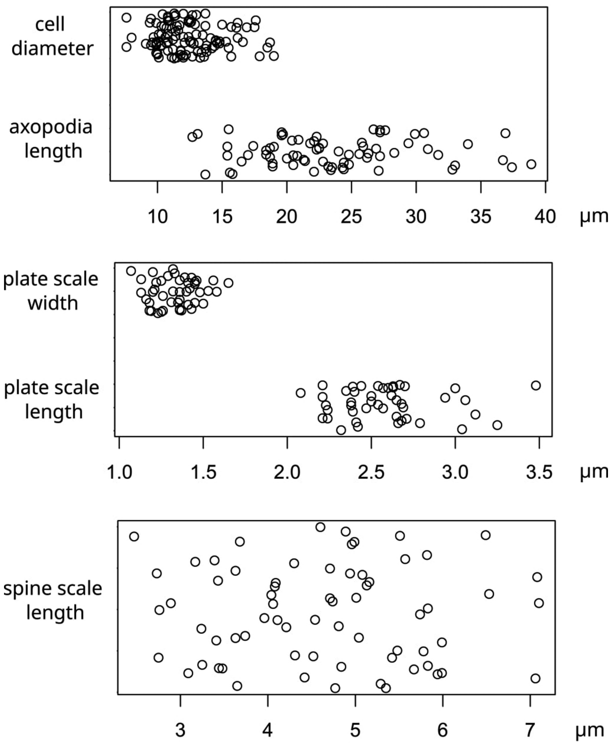

Fig. 3. Scatterplots with jitter, visualizing the size variability of cell and skeleton structures in *Pterocystis caudata* sp. n.

bulbous ending (Fig. 2, B-F). The shaft could be almost perfectly straight (Fig. 2, B), but often it was markedly curved or even wavy (Fig. 2, B). The midrib of the shaft going from the funnel towards the tip of a spine scale was only detectable when observed with TEM (Fig. 2, D, F). The small slits parallel to the shaft axis were present near the shaft base and on the funnel surface (Fig. 2, C). In some of the spine scales, the shaft was rudimentary, more or less shortened or even completely absent (Fig. 2, E, F). The length of spine scales was 2.47–7.1 (av. 4.6) μm [N = 67] (Fig. 3). The funnel part and the shaft were outlined with a distinct marginal border (Fig. 2, A, B, C).

Taxonomic description

● Diaphoretickes Adl et al., 2012
●● Haptista Cavalier-Smith, 2003
●●● Centroplasthelida Febvre-Chevalier et Febvre, 1984
●●●● Pterocystida Cavalier-Smith et von der Heyden, 2007 sensu Shishkin et Zlatogursky, 2018
●●●●● Pterista Shishkin et Zlatogursky, 2018
●●●●●● Pterocystidae Cavalier-Smith et von der Heyden, 2007
●●●●●●● *Pterocystis* Siemensma et Roijackers, 1988
●●●●●●●● *Pterocystis caudata* Udalov et Zlatogursky sp. n.

Diagnosis: The cell body diameter usually 10–20 μm. Axopodia 2–3 cell diameters long, granulated. Plate scales oval, sometimes slightly sharpened towards one end with parallel edges, axial thickening and thin marginal border, typically 2–3.5 μm long and 1–1.6 μm wide. Spine scales (2.5–7 μm long) with elongated shaft and funnel-shaped base, outlined with marginal border. Funnel with circular or drop-shaped outline, separated from the shaft indistinctly but noticeably. Shaft with midrib going from the funnel up to scale tip. Funnel with short (not longer than its own diameter) bulbous stalk. Proximal part of shaft and funnel with some amount of slits parallel to scale axis. Some spine scales with rudimental shaft or without shaft. In marine waters.

Etymology: from L. *cauda* – tail; for often wavy or curved shafts of spine scales reminiscent of tails in spermatozoa or tadpoles.

Type locality: Bay of Villefranche, Mediterranean Sea, France (N43.683; E7.317).

Syntypes (hapantotype): air-dried preparation has been deposited in the Collection of Preparations of the Laboratory of Cytology of Unicellular Organisms, Institute of Cytology of the Russian Academy of Sciences, St. Petersburg; Accession No. 1063.

Zoobank LSID of the publication: urn:lsid:zoobank.org:pub:2BA73C0E-8EC2-4BE4-B4D4-5313FBFA7D1C.

Zoobank LSID of the species: urn:lsid:zoobank.org:act:8D6112FA-FBD1-4884-826A-0EA254AB1C8F.

Discussion

Since the pioneering publications of Monika Dürrschmidt (Dürrschmidt, 1985, 1987b), containing the description of *Pterocystis foliacea* (Dürrschmidt, 1985) Siemensma, 1991 and *P. pyriformis* (Dürrschmidt, 1987) Siemensma, 1991, several species with more or less similar scale structure were described. This made the subsection of *Pterocystis* with leaf-like spine scales (probably deserving a separate genus level, but to establish that, a molecular phylogenetic proof of its monophyly is necessary) somewhat difficult to identify, which leads to multiple misidentifications and questionable status of some taxa. Even *P. foliacea* itself from the very beginning obviously was a heterogeneous entity, which finally led Dürrschmidt to split it into three "subspecies" (Dürrschmidt, 1987b) that differ much more clearly than many of the subsequently described species. Thus, contrary to Mikrjukov who listed them as subjective synonyms of *P. foliacea* (Mikrjukov, 2002), here we raise their rank to the species level:

Pterocystis truncata (Dürrschmidt, 1987) Udalov et Zlatogursky comb. nov.

Basionym: *Acanthocystis foliacea* ssp. *truncata* Dürrschmidt, 1987

Objective synonym: *Pterocystis foliacea* ssp. *truncata* (Dürrschmidt, 1987) Wujek et Saha, 2006

Non *Pterocystis foliacea* ssp. *truncata* (Dürrschmidt, 1985) Siemensma, 1991 as in Prokina and Mylnikov (2019).

Pterocystis elongata (Dürrschmidt, 1987) Udalov et Zlatogursky comb. nov.

Basionym: *Acanthocystis foliacea* ssp. *elongata* Dürrschmidt, 1987

Objective synonym: *Pterocystis foliacea* ssp. *elongata* (Dürrschmidt, 1987) Wujek et Saha, 2006

After the initial description of *P. pyriformis* found in Chile (type location) and New Zealand (Dürrschmidt, 1987b), almost identical scales by both the size and morphology were reported from soil in Scotland (Esteban et al., 2006), erroneously labelled as "*Pterocantha pyriformis*" (not an officially introduced combination) in their Fig. 5, C. Subsequently, the scales of similar morphology were again reported from Chile (Prokina and Mylnikov, 2019). In this material, the spine scales were slightly longer (2–2.5 μm compared to 1.5–2 μm in the type specimen), while the plate scales were of the same size. Recently, the material nearly identical to the type description was also reported from Ontario, Canada (Nicholls, 2023). There are no other reports of this species in literature we are aware of, which may indicate its extreme rarity. The micrograph of the single scale from Florida, USA (Wujek, 2006) is definitely not *P. pyriformis*, as it is labelled on his Fig. 14, and its status will be discussed below. At the same time it is possible that this species adapted to soil (as Esteban's finding suggests) and could be rarely found due to the shortage of studies on soil centrohelids. Anyway, this species seems to be well outlined and easily distinguished by the presence of a considerable part of the shaft devoid of wings, which is not observed in any other *Pterocystis* with leaf-like spine scales (Fig. 4, I).

Four other *Pterocystis* with leaf-like scales were described with a combination of molecular and morphological data (Cavalier-Smith and von der Heyden, 2007), but both the quality of the published micrographs and the clarity of in text descriptions make it difficult to integrate these data with the subsequent and previous findings. The comparison of this material with other micrographs is even more problematic due to some obvious issues concerning the size of scales. Specifically, the text descriptions of size often contradict the size based on scale bars as it was noted previously (Prokina et al., 2018; Nicholls, 2023). They also contradict what we can confirm for the four species that are under discussion here (*Pterocystis contorta* von der Heyden et Cavalier-Smith, 2007; *P. cuspidata* Cavalier-Smith et von der Heyden, 2007; *P. ovata* Cavalier-Smith et von der Heyden, 2007; *P. polymorpha* Cavalier-Smith et von der Heyden, 2007). These species are similar to *P. foliacea, P. elongata* and *P. truncata* and need to be reisolated and studied in more details to establish more practically applicable morphological criteria.

P. contorta and *P. cuspidata* are likely distinct from *P. foliacea* by the more cuspidate shape of their spine scales (Fig. 4, A, F, K). They hardly can be confused with *P. truncata* (Fig. 4, B), because of contrastingly different proportions. However, their distinction from each other and *P. elongata* (Fig. 4, E) appears to be quite problematic. The scales shown on the type micrographs for these three species look very similar in shape and size. The scales reported as *P. foliacea* by several authors (Gaponova, 2008; Prokina et al., 2018; Gerasimova, 2021) also seem to belong to this problematic group, as well as single scale reported by Wujek (2006) as *P. pyriformis*. The main difference between *P. contorta* and *P. cuspidata* is a slightly twisted tip of the shaft in the first (Fig. 4, F) and a more flattened base of the spine scales in the second (Fig. 4, K). At the same time, the scales both with twisted and straight tips as well as both scales with more rounded and more flattened base are often present on the same cell (see Fig. 1 in Gaponova, 2008; Fig. 5I in Prokina et al., 2018; Fig. 1g in Gerasimova, 2021).

P. ovata (Fig. 4, C) and *P. polymorpha* (Fig. 4, D) are clearly distinct from *P. foliacea* (Fig. 4, A) and *P. elongata/contorta/cuspidata* (Fig. 4, E, F, K) by the shortened and broadened shape of some (not all) spine scales. They also seem to be different from *P. truncata* (Fig. 4, B) by the presence of more elongated scales along with truncated ones. At the

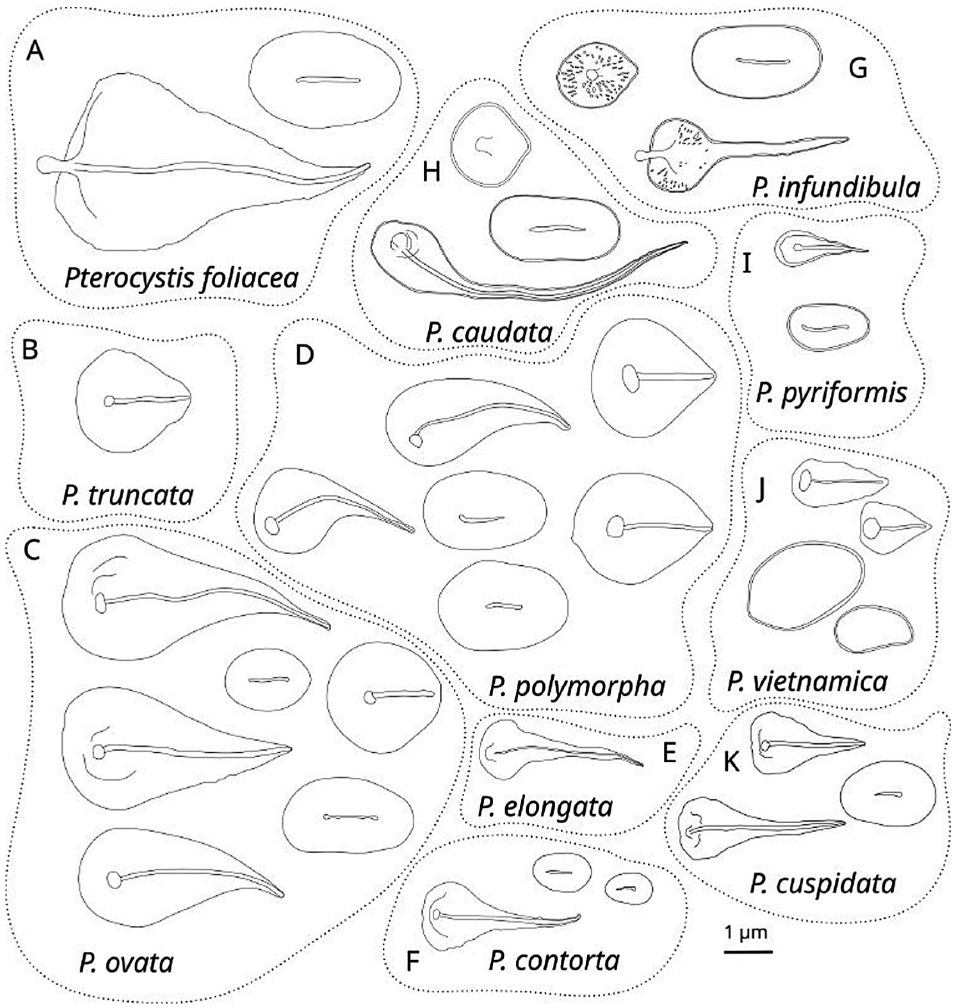

Fig. 4. Line drawings of siliceous scales (*Pterocystis caudata* sp. n. and other *Pterocystis* spp. discussed in the text are shown). All drawings are based on the type micrographs of the described species, all drawn to scale, but note that the dimensions for *P. contorta, P. cuspidata, P. ovata* and *P. polymorpha* were chosen arbitrarily (see text).

same time, the distinction between *P. ovata* and *P. polymorpha* is quite unclear. The finding of Prokina and Mylnikov (2019) on their Fig. 2h designated as *Pterocystis foliacea* subsp. *truncata* is quite similar to *P. ovata/polymorpha*, but a combination of more bluntly ending (diagnostic criterion of *ovata*) and more sharpened (diagnostic criterion of *polymorpha*) is observed in the coverings of the same individual.

P. vietnamica Prokina, Radaykina et Mylnikov, 2020 (Fig. 4, J) is easily distinguishable from the species described above by the absence of the conical stalk and the presence of the broad roundish depression at the scale base instead (Prokina et al., 2020).

All the above-mentioned species are exclusively freshwater without reliable reports from marine environments, except the finding of *P. elongata*-like organism at 2‰ salinity by Gerasimova (2021). *P. caudata*, described here, was found and kept at 40‰ and therefore needs to be considered a truly marine species. Its spine scales (Fig. 4, H) are much more obviously elongated than in *P. foliacea, P. truncata* and *P. ovata/polymorpha*. The problematic group of *P. elongata/contorta/cuspidata* (Fig. 4, E, F, K) is also dissimilar from it by the shape of spine scales. In these species, spine scales have a shape of the more or less distorted and/or elongated triangle, while spine scales of *P. caudata* are scoop-shaped with easily recognizable shafts. The same is true when comparing spine scales of *P. caudata* to those of *P. vietnamica* (Fig. 4, J).

Thus, the newly described *P. caudata* is well separated from *P. foliacea* and other *P. foliacea*-like species. At the same time, there are several findings reported in the literature as *P. foliacea*, which are quite similar to *P. caudata* by the shape of scales.

This confusion has started from Siemensma, who published a drawing of a scoop-shaped spine scale in his identification guide (Siemensma, 1991) under the name *P. foliacea*, without any micrographs or habitat description. Gerasimova and Plotnikov reported a centrohelid with scoop-shaped scales from Solyanka river (Volgograd region, Russia) at 16‰ and designated it as *Pterocystis foliacea* Siemensma, 1991 (which is not correct, since Siemensma is not the author of *foliacea*) (Plotnikov and Gerasimova, 2017). Prokina and coauthors found a heliozoan with similar scoop-shaped scales in Black sea at salinity 16–18‰ (Prokina et al., 2019). They designated their finding as *P. foliacea* and mentioned previous findings by Siemensma as well as by Plotnikov and Gerasimova, which do not match the diagnosis of *P. foliacea*. Nevertheless, their conclusion was that "observed morphological variation is probably within the intraspecific variability" (Prokina et al., 2019). The scales on their Figs. 2a-c are very similar to *P. caudata* by size and shape, which applies to both plate and spine scales. At the same time, we can hardly consider them conspecific. In *P. caudata,* we detected the presence of the midrib, which is only visible when observed with TEM (not SEM) and it is not clearly recognizable in the spine scales on Figs. 2a-c of Prokina and coauthors (2019), which is also a TEM micrograph. Additionally, in *P. caudata* the spine scales with a shaft reduced or even absent were found, but such scales are not visible on micrographs of the Black sea specimen. Thus, we consider the finding of Prokina and coauthors a potentially related, but separate undescribed species, not *P. caudata*. We also included a micrograph of another finding of a similar marine heliozoan, which was isolated from a marine aquarium at 40 ppt back in 2008 (Fig. 2, G). It was only studied with SEM and the presence of the midrib is impossible to determine, but judging by the absence of scales with reduced shaft, it is probably more related to Black Sea isolate, but not to *P. caudata*.

Finally, it is important to compare *P. caudata* and another recently described marine species *Pterocystis infundibula* Shɨshkin et Zlatogursky, 2022. The two are similar in having scoop-shaped spine scales with the shaft demonstrating different levels of reduction (Shɨshkin et al., 2022). Nevertheless, the two species are clearly distinct by the structure of the stalk. The spine scale stalk in *P. infundibula* is much longer, having a thin tube-like part and bulbous ending (Fig. 4, G). In contrast, the stalk of *P. caudata* spine scale has no tube-like part (Fig. 4, H), it is quite short and usually not longer than its own diameter. The other well-expressed difference is the reticulate structure of *P. infundibula* spine scales (Fig. 4, G), which was not observed in *P. caudata* (Fig. 4, H) except for the presence of non-numerous slits. The presence of the midrib in *P. infundibula* is unclear, because it was never studied with TEM.

Summarizing the species diversity described above we can note that two groups of species are starting to be outlined. *Pterocystis* spp. with leaf-like spine scales (*P. pyriformis, P. foliacea, P. elongata/ contorta/cuspidata, P. truncata, P. polymorpha/ ovata, P. vietnamica*) are restricted to freshwater and have never been reported in marine locations. In contrast, all the findings of *Pterocystis* spp. with scoop-like scales (*P. infundibula, P. caudata*, undescribed Black Sea species) are not in freshwater but only in continental saline habitats and sea. Erroneously, both groups are still oftentimes reported under the same species name — *Pterocystis foliacea*. This example indicates that many "species" that may seem to be euryhaline actually just suffer from lumping and misidentifications.

Acknowledgments

Plankton sampling was performed using the facilities of the Villefranche-sur-mer Oceanology Observatory. AK and EV are grateful to Alessandra Contento and Régis Lasbleiz for help at the Villefranche station, and Jean Yves Carval for operating the research vessel and performing plankton sampling. The reported study was funded by a Russian Science Foundation (RSF) grant (20-74-10068) (all the studies except sampling). Sampling was supported by the grant (agreement no. 730984, ASSEMBLE+ project) from the European Union's Horizon 2020 research and innovation program to AK. The present study utilized equipment of the core facility centres "Interdisciplinary Resource Centre for Nanotechnology" and "Centre for Molecular and Cell Technologies" of Saint Petersburg State University.

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