Morphological and molecular reinvestigation of acanthoecid species II. – **Pseudostephanoeca paucicostata (Tong et al.) gen. et comb. nov. (= Stephanoeca diplocostata var. paucicostata Throndsen 1969) including also the description of Pseudostephanoeca quasicupula sp. nov. and Stephanoeca ellisfiordensis sp. nov**

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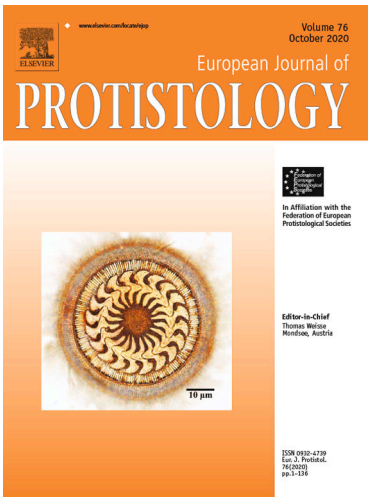

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Morphological and molecular reinvestigation of acanthoecid species II. – *Pseudostephanoeca paucicostata* (Tong et al., 1998) gen. et comb. nov. (= *Stephanoeca diplocostata* var. *paucicostata* Throndsen, 1969) including also the description of *Pseudostephanoeca quasicupula* sp. nov. and *Stephanoeca ellisfiordensis* sp. nov.

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Abstract

The present study has revealed morphological differences of species assigned to the acanthoecid choanoflagellate genus *Stephanoeca* based on the relative position of transverse and longitudinal costae in the anterior lorica chamber. A detailed re-examination of published material of *S. paucicostata* Tong et al., 1998 combined with new data has shown that the species characteristic, a double transverse costa, is clearly located outside of the longitudinal costae excluding this taxon from an affiliation to *Stephanoeca* sensu stricto, confirmed also by molecular data of a clonal culture from Kos Islands, Greece. Two specimens of *S. paucicostata* with only a single, interiorly positioned transverse costa, namely from Marchant et al. (1987) and Nitsche et al. (2011) were erroneously assigned to this species. These facts has here led to the establishment of a new genus, *Pseudostephanoeca* with its type species *P. paucicostata* and the description of *S. ellisfiordensis* for the two specimens mentioned above corroborated by new material from Antarctica. In addition, two specimens previously assigned to *S. cupula* have been shown to share the core characteristic of *Pseudostephanoeca* with exteriorly positioned transverse elements what has led to the description of the new species *P. quasicupula* including also new material from Iceland.

Keywords

Acanthoecida; Choanoflagellatea; Revision; *Stephanoeca*; Stephanoecidae; Tectiform division mode

Introduction

Acanthoecid (loricate) choanoflagellates are characterized by one of the most distinctive extracellular coverings present in any protistan taxon. The formation of the lorica, a siliceous basket-like investment, is a precise sequence of developmental stages resulting in unique and species-specific costal strip arrangements (Leadbeater 2015). Until now, more than 150 loricate species have been described from all regions of the world's oceans. The species distribute themselves within two families differentiated according to the relationship between stages in lorica production and the cell cycle, i.e. nudiform (Acanthoecidae) and tectiform (Stephanoecidae) division. In the 'nudiform' mode, naked juveniles result from a diagonal cell division and subsequently produce and assemble costal strips for their own lorica. Contrastingly, in the 'tectiform' mode, a parent cell, already with a lorica, produces and accumulates a complete set of costal strips that are subsequently passed on to the juvenile cell following an inverted division with flagellar poles of the two daughter cells facing each other. These developmental differences are well supported by some multigene phylogenies resulting in two distinct but evolutionarily related lineages reflecting the two families (Carr et al. 2017, 2008; Schiwitza and Thomsen 2022; Schiwitza et al. 2019). However, depending on the phylogenetic model and number of investigated species, tectiform paraphyly was also recovered in other multigene analyses (e.g. Carr et al. 2017, 2008; López-Escardó et al. 2019; Nitsche and Arndt 2008; Nitsche et al. 2017; Paps et al. 2013; Tikhonenkov et al. 2020).

Kent (1878, 1880-1882) described *Salpingoeca ampulla* characterized by a voluminous and longitudinally ridged 'superstructure' that enclosed the cell. This species was later transferred to the loricate genus *Stephanoeca* (Ellis 1929) which displays a distinct pattern of longitudinal and transverse costae. Members of the genus *Stephanoeca* (currently 19 species described) are characterized by loricae composed of a larger anterior and smaller posterior lorica chamber divided by a waist. In *Stephanoeca* sensu stricto, the anterior lorica

chamber comprises an outer layer of longitudinal costae and interior positioned transverse costae (except for the anterior costa) forming various numbers of transverse rings.

A classification of species of *Stephanoeca* based exclusively on morphological traits has proved to be problematic and is further seriously challenged by molecular phylogenetic analyses from which no monophyly can be inferred (Carr et al. 2017; Nitsche et al. 2017; Schiwitza et al. 2019). A striking example is *S. arndti* Nitsche, 2014, which clearly shows a *Stephanoeca* morphology but clusters with high bootstrap support within the genus *Didymoeca* (Nitsche 2014). There is obviously an urgent need for a revision of the genus *Stephanoeca*, aiming for (1) a rigorous circumscription of the *Stephanoeca* core species that cluster around the unfortunately insufficiently known generic type species, *S. ampulla*, as well as *S. diplocostata*, which on the contrary is by far the most intensively examined single species of *Stephanoeca* (e.g. Leadbeater 1989, 1987, 1985, 1979a, b), and (2) an overall clarification of its relationships to other loricate species. Based on published material and new morphological and molecular data, we are here able to provide small pieces of evidence that in the long-term will contribute to solving the *Stephanoeca* enigma.

Stephanoeca diplocostata var. *paucicostata* was described by Throndsen (1969) based on material from Norway. The new variety described appeared similar to *S. diplocostata* Ellis, 1929 except for the presence of an anterior lorica chamber which had a double transverse costa at the level of the maximum lorica chamber diameter, and a significantly broader anterior lorica chamber. While the first description of this taxon (Throndsen 1969) was based exclusively on light microscopy, it subsequently became clear that this form was easily distinguished from *S. diplocostata* var. *diplocostata* in choanoflagellate surveys (e.g. Hara et al. 1997; Thomsen 1982, 1973). Tong (1997) discussed the inherent logic in henceforward dealing with this well-defined form at the specific level, and a binomial species name, *S. paucicostata* Tong et al., 1998, was in fact introduced in a subsequent publication (Tong et al. 1998).

A detailed reinvestigation of published material of *S. paucicostata* Tong et al., 1998 (Table 1), combined with new morphological and molecular data, has revealed that the double transverse costa is located outside of the longitudinal costae, precluding an affiliation of this taxon to *Stephanoeca* sensu stricto. This is further corroborated by our molecular data. Therefore, we here establish the new tectiform genus *Pseudostephanoeca* gen. nov. to accommodate *P. paucicostata* (Tong et al., 1998) comb. nov.

Material referred to as *S. diplocostata* var. *paucicostata* by Marchant et al. (1987, loc. cit. Figs 9-10) and Nitsche et al. (2011, loc. cit. Fig. 8) has in retrospect been shown to have a costal strip pattern in accordance with *Stephanoeca* sensu stricto, and there is also a significant difference on a molecular basis when compared to both *S. paucicostata* sensu stricto and *S. diplocostata*. As a consequence of the observations summarized above, we here describe the Marchant et al. (1987) and Nitsche et al. (2011) material as a new species of the genus *Stephanoeca*, *S. ellisfiordensis* sp. nov., named after the Antarctic site where this taxon was first observed.

Two specimens of *Stephanoeca cupula* Leadbeater, 1972, sensu Thomsen, 1988 (loc. cit. Figs 16-19) share the core characteristic of *Pseudostephanoeca* with an exteriorly positioned transverse costa on the anterior lorica chamber. This material was already found to differ from the original description of *S. cupula* sensu Leadbeater, 1972 by the number of anterior transverse rings (Leadbeater 2015). We here redescribe the material erroneously identified as *S. cupula* by Thomsen (1988) as *P. quasicupula* sp. nov., including also new material from Iceland.

Material and methods

Sampling

Water samples were collected at various sampling stations and processed for further morphological analyses (Fig. 1, Table 2). Sampling (BSCL) from tide pools on a rocky shore

outside Concarneau Marine Station, France, and from an extensive saltmarsh at Freiston Shore, Lincolnshire, UK were carried out by hand using sterile polypropylene sampling bottles. Samples were returned to the laboratory within two hours for processing. Sampling (BSCL) of seawater from around Ólafsvik, Iceland was carried out on board R/V 'Bjarni Sæmundsson' using PVC Niskin bottles on a CTD rosette sampler at a depth of 30 metres. Sampling (HAT) was either directly from the shore (Tempelkrogen, Isefjorden, Denmark) or from a variety of research vessels using PVC bottles either singly attached to a cable or being part of a rosette sampler (Denmark: R/V 'Martin Knudsen'; Finland: R/V 'Aranda'; Greenland: R/V 'Porsild'; Antarctica: R/V 'Polarstern'). Occasionally locally available boats were used (Isefjord, outer broad, Denmark). Wherever possible the samples were kept in Duran© water bottles at ambient temperature until further processed. Surface water samples (SS) from Kos Island, Greece were taken at a pier and kept at ambient temperature.

Sample processing

BSCL protocol: On all occasions, sampled water was rough filtered through a 25-µm mesh plankton net and allowed to stand in sterile glass bottles for one day at ambient temperature. Suspended nanoplankton within the clarified water was concentrated over a 3.0 µm membrane filter and subsequently pelleted by centrifugation. A few drops of 2% osmium tetroxide in 0.1M cacodylate buffer at pH 7.0 were added to the resuspended pellet. Fixed cells were washed three times in distilled water and deposited on to Formvar coated copper grids. Grids were coated with gold/palladium and viewed on a Philips EM300 microscope (Birmingham, England).

HAT protocol: The general protocol for processing water samples for the transmission electron microscope (TEM) was according to Moestrup and Thomsen (1980). The nanoplankton community was typically concentrated for further processing by means of 1) either repeated cycles of centrifugation to produce pelleted nanoflagellate material, or 2) centrifugation of prefiltered material resuspended from an initial filtration of cells on top of

e.g., a 1 µm Nuclepore filter (range of sizes used is 1-3 µm). The volume of water processed was around 0.5 litres, however, according to the site the volume was specifically adjusted as deemed necessary to produce a workable suspension of cells. Small droplets of cells from the resuspended final pellet of material were placed on carbon coated grids for the TEM. Cells were subsequently fixed for ca. 30 seconds in the vapour from a 1-2% aqueous solution of OsO4. After drying the grids were carefully rinsed in distilled water to remove salt crystals. Grids were shadow cast with either gold/palladium or chromium prior to the examination in JEOL electron microscopes property of the Botanical Institute at the Univ. of Copenhagen.

SS protocol: Water samples from Kos Island were transferred to 50 ml culture flasks (Falcon, Durham, USA) and cultured in artificial seawater (Instant Ocean, Aquarium Systems, Strasbourg, France) at 40 PSU (practical salinity unit) enriched with sterilized wheat grains as a carbon source for bacterial growth. Culture flasks were regularly monitored for acanthoecid choanoflagellates by light microscopy (Zeiss Axiovert S 100). Culture flasks containing target species were further processed by LAM (liquid aliquot method; Butler and Rogerson 1995) to obtain a monoclonal culture. The acanthoecid choanoflagellate species was further processed for morphological information (SEM) and molecular analysis of SSU and LSU rRNA for phylogenetic data.

Morphological analyses (SEM)

For scanning electron microscopy of samples from Kos Island, cultures were maintained in culture flasks for preparation (see Schiwitza and Thomsen (2022)). In brief, samples were fixed with 2.5 % cacodylate buffered glutaraldehyde (final concentration) at 4°C for 100 min. and afterwards dehydrated in an ascending ethanol series. Samples were washed twice with each ethanol concentration and incubated for 10 min. in the equivalent ethanol concentration. For final dehydration, samples were treated with a 50:50 hexamethyldisilazane (HMDS)-ethanol solution for 10 min., followed by two washing steps with pure HMDS and 5 min. incubation. Finally, samples were dried and the bottom of each culture flask cut to size and

stuck to a sample holder. Mounted samples were sputter coated with a layer of gold (12 nm) and examined by SEM (Fei Quanta 250 FEG, Biocenter Cologne, University of Cologne, Germany).

rRNA gene sequencing and phylogenetic analyses

For molecular analyses of the partial SSU and LSU rRNA of the monoclonal culture HFCC 1361 (Kos Island), DNA was extracted using the Quick-gDNA™ MiniPrep kit (Zymo Research, Ca, USA). The SSU rRNA fragment was amplified with the primers 18S-42F and 18S-nested-rev at a concentration of 0.1 µM using a PCR Mastermix (2x) (VWR Life Science, Red Taq DNA Polymerase, Hassrode, Belgium). The LSU rRNA fragment was amplified with the primer combination NLF184F/21 and NLR2098R/24 at the same concentration mentioned above. Both PCRs were done according to the first amplification round mentioned in Schiwitza and Thomsen (2022). All primer sequences are listed in Table 3.

The PCR products were purified by the FastGene Gel/PCR Extraction Kit (Nippon Genetics, Düren, Germany) and sequenced by the Eurofins Genomics sequencing service (Cologne, Germany) using the primer 18S-42F, 18S-nested-rev (SSU rRNA) and the primer NLF184/21, NLR2098/24 (LSU rRNA). Sequences were assembled by BioEdit (Hall 1999).

Molecular data (SSU and LSU rRNA) of HFCC 1361 with additional closely related environmental sequences of the SSU rRNA were aligned with sequences from the latest analysis of acanthoecid choanoflagellate phylogeny (Schiwitza and Thomsen 2022) using MAFFT (Katoh et al. 2002) (see supplementary material Table S1 for accession numbers) to create a concatenated six-gene tree (SSU and LSU rRNA, Hsp90, αtub, Efl and Ef1α) containing a manually masked alignment of 9851 bp. The alignment was analysed using Maximum likelihood (ML) and Bayesian inference (BI) methods. ML analysis was done by RAxML v.8.2.12 (Stamatakis 2014) using the GAMMA + P-Invar model of rate heterogeneity with 1000 replicates for thorough bootstrapping. The parameter N, number of

alternative runs on distinct starting trees, was set to autoMRE as proposed by the program (i.e. 10 starting trees). The Bayesian analysis was performed by MrBayes v.3.2.6 (Ronquist et al. 2012) running a GTR + I + Γ model and a four-category gamma distribution to correct for among site rate variation. The search consisted of two parallel chain sets run at default temperatures with a sample frequency of 10 and run until the average standard deviation of split frequencies dropped below 0.01. The analysis consisted of 3,500,000 generations with a burnin of 87,500 before calculating posterior probabilities. The acanthoecid choanoflagellate phylogeny was rooted with a two-taxa ichthyosporean clade. The pairwise distances (based on SSU rRNA) were calculated using BioEdit (Hall 1999). The alignment is available from the author upon request.

Results and discussion

The morphological examination of *S. paucicostata* specimens from a wide range of geographical sites (Table 2, Figs 1, 2) and in particular the establishment of a clonal culture from Kos Islands, Greece (Fig. 3) has considerably expanded the morphological and molecular (SSU and LSU rRNA) knowledge base with reference to *S. paucicostata*. It has become evident, with the exception of material documented by Marchant et al. (1987) and Nitsche et al. (2011) (see further below), that the characteristic double transverse costa in the anterior lorica chamber of *S. paucicostata* is located exterior to the longitudinal costae in all specimens examined. The relative positions of transverse and longitudinal elements were not noticed in past taxonomical surveys dealing with *Stephanoeca* species, and only recently received attention in the wake of the reinvestigation of *S. urnula* Thomsen, 1973, which was shown to have exteriorly positioned transverse costae in the anterior lorica chamber (Schiwitza and Thomsen 2022). This morphological divergence of *S. urnula* from *Stephanoeca* sensu stricto was confirmed by molecular analyses inferring an affiliation to the genus *Enibas*. The nudiform genus *Enibas* (resembling the tectiform genus *Stephanoeca* in a

vase-shaped lorica appearance) also has anterior lorica chamber transverse elements located exterior to the longitudinal elements.

The external or internal positioning of transverse costae in loricate choanoflagellates is considered to be a keystone morphological trait, because the relative positions of costae are defined as an integrated part of the lorica assembly protocol (Leadbeater 2015). It should be mentioned also that in the best documented forms with an external mid-lorica transverse costa (i.e. species of *Pleurasiga* (except *P. orculaeformis* Schiller, 1925) and core species of *Parvicorbicula*; see e.g. Leadbeater 2015, Thomsen et al. 2020) there is not a single known deviation from the basic lorica assembly principle. Considering that costal strip patterns and lorica assembly protocols are genetically controlled, it is, based on our current knowledge base, hard to accept that species with reversed relative positions of costae can be accommodated within the same genus. However, it is obvious that only a much improved molecular screening of a multitude of loricate choanoflagellates can contribute to a more final understanding of the significance of this trait with reference to classification schemes. Based on this clear morphological characteristic (Figs 2, 3) combined with molecular data of the SSU and LSU rRNA (Fig. 6), we have erected the new tectiform genus *Pseudostephanoeca* gen. nov. with its type species *P. paucicostata* (Tong et al., 1998) comb. nov. The species is characterized by a certain variability in the number of longitudinal costae (10-16) in the anterior lorica chamber (Figs 2, 3). A double transverse costa is located at the widest part of the anterior chamber. However, this duplication of a costa, which is not an uncommon phenomenon in loricae of species with many costae, may in some specimens observed be reduced (Fig. 2B) and appear as just a single costa. An additional transverse costa is located midway between the double transverse costa and the waist separating the lorica chambers. While this costa is consistently and distinctly present in our material, it is not quite as evident in the type drawing where it is more posteriorly located (Throndsen 1969; reproduced here as

Fig. 2A). We interpret that this minor morphological difference reflects the observation techniques applied (LM versus TEM/SEM).

The above-mentioned specimens assigned to *S. paucicostata* by Marchant et al. (1987) and Nitsche et al. (2011) differ in their appearance by possessing only a single transverse costa, which is located inside to the longitudinal costae of the anterior chamber. Our present material from Antarctica (Fig. 5) strikingly resembles both specimens in costal strip arrangement and lorica size, which is overall (about 1.5 times) larger than *P. paucicostata*. The Antarctic material thus shares critical lorica morphological details with core species of the genus *Stephanoeca* and is therefore here redescribed as a new species, *S. ellisfiordensis* sp. nov. An additional feature of *S. ellisfiordensis* sp. nov. is that longitudinal costal strips at the base of the anterior chamber overlap significantly, forming triangular buttress-like formations in the waist region (Fig. 5A, B). Nitsche et al. (2011) provided molecular data (SSU rRNA) of their material (i.e. *S. ellisfiordensis*), which was found to differ by 1.7 % pairwise distance (Table 4) to our *P. paucicostata* material from Kos Island. This adds molecular support to differentiate *P. paucicostata* (Tong et al., 1998) comb. nov. from *S. ellisfiordensis* sp. nov. Within the phylogenetic analyses, molecular data of the SSU rRNA of several uncultured eukaryotic sequences were implemented for a possible higher resolution of phylogenetic relationships. *Pseudostephanoeca paucicostata* and *S. ellisfiordensis* cluster together with *S. cauliculata* but polytomous branching and weak bootstrap support indicate still low resolutions within this clade (Fig. 6). The present inconsistency of pairwise to patristic distances might result from the use of a masked alignment for phylogenetic analyses whereas pairwise distances were calculated from complete, uncorrected sequences.

A re-examination of the material published by Thomsen (1988) and assigned to *S. cupula*, reveals differences in several specimens illustrated that were not appreciated at the time of publication and which render conspecificity highly unlikely. Two specimens illustrated (Thomsen 1988, loc. cit. Figs 16-19) clearly have the first intermediate transverse

costa located exterior to the longitudinal costae allowing for a reassignment of the material (Fig. 4) to the newly established genus *Pseudostephanoeca*. In the type material of *S. cupula* only the anterior transverse ring of the two transverse costae in the anterior lorica chamber is outside the longitudinal costae (Leadbeater 2015, 1972). The Thomsen (1988) specimens differ in their number of anterior lorica chamber transverse costae, i.e. four (Fig. 4 A; Thomsen 1988, loc. cit. Figs 16, 18) and three (Fig. 4 F; Thomsen 1988, loc. cit. Figs 17, 19) transverse costal rings. Additional material collected at Ólafsvik, Iceland (Fig. 4 B-E, G-K) strikingly resembles the two forms of the original specimens. Unfortunately, the Ólafsvik material shows no clear position of the transverse elements. Being well aware of missing molecular information that might distinguish between both specimens (Thomsen 1988; loc. cit. Figs 17, 18), we here describe both morphotypes as a natural variability within one species as already the number of longitudinal costae is known for intraspecific variations (summarized in Leadbeater 2015).

Nomenclatural history and resolution of *Stephanoeca diplocostata* var. *paucicostata* Throndsen, 1969

Stephanoeca diplocostata var. *paucicostata* Throndsen, 1969 was originally described based on botanical rules of nomenclature (ICBN) as choanoflagellates at that time were classified among the Chrysophyceae (e.g. Christensen 1962). Hibberd (1976) summarized ultrastructural analyses of choanoflagellates and gave evidence for the correct assignment according to zoological classifications. *Stephanoeca diplocostata* var. *paucicostata* Throndsen, 1969 is not compliant with the zoological code since after 1960 a sub-specific term such as *variety* is not formally recognized in zoological nomenclature (see ICZN Article 45.5 and 45.6). The first usage of *S. paucicostata* Throndsen, 1969 (at the level of species) is by Tong et al. (1998) and this thereby made the binomial species name available. The description is in accordance with the current criteria for compliance and therefore the

authorship of the binomial adopts the name and date after this 'subsequent usage' resulting in *S. paucicostata* Tong et al., 1998 being the correct designation. A regulation to explicitly state the new proposed name as 'sp. nov.' or similar was introduced in 1999 and is therefore not relevant in the case of *S. paucicostata*.

Conclusion

The presented re-examination of species assigned to the genus *Stephanoeca* enables a refined taxonomical delineation based on a distinct morphological criterion, i.e. the relative position of transverse to longitudinal costae to distinguish the two genera *Stephanoeca* and *Pseudostephanoeca* sensu stricto. This clear morphological characteristic for species delineation needs further phylogenetical support as for now, only molecular data of the type species *P. paucicostata* are available. Enhanced studies on specimens of the genus *Stephanoeca* integrating morphological and molecular data will help to solve the taxonomical conundrum of this genus.

Taxonomic summary

Order: Acanthoecida (Cavalier-Smith, 1997)

Family: Stephanoecidae Leadbeater in Nitsche et al. 2011

Genus: ***Pseudostephanoeca* gen. nov.**

Diagnosis: Lorica (7-17 µm long, 5-15 µm maximum width) comprising anterior and posterior chambers. The protoplast is located in the posterior lorica chamber while the collar and flagellum extend into the anterior chamber. The anterior lorica chamber is constructed from up to four transverse costae and 8-16 longitudinal costae. Both the anterior transverse ring and the first intermediate transverse costa are located exterior to the longitudinal costae. The subsequent anterior lorica chamber transverse costae are located inside the longitudinal

costae. The posterior lorica chamber comprises closely spaced helical transverse costae. Cell division tectiform.

Etymology: Genus name derived from '*stephanoeca*' and '*pseudo-*' (Greek; meaning fake), and referring to the fact that the generic type species has previously been associated with the genus *Stephanoeca*.

Generic type species: ***Pseudostephanoeca paucicostata*** **(Tong et al., 1998) comb. nov. (Figs 2, 3)**

Protonym: *Stephanoeca diplocostata* var. *paucicostata* Throndsen, 1969; *Stephanoeca paucicostata* Tong et al., 1998 (erroneously accredited to Throndsen, 1969; see nomenclatural history above)

Improved diagnosis: Lorica divided into two chambers, where the small posterior lorica chamber surrounds the protoplast, while the collar and flagellum expand into the larger, bulbous anterior lorica chamber. The overall lorica height ranges from 9.0-16.6 µm. There are 10-16 (with a mode of 11) longitudinal costae. The anterior lorica chamber is characterized by a double transverse costa in the widest part of the lorica chamber, sometimes reduced and appearing as a single costa with just the occasional doubling of individual strips, and a single transverse costa slightly above the level of the constriction between the lorica chambers. The maximum lorica diameter ranges from 7.2-15.0 µm. The organic lining of the posterior lorica chamber extends to the level of the anterior lorica chamber posteriormost transverse costa.

Voucher material: The culture from Kos Island is strain 1361 (Fig. 3) of the Heterotrophic Flagellate Collection Cologne (HFCC) and has been deposited as SEM material at the Oberösterreichischem Landesmuseum Linz (LI), Austria (deposition nr. EVAR 2022/x).

Sequence data: The sequences (SSU and LSU rRNA) of the strain HFCC 1361 have been deposited in GenBank with the accession numbers OP038903 (SSU rRNA) and OP038902 (LSU rRNA).

Species: ***Pseudostephanoeca quasicupula* sp. nov. (Fig. 4)**

Protonym: *Stephanoeca cupula* (Leadbeater, 1972) Thomsen, 1988 sensu Thomsen 1988 (loc. cit. Figs 16-19; see also Leadbeater 2015)

Diagnosis: Lorica divided into two chambers, where the small posterior lorica chamber surrounds the cell, while the collar and flagellum expand into the larger anterior lorica chamber. The overall lorica height ranges from 6.9-11.9 µm. There are 7-10 (with a mode of 8) longitudinal costae each comprising 4 costal strips with nib-like points of the anteriormost costal strips. The anterior lorica chamber contains 3-4 transverse costae with an organic lining from the posteriormost anterior chamber transverse costa and downwards. One transverse ring closes the lorica anteriorly. The maximum lorica diameter (flattened), at the level of the intermediate transverse costae, ranges from 4.9-8.1 µm. The posterior lorica chamber is formed by the converging longitudinal costae and also a distinct helical costal element.

Type specimen: The specimen illustrated in Fig. 17 of the original material by Thomsen 1988 is fixed as type specimen.

Type locality: Surface water sample (ca. 12 °C / ca. 19 PSU) from a near coastal site in the innermost part of the Isefjord, Denmark, collected October 1976.

Voucher material: The specimens of Fig. 4B, C, J, K of water samples from Iceland (64°53’44’’N 23°42’14’’W), collected June 1995.

Etymology: The species name is chosen to emphasize (‘*quasi*’ (Latin) meaning as if) that this taxon has previously been considered identical to *S. cupula*.

Remarks: The original material by Thomsen (1988; loc. cit. Figs 16-19) was embedded in an extended description of *Stephanoeca cupula* disregarding the morphological difference of the mid-anterior lorica chamber transverse costa being located exterior to the longitudinal costae.

Species: ***Stephanoeca ellisfiordensis* sp. nov. (Fig. 5)**

Diagnosis: Lorica divided into two chambers, where the small posterior lorica chamber surrounds the cell, while the collar and flagellum expand into the larger anterior lorica chamber. The overall lorica height ranges from 16-19.3 µm. There are 12-15 longitudinal costae with only two longitudinal costal strips between the anterior lorica end and the waist. The prominent mid-anterior chamber transverse costa is internal to the longitudinal costae. The maximum lorica diameter, at the level of the mid-anterior chamber transverse costae, ranges from 9-18 µm. Longitudinal costal strips overlap significantly forming triangular structures (buttress-like formations) in the waist region.

Type specimen: The specimen illustrated in Fig. 10 of Marchant et al. 1987 is fixed as type specimen.

Type locality: Water sample (ca. 2.6 °C / 37.6 – 38.4 PSU) from a basin at the head of Ellis Fjord, Antarctica (68°36'S 78°13'E), collected January 1986.

Voucher material: The specimens of Fig. 5 A-C of Antarctic water samples (70°30'06''S 07°59'00''W), collected April 1992.

Etymology: *ellisfiordensis* (adjective) alluding to the type locality.

Sequence data: The sequence data of the SSU rRNA with the accession number HQ026769 by Nitsche et al. (2011).

Remarks: Specimen examined by Marchant et al. (1987) and Nitsche et al. (2011) were incorrectly assigned to *Stephanoeca diplocostata* var. *paucicostata* and *Stephanoeca paucicostata*, respectively. In contrast to all other material previously associated with the epithet '*paucicostata'* the Marchant et al. (1987) and Nitsche et al. (2011) specimens share basic morphological lorica characteristics with *Stephanoeca* sensu stricto rather than *Pseudostephanoeca*.

CRediT authorship contribution statement

SS: Conceptualization, Data curation, Formal analysis, Investigation, Visualization, Writing – original draft; Writing – review & editing. HAT: Conceptualization, Data curation, Formal analysis, Investigation, Writing – review & editing. BSCL: Conceptualization, Data curation, Formal analysis, Investigation, Writing – review & editing.

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References

Butler, H., Rogerson, A., 1995. Temporal and spatial abundance of naked amoebae (Gymnamoebae) in marine benthic sediments of the Clyde Sea area, Scotland. J. Eukaryot. Microbiol. 42, 724–730. https://doi.org/10.1111/j.1550-7408.1995.tb01624.x

Carr, M., Leadbeater, B.S.C., Hassan, R., Nelson, M., Baldauf, S.L., 2008. Molecular phylogeny of choanoflagellates, the sister group to Metazoa. PNAS 105, 16641–16646. https://doi.org/10.1073/pnas.0801667105

Carr, M., Richter, D.J., Fozouni, P., Smith, T.J., Jeuck, A., Leadbeater, B.S.C., Nitsche, F., 2017. A six-gene phylogeny provides new insights into choanoflagellate evolution. Mol. Phylogenet. Evol. 107, 166–178. https://doi.org/10.1016/j.ympev.2016.10.011

Christensen, T., 1962. Alger, in: Böcher, T.W., Lange, M., Sørensen, T. (Eds.), Botanik, 2, Systematisk Botanik. Copenhagen: Munksgaar, pp. 1–178.

Ellis, W.N., 1929. Recent researches on the Choanoflagellata (Craspedomonadines). Ann. Soc. R. Zool. Belg. 60, 49–88.

Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp. 41, 95–98.

Hallegraeff, G.M., 1983. Scale-bearing and loricate nanoplankton from the East Australian current. Bot. Mar. 26, 493–515. https://doi.org/10.1515/botm.1983.26.11.493

Hara, S., Sheu, J.-C., Chen, Y.L, Takahashi, E., 1997. Choanoflagellates (Sarcomastigophora, Protozoa) from the coastal waters of Taiwan and Japan (II): species composition and biogeography. Zool. Stud. 36, 98–110.

Hibberd, D.J., 1976. The ultrastructure and taxonomy of the Chrysophyceae and Prymnesiophyceae (Haptophyceae): a survey with some new observations on the ultrastructure of the Chrysophyceae. Bot. J. Linn. 72, 55–80. https://doi.org/10.1111/j.1095-8339.1976.tb01352.x

ICZN (The International Commission on Zoological Nomenclature), 1999. International Code of Zoological Nomenclature, 4th ed. The International Trust for Zoological Nomenclature, London, pp. 1–306.

Katoh, K., Misawa, K., Kuma, K., Miyata, T., 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Res. 14, 3059–3066. https://doi.org/10.1093/nar/gkf436

Kent, W.S., 1880. A manual of the Infusoria. Vols 1-3. London: D.Bogue.

Leadbeater, B.S.C., 1972. Ultrastructural observations on some marine choanoflagellates from the coast of Denmark. Br. Phycol. J. 7, 195–211. https://doi.org/10.1080/00071617200650211

Leadbeater, B.S.C., 1979a. Developmental studies on the loricate choanoflagellate *Stephanoeca diplocostata* Ellis I. Ultrastructure of the non-dividing cell and costal strip production. Protoplasma 98, 241–262.

Leadbeater, B.S.C., 1979b. Developmental studies on the loricate choanoflagellate *Stephanoeca diplocostata* Ellis II. Cell division and lorica assembly. Protoplasma 98, 311–328.

Leadbeater, B.S.C., 1985. Developmental studies on the loricate choanoflagellates *Stephanoeca diplocostata* Ellis IV. Effects of silica deprivation on growth and lorica production. Protoplasma 127, 171–179. https://doi.org/10.1007/BF01322467

Leadbeater, B.S.C., 1987. Developmental studies on the loricate choanoflagellates *Stephanoeca diplocostata* Ellis V. The cytoskeleton and the effects of microtubule poisons. Protoplasma 136, 1–15. https://doi.org/10.1007/BF01276313

Leadbeater, B.S.C., 1989. Developmental studies on the loricate choanoflagellates *Stephanoeca diplocostata* Ellis VI. Effects of silica replenishment on silica impoverished cells. Protoplasma 153, 71–84. https://doi.org/10.1007/BF01322467

Leadbeater, B.S.C., 2015. The Choanoflagellates: Evolution, Biology and Ecology. Cambridge University Press, Cambridge.

Lee, W.J., Brandt, S.M., Vørs, N., Patterson, D.J., 2003. Darwin's heterotrophic flagellates. Ophelia 57, 63–98. https://doi.org/10.1080/00785236.2003.10409506

López-Escardó, D., Grau-Bové, X., Guillaumet-Adkins, A., Gut, M., Sieracki, M.E., Ruiz-Trillo, I., 2019. Reconstruction of protein domain evolution using single-cell amplified genomes of uncultured choanoflagellates sheds light on the origin of animals. Phil. Trans. R. Soc. B. 374, 20190088. https://doi.org/10.1098/rstb.2019.0088

López-García, P., Rodríguez-Valera, F., Pedrós-Alió, C., Moreira, D., 2001. Unexpected diversity of small eukaryotes in deep-sea Antarctic plankton. Nature 409, 603–607. https://doi.org/10.1038/35054537

López-García, P., Philippe, H., Gail, F., Moreira, D., 2003. Autochthonous eukaryotic diversity in hydrothermal sediment and experimental microcolonizers at the mid-Atlantic Ridge. PNAS 2, 697–702.

Marchant, H.J., van den Hoff, J., Burton, H.R., 1987. Loricate choanoflagellates from Ellis Fjord, Antarctica including the description of *Acanthocorbis tintinnabulum* sp. nov. Proc. NIPR Symp. Polar Biol. 1, 10–22.

Menezes, S., 2005. Nanoplankton biodiversity in the Pettaquamscutt River estuary, Rhode Island, U.S.A. PhD thesis, University of Rhode Island.

Moestrup, Ø., Thomsen, H.A., 1980. Preparation of shadow-cast whole mounts, in: Gantt, E. (Ed.), Handbook of Phycological Methods, Vol. III. Cambridge, pp. 385–390.

Nitsche, F., 2014. *Stephanoeca arndti* spec. nov. – First cultivation success including molecular and autecological data from a freshwater acanthoecid choanoflagellate from Samoa. Eur. J. Protistol. 50, 412–421. https://doi.org/10.1016/j.ejop.2014.03.004

Nitsche, F., Arndt, H., 2008. A new choanoflagellates species from Taiwan: Morphological and molecular biological studies of *Diplotheca elongata* nov. spec. and *D. costata*. Eur. J. Protistol. 44, 220–226. https://doi.org/10.1016/j.ejop.2008.01.002

Nitsche, F., Carr, M., Arndt, H., Leadbeater, B.S.C., 2011. Higher level taxonomy and molecular phylogenetics of the Choanoflagellatea. J. Eukaryot. Microbiol. 58, 452–462. https://doi.org/10.1111/j.1550-7408.2011.00572.x

Nitsche, F., Thomsen, A.H., Richter, D.J., 2017. Bridging the gap between morphological species and molecular barcodes – exemplified by loricate choanoflagellates. Eur. J. Protistol. 57, 26–37. https://doi.org/10.1016/j.ejop.2016.10.006

Paps, J., Medina-Chacón, L.A., Marshall, W., Suga, H., Ruiz-Trillo, I., 2013. Molecular phylogeny of unikonts: new insights into the position of apusomonads and ancyromonads and the internal relationships of opisthokonts. Protist 164, 2–12. https://doi.org/10.1016/j.protis.2012.09.002

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

Schiwitza, S., Thomsen, H.A., 2022. Morphological and molecular reinvestigation of acanthoecid species I. – *Enibas urnula* (Thomsen, 1973) comb. nov. (=*Stephanoeca urnula* Thomsen, 1973). Eur. J. Protistol. 83, 125865. https://doi.org/10.1016/j.ejop.2022.125865

Schiwitza, S., Arndt, H., Nitsche, F., 2019. First description of an euryoecious acanthoecid choanoflagellate species, *Enibas tolerabilis* gen. et sp. nov. from a salar in the Chilean Andes based on morphological and transcriptomic data. Eur. J. Protistol. 67, 106–113. https://doi.org/10.1016/j.ejop.2018.11.004

Schlitzer, R., 2021. Ocean Data View, http://odv.awi.de.

Stamatakis, A., 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30, 1312–1313. https://doi.org/10.1093/bioinformatics/btu033

Thomsen, H.A., 1973. Studies on marine choanoflagellates I. Silicified choanoflagellates of the Isefjord (Denmark). Ophelia 12, 1–26. https://doi.org/10.1080/00785326.1973.10430116

Thomsen, H.A., 1979. Electron microscopical observations on brackish-water nannoplankton from the Tvärminne area, SW coast of Finland. Acta Bot. Fennica 110, 11–37.

Thomsen, H.A., 1982. Planktonic choanoflagellates from Disko Bugt, West Greenland, with a survey of the marine nanoplankton of the area. Meddr. Grønland, Biosci. 8, 3–35.

Thomsen, H.A., 1988. An electron microscopical study of marine loricate choanoflagellates: *Nannoeca minuta* (Leadbeater) gen. et comb.n. and *Stephanoeca cupula* (Leadbeater) comb.n. Zool. Scripta 17, 315–323. https://doi.org/10.1111/j.1463-6409.1988.tb00107.x

Thomsen, H.A., Østergaard, J.B., 2019. Loricate choanoflagellates (Acanthoecida) from warm water seas. III. *Acanthocorbis* Hara and Takahashi and *Stephanoeca* Ellis. Eur. J. Protistol. 69, 52–69. https://doi.org/10.1016/j.ejop.2019.02.001

Thomsen, H.A., Buck, K.R., Chavez, F.P., 1991. Choanoflagellates of the Central California waters: taxonomy, morphology and species assemblages. Ophelia 33, 131–164. https://doi.org/10.1080/00785326.1991.10429736

Thomsen, H.A., Kamennaya, N.A., Zubkov, M.V., Østergaard, J.B., 2020. Loricate choanoflagellates (Acanthoecida) from warm water seas. VI. *Pleurasiga* Schiller and *Parvicorbicula* Deflandre. Eur. J. Protistol. 75, 125717. https://doi.org/10.1016/j.ejop.2020.125717

Tikhonenkov, D.V., Mikhailov, K.V., Hehenberger, E., Karpov, S.A., Prokina, K.I., Esaulov, A.S., Belyakova, O.L., Mazei, Y.A., Mylnikov, A.P., Aleoshin, V.V., Keeling, P.J., 2020. New lineage of microbial predators adds complexity to reconstructing the evolutionary origin of animals. Curr. Biol. 30, 4500–4509. https://doi.org/10.1016/j.cub.2020.08.061

Tong, S.M., 1997. Heterotrophic flagellates from the water column in Shark Bay, Western Australia. Mar. Biol. 128, 517–536. https://doi.org/10.1007/s002270050118

Tong, S.M., Nygaard, K., Bernard, C., Vørs, N., Patterson, D.J., 1998. Heterotrophic flagellates from the water column in Port Jackson, Sydney, Australia. Eur. J. Protistol. 34, 162–194. https://doi.org/10.1016/S0932-4739(98)80027-8

Throndsen, J., 1969. Flagellates of Norwegian coastal waters. Nytt. Mag. Bot. 16, 161–216.

Throndsen, J., 1974. Planktonic choanoflagellates from North Atlantic waters. Sarsia 56, 95–122. https://doi.org/10.1080/00364827.1974.10411265

van der Auwera, G., Chapelle, S., de Wachter, R., 1994. Structure of the large ribosomal subunit RNA of *Phytophthora megasperma*, and phylogeny of the oomycetes. FEBS Letters 338, 133–136. https://doi.org/10.1016/0014-5793(94)80350-1

Vørs, N., 1992. Heterotrophic amoebae, flagellates and Heliozoa from the Tvärminne area, Gulf of Finland, in 1988-1990. Ophelia 36, 1–109. http://dx.doi.org/10.1080/00785326.1992.10429930

Wylezich, C., Meisterfeld, R., Meisterfeld, S., Schlegel, M., 2002. Phylogenetic analyses of small subunit ribosomal RNA coding regions reveal a monophyletic lineage of euglyphid testate amoebae (Order Euglyphida). J. Eukaryot. Microbiol. 49, 108–118. https://doi.org/10.1111/j.1550-7408.2002.tb00352.x

Figures and Tables

Figure 1: Map showing sampling stations where *Pseudostephanoeca* spp. and *Stephanoeca ellisfiordensis* occurred; created with ODV (Ocean Data View), Schlitzer 2021. Colour code: red – *P. paucicostata*; green – *P. quasicupula*; blue – *S. ellisfiordensis*

Figure 2: Morphology of *Pseudostephanoeca paucicostata* (A, original drawing from Throndsen 1969; notice that tectiform division (costal strip accumulation at the top of the collar, see arrow) is indicated; B-K transmission electron microscopy). (B-J) TEM images showing variability across specimens from a range of habitats. See Table 2 for information on the origin of the material illustrated here. (B) Weakly developed double transverse costa. (D, G) Tectiform division signature, i.e. costal strip accumulation in the collar region. (K) Close-up of TEM image J (negative), arrows point to places where the outside location of the mid-lorica transverse costa relative to the longitudinal costae is easily ascertained. Scale bar: 5 µm.

Figure 3: Morphology of *Pseudostephanoeca paucicostata* strain HFCC 1361 (A-F, scanning electron microscopy). (A-F) Images showing variability across individuals from the culture. (F) Tectiform division signature. Arrows point to places where the outside location of the

mid-lorica transverse costa relative to the longitudinal costae is easily ascertained. Scale bar: 5 µm.

Figure 4: (A-K, transmission electron microscopy) Morphology of *Pseudostephanoeca quasicupula* (A-E) Form with four transverse costae. (F-K) Form with three transverse costae. (A) Original specimen (Thomsen 1988, loc. cit. Fig. 18). (F) Original specimen (Thomsen 1988, loc. cit. Fig. 17). (B-E, G-K) Icelandic material. White arrows point to places where the outside location of the mid-lorica transverse costa relative to the longitudinal costae is indicated, black arrows for inside location of the subsequent anterior lorica transverse costa relative to the longitudinal costae. White rectangles highlight anterior terminal nib-like points in the longitudinal costae. Scale bar: 2 µm in A, B, C, F, G, H and 1 µm in D, E, I, J, K.

Figure 5: Morphology of *Stephanoeca ellisfiordensis* (A-D, transmission electron microscopy; reversed printing). (A-C) Images showing the variability encountered. (C) Tectiform division signature. (D) Close-up of image B, arrows point to places where the interior positioning of the transverse costa is evident. White rectangle in A highlights triangular buttress-like formations in the waist region. Scale bar in B: 5 µm (applies to A, B, C), D: 2 µm.

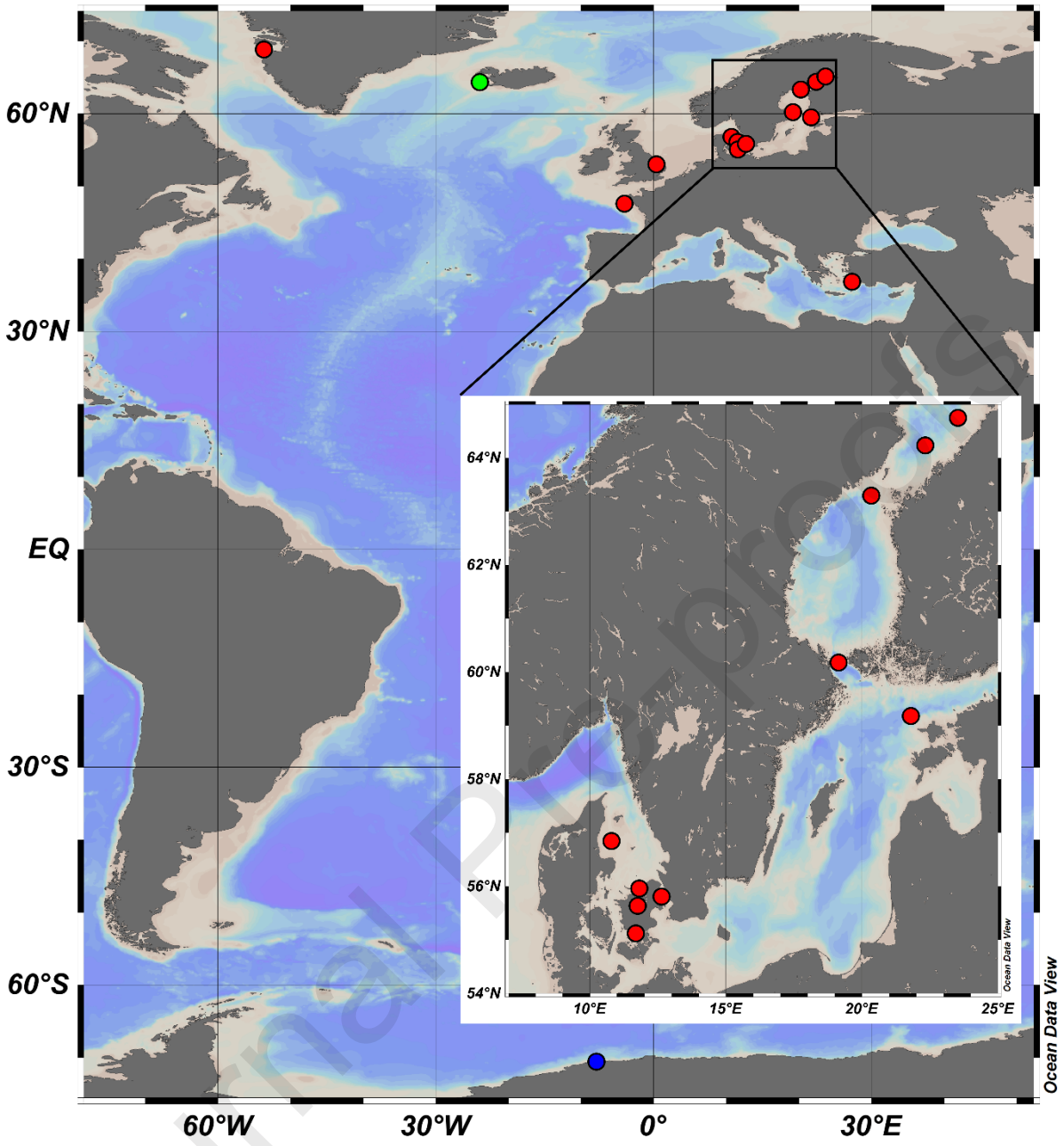
Figure 6: Concatenated six-gene (SSU and LSU rRNA, Hsp90, αtub, Efl and Ef1α) maximum likelihood phylogeny of Acanthoecida (Choanoflagellatea) based on a manual corrected alignment (9851 nt). Accession numbers of implemented strains containing only SSU and LSU rRNA data are displayed within the tree. For accession numbers of species with full molecular data of transcriptomes (TR) see supplementary material (Table S1). Support values are given for RAxML/BI at each branch. 100 % RAxML bootstrap percentage support (mlBP) and 1.00 BI posterior probabilities (biPP) are denoted by a '*'. Support values below 50% mlBP and 0.7 biPP are indicated by a '-'. Scale bar in the lower left refers to a phylogenetic distance of 0.05 nucleotide substitutions per site. The investigated species are marked by bold letters (molecular data of *Stephanoeca ellisfiordensis* from Nitsche et al. (2011)).

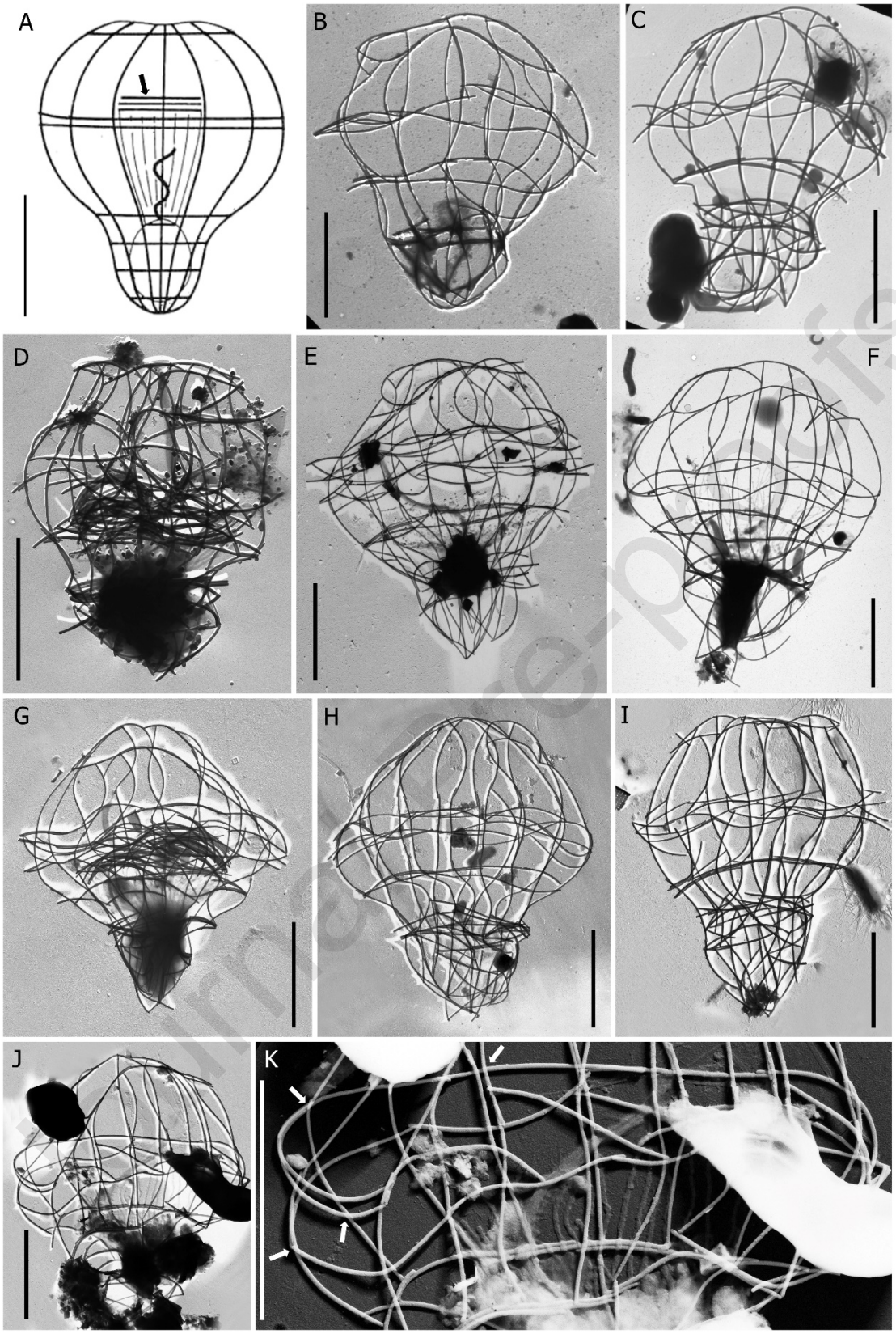
Table 1. Literature records of *Pseudostephanoeca paucicostata* and *Stephanoeca ellisfiordensis* mentioned in original publication as [1]*Stephanoeca diplocostata* Ellis var. *paucicostata* (Throndsen, 1969) or [2]*Stephanoeca paucicostata*.

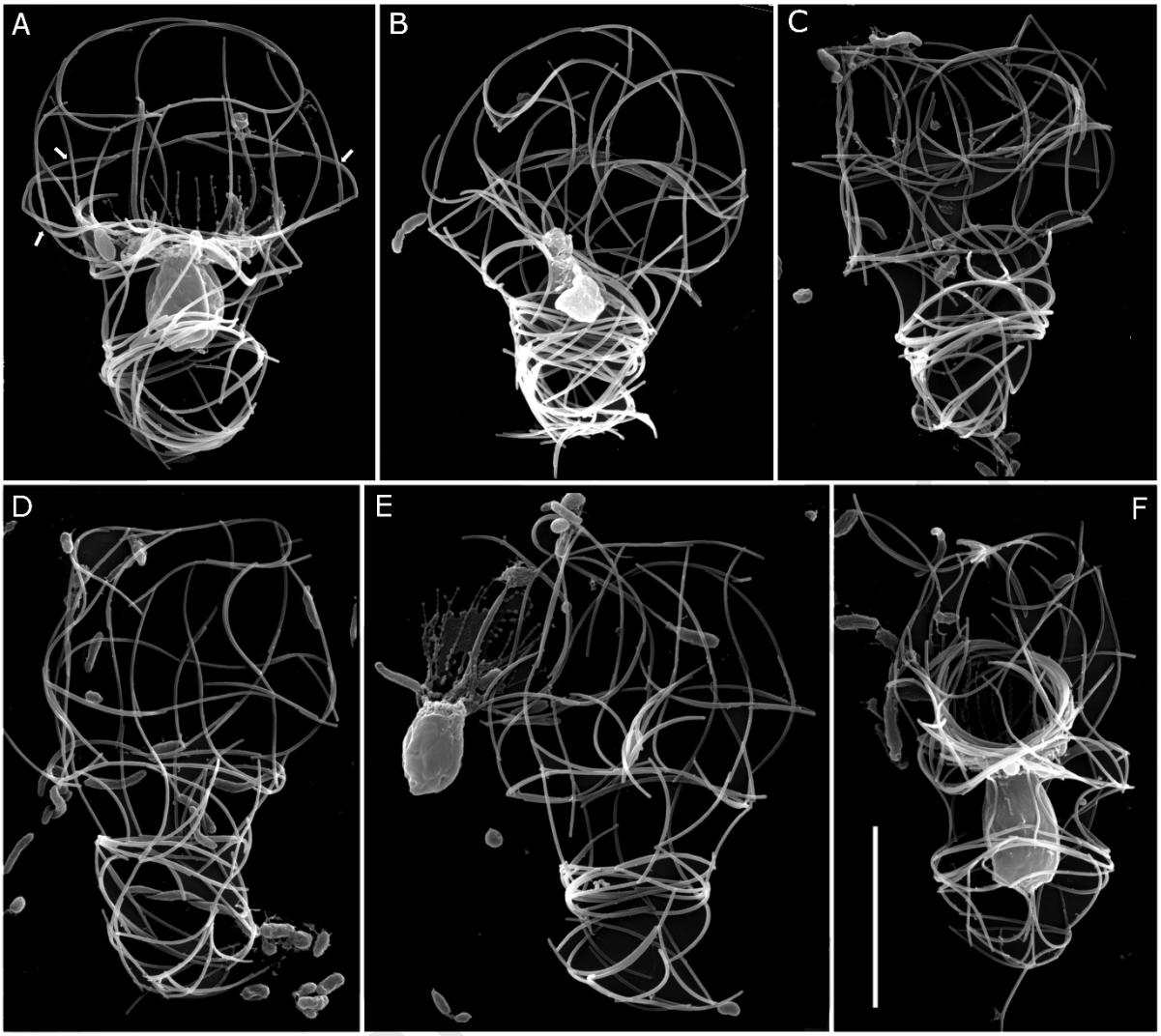
Table 2. Occurrence of species from the genus *Pseudostephanoeca* and *Stephanoeca ellisfiordensis* with basic parameters of investigated sampling areas.

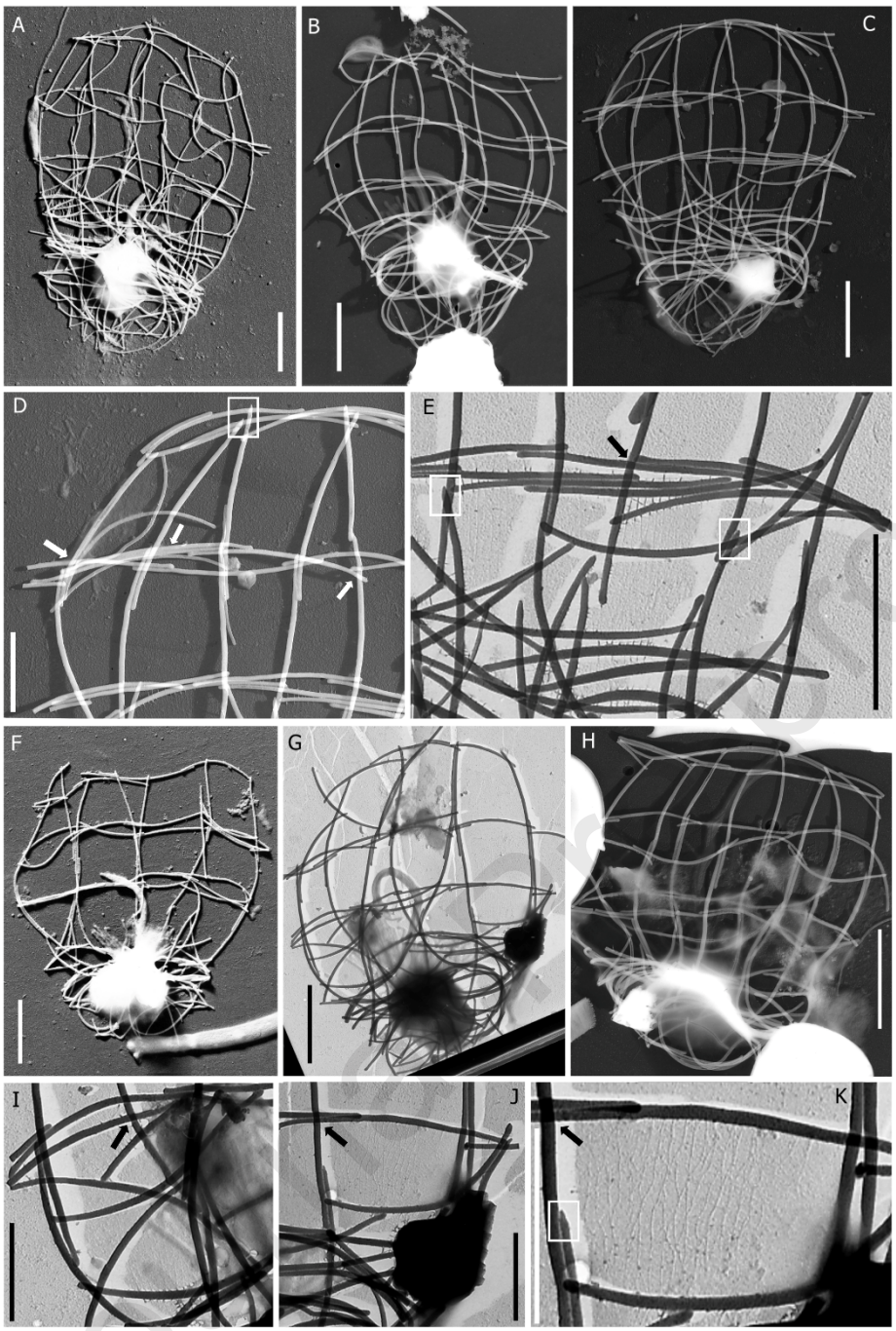
Table 3. Primer sequences used for molecular analyses of the SSU and LSU rRNA.

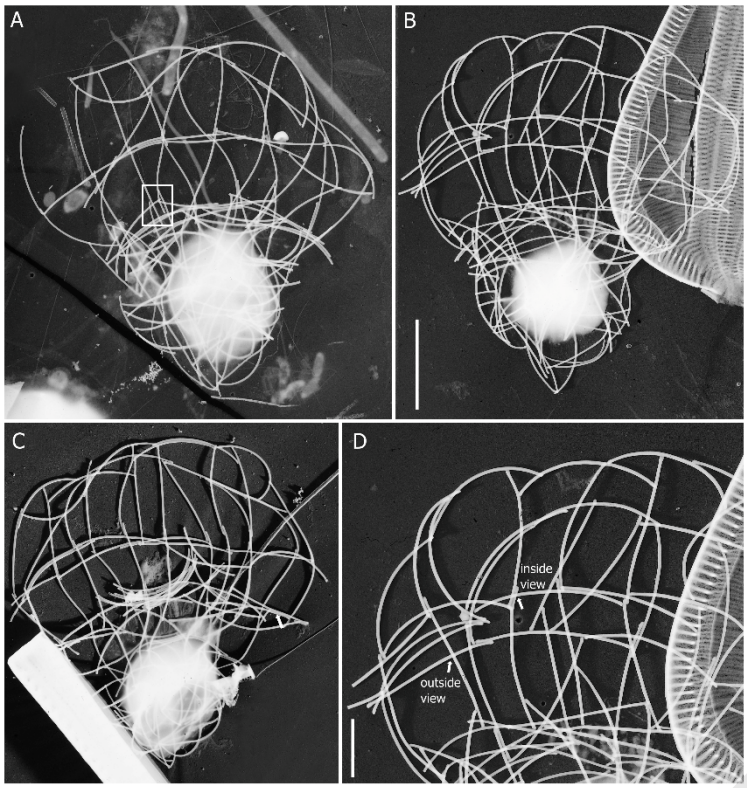
Table 4. Pairwise distances in % of *Pseudostephanoeca paucicostata* (strain HFCC 1361) and *Stephanoeca ellisfiordensis* (= *S. paucicostata* Nitsche et al. 2011) to closest molecular relatives based on SSU rRNA.

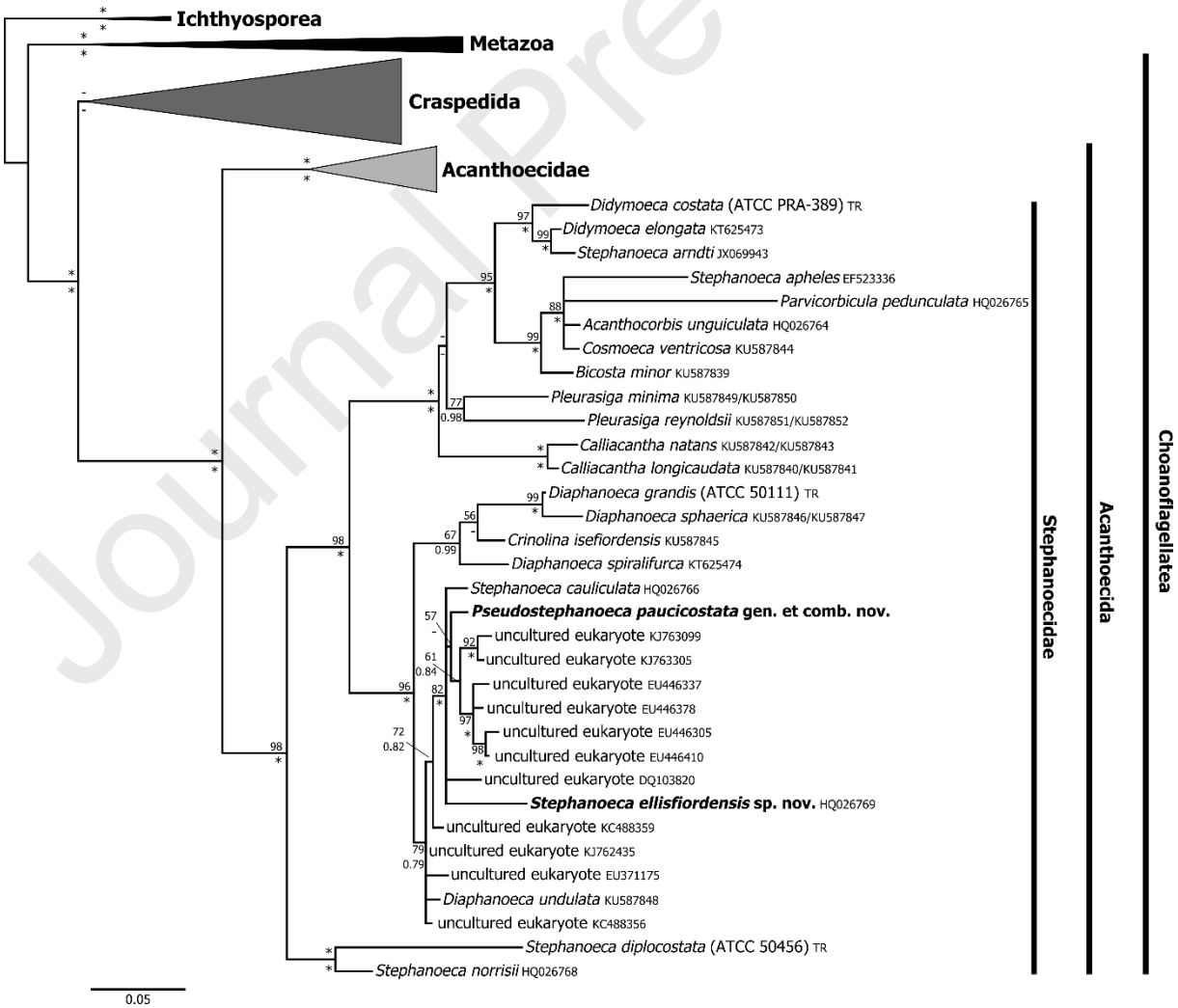












	Pseudostephanoeca paucicostata	*Stephanoeca ellisfiordensis*
Norwegian coastal waters, inner Oslofjorden Throndsen 1969	loc. cit. Fig. 14[1]	
Isefjord Denmark Thomsen 1973	loc. cit. Fig. 21[1]	
Norwegian coastal waters, southern Norway Throndsen 1974	loc. cit. Fig. 44[1]	
Tvärminne area, SW coast of Finland Thomsen 1979	loc. cit. Fig. 38[1]	
Disko Bay, West Greenland Thomsen 1982	loc. cit. Fig. 94[1]	
Port Hacking Sydney, Australia Hallegraeff 1983	loc. cit. Fig. 30[1]	
Ellis Fjord, Antarctica Marchant et al. 1987		loc. cit. Figs 9, 10[1]
California, USA Thomsen et al. 1991	loc. cit. Fig. 52[1]	
Tvärminne area, Gulf of Finland, Baltic Sea Vørs 1992	loc. cit. Fig. 15g[1]	

Coastal waters of Taiwan and Japan **Hara et al. 1997**			loc. cit. Fig. 29[1]			
Shark Bay, Western Australia **Tong 1997**			loc. cit. Fig. 5m[1]			
Port Jackson, New South Wales, Australia **Tong et al. 1998**			loc. cit. Fig. 3e[2]			
Darwin, Northern Territory, Australia **Lee et al. 2003**			loc. cit. Fig. 2j[2]			
Rhode Island, East coast USA **Menezes 2005**			loc. cit. Fig. XIId, e[2]			
Mediterranean Sea, Pula, Italy **Nitsche et al. 2011**					loc. cit. Fig. 8[2]	
West Australia **Thomsen and Østergaard 2019**			loc. cit. Fig. 7h-j[1]			

Species	**Sampling station**	**Sampling time**	**Coordinates**	**Salinity**	**Temperature [°C]**	**Figure**
P. paucicostata	Baltic Sea	08/1979	59°11’00’N 21°45’00’’E 60°11’05’’N 19°09’00’’E 63°18’05’’N 20°18’00’’E	3.3-7.6 PSU	1.4-16.5	2 I

			64°14’00’’N 22°21’00’’E 64°47’05’’N 23°28’8’’E			
P. paucicostata	Disko Bay, Greenland	1990	69°12’30’’N 53°30’00’’W	-	-	2 E
P. paucicostata	Dybsoe Fjord	03/1977	55°08’25’’N 11°46’37’’E	brackish	-	2 H
P. paucicostata	Kattegat	10/1981	56°51’00’’N 10°48’00’’E	-	-	2 G
P. paucicostata	Isefjord, Tempelkrogen	1973-1983	55°40’45’’N 11°48’50’’E	-	-	2 F
P. paucicostata	Isefjord, outer broad	1973-1983	55°51’45’’N 11°49’00’’E	-	-	-
P. paucicostata	Taarbaek Rev, Øresund	01/1976	55°47’15’’N 12°37’50’’E	13.7 PSU	3.2	2 J, K
P. paucicostata	Freiston Shore, Lincolnshire, England	08/1972	52°57’28’’N 00°05’32’’E	36.2 ppt	21.5	2 D
P. paucicostata	Concarneau, Brittany, France	08/1976	47°54’4.8’’N 03°55’2.6’’W	-	17.1	2 B, C
P. paucicostata **strain HFCC 1361**	Kos Island, Greece	09/2019	36°53’42.8’’N 27°17’36.7’’E	40 PSU	ca. 25	3
P. quasicupula	Ólafsvik, Iceland	06/1995	64°54’09’’N 23°42’11’’W	36 PSU	7	4 B-E, G-K
S. ellisfiordensis	Antarctica	04/1992	70°30’06’’S 07°59’00’’W	-	-	5

Primer	**Sequence 5‘-3‘**	**Reference**
18S-42F	CTC AAR GAY TAA GCC ATG CA	López-García et al. 2003
18S-nested-rev	ACC TAC GGA AAC CTT GTT ACG	Wylezich et al. 2002
NLF184/21	ACC CGC TGA AYT TAA GCA TAT	van der Auwera et al. 1994
NLR2098/24	AGC CAA TCC TTW TCC CGA AGT TAC	van der Auwera et al. 1994

%	Strain	1	2	3	4	5	6	7	8	9	10
1	***P. paucicostata***	X									
2	***S. ellisfiordensis***	1.7	X								
3	***S. cauliculata***	2.3	3.7	X							
4	**KJ763099**	2.9	4.2	2.9	X						
5	**KJ763305**	2.8	4.3	3.0	0.5	X					
6	**EU446337**	2.9	4.1	3.1	3.1	3.0	X				
7	**EU446378**	2.7	3.9	3.0	3.0	2.8	0.8	X			
8	**EU446305**	2.9	3.8	2.9	3.3	3.2	1.2	0.8	X		
9	**EU446410**	2.7	3.8	2.9	3.1	3.0	0.9	0.7	0.3	X	
10	**DQ103820**	2.6	3.8	2.6	3.0	3.0	3.4	3.2	3.4	3.2	X