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Protistology

ORIGINAL ARTICLE

Pterocystis infundibula sp. n. (Pterocystidae, Pterocystida, Centroplasthelida) – the new species of centrohelid heliozoans from the Gulf of Aqaba, Israel

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Summary

A new species of centrohelid heliozoans *Pterocystis infundibula* sp. n. from the Gulf of Aqaba (Israel) was studied with light and electron microscopy. The cell diameter is 10.53–19.03 (average 13.73) µm. *P. infundibula* has scales of two distinct types. Plate scales are 1.99–3.02 (av. 2.53) × 1.10–1.54 (av. 1.35) µm in size, flat, oval or slightly ovoid with an axial thickening. Spine scales are 1.39–5.22 (av. 2.88) µm long, with a funnel-shaped base, which is indistinctly continuous with a shaft, demonstrating gradual levels of reduction to the extent of the complete absence. Spine scales are fenestrated with holes of irregular shape and have a thin marginal border. The cells are floating or attached to the substratum, sometimes in aggregates of 2-5 cells. The similarities with and differences from other *Pterocystis* spp. as well as other centrohelids are discussed.

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

Introduction

Centroplasthelida Febvre-Chevalier et Febvre, 1984, colloquially known as centrohelids, form a holophyletic group of heterotrophic protists (Cavalier-Smith and Chao, 2003), together with haptophytes representing Haptista supergroup (Cavalier-Smith, 2003; Cavalier-Smith et al., 2015; Burki et al., 2016; Adl et al., 2019). Despite being challenged with a potential presence of the cryptic speciation (Zlatogursky and Klimov, 2016) as well as dimorphism in the course of a complex life cycle (Zlatogursky et al., 2018; Drachko et al., 2020; Zagumyonnyi et al., 2021), the morphology, especially the ultrastructure of the external siliceous scales, remains the main criterion for species description and identification in centrohelids (Zlatogursky, 2010, 2014; Gerasimova and Plotnikov, 2016; Shishkin et al., 2021). In several genera, the external skeleton is represented by two layers of scales: the layer of plate scales closer to the cell surface and the layer of spine scales

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located externally. In *Acanthocystis* Carter, 1864 and *Choanocystis* Penard, 1904, each spine scale is distinctly differentiated into the flat base and cylindrical shaft (Siemensma, 1991). The genus *Pterocystis* Siemensma et Roijackers, 1988 was introduced as a taxonomic vehicle for centrohelids with bilaterally symmetrical spine scales, where the shaft forms membranous extensions (wings) that are continuous with the flat scale base (Siemensma and Roijackers, 1988). Later Mikrjukov (1997) distinguished two separate genera within *Pterocystis.* The species, in which the shaft is rudimentary and the spine scales are tangentially oriented, were grouped into the genus *Pseudoraphidiophrys* Mikrjukov, 1997. The species, in which the base is more distinct from the shaft and perpendicularly oriented to it, were grouped into the genus *Echinocystis* Mikrjukov, 1997 (subsequently changed to *Raineria* Mikrjukov, 1999 and then eventually to *Raineriophrys* Mikrjukov, 2001 after finding out that *Echinocystis* and *Raineria* had been preoccupied (Mikrjukov, 1999, 2001)). After these transfers, *Pterocystis sensu* Mikrjukov, 1997 includes 22 species with spine scales having a prominent winged shaft, where wings are indistinctly continuous with the scale base.

Here we describe *Pterocystis infundibula* sp. n. — another species of this genus with unusual morphology of the spine scales demonstrating a continuum from typical winged bilaterally symmetric *Pterocystis*-type scales to radially symmetric funnel-shaped scales resembling those in the genus *Pseudoraphidocystis* Mikrjukov, 1997.

Material and methods

Cultures and samples

Samples were collected from the shore of the Gulf of Aqaba, Israel (N29.50; E34.92) on 13 September 2016. Aliquots (1 ml) of sea-water samples (34‰) were inoculated into artificial sea water with the addition of 0.05% cerophyl as a nutrient substrate. The clonal cultures were obtained by isolation of single cells on the same nutrient medium with the addition of *Neobodo saliens* (Larsen et Patterson, 1990) flagellates as food. The bacterial community was unregulated.

Microscopy and morphometry

Light microscopical photos (Fig. 1, A, E, F) were taken using the temporary preparation on an object slide with Leica DM 2500 (100× objective) microscope, equipped with differential interference contrast (DIC) and a Nikon DS-Fi1 camera. The morphometry was performed using the photos of 97 cells taken with Leica DMI3000 inverted microscope (63× objective) equipped with integrated modulation contrast (IMC) optics and a Leica DFC295 camera. Preparation of the scales for scanning electron microscopy (Fig. 2) was conducted according to Zlatogursky (2014). The cells were air-dried on the surface of a cover-slip. The cover-slips were washed with distilled water, attached to the specimen stubs, carbon-coated and observed with a Zeiss Auriga Laser working station operated at 5 kV. Scales were measured in EM images.

Results

Light microscopy

The cells are spherical (Fig. 1, A-E). Most of the cells are floating and single, although colonies of 2–5 cells have sometimes been observed (Fig. 1, D). The cell diameter is 10.53–19.03 (av. 13.73) µm (*n*=97). Axopodia are more often 2–3 cell diameters long and are distinctively granulated. The scales form a well visible loose layer surrounding the cells and sometimes extending along with the axopodial bases.

Electron microscopy

Most of the plate scales are oval or slightly ovoid (Fig. 2, A, C), with a well-developed axial thickening and thin marginal border. The length of plate scales is 1.99–3.02 (av. 2.53) µm (*n*=76) and the width is 1.10–1.54 (av. 1.35) µm (*n*=79). Spine scales are elongated with a well-developed shaft, which is indistinctly continuous with the funnel-shaped scale base (Fig. 2, D). The funnel of the base has a conical stalk with a bulbous ending. In some of the spine scales, the shaft is rudimentary, sometimes present only in a form of a sharpened edge of a funnel-shaped base (Fig. 2, B). Finally, in some of the spine scales, the shaft is absent completely and the scale acquires the radially symmetric funnel shape (Fig. 2, A). The elongated spine scales extend along the axopodia, while the funnel-shaped ones form a layer on the cell surface (compare Fig. 1, A and Fig. 2). The length of spine scales is 1.39-5.22 (av. 2.88) µm (*n*=47). In all types of spine scales, the funnel is fenestrated with holes of irregular shape (Fig. 2, A, B, D). Both the shaft and the funnel are edged with a thin marginal border.

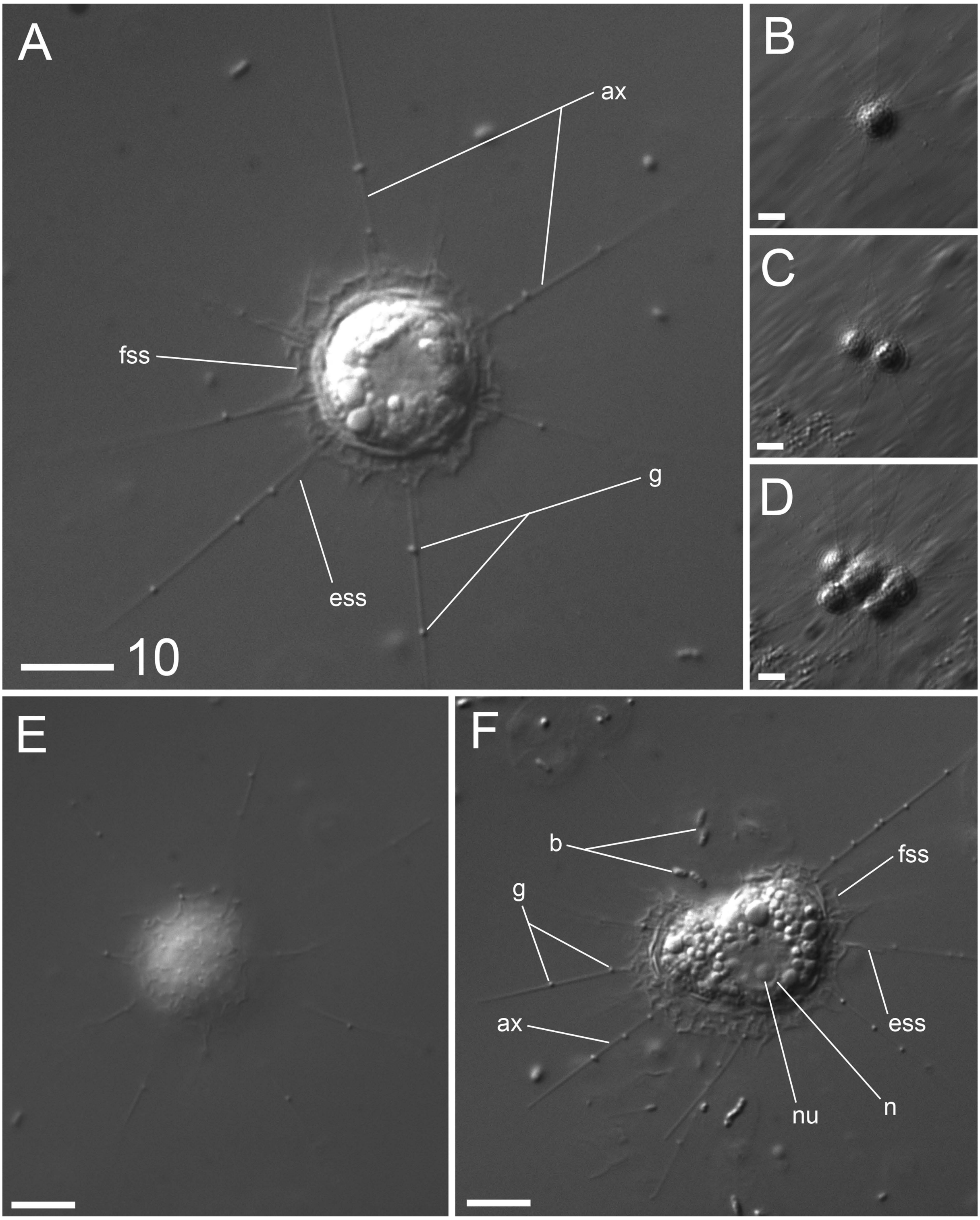

Fig. 1. Light microscopic appearance of *Pterocystis infundibula* sp. n., A, E, F – DIC, B, C, D – IMC. A – General view of the cell under the cover slip, optical section through the center; B – solitary cell in a Petri dish; C – a pair of cells in a Petri dish; D – cells aggregation in a Petri dish; E – general view of the cell surface under the cover slip; F – the cell squished under the cover slip. *Abbreviations*: ax – axopodium, b – bacteria, ess – elongated spine scale, fss – funnel-shaped spine scale, g – granules, n – nucleus, nu – nucleolus. Scale bars: 10 µm.

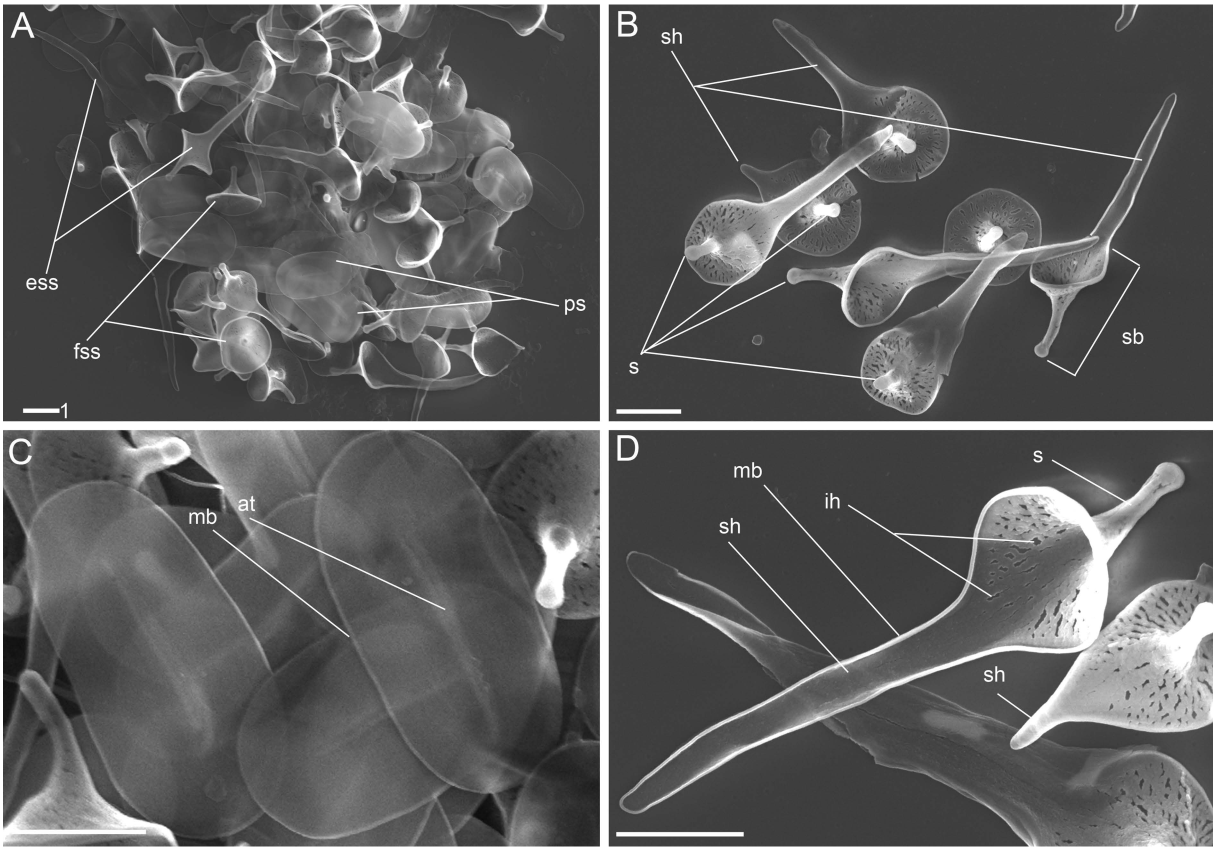

Fig. 2. *Pterocystis infundibula* sp. n. scales observed with SEM. A – Different types of scales under low magnification; B – different types of spine scales; C – close up of the plate scales; D – close up of the spine scales. *Abbreviations*: at – axial thickening, ess – elongated spine scales, fss – funnel-shaped spine scales, ih – holes of irregular shape, mb – marginal border, ps – plate scales, s – stalk, sb – scale base, sh – shaft. Scale bars: 1 µm.

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 stat. n. & em. Shɨshkin et Zlatogursky, 2018

●●●●● Pterista Shɨshkin et Zlatogursky, 2018

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

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

●●●●●●●● *Pterocystis infundibula* Shɨshkin et Zlatogursky sp. n.

Diagnosis: The cell body diameter is 11–19 (av. 14) µm. Axopodia are more often 2–3 cell diameters long, granulated. The cell body is covered with two layers of siliceous scales of two distinct types. Plate scales are more or less oval or slightly ovoid with an axial thickening and thin marginal border. The length of plate scales is 1.99–3.02 (av. 2.53) µm and the width is 1.10–1.54 (av. 1.35) µm. Spine scales (1.39–5.22 (av. 2.88) µm) with an elongated shaft and a funnel-shaped base, fenestrated with holes of irregular shape and edged with a thin marginal border. In some of the scales, the shaft is more or less rudimental or absent.

Etymology: from Latin infundibulum (a funnel); for the funnel-shaped scales.

Type locality: Gulf of Aqaba shore, Israel (N29.50; E34.92).

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 Number is 1053.

Zoobank LSID of the publication: urn:lsid:zoobank.org:pub:D0F43C99-34DE-4C1C-9F9E-C3F9231D3207.

Zoobank LSID of the species: urn:lsid:zoobank.

org:act:D92DE9C1-FFEA-41C5-A98B-8DC159F613BC.

Discussion

The newly described species clearly belongs to the genus *Pterocystis* due to the presence of the well-expressed winged shaft, with wings indistinctly continuous with a spine scale base. At the same time, among the representatives of *Pterocystis* (sensu Mikrjukov, 1997), this species is unique in having funnel-shaped spine scales, similar to those of *Pseudoraphidocystis* spp. Almost funnel-shaped, referred to as "calyx-shaped", spine scales are known in *P. pulchra* (Dürrschmidt, 1985) Siemensma, 1991; however, unlike those in *Pseudoraphidocystis* spp., they have a prominent sternum in the form of a dark narrow ridge (Dürrschmidt, 1985). There is a continuum between elongated spine scales with a well-developed shaft through the spine scales with a more or less reduced shaft to the funnel-shaped ones, where a shaft is totally missing. The presence of the shaft, at least in some form, in most of the spine scales of *P. infundibula* prevents the attribution of this organism to the genus *Pseudoraphidocystis*. Nevertheless, the similarity between *P. infundibula* and *Pseudoraphidocystis* spp. is noteworthy and points to the close relationships between these two genera. The fenestrated and sometimes almost reticulated (Fig. 2, D) nature of *P. infundibula* spine scales is also unique for *Pterocystis* and reminiscent of the reticulated structure of the plate scales in *Raphidocystis ambigua* (Penard, 1904) Zlatogursky, 2018, *R. symmetrica* (Penard, 1904) Zlatogursky, 2018, and *R. tubifera* Penard, 1904 (Zlatogursky et al., 2018). The elongated spine scales of *P. infundibula* are similar to those of *P. foliacea* (Dürrschmidt, 1985) Siemensma, 1991, *P. ovata* Cavalier-Smith et von der Heyden, 2007, *P. polymorpha* Cavalier-Smith et von der Heyden, 2007, and *P. contorta* Cavalier-Smith et von der Heyden, 2007 in the elongated form of the wings that reach the tip of a shaft and a presence of the funnel-shaped bases with stalks. However, in these species, spine scales are never reticulated. In addition, the spine scales stalks in *P. infundibula* are resembling those of *P. pteracantha* (Siemensma, 1981) Siemensma et Roijackers, 1988; however, in the latter species, spine scales are ribbed, which was never observed in *P. infundibula*.

The molecular data are necessary to clarify the phylogenetic position of this remarkable species. Moreover, to test its hypothetical relationships with *Pseudoraphidocystis* spp., the sequences from representatives of this genus would be crucial as well, while currently, those data are missing. Except for questionable finding of *Pterocystis* sp., most probably belonging to *P. paliformis* (Dürrschmidt, 1987) Siemensma, 1991, by Tong (1994) and recently described *P. pontica* Prokina, Zagumyonnyi et Mylnikov, 2019 and *P. jongsooparkii* Zagumyonnyi, Prokina et Tikhonenkov, 2020 (Prokina et al., 2019; Zagumyonnyi et al., 2020), there are almost no records of *Pterocystis* spp. in marine habitats. Interestingly, according to environmental sequencing data, *Pterocystis* diversity is restricted to freshwater – see Fig. 2 in Cavalier-Smith and Chao (2012). Despite that, the careful studies in recent years revealed three new species, isolated from marine habitats, including *P. infundibula* described here. This suggests that almost exclusively the freshwater nature of *Pterocystis* spp. is a result of a research bias against marine environments in both morphological and metabarcoding surveys, and further research most probably will reveal higher diversity of these centrohelids in brackish-water and marine habitats.

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