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Two New Species of Centrohelid Heliozoans: *Acanthocystis costata* sp. nov. and *Choanocystis symna* sp. nov.

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Abstract. Two new species of centrohelid heliozoans *Acanthocystis costata* and *Choanocystis symna* from a freshwater lake on Valamo Island and a freshwater pool in St. Petersburg (North-Western Russia) were studied with light and electron microscopy. Sequences of 18S rDNA were obtained for both species. *Choanocystis symna* has dumbbell-shaped plate scales (4.4–5.0 × 1.62–1.90 μm) and spine scales (3.9–6.7 μm) bearing from 3 to 5 (usually four) short teeth on the distal end. *Acanthocystis costata* has oval plate scales (1.3–3.7 × 0.9–1.9 μm), bearing numerous granules as well as radial slits and spine scales (2.1–9.5 μm) with 4–6 teeth on the distal end. *Acanthocystis costata* and *Acanthocystis nichollsi* are similar in having slit-bearing plate scales and group together on the 18S rDNA tree. The presence of large particles of unknown nature was observed in food vacuoles of *Acanthocystis costata*.

Key words: Centrohelids, Heliozoa, protists, systematics, ultrastructure, external skeleton, *Acanthocystis costata* sp. nov., *Choanocystis symna* sp. nov.

INTRODUCTION

The Centroplasthelida Febvre-Chevalier et Febvre, 1984 is a monophyletic group of free-living predatory protists (Cavalier-Smith and von der Heyden 2007). Species identification and systematics of this group are based mostly on the morphology of scales – siliceous external skeletal elements covering their cells (Siemensma 1991).

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Acanthocystis Carter, 1863 and *Choanocystis* Penard, 1904 are the most abundant centrohelid genera and several new species have been described recently (Wujek and Saha 2006, Cavalier-Smith and von der Heyden 2007, Leonov 2010, Leonov and Mylnikov 2012). At present there are 29 described species of *Acanthocystis* and 18 described species of *Choanocystis*, including two species described here. Both genera include heliozoans with two types of siliceous scales: tangentially oriented (plate scales) and radially oriented (spine scales). *Acanthocystis* is characterized by spine scales having radially symmetrical plate-shaped bases. By contrast, representatives of *Choanocystis* have bilaterally symmetrical heart-shaped bases of spine scales.

Major characteristics for the classification of these two genera are the shape and texture of plate scales and morphology of spine scales apexes. A short comparison of described species is given in the Table 1.

Centrohelid heliozoans are very common in both freshwater and marine habitats and their high biomass has been reported in many works (Stensdotter-Blomberg 1998, Biyu 2000, Kiss *et al*. 2009). Therefore, their role in ecological processes is very important. But currently ecological studies are hard to perform due to poor knowledge of biodiversity. In this paper two new species of Centroplasthelida in two genera *Choanocystis* and *Acanthocystis* are described.

MATERIALS AND METHODS

Bottom layer of sediments at 30–40 cm water depth was collected with 500 ml sterile containers in lakes on Valamo archipelago (North-Western Russia) and in St. Petersburg. For details see type localities.

Samples were transported to the laboratory and inoculated into 60 mm Petri dishes filled with PJ mineral medium (Prescott and James 1955).

For *A. costata* and *C. symna* clonal cultures on PJ medium-based 0.025% cerophyl extract were established (Page 1988). For that, heliozoan cells were individually collected with Pasteur pipette, washed in fresh sterile medium and inoculated into 60 mm dishes with cerophyl extract. Cultures were stored at + 15ºC under room light conditions. *Bodo saltans* was added as a food source. All further studies (light and electron microscopic morphology, molecular analyses) were performed on the same clone originated from a single individual.

Light microscopy and measurements of the living and air dried cells was performed using inverted Nikon Eclipse TS 100 microscope equipped with phase contrast optics and Leica DM 2500 microscope equipped with phase contrast and DIC optics.

Preparation of skeleton for light and electron microscopy was described in Zlatogursky (2012). Scales were measured in EM images and mean scale diameter was calculated for each individual separately (Table 2). Preparations for EM were made several times on different stages of culture growth, to prove the stability of this structure.

For molecular study the genomic DNA was isolated from the cell cultures using the guanidine isothiocyanate (Maniatis *et al*. 1982). SSU rDNA was amplified with eukaryote-specific forward primer (Thx25F 5' > cat atg ctt gtct caa aga tta agc ca < 3') and centrohelid-specific reverse primer (Helio1979R: 5' > cac actt acw agg ayt tcc tcg tts aag acg < 3') (Cavalier-Smith and von der Heyden 2007). PCR amplification and sequencing were performed as in Kudryavtsev *et al*. 2009. Then sequences were manually aligned using SeaView v. 4.3.5. (Gouy *et al*. 2010). Maximum-likelihood reconstruction was done using RaxML Version 7.2.6 (Stamatakis 2006).

For authors of species, see Table 1.

RESULTS

Acanthocystis costata sp. nov. (Figs 1, 2)

Diagnosis: Cell body ca 9.5 µm in diameter. Axopodia 2–3 times longer than cell diameter. Length of spine scales 2.1–9.5 µm (on average 5.1 µm). There are straight scales as well as curved ones. Spine scales with four, five or six teeth. Plate scales with concave or, sometimes, parallel edges and rounded ends. Length of plate scales 1.3–3.7 (ca. 2.6) µm, width 0.9–1.9 (ca. 1.4) µm. Periphery of scale with a thickened border. Axial thickening and about fifty radial slits are present. Some scales with numerous small granules, probably present on one side of the scale only and therefore visible not on all scales. Cells actively move at the bottom of the culture dish or float in the culture medium. Sometimes fusions of two or three cells with a common food vacuole were observed. Large particles of unknown nature were observed in vacuoles of some individuals.

Etymology: The species group name *costata* (from the Latin *costatus*; ribbed), refers to the texture of plate scales.

Type locality: Freshwater pool in the greenhouse of the botanical garden of St. Petersburg State University, St. Petersburg, North-Western Russia, 59°56′N, 30°17′E. Collected 22.12.2011.

Hapantotype: preparation (Fig. 1A) has been deposited in the Natural History Museum UK, accession number NHMUK 2013.6.28.2.

Type sequence: GenBank accession number KF990486.

Culture: CCAP 1504/2 – the clonal culture from which type material and all the data provided in this paper were obtained.

ZooBank LSID: urn:lsid:zoobank.org:act:CC3A84B0-1699-46BD-89E1-68A633AA7120.

Comparative diagnosis: The sculpture of the plate scales is a very characteristic feature of this species. Plate scales with similar radial slits are also described in *A. myriospina* and *A. nichollsi*. In *A. myriospina* these slits are observed only by Roijackers (cit. from Siemensma 1991) and only line drawing of the plate scale is published. But the quantity of slits on this drawing is nineteen, while in *A. costata* there are about fifty slits per scale. Probably this finding of Roijackers represents not an *A. myriospina,* but a new not yet described species. Anyway, even assuming, that slit-bearing scales are characteristic for *A. myriospina*, this species differs

Table 1. Summary of characteristics of all described species of *Acanthocystis* and *Choanocystis*.

Species	Plate scales	Spine scales
1. *A. antonkomolovi* Leonov, 2010	oval, radial openings	2 long teeth
2. *A. astrakhanensis* Mikrjukov, 1993	oval	2 short teeth
3. *A. bicornis* Dürrschmidt, 1987	oval	2 types: short, secondary bifurcations; long, branches with inner denticles
4. *A. clavata* Dürrschmidt, 1987	oval	tapering
5. *A. cornuta* Dürrschmidt, 1987	oval	2 branches with inner denticles
6. *A. costata* sp. nov	oval, radial slits, granules	4–6 short teeth
7. *A. dentata* Leonov et Mylnikov, 2012	dumbbell	2 branches with 1 inner tooth each
8. *A. dresscheri* Siemensma et Roijackers, 1988	dumbbell, granules	truncated 5–8 short teeth
9. *A. dürrschmidtidae* Wujek, 2003	oval, granules, marginal rim	truncated
10. *A. elenazhivotovae* Leonov, 2010	oval, radial openings	2–3 short teeth
11. *A. mikrjukovi* Leonov, 2010	pear-shaped	2 branches with secondary bifurcations
12. *A. mylnikovi* Leonov, 2010	oval, granules	2–6 short teeth
13. *A. myriospina* Penard, 1890 First EM: Dürrschmidt, 1985	oval	3 sharp teeth
14. *A. nichollsi* Siemensma et Roijackers, 1988	oval, radial slits	about 6 teeth with flarings
15. *A. olgashelestae* Leonov, 2010	oval or pear-shaped, granules	2 short teeth
16. *A. pectinata* Penard, 1889 First EM: Siemensma, Roijackers, 1988	oval, granules	2 types: short, 4–6 teeth, flarings, numerous; long, 2–3 teeth, rare
17. *A. penardi* Wailes, 1925 First EM: Nicholls, 1983	oval	truncated 8–14 short teeth
18. *A. polymorpha* Dürrschmidt, 1985	dumbbell, granules	two types: short, 5–8 teeth, flarings; long, with about 3 long teeth
19. *A. pusilla* (Dürrschmidt, 1987) Siemensma, 1991	dumbbell or pear-shaped, granules	4–7 teeth
20. *A. quadrifurca* Nicholls, 1983	oval, radial openings	4 sharp teeth
21. *A. saphonovi* Mikrjukov, 1994	bean-shaped	10–12 short teeth, spiral rib
22. *A. spinifera* Greeff, 1869 First EM: Siemensma et Roijackers, 1988	dumbbell	2–3 obtuse teeth
23. *A. spinosa* Cavalier-Smith et von der Heyden, 2007	oval	3 short teeth
24. *A. takahashii* Dürrschmidt, 1987	oval or pear-shaped	3 branches with small denticles
25. *A. taurica* Mikrjukov, 1997	oval	5 sharp teeth
26. *A. tubata* Dürrschmidt, 1987	oval	about 12 short teeth, broad apex
27. *A. turfacea* Carter, 1863 First EM: Dürrschmidt, 1987	oval	2 long teeth
28. *A. valdiviense* Dürrschmidt, 1987	oval	4 teeth two types: short with long teeth; long with short teeth
29. *A. wiasemskii* Ostroumoff, 1917 First EM: Mikrjukov, 1994	bean-shaped	tapering

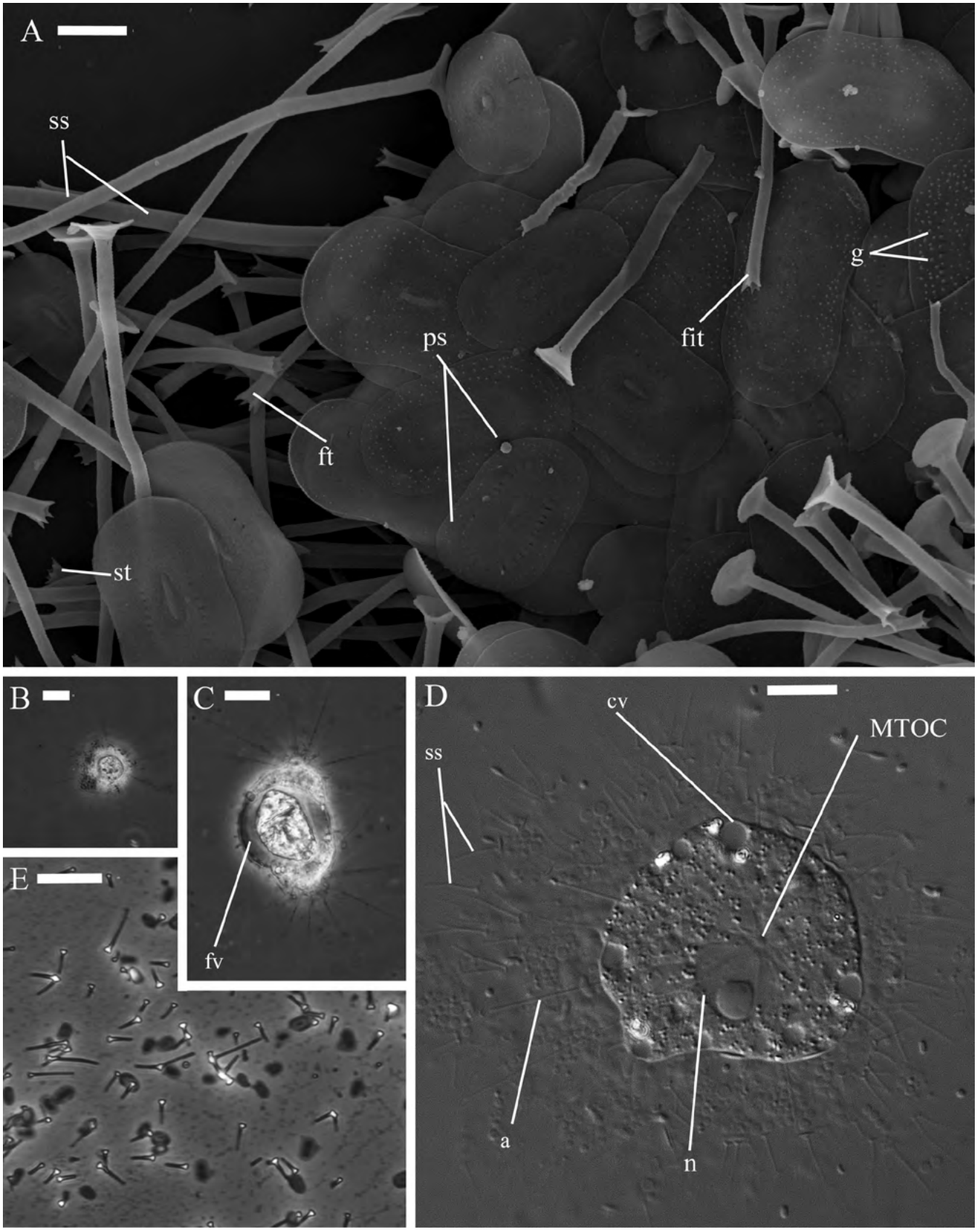
Species	Plate scales	Spine scales
1. *Choanocystis aculeata* (Hertwig et Lesser, 1874) Siemensma et Roijackers, 1988	dumbbell	papillate, truncated
2. *Choanocystis antarctica* Tikhonenkov et Mylnikov, 2010	oval	spur at the bend point of the shaft, curved toward base
3. *Choanocystis bicornioides* Mikrjukov, 1995	dumbbell	2 branches with secondary bifurcations
4. *Choanocystis curvata* Cavalier-Smith et von der Heyden, 2007	oval	2–3 teeth, curved toward base
5. *Choanocystis ebelii* (Wujek et Elsner, 1992) Mikrjukov, 1997	rod-shaped	pointed, granules
6. *Choanocystis kareliensis* Mikrjukov, 1994	dumbbell, granules	pointed
7. *Choanocystis lepidula* Penard, 1904 First EM: Siemensma et Roijackers, 1988	oval, marginal rim	truncated, curved toward base
8. *Choanocystis malyutini* Mikrjukov, 1995	oval, granules	3 branches
9. *Choanocystis pantopoda* (Penard, 1904) Siemensma, 1991 First EM: Dürrschmidt, 1987	oval	4–6 teeth
10. *Choanocystis pelagica* (Ostenfeld, 1904) Mikrjukov, 1994	oval, granules	pointed, curved toward base
11. *Choanocystis perpusilla* (Petersen et Hansen, 1960) Siemensma, 1991	oval	2 (more rarely 3–4) small teeth
12. *Choanocystis pertusa* (Dürrschmidt, 1987) Siemensma, 1991	oval, radial openings	4 teeth
13. *Choanocystis rhytidos* (Dürrschmidt, 1987) Siemensma, 1991	oval or pear-shaped; radial slits	2–4 teeth
14. *Choanocystis roijackersi* Wujek, Saha, 2006	oval or slightly dumbbell	2–4 teeth, constriction near base-plate
15. *Choanocystis rossica* (Mikrjukov, 1995) Mikrjukov, 2002	oval or slightly dumbbell	truncated
16. *Choanocystis rotoairense* (Dürrschmidt, 1987) Mikrjukov, 1995	oval, granules	4 teeth
17. *Choanocystis rotundata* (Nicholls, 1983) Dürrschmidt, 1987	oval	10–20 small teeth
18. *Choanocystis symna* sp. nov.	dumbbell	3–5 teeth

Table 2. Morphometry of *Acanthocystis costata* and *Choanocystis symna*.

	Parameter	Min	Max	Mean unweighted +/– SE	n (i)	n
A. costata sp. nov., CCAP 1504/2	Cell diameter	5.8	12.0	9.5 ± 0.19	58	58
	Radial scales length	2.1	9.5	5.1 ± 0.14	5	149
	Plate-scales length	1.3	3.7	2.6 ± 0.05	5	83
	Plate-scales width	0.9	1.9	1.4 ± 0.02	5	94
C. symna sp. nov., CCAP 1597/1	Cell diameter	5.3	8.1	6.7 ± 0.10	50	50
	Radial scales length	3.9	6.7	5.1 ± 0.49	6	111
	Plate-scales length	4.4	5.0	4.7 ± 0.17	3	33
	Plate-scales width	1.62	1.90	1.71 ± 0.092	3	33

All measurements in μm. *Abbreviations*: Max – maximum, Mean – arithmetic mean, Min – minimum, n – number of measurements, n (i) – number of individuals and SE – standard error of the mean.

Fig. 1. *Acanthocystis costata* spec. nov strain CCAP 1504/2. (**A**) Air-dried whole-mount of the cell. Type-slide NHMUK 2013.6.28.2. SEM (Scanning electron micrograph). Spine scales (ss) and plate scales (ps) are seen. Spine scales with four (ft), five (fit), and six (st) teeth can be found. Surface of plate scales is covered by numerous granules (g). (**B**) Living cell. Phase contrast. (**C**) Two individuals with a common food vacuole (fv), containing some vegetable material. Phase contrast. (**D**) Living cell compressed with a cover-slip. DIC (Differential interference contrast). Axopodia (a), contractile vacuoles (cv), microtubule-organizing center (MTOC), nucleus (n) and spine scales can be seen. (**E**) Scales of the air-dried specimen. Phase contrast. Scale bars: 1 μm (A), 10 μm (B–E).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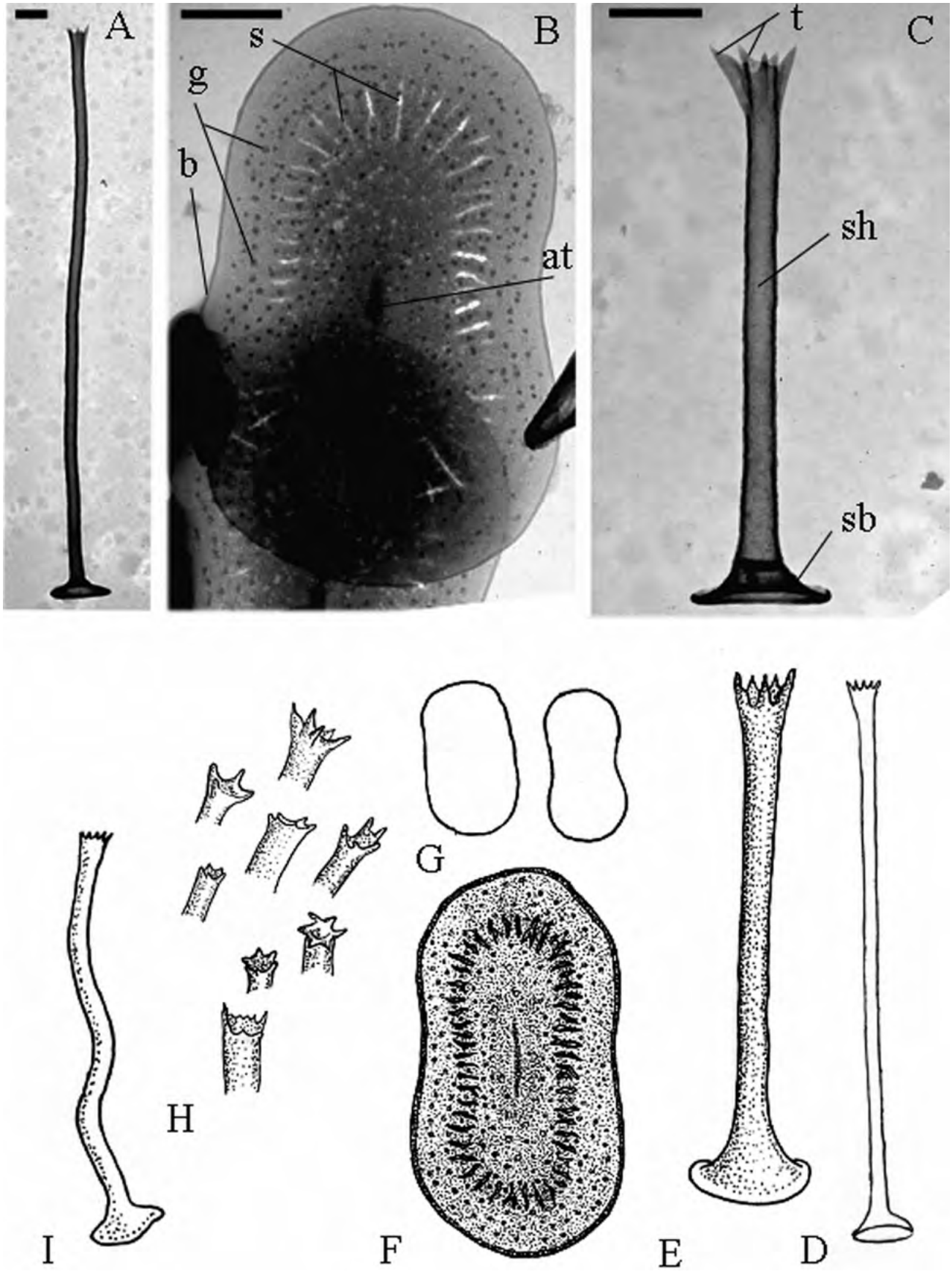
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from *A. costata* in the quantity of slits and the morphology of spine scales. The similar quantity of slits is characteristic for scales of *A. nichollsi*. The size of plate scales is comparable, but not the same: 1.4–2.2 × 2.3–3.8 (*A. nichollsi* from Siemensma 1991) vs 1.3–3.7 × 0.9–1.9 and appearance is alike, except of the presence of granules in *A. costata*. But morphology of spine scales between those two species is clearly different, because in *A. nichollsi* short spine scales with apexes, separated into arms and connected with flarings of scale shaft are present (Nicholls 1983). Contrary, there is no any flarings on spine scales of *A. costata*.

A. mylnikovi is similar to the new species in having spine scales with four, five or six teeth (however two or three teeth are also reported for *A. mylnikovi*). But spine scales of *A. costata* are much shorter: 2.8–11.3 μm vs 5–17 μm for *A. mylnikovi* and plate scales of *A. mylnikovi* have no slits (Leonov 2010).

A. pectinata, *A.polymorpha* and several other species (see Table 1) have granules on plate scales similar to granules in *A. costata*. But in these species there are no slits on plate scales and morphology of spine scales is very different.

***Choanocystis symna* sp. nov. (Figs 3, 4)**

Diagnosis: Cell body ca. 6.7 μm in diameter. Axopodia three–five times longer than a cell diameter. Plate scales dumbbell-shaped with a median constriction. Circlular lines, parallel to the border of the scale sometimes can be seen. The shafts of spine scales can be straight, but usually it is slightly curved towards the scale base. Length of plate scales 4.4–5.0 μm (ca. 4.7 μm); width 1.62–1.90 μm (ca. 1.71 μm). Spine scales with heart-shaped bases. Sometimes a small circular depression is located on the base at the place of the shaft location. Distal ends of spine scales bear from three to five (usually four) short teeth. Length of spine scales 3.9–6.7 μm (ca. 5.1 μm). Cells tightly attach to the bottom of Petri dish or, very rarely, float. No rolling movement was observed.

Etymology: The species–group name *symna* refers to the name of type location – Lake Symniakhovskoe.

Type locality: Lake Symniakhovskoe, Valamo island, North-Western Russia, 61°22′912″N, 30°58′503″E. Collected 03.08.2010.

Culture: CCAP 1597/1 – the clonal culture from which type material and all the data provided in this paper were obtained.

Hapantotype: preparation (Fig. 3) has been deposited in the Natural History Museum UK, accession number NHMUK 2013.6.28.1.

Type sequence: GenBank accession number KF990487.

ZooBank LSID: urn:lsid:zoobank.org:act:C538EF0A-BF25-4539-8C62-CACE552B9C97

Comparative diagnosis: There are only two more described species of *Choanocystis* with dumbbell-shaped plate scales and teeth-bearing spine scales: *C. pantopoda* and *C. aculeata*. *C. aculeata* is a much larger heliozoan (cell diameter 23–60 μm) and has very different spine scales which are notably tapering and have short protrusions (“nodules”) in proximal part of the shaft. This species also has from five to eleven teeth on the spine scale tip (Dürrschmidt 1985, Siemensma and Roijackers 1988). *C. pantopoda* spine scales are three times longer than plate scales, while in *C. symna* only two times longer. The length of scales is 25–30 μm in *C. pantopoda*, while in *C. symna* it is only about 5 μm. The number of teeth in *C. pantopoda* is four to six (Siemensma 1991), but six teeth have never been observed in *C. symna*.

DISCUSSION

In this paper two species (*A. costata* and *C. symna*) are described. Newly described *A. costata*, a known species *A. nichollsi* and possibly some undescribed *A. myriospina*–like species observed by Roijackers

Fig. 2. *Acanthocystis costata* spec. nov. strain CCAP 1504/2. (**A**) Air-dried spine scale. TEM (Transmission electron micrograph). (**B**) Air-dried plate scale. TEM. Radial slits (s), granules (g), thickened border (b) and axial thickening (at) can be seen. (**C**) Air-dried spine scale. TEM. Teeth (t), shaft (sh) and scale base (sb) are seen. (**D**) Spine scale. Line drawing. (**E**) Spine scale. Line drawing. (**F**) Plate scale. Line drawing. (**G**) Different shapes of plate scales. Line drawing. (**H**) Variation of spine scale apex morphology. Line drawing. (**I**) Curved spine scale. Line drawing. Scale bars: 500 nm.

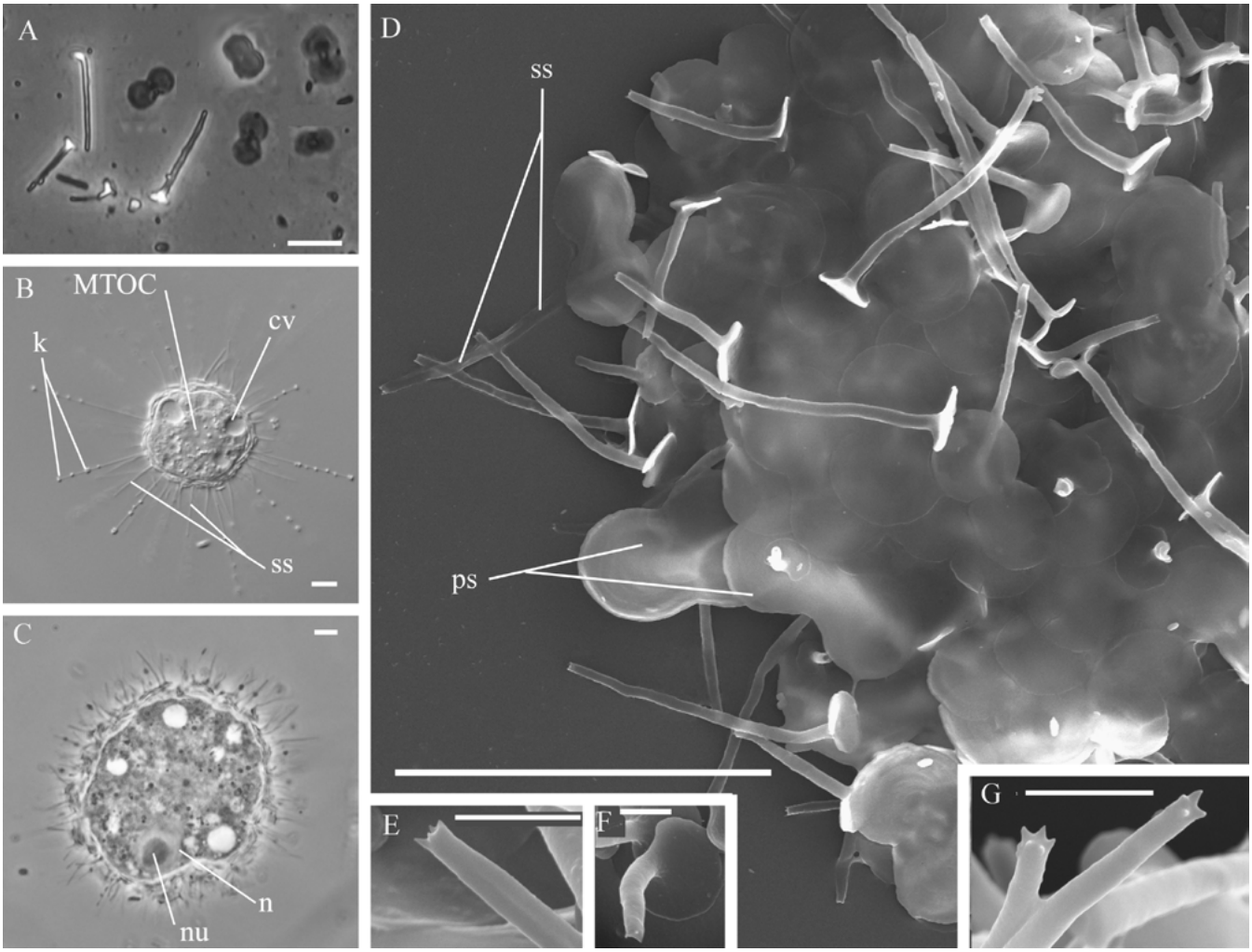

Fig. 3. *Choanocystis symna* spec. nov., strain CCAP 1597/1. (**A**) Scales of the air-dried specimen. Phase contrast. (**B**) Living cell. DIC. Contractile vacuoles (cv), kinetocysts (k), microtubule-organizing center (MTOC) and spine scales can be seen. (**C**) Living cell compressed with a cover-slip. Phase contrast. Nucleus (n) with a central nucleolus (nu) is visible. (**D**) Air-dried whole-mount of the cell. Type-slide NHMUK 2013.6.28.1. SEM. Spine scales and plate scales are visible. (**E**) The most common quantity of teeth on the tip of spine scale is four. SEM. (**F**) Spine scale with three teeth. SEM. (**G**) Tips of spine scales with five (left) and four (right) teeth. SEM. Scale bars: 5 μm (A–C), 10 μm (D), 1 μm (E–G).

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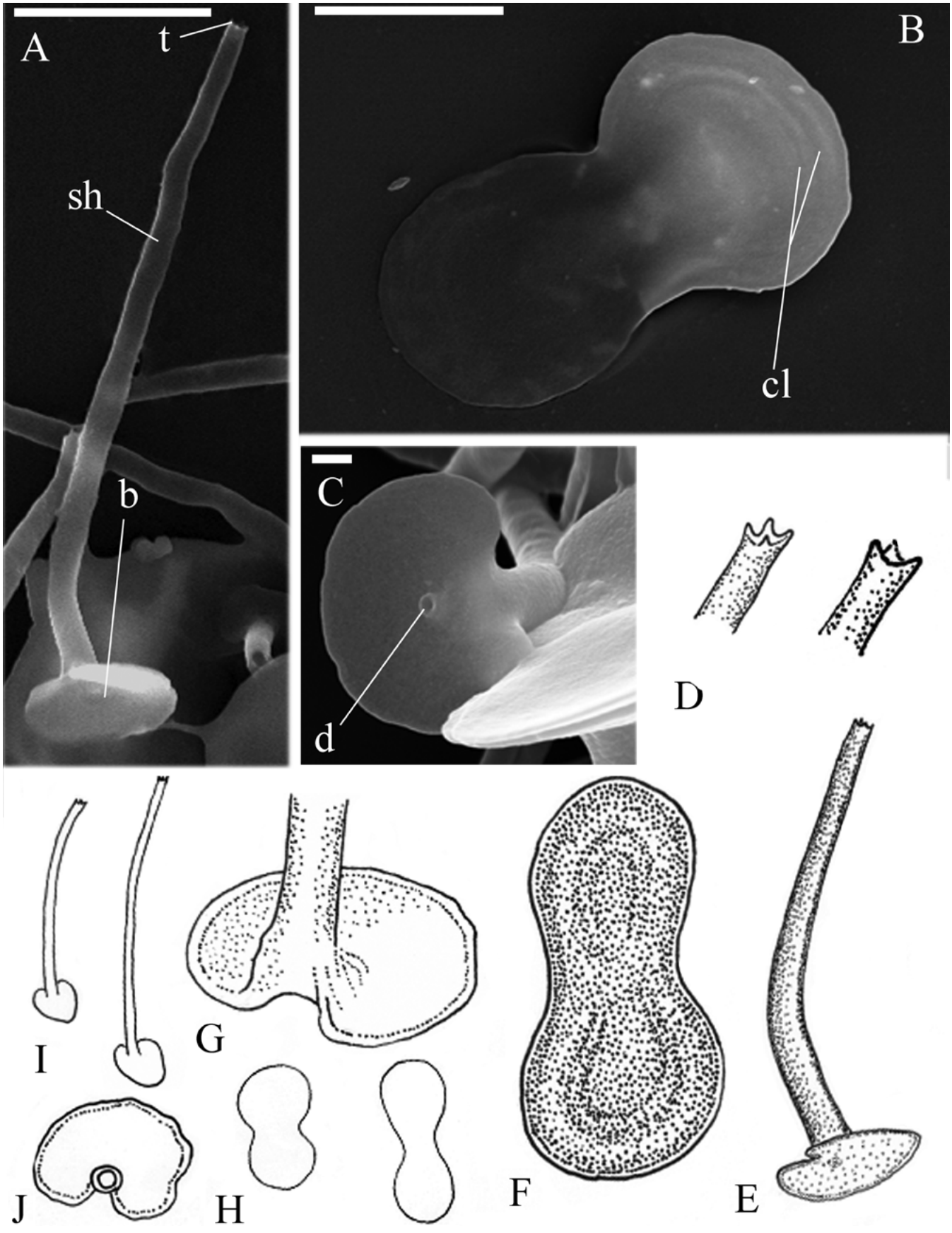
Fig. 4. *Choanocystis symna* spec. nov., strain CCAP 1597/1. (**A**) Spine scale. SEM. The base (b), shaft (sh) and apical teeth (t) are visible. (**B**) Plate scale. SEM. Note circular lines (cl). (**C**) The base of spine scale with circular depression (d). SEM. (**D**) Variation of spine scale apex morphology. Line drawing. (**E**) Spine scale. Line drawing. (**F**) Plate scale. Line drawing. (**G**) Spine scale base. Line drawing. (**H**) Different shapes of plate scales. Line drawing. (**I**) Spine scales with different length of the shaft. Line drawing. (**J**) The heart-like shape of the spine scale base. Line drawing. Scale bars: 2 μm (A, B) and 200 nm (C).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(cit. from Siemensma 1991) are similar in having very distinctive plate scales with radial slits. Phylogenetic analysis has shown that slit-bearing *Acanthocystis* form a monophyletic cluster (*A. nichollsi* + *A. costata*, Fig. 5), but bootstrap support for this clade is only 75%. This cluster in its part is sister to a clade, containing beside some unidentified taxa *A.* aff. *polymorpha* and *A.* aff. *pectinata*. These two species are similar to *A. nicholl-si* in having radial scales with flarings (see Table 1). Probably this feature was secondarily lost in *A. costata*.

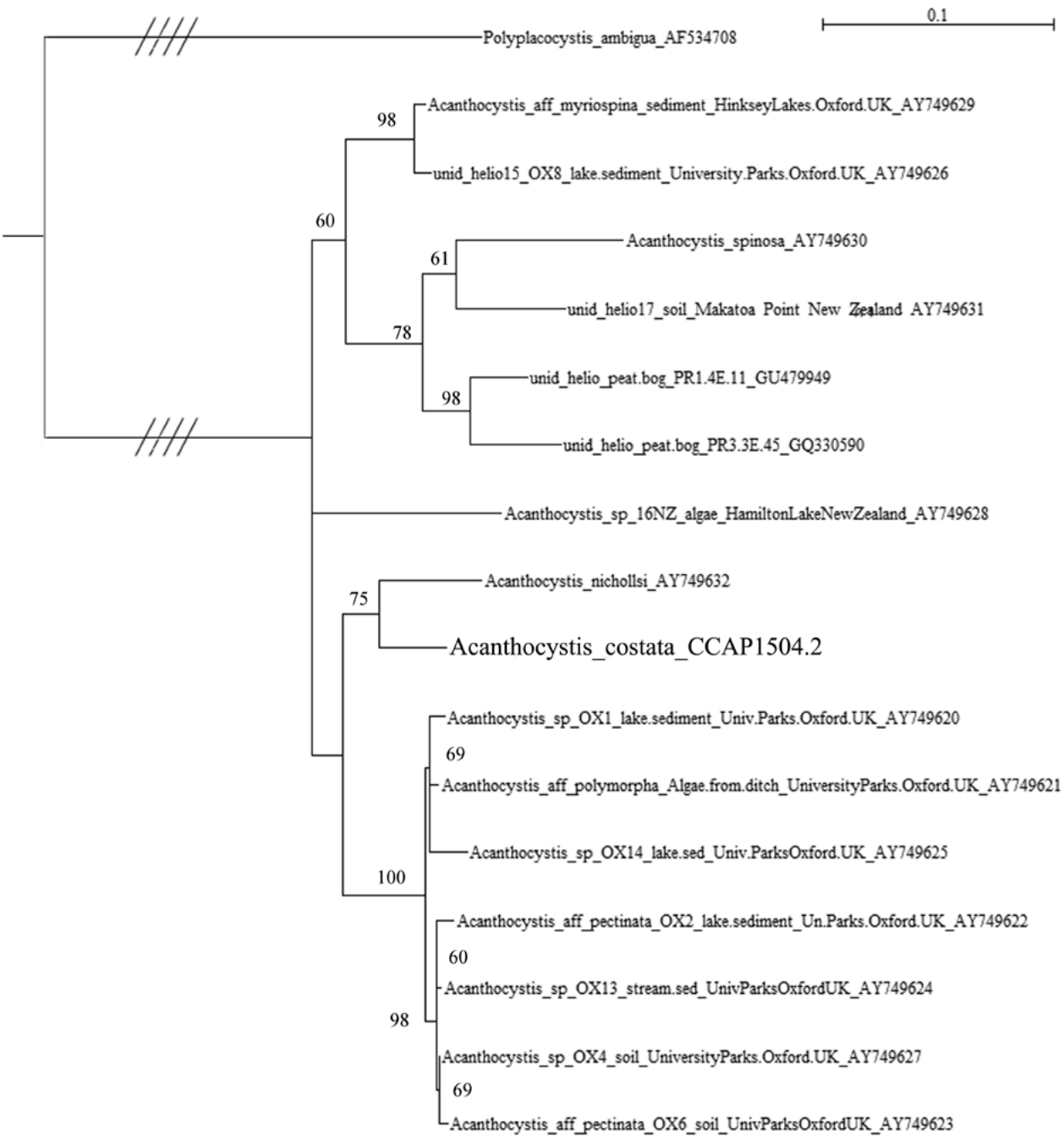

Fig. 5. RAxML tree for 18S rRNA genes of 16 heliozoans from the genus *Acanthocystis* and *Polyplacocystis ambigua* as an outgroup (1520 nucleotide positions). Only bootstrap values more than 50% are shown. New sequence is in bold. Crossed branches were shortened fourfold.

It is also interesting that *A.* aff. *myriospina* grouped in our analysis with *A. spinosa*. These two species were usually separate on previous 18S phylogenetic trees (Cavalier-Smith and von der Heyden 2007, Cavalier-Smith and Chao 2012) but their grouping together is supported with morphological features such as oval plate scales and three teeth on the spine scale apexes. *Choanocystis symna* form a weakly (53%) supported clade with *Choanocystis curvata* AY749616 (Fig. 6).

The species of *Acanthocystis* described here and all known species which I could observe (*A. nichollsi*, *A. olgashelestae*, *A. turfacea*, *A. takahashii*, *A. penardi*, *A. bicornis*) are capable of rolling movement and demonstrate this type of locomotion in the cultures most of the time. So it seems to be an additional generic character of *Acanthocystis*. In *Choanocystis,* according to our observations, rolling movement is absent, or at least much rarer. *Choanocystis symna* described here tightly

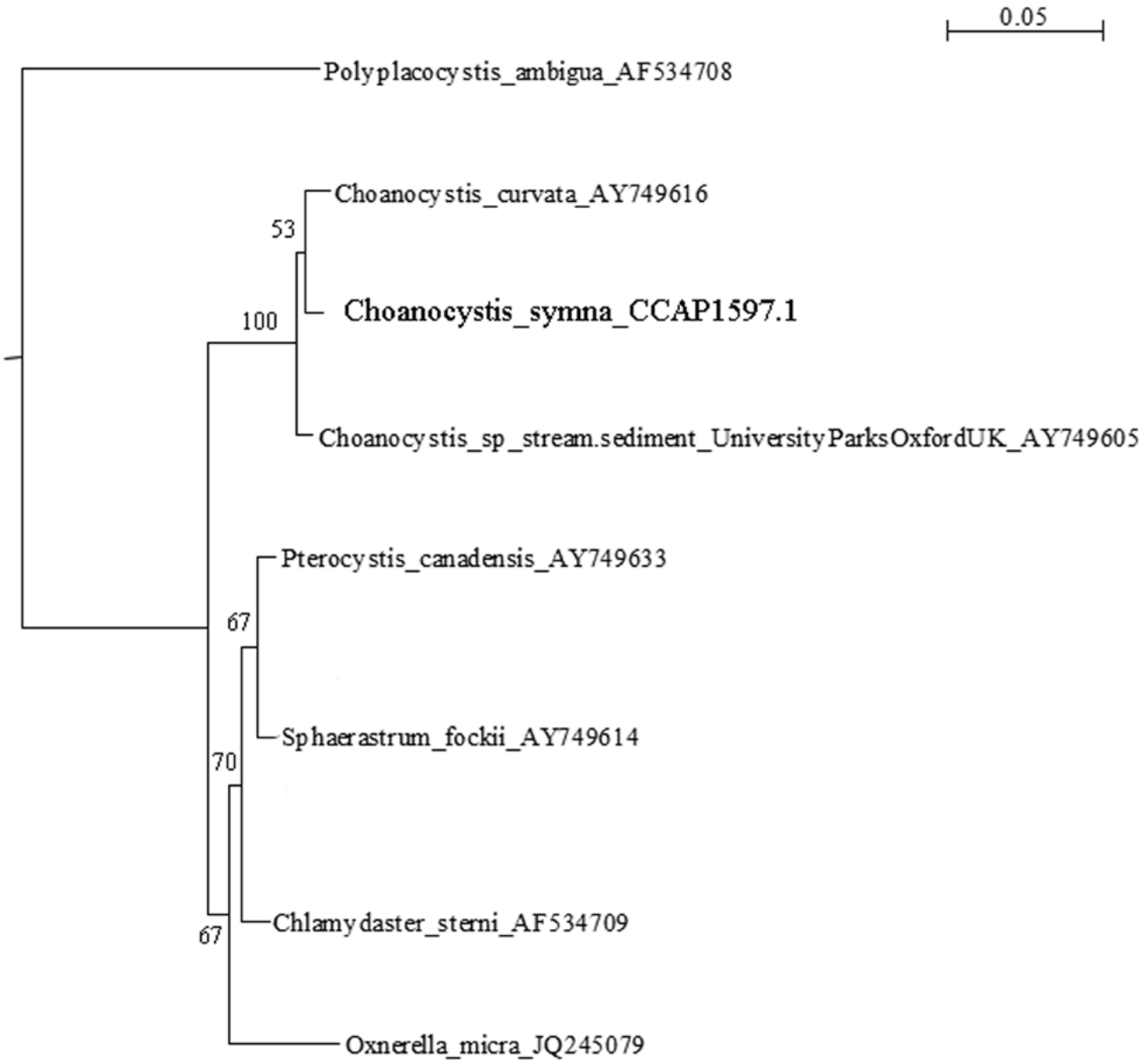

Fig. 6. RAxML tree for 18S rRNA genes of 8 pterocystid heliozoans (1320 nucleotide positions). Only bootstrap values more than 50% are shown. New sequence is in bold.

attach to the bottom of the Petri dish under the culture conditions. But our observations of the type species *Choanocystis lepidula* show, that it is neither a rolling nor an attaching but rather a floating species. So in this case it is questionable whether behavior is a generic characteristic.

The large particles which were observed in food vacuoles of *Acanthocystis costata* are very similar to microscopic leaf fragments, because of the similar size and general apperance. Such fragments are always present in the cerophyl medium. The similar observations were also made in cultures of *A. penardi*. Otherwise it seems to be the first observation of the heliozoans feeding on anything but living bacterial/protist/metazoan organisms.

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