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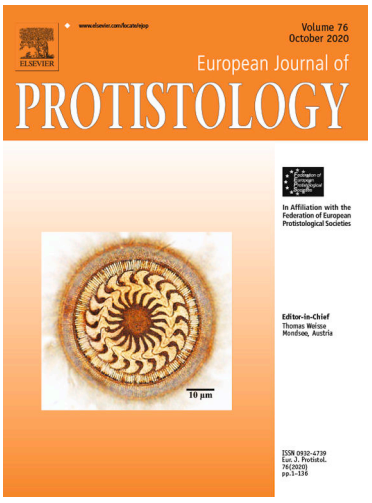

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Loricate choanoflagellates (Acanthoecida) from warm water seas. IX. *Coronoeca* gen. nov., *Polyfibula* Manton and spiny forms of *Parvicorbicula* Deflandre

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Abstract

The ambition to generate an overview of warm water loricate choanoflagellate biodiversity, based on a classic morphometric approach, is here completed by analyses of a range of tiny forms with anterior spines or projections and in most cases also a posterior pedicel. The warm water study complements previously obtained results from the more extensively studied temperate and polar regions of the world's oceans. It thus contributes to a significantly more balanced approach to global diversity patterns for these organisms. The current survey includes taxa such as *Polyfibula elatensis*, *Parvicorbicula pedicellata*, as well as a range of primarily undescribed and taxonomically challenging species, that are in an interim approach allocated to *Coronoeca* gen. nov. (*C. kosmaniae* sp. nov., *C. conicella* sp. nov., *C. superpositus* (Booth) comb. nov., *C. marchantii* sp. nov., *C. tongiae* sp. nov., and *C. patongiensis* sp. nov.). The analysis of warm water acanthoecid biodiversity has revealed in total 80 species from the six geographic regions sampled, corresponding to approximately 50% of all loricate species described. Nineteen species are

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previously undescribed forms. The Andaman Sea, Thailand, and West Australia are in a global context the most species-rich regions with 62 and 64 species respectively.

Keywords: Acanthoecida; Biogeography; Loricate choanoflagellates; Taxonomy; Ultrastructure

Introduction

In an ongoing effort (Thomsen and Østergaard 2019a-e, 2021; Thomsen et al. 2020a, b) to provide a first comprehensive overview of warm water loricate choanoflagellate diversity, based on a traditional microscopical approach, we here primarily deal with a contingent of small, spiny and often pedicellated species. While a single species (i.e. *Polyfibula elatensis* (Thomsen, 1978) Manton, 1981), belongs to an unambiguously defined genus, the remaining taxa have unresolved phylogenetic positions within the loricate choanoflagellate species matrix. It is in our opinion beneficial to future research, to be able to refer to a morphospecies by using a specific taxon name, rather than an ad hoc labelling of forms that will not to the same extent, systematically and rigorously, serve as a reference point for future observations of the specific taxon. We have therefore created the genus *Coronoeca* gen. nov., that can serve as an interim 'parking space' for these forms, while awaiting an in-depth analysis aided by state-of-the-art molecular tools. The generic type-species, *C. kosmaniae* sp. nov., has unique morphological features, that irrespective of the future fate of other species currently allocated to the genus (i.e. *C. conicella* sp. nov., *C. superpositus* (Booth, 1990) comb. nov., *C. marchantii* sp. nov., *C. tongiae* sp. nov., and *C. patongiensis* sp. nov.) will likely remain a phylogenetically valid unit. *Parvicorbicula superpositus* Booth, 1990, is transferred to *Coronoeca* (*C. superpositus* (Booth, 1990) comb. nov.) based on critical discrepancies, between this species and a core group species of *Parvicorbicula* as defined

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54 by Leadbeater (2015), relating to in particular mutual positions of transverse and longitudinal
55 costae.

56 The current paper rounds off the survey of warm water loricate choanoflagellate diversity with
57 a brief global perspective of the results obtained, and an inter-comparison of the geographic
58 regions sampled.

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Material and Methods

The material that constitutes the background for this and a series of papers on warm water acanthoecid choanoflagellates was collected over a period of 35 years. The geographic origin of samples is recorded in Fig. 1. See Thomsen and Østergaard (2019a) for information on each of the collection sites and sampling campaigns. In order to substantiate the analysis of *Coronoeca superpositus* (= *Parvicorbicula superpositus*) we have added material (Fig. 12) from Danish coastal waters. In order to further corroborate observations from our standard regions (Thomsen and Østergaard 2019a) we have appended to this paper wherever relevant, also historical data from Japan (Seto Inland Sea) to species descriptions, as well as the global analysis of loricate choanoflagellate biogeography (Figs. 16, 17).

The general protocol for processing water samples for light (LM) and (TEM/SEM) electron microscopy was according to Moestrup and Thomsen (1980) and Thomsen (1982). For details on sample processing, preparational issues and microscopes used see Thomsen and Østergaard (2019a). The Japanese material from the Shioya Coast (Seto Inland Sea, Kobe, Japan) comprises surface water samples collected from April 1979 to September 1982 (7.5-27.3°C; 27-33 PSU). The processing of the Japanese samples for electron microscopy followed the basic principles outlined by Moestrup and Thomsen (1980).

The material examined here is dried, which means that the natural 3-D structures have collapsed to become 2-D structures, leading to a partial dislocation of costal strips, and an artefactual expansion of in particular the lorica width. While several structures can still be measured with confidence, e.g. lorica height and the length of spines and pedicels, it does imply that certain values such as lorica diameter, typically at the level of the transverse costa(e), cannot

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be measured directly but only calculated from measurements of the circumference. This approach was used in the species descriptions below.

Efforts were made when describing lorica features to follow the terminology standards that have developed in the course of dealing with these organisms; see e.g. Leadbeater (2015; loc. cit. chapter 4 and glossary p. 278) and Thomsen and Buck (1991).

The unfortunate existence of an electron microscope specific problem causing negatives to appear horizontally flipped, was discussed in Thomsen and Østergaard (2019d). The evidence in favour of dealing with this problem as a purely technical issue is overwhelming. Scans of the affected negatives have accordingly been flipped horizontally to produce non-inverted images. In the current publication this applies to: Fig. 2a; Fig. 3c; Fig. 5d-f; Fig. 6a; Fig. 8a, b; Fig. 14a, b; Fig. 15a, b, g.

The shade matrix (Fig. 17) is constructed using the PRIMER software package (ver. 7) that has a built-in wizard that carries out the sequences of routines needed to perform the analysis (Clarke et al. 2014; Clarke and Gorley 2015).

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Results and Discussion

Polyfibula Manton in Manton and Bremer, 1981

Species of *Polyfibula* are characterized by small, barrel-shaped loricae that comprise 6-8 longitudinal costae and three transverse costae. The transverse costae are all located inside the longitudinal costae. The individual species may have a posterior pedicel and short to medium-sized spines anteriorly. Most characteristically, the transverse costal strips that form the anterior and intermediate costal rings, are all furnished with centrally placed enlargements (facets) that anteriorly secures the fusion (T-joins) of longitudinal and transverse costal strips. A single costal strip from the posterior transverse costa may also be furnished with a median central facet (Fig. 2a; arrow). The protoplast is located posteriorly in the lorica. The genus is characterized by tectiform division (Fig. 2J; see also Manton and Bremer 1981; loc. cit. Figs. 2, 18, 31).

The genus currently comprises five formally described species (i.e. *P. sphyrelata* (Thomsen, 1973) Manton, 1981; *P. elatensis* (Thomsen, 1978) Manton, 1981; *P. caudata* (Leadbeater, 1975) Manton, 1981; *P. hexacostata* Manton, 1981, and *P. stipitata* Manton, 1981). A comprehensive description of the genus is provided by Manton and Bremer (1981). Tong (1997a) advocated that *P. caudata*, *P. hexacostata* and *P. stipitata* should be relegated to synonyms of *P. sphyrelata*. The distinguishing lorica features are merely the number of longitudinal costae, and the presence or absence of a pedicel. Such lorica features are generally accepted to show some level of variability at the intra-specific level (*P. elatensis* (see below) typically has seven longitudinal costae, however occasionally eight; *P. sphyrelata* usually has seven longitudinal costae (Thomsen 1973) but is also found with eight longitudinal costae (Thomsen et al. 1991)). It is thus likely that the species circumscriptions provided by Manton and Bremer (1981) are too narrow.

While three morphotypes (i.e. *P. caudata*, *P. hexacostata*, *P. stipitata*) are sparingly recorded and almost exclusively from North Pacific and North Atlantic sub-arctic seas (Manton and Bremer 1981), the generic type species *P. sphyrelata* is more widely reported from in particular mid-latitude locations on both hemispheres (Thomsen 1973; Moestrup 1979; Hallegraeff 1983; Thomsen et al. 1991; Tong 1997a; Tong et al. 1998; Bérard-Therriault et al. 1999; Menezes 2005). The species also extends into brackish waters (Thomsen 1979; Ikävalko and Thomsen 1997), Antarctic circumpolar waters (Marchant 1985; Thomsen et al. 1990), and is further recorded from the Beagle Channel, Patagonia (Thomsen, unpublished).

P. elatensis (Thomsen, 1978) Manton in Manton and Bremer, 1981 (Fig. 2)

Polyfibula elatensis is unambiguously distinguished from other species of *Polyfibula* based on the presence of prominent anterior spines (Fig. 2a, d), each one-half costal strip in length. The material examined here (Fig. 2; Table 1) is morphometrically in good agreement with the type material from the Gulf of Aqaba (Thomsen 1978). While the facets on transverse costal strips stand out clearly in material from the Gulf of California (Fig. 2a, e), these structures are, however, in material from the Andaman Sea (Fig. 2d, f, g) often very little pronounced, and appearing as just a minor median bulging of the transverse costal strip (Fig. 2f, g; arrows). Such specimens are hard to distinguish from *Parvicorbicula pedicellata* (Fig. 3; see further below). The vast majority of specimens examined have seven longitudinal costae. However, a few cells with eight longitudinal costae were also registered (e.g. Fig. 2h). While the type material (Thomsen 1978) was characterized by a posterior pedicel comprising just a single costal strip, it is evident that specimens examined here may occasionally, and most likely depending on environmental nuances, carry a considerably longer pedicel (Fig. 2j, l) that is up to five costal strips long.

Polyfibula elatensis was frequently observed at all sites here examined (Table 2) with the exception of the East Mediterranean Sea. It is also known from Japanese coastal waters and has furthermore been recorded from the Gulf of Aqaba, Israel (Thomsen 1978), Galapagos and N. Alaska (Manton and Bremer 1981), California (Thomsen et al. 1991), Sydney, Australia (Tong et al. 1998), Pettaquamscutt River Estuary, Rhode Island, USA (Menezes 2005), Brazil (Bergesch et al. 2008), and also from Beagle Channel, Chile, and Friday Harbor, Puget Sound, USA (Thomsen, unpublished), and from New Zealand (Moestrup, unpublished).

Parvicorbicula Deflandre, 1960

Non-spiny warm water forms of *Parvicorbicula* were commented on in Thomsen et al. (2020a), and overall concerns with reference to a proper circumscription of the genus discussed at length. The generic type species (*P. socialis* (Meunier, 1910) Deflandre, 1960) forms, together with a small group of species of *Parvicorbicula* and *Pleurasiga* Schiller, 1925, i.e. *Pa. quadricostata* Throndsen, 1970; *Pa. circularis* Thomsen, 1976; *Pa. corynocostata* Thomsen, Garrison and Kosman, 1997; *Pl. minima* Throndsen, 1970; *Pl. reynoldsii* Throndsen, 1970, *Pl. tricaudata* Booth, 1990, what appears to constitute a phylogenetically coherent cluster of species, that in the future should be singled out as a separate genus. A feature that characterizes this group of species is first and foremost the fact that they have both the anterior and the mid-lorica transverse costae on the outer surface of the lorica. Additional shared morphological features are longitudinal costae (4-10) composed of three costal strips, two transverse costae separated by a single longitudinal costal strip, anterior T-joins between longitudinal and transverse costal strips, a flattened and slightly biforked termination of the tip of the anterior longitudinal costal strips, and the presence of a membrane, suspended from the free posterior tips of the middle layer of costal strips, and enveloping the

posteriorly positioned protoplast. None of the species have anterior spines or projections, and a pedicel is only reported from *Pl. tricaudata*.

The genus *Parvicorbicula* additionally comprises eight morphologically diverse species, including *P. pedicellata* (see below), that in different ways do not conform to the morphological circumscription of the core group of taxa referred to above. Because of uncertainties with respect to selecting, within the loricate choanoflagellate species matrix at large, more appropriate taxonomic positions for these outlier species, Thomsen et al. (2020a) opted for maintaining status quo while awaiting in particular molecular data to support the decisions needed.

P. pedicellata Leadbeater, 1973 (Fig. 3)

There is overall good agreement between the *P. pedicellata* material examined here (Fig. 3; Table 1) and the type material from Bay of Kotor, Montenegro (Leadbeater 1973). The only noticeable difference is with respect to the number of longitudinal costae. Leadbeater (1973) reports that there are 6-9 longitudinal costae, whereas in our material there is consistently seven longitudinal costae. In *P. pedicellata* (Table 3) the anterior transverse costa is located inside the longitudinal costae (Fig. 3d) while the mid-lorica transverse costa (Fig. 3e) is exterior. The posterior transverse costa is interior.

The only *P. pedicellata* morphological feature that ties-up this species to the *Parvicorbicula* core group of species as circumscribed above, is the fact the mid-lorica transverse costa is located outside the longitudinal costae. Features that separate *P. pedicellata* from *Parvicorbicula* sensu stricto are the presence of three transverse costae and anterior spines, and also the fact that each longitudinal costa consists of four costal strips.

190 *Parvicorbicula pedicellata* shares features with *Coronoeca* spp. (see below), e.g. anterior spines
191 and four costal strips in each longitudinal costa, but is still clearly differentiated because of the
192 external position of the mid-lorica transverse costa. While awaiting supporting molecular evidence
193 it appears most relevant to make no taxonomic changes with reference to *P. pedicellata*.
194 *Parvicorbicula pedicellata* is easily mistaken for *Polyfibula elatensis* (Fig. 2). Decisive differences
195 between these taxa, that can only be resolved using electron microscopy, are the presence in *Pol.*
196 *elatensis* of facets in the middle of transverse costal strips, and the fact that all transverse costae
197 are located inside the longitudinal costae in *Pol. elatensis* (Table 3). At the light microscopical level
198 the best distinguishing feature is the overall lorica size (*Pa. pedicellata* is 25% smaller) and the
199 shape of the lorica chamber, which is strictly conical in *Pa. pedicellata* (Fig. 3f-j) while anteriorly
200 more parallel sided in *Pol. elatensis* (Fig. 2b, c, h-m).
201 *Parvicorbicula pedicellata* is here recorded from the Andaman Sea, Thailand, West Australia,
202 the Equatorial Pacific Ocean, and the Gulf of California, Mexico (Table 2). The species is also
203 observed in Japanese coastal samples and has previously been reported from Bay of Kotor,
204 Montenegro, and Bay of Algiers (Leadbeater 1973), the Gulf of Aqaba, Israel (Thomsen 1978),
205 equatorial Pacific (Vørs et al. 1995), Sydney, Australia (Tong et al. 1998), and the Pettaquamscutt
206 River, Rhode Island, USA (Menezes 2005).
207

208 *Coronoeca* gen. nov.
209
210 Although the species dealt with below under the heading '*Coronoeca'* do share certain basic
211 morphological features, we are painfully aware of that a future in depth analyses of these taxa,
212 including also a multifaceted molecular probing, will undoubtedly unveil a need for a
213 redistribution of these within a number of separate genera. Realizing that none of these forms can

be smoothly and convincingly allocated to an existing genus, and further in an attempt to avoid establishing too many monotypic genera created on an uncertain knowledge basis, we here opt for creating a pro forma genus that can at least temporarily harbour these forms (Fig. 4). Choosing *C. kosmaniae* as the generic type species reflects the fact, that this species has a large range of unique morphological features, that makes it a good long-term candidate for genus survival.

Diagnosis: Lorica conical or barrel-shaped and comprising 4-10 longitudinal costae and 2-4 transverse costae. There are anterior projections or spines, and the lorica may have a compound pedicel, a simple pedicel or no pedicel. Each longitudinal costa comprises four costal strips (including anterior projection). Anterior junctions between longitudinal and transverse costal strips can be either T-junctions or E-junctions. All costal strips are narrow rods. Minor morphological modifications can apply to anterior projections or costal strips from the pedicel. The longitudinal costae are external relative to the mid-lorica transverse costa. Tectiform division presumed but not yet verified in the type species.

Generic type species: *Coronoeca kosmaniae* sp. nov.

Etymology: Genus name derived from ‘corona’ (Latin) meaning ‘crown’ and referring to the prominent anterior spines or projections, and ‘oicos’ (Greek) meaning ‘house’.

Coronoeca kosmaniae sp. nov. (Figs. 4a, 5, 6)

Diagnosis: Lorica barrel- or bell-shaped (lorica height: 25.5 ±1.2 µm incl. anterior projections and excl. posterior pedicel), constructed of rod-shaped costal strips, and with modified attenuating anterior projections (seven) that have a proximal swelling ca. 0.2 µm from the posterior tip (Fig. 5e, f). Anterior transverse costal strips attach to longitudinal costae at the level of the swellings

238 creating T-junctions. The lorica chamber is composed of seven longitudinal costae and two
239 transverse costae (Fig. 5a-d). The anterior transverse costa rests in a fork created by the
240 overlapping longitudinal costal elements (Fig. 5e, f) and is exterior relative to the tip of the
241 penultimate longitudinal costal strip, but interiorly located relative to the anterior projection. The
242 mid-lorica transverse costa is inside the longitudinal costae. A longitudinal costa is composed of
243 four costal strips (including the anterior projections). Longitudinal costae converge posteriorly,
244 with the posteriormost longitudinal costal strips being noticeably curved and sometimes
245 duplicated. Transverse costae each comprising seven costal strips, and of approximately the same
246 size (anterior ring: 15.5 ±0.6 µm / mid-lorica ring: 15.2 ±0.7 µm). Lorica with a compound pedicel,
247 usually with 1-3 diverging costal strips. Both anterior and posterior projections tapering, and ca.
248 1.0 µm longer than all other costal strips (7.2 ±0.6 µm versus 6.2 ±0.6 µm). The protoplast is
249 located at the posterior end of the lorica chamber, within a suspensory membrane (Figs. 5c, d; 6a,
250 d). Tectiform division presumed but not verified from an accumulation of newly formed costal
251 strips in the collar region.
252 **Holotype**: The specimen illustrated in Fig. 5d of the present work is fixed as holotype (ICZN 1999,
253 Article 73.1.4).
254 **Type locality**: Surface water sample (ca. 18°C / 35 PSU) from a near coastal site at Bahia de los
255 Ángeles, Gulf of California, Mexico, collected 7 January 1990.
256 **Etymology**: The species-group name is chosen to acknowledge significant contributions to the
257 microscopical examination of the Gulf of California material made by Carol Kosman. Carol was
258 during the period 1991-1992 working at the Univ. of Copenhagen as a Fulbright Research Scholar.
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The spacious *C. kosmaniae* lorica has an uncomplicated and fairly standard lorica construction, but is nevertheless uniquely defined, in particular because of the costal strip swellings (Fig. 5e, f) at the base of the anterior projections, and at a point where the anterior transverse costa crosses. These localized swellings are reminiscent of the median facets on *Polyfibula* transverse costal strips (Manton and Bremer 1981; see also Fig. 2e) and also the less confined swellings in the posterior one third of anterior longitudinal costal strips in *Saroeca attenuata* (Thomsen 1979).

There is in *C. kosmaniae* a variable degree of costal strip duplication at the posterior lorica end. The TEM image (Fig. 6a) thus shows a lorica in which all posterior longitudinal costal strips are duplicated (or even triplicated; Fig. 6a arrows) except for one costal strip (Fig. 6a; arrowhead). Duplications are also easily detected from LM (Fig. 6b). The anterior projections and costal strips from the compound pedicel are basically similar and deviate from mid-lorica longitudinal costal strips by being larger and attenuating in width from one end to the other (Fig. 5d). This precludes both the possibility that the posterior lorica end costal strip duplication could in part be explained by a displacement of costal strip elements from the compound pedicel, and likewise that the compound pedicel could be interpreted as a simple displacement of costal strips from the posterior lorica end duplications. A compound pedicel is a feature that is otherwise shared among several species, e.g. *Didymoeca tricyclica* (Bergesh et al. 2008), *Pleurasiga tricaudata* (Booth 1990; Thomsen et al. 2020a), *Thomsenella cercophora* (Thomsen and Boonruang 1983; Thomsen and Østergaard 2019e), and *Syndetophyllum pulchellum* (Thomsen and Moestrup 1983; Thomsen et al. 2020b).

The costal strip pattern at the level of the anterior transverse costa, where the transverse ring rests in a fork created by an overlap between neighbouring longitudinal costal strips (the ring is external relative to the penultimate longitudinal costal strip, however, overlaid externally by the

anterior projection), is basically similar to what is observed in species of *Diaphanoeca,* e.g. *Diaphanoeca grandis* (Leadbeater 2015; loc. cit. page 80 and Figs 4.29-4.33) and *Conion groenlandicum* (Thomsen 1982), and also mirrored in species discussed below (*C. conicella*, *C. superpositus*, and *C. marchantii*).

Coronoeca kosmaniae is superficially reminiscent of *Diaphanoeca throndsenii* (Thomsen and Østergaard 2021). However, in this species there is consistently eight longitudinal costae, anterior E-junctions between longitudinal and transverse costal elements, no swelling at the posterior end of a projection, a pedicel that comprises a single costal strip, and no indication of costal strip duplications at the posterior lorica end.

The combination of lorica features in *C. kosmaniae* as accounted for above calls for the description of a new genus.

Coronoeca kosmaniae was frequently observed in samples from the Gulf of California, Mexico, and has also been observed in samples from the Seto Inland Sea, Japan. The Japanese specimens are morphometrically exactly similar to the Mexican type material.

Coronoeca conicella sp. nov. (Figs. 4b, c, 7-9)

Diagnosis: Lorica funnel-shaped (lorica height: 16.4 ±2.5 µm incl. anterior projections and excl. posterior pedicel) and constructed of rod-shaped costal strips organized in 4-(5) longitudinal costae and two transverse costae. There are anterior attenuating projections and a pedicel of highly variable length. The anterior transverse costa (diam. 11.1 ±1.7 µm) comprises twice as many costal strips as there are longitudinal costae (T-junctions). The mid-lorica transverse costa (diam. 6.8 ±1.4 µm) comprises (4)-5-(6) costal strips. The anterior transverse costa rests in a fork created by overlapping longitudinal costal elements (Fig. 8b). It is exterior relative to the tip of the

307 penultimate longitudinal costal strip, but interiorly located relative to the anterior projections (Fig.
308 7a, Fig. 8b). The mid-lorica transverse costa is inside the longitudinal costae. A longitudinal costa is
309 composed of four costal strips (including the anterior projections). The protoplast is located at the
310 posterior end of the lorica chamber, within a suspensory membrane (Fig. 7a, b, j; Fig. 8f, h).
311 Tectiform division evidenced from an accumulation of newly formed costal strips in the collar
312 region (Fig. 9b).
313 **Holotype**: The specimen illustrated in Fig. 7a of the present work is fixed as holotype (ICZN 1999,
314 Article 73.1.4).
315 **Type locality**: Surface water sample (ca. 28°C / 35 PSU / 2 meters depth) from a near coastal site
316 at Ao Patong, Phuket Island, Thailand (7° 53'56'' N / 98° 17'36'' E), collected 8 September 1981.
317 **Etymology**: The species-group name refers to the perfect conical shape of the lorica; -ella =
318 diminutive.
319
320 Ignoring the anterior projections and the pedicel, *C. conicella* does superficially appear similar
321 to e.g. *Parvicorbicula circularis* (Thomsen 1976). Shared features include four longitudinal costae
322 each comprising three costal strips, and two transverse costae where the anterior costa has eight
323 costal strips with every second strip forming T-junctions with the longitudinal costae, while the
324 posterior transverse costa is formed by 5-6 costal strips. The decisive factor when considering a
325 possible phylogenetic linkage between the two species is, however, that while the mid-lorica
326 transverse costa is located outside the longitudinal costae in *P. circularis* it is inside in *C. conicella*
327 (Table 3). This is a fundamental difference that is intimately linked to processes being part of the
328 lorica assembly following cell division. Despite a superficial resemblance, *P. circularis* and *C.*
329 *conicella* must therefore be kept apart at the supra-generic level.

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In *C. conicella* the anterior transverse costa is external relative to the tip of the penultimate longitudinal costal strip, however, overlaid by the anterior projections (Figs 7b, 8a, b). A similar set up is observed in *C. kosmaniae* (see above) and *C. superpositus* (see below).

Coronoeca conicella is, as defined here, a highly variable taxon, and has accordingly been lavishly illustrated from various geographic regions. We have at all sites examined observed two morphotypes of the species, i.e. forms with a short pedicel comprising just a single costal strip (e.g. Fig. 7c, k; 8j), and others that have an extended pedicel comprising multiple costal strips (e.g. Fig. 7d, g, j; 8f). There is an obvious linear linkage (Fig. 10A; r^2= 0.77) between lorica height and length of pedicel in the material examined (> 60 specimens), as well as a non-patchy distribution, indicating that this element of variability is likely an inherent characteristic of the species. It can be further noticed from Fig. 10A that there are no obvious regional differences, although specimens from the Gulf of California are much less variable in size than e.g. specimens from West Australia.

Somewhat surprisingly it has been noticed that approximately 10% of all loricae examined have five rather than four longitudinal costae (Fig. 7g; 8a, f). The Gulf of California (Mexico) has a marked overrepresentation of such forms with five longitudinal costae (Fig. 10B). In material from Japanese coastal sites the only morphotype observed has five longitudinal costae (Fig. 9a, 10B).

All costal strips are narrow rods and quasi-identical throughout the lorica. However, the anterior projections do differ in being anteriorly attenuating and pointed and markedly thickened posteriorly (Fig. 8b). The maximum diameter of the rod is at the crossing of the transverse costa. Costal strips from extended pedicels generally appear somewhat more compact than costal strips elsewhere.

Coronoeca conicella was observed at all sites sampled (Table 2) and the form with an extended pedicel also occurred in Japanese coastal samples. The species has previously been observed

(specimens with five longitudinal costae) in samples from the Galapagos Islands, Peru (I. Manton (†), pers. com.) and Sydney, Australia (Tong et al. 1998, loc. cit. Fig. 2l; referred to as *Parvicorbicula superpositus*).

Coronoeca superpositus (Booth, 1990) comb. nov. (Figs. 4d, e, 11, 12)
Basionym: *Parvicorbicula superpositus* Booth, 1990

When first described (Booth 1990) this species was primarily discussed with reference to *Parvicorbicula socialis* (*Meunier*, 1910) Deflandre, 1960, with which it does share a striking resemblance (e.g. ten longitudinal costae, two transverse costae separated by a single longitudinal costal strip, anterior T-junctions), when ignoring the *C. superpositus* anterior projections and pedicel. However, a close inspection of the material from Danish coastal waters that have been included here to consolidate the *C. superpositus* species circumscription, clearly unveils that the mid lorica transverse costa is located inside the longitudinal costae (Table 3), while it is clearly exterior in *Parvicorbicula socialis* (see e.g. Buck 1981, loc. cit. Figs. 9, 11). This is perhaps even more evident when consulting the excellent SEM images of *C. superpositus* published by Bérard-Therriault et al. 1999; loc. cit. Pl. 142a, b). An exterior versus interior placement of the mid-lorica transverse costa is, as previously emphasized, a fundamental distinguishing characteristic, which is coupled to intrinsic processes of lorica assembly, and hence being a feature only applicable at the supra-generic level. The anterior transverse costa is exterior relative to the tips of the longitudinal costal strips from the tier below, however externally overlaid by the anterior projections (Fig. 11a, 12c). The costal strip configuration at the level of the anterior transverse costa is thus similar to what is observed in both *C. kosmaniae* and *C. conicella*. The actual morphological elaboration of the anterior projection is also identical in the species *C. kosmaniae*, *C. conicella* and *C.*

superpositus. The projection is anteriorly sharply attenuated while bulging posteriorly (Fig. 12c). The maximum width is attained at exactly the level of the anterior transverse costa.

Specimens resembling *C. superpositus* (Fig. 11) are only sparingly observed in our warm water samples (Andaman Sea, Thailand). While the mid-lorica transverse costa is also in these specimens internal relative to the longitudinal costae, they deviate from *C. superpositus* sensu stricto by having fewer longitudinal costae (eight) and by the fact that all and not just a sub-set of longitudinal costae are here terminated by anterior projections (Fig. 11a, b). For the time being the variability encountered in Thailand specimens, when compared with *C. superpositus* sensu stricto (Fig. 12), is considered to represent intraspecific variability. The Thailand morphotype of *Coronoeca superpositus* was sparingly observed also in samples from Japanese coastal waters.

Coronoeca superpositus sensu stricto has previously been reported from the subarctic North Pacific Ocean (Booth 1990; type locality), Kaikoura, New Zealand (Moestrup 1979, loc. cit. Fig. 46; referred to as *Parvicorbicula* sp.), Kilsfjorden, Norway (Espeland and Throndsen 1986, loc. cit. Fig. 36; referred to as *Parvicorbicula socialis* aff.), California, USA (Thomsen et al. 1991), Southampton, UK (Tong 1997a), Taiwan (Hara et al. 1997), Sydney, Australia (Tong et al. 1998; only loc. cit. Fig. 2k), St. Lawrence Estuary, Canada (Bérard-Therriault et al. 1999), and Danish coastal waters (Thomsen et al. 2016). Soto-Liebe et al. (2007) reports *C. superpositus* from the Chilean coast. However, the resolution of the image provided (loc. cit. Fig. 1d) does not allow for a positive identification. The overall picture of the distribution pattern is thus that *C. superpositus* sensu stricto has a preference for the temperate climate zone avoiding high and low latitude habitats.

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Coronoeca marchantii sp. nov. (Figs. 4h, 13)

Diagnosis: Lorica funnel-shaped (lorica height: 14.2 ±1.4 µm incl. anterior projections and excl. posterior pedicel) and constructed of rod-shaped costal strips organized in (7)-8 longitudinal costae and three transverse costae separated by single tiers of longitudinal costal strips. There are anterior attenuating projections and a pedicel comprising a single costal strip. A longitudinal costa is composed of four costal strips (including the anterior projections). The anterior transverse costa (diam. 8.9 ±0.8 µm) comprises as many costal strips as there are longitudinal costae (E-junctions). The mid-lorica transverse costa (diam. 6.6 ±0.5 µm) has the same number of costal strips with much larger overlap between neighbouring strips. The posterior transverse costa (diam. 2.9 ±0.4 µm) comprises 4-5 costal strips. All transverse costae are internal relative to the longitudinal costae. The anterior transverse costa rests in a fork created by overlapping longitudinal costal elements. It is exterior relative to the tip of the penultimate longitudinal costal strip, but interiorly located relative to the anterior projections (Fig. 13a). The protoplast is located at the posterior end of the lorica chamber, within a suspensory membrane (Fig. 13a). Tectiform division evidenced from the accumulation of costal strips in the collar region (Fig. 13e, h).

Holotype: The specimen illustrated in Fig. 13a of the present work is fixed as holotype (ICZN 1999, Article 73.1.4).

Type locality: Surface water sample (ca. 28°C / 35 PSU / 2 metres depth) from a near coastal site at Ao Patong, Phuket Island, Thailand (7° 53’56’’ N / 98° 17’36’’ E), collected 8 September 1981.

Etymology: The species-group name acknowledges the great contributions that Dr. Harvey Marchant (Australian Antarctic Division, Hobart) has made to loricate choanoflagellate research.

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There is little to add to the formal description of *C. marchantii* which has a well-defined costal strip configuration of its tiny conical lorica. In most cases this species can be unambiguously identified also from LM of dried specimens (e.g. Fig. 13c-e, j, k). A few slightly deviant forms were encountered in samples from West Australia and the Sargasso Sea (Fig. 13f-i). While these specimens obviously have eight longitudinal costae and three transverse costae, the difference noted is a slight pushing forward of the anterior transverse costa. Arrows (Fig. 13f-i) indicate where to find the transition between the anterior projections and the first tier of longitudinal costal strips. The overall appearance of these forms is reminiscent of *Parvicorbicula pedicellata* (Fig. 3). However, the pattern of crossing between longitudinal costae and costal strips from the anterior transverse costa (T- versus E-junctions) and the mere number of longitudinal costae (seven versus eight) adequately separates the two forms. For now, it thus appears relevant to treat this small deviation in lorica morphology as merely *C. marchantii* intraspecific variability. However, the validity of this approach will need to be tested using molecular tools.

Coronoeca marchantii was observed at all sites sampled with the exception of the eastern Mediterranean Sea (Table 2). It is additionally observed from the Galapagos Islands, Peru (I. Manton (†), pers. comm.).

Coronoeca tongiae sp. nov. (Figs. 4f, 14a-g)

Diagnosis: Lorica funnel-shaped (lorica height: 14.6 ±0.9 µm) and constructed of rod-shaped costal strips organized in 9-10 longitudinal costae and four transverse costae. There are anterior spines up to one half longitudinal costal strip in length. A longitudinal costa is composed of four costal strips. The mid-lorica and posterior transverse costae are separated by single tiers of longitudinal

450 costal strips. The three anterior transverse costae (diam. 12.0 ±1.1 µm, 9.9 ±0.5 µm, and 7.4 ±0.8
451 µm) comprise as many costal strips as there are longitudinal costae. While longitudinal costal
452 strips attach to the middle of costal strips from the anterior ring, transverse costal strips from the
453 mid-lorica costae span the width between neighbouring longitudinal costae. The reduction in
454 diameter is occasioned by increasing overlaps between neighbouring transverse costal strips. The
455 posterior transverse costa (diam. 3.1 ±0.5 µm) comprises 4-5 costal strips. All transverse costae
456 are internal relative to the longitudinal costae. The protoplast is located at the posterior end of
457 the lorica chamber, within a suspensory membrane. Tectiform division assumed but not yet
458 confirmed.
459 **Holotype**: The specimen illustrated in Fig. 14a of the present work is fixed as holotype (ICZN 1999,
460 Article 73.1.4).
461 **Type locality**: Water sample (ca. 28°C / 35 PSU / 45 metres depth) from a continental shelf station
462 (#31) in the Andaman Sea, Thailand (7° 24' N / 97° 27' E), collected March 1996.
463 **Etymology**: The species-group name acknowledges the great contributions that Dr. Susan M. Tong
464 has made to loricate choanoflagellate research.
465
466 This species was first observed by Leadbeater (1974; loc. cit. Pl. 2E / referred to as
467 *Parvicorbicula* sp.) in samples from the Mediterranean (Split, Croatia and the Bay of Algiers). The
468 single specimen illustrated is unmistakably morphometrically identical to the material examined
469 here (Table 1). The generic affiliation of this species was uncertain when first examined by
470 Leadbeater (1974), and it has remained so ever since. It therefore seems appropriate at this stage,
471 and while awaiting a molecular based unravelling of its phylogenetic connections, to at least
472 formally describe this form, and as an interim solution allocate it to the 'pro forma' genus

473 *Coronoeca* described here. The possibility initially indicated by Leadbeater (1974) of a certain
474 resemblance between this form and species of *Parvicorbicula* is no longer valid considering the
475 recent focus on regrouping selected species of *Pleurasiga* and core group members of
476 *Parvicorbicula* species that are closely affiliated with the *Parvicorbicula* type species *P. socialis*
477 (Leadbeater 2015, Thomsen et al. 2020a).

478 *Coronoeca tongiae* is here (Fig. 14a-g; Table 2) reported from the Andaman Sea, Thailand, West
479 Australia, the eastern Mediterranean Sea, Alexandria, and the Gulf of California, Mexico.

480
481 *Coronoeca patongiensis* sp. nov. (Figs. 4g, 14h, i)
482
483 **Diagnosis**: Lorica funnel-shaped (lorica height ca. 15 µm) and constructed of rod-shaped costal
484 strips organized in ten longitudinal costae and three transverse costae. There are anterior spines
485 corresponding in length to two thirds of an anterior longitudinal costal strip. A longitudinal costa is
486 composed of four costal strips. Both the anterior transverse costa (diam. 12.5 µm) and the mid-
487 lorica costa (diam. 8.0 µm) comprise as many costal strips as there are longitudinal costae, yet
488 with a significantly larger overlap between neighbouring strips in the mid-lorica transverse costa.
489 Transverse costal strips from both the anterior and the mid-lorica costa span the width between
490 neighbouring longitudinal costae. The posterior transverse costa (diam. ca. 3 µm) comprises 4-5
491 costal strips. The anterior transverse costa is external relative to the longitudinal costae while the
492 mid-lorica and the posterior transverse costae are internally located. The protoplast is located at
493 the posterior end of the lorica chamber. Tectiform division presumed but not yet evidenced from
494 the accumulation of costal strips in the collar region.
495 **Holotype**: The specimen illustrated in Fig. 14i of the present work is fixed as holotype (ICZN 1999,
496 Article 73.1.4).

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Type locality: Surface water sample (ca. 28°C / 35 PSU / 2 metres depth) from a near coastal site at Ao Patong, Phuket Island, Thailand (7° 53'56'' N / 98° 17'36'' E), collected 8 September 1981.

Etymology: The species-group name refers to the type locality Ao Patong, Phuket Island, Thailand.

While this species is superficially reminiscent of *C. tongiae* with reference to lorica shape, dimensions and costal strip configuration, there are, however, a number of distinguishing features, i.e. the pattern of junctions between transverse and longitudinal costal strips at the level of the anterior transverse costa (E-joins in *C. patongiensis* and T-joins in *C. tongiae*), and the placing of the anterior transverse costa (internal in *C. tongiae* and external in *C. patongiensis*). It is thus credible that the two species, once further studied using also molecular tools, cannot reside within the same genus. However, in an interim period it is convenient to be able to refer to a well-defined morphotype such as *C. patongiensis* using a formally established scientific name.

Coronoeca patongiensis has also been observed in samples from West Australia (Fig. 14h) and from the Sargasso Sea (Paul Davis; pers. com.).

Miscellaneous forms (Fig. 15)

Under this heading we have assembled material of loricate choanoflagellate taxa that have either been observed too sparingly to allow for a formal species description, or unintentionally left out while dealing with the relevant genus in a previous paper (*Stephanoeca urnula*).

Choano sp. 1 (Fig. 15a)

Lorica funnel shaped (lorica height: 10.1 µm) comprising eight longitudinal costae and two transverse costae (diam. 7.6 and 4.8 µm). The anterior tier of longitudinal costal strips project as

spines. Costal strips from the anterior ring span the width between neighbouring longitudinal costal strips. This taxon resembles *C. patongiensis* but differs in having fewer transverse and longitudinal costae. Only observed (single specimen) in a water sample from the equatorial Pacific Ocean.

Choano sp. 2 (Fig. 15b)

Lorica funnel shaped (lorica height: 14.8 µm) comprising ten longitudinal costae and two transverse costae (i.e. an anterior ring (diam. 12.0 µm) forming E-junctions with the longitudinal costae, and a posterior costa (diam. 4.6 µm) located at the joints between longitudinal costal strip number two and three counted from the anterior lorica end). This taxon, which is only observed once in a sample from the equatorial Pacific Ocean, resembles *Cosmoeca phuketensis* (see Thomsen and Østergaard 2019d) but differs because of a different number of longitudinal costae (there are consistently nine in *C. phuketensis*) and also by having a more complex posterior transverse costa.

Choano sp. 3 (Fig. 15g)

A single cell observed in a sample from the Andaman Sea, Thailand. The funnel-shaped lorica (lorica height: 19.5 µm) is constructed from eight longitudinal costae and two transverse costae. The anterior ring (diam. 12 µm) closes the lorica (E-junctions). The mid lorica transverse costa (diam. 9 µm) crosses posterior to the joints between the first and second longitudinal costal strips (counted from the anterior lorica end). Notice that the longitudinal costal strips are exceptionally long (6-7 µm). The lorica chamber is pedicellate and posteriorly terminated by a basket-like configuration of obliquely oriented costal strips.

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Stephanoeca urnula Thomsen, 1973 (Fig. 15c, d, h)

This species was observed repeatedly in samples from the Eastern Mediterranean Sea outside Alexandria, Egypt. It is morphometrically (lorica height: 11 µm; diam. 3.7 µm (anterior opening), 7.2 µm (max. lorica width), and 4.5 µm (lorica waist)) in overall good agreement with the Danish type material (Thomsen 1973), and also material sampled from the innermost parts of the Baltic Sea (Thomsen, 1979, Vørs 1992, Ikävalko and Thomsen 1997, Ikävalko 1998). *Stephanoeca urnula* has additionally been recorded from Lake Saroma, Japan (Takahashi 1981) and the South Atlantic coastal zone, Brazil (Bergesh et al. 2008; 14-15 PSU). The majority of samples yielding *S. urnula* are from low-saline regions such as the Baltic Sea. The occurrence of this form in an eastern Mediterranean high salinity environment is thus surprising. The exact habitat sampled is perhaps impacted by the river Nile freshwater outflow creating brackish water niches that may allow *S. urnula* to thrive, or is it rather that the Egyptian material, despite an overall similarity, does represent a separate taxon with markedly different habitat demands. It is in this connection relevant to refer to recently described nudiform *Stephanoeca*-like species (*Enibas tolerabilis* and *E. thessalia*; Schiwitza et al. 2019; Schiwitza and Nitsche 2021) that are morphologically reminiscent of *S. urnula* yet with a significantly different autecological signature. Based on laboratory tests is was found (Schiwitza et al. 2019) that *E. tolerabilis* is able to survive within a salinity range of 0-70 PSU.

Savillea sp. (Fig. 15e, f)

Two cells observed in samples from the Sargasso Sea. They differ from *Savillea parva*(Ellis, 1929)Loeblich III, 1967, by having a significantly more open-meshed pattern of spiralling and

longitudinal costae. The lorica is 13-15 µm high, and the diameter at the orifice 4-5 µm and in the mid-lorica region 8-9 µm. Similar material has previously been observed in Sargasso Sea samples (Paul. G. Davis, pers. com.; lorica height 14.2 µm, diam. at orifice 4.2 µm, max. diam. 7.3 µm).

Species diversity

The total number of species encountered in our warm water samples is 80 (Table 2). This represents close to 50% of all loricate choanoflagellate species described so far. There is a fairly obvious link between the material available to us, in terms of the mere number of water samples and LM/TEM preparations, and the total number of species listed from each individual site. The most thoroughly screened locations (West Australia and the Andaman Sea) are each represented by more than 60 species, whereas for the least intensively sampled regions (the Caribbean Sea, and the Eastern Mediterranean Sea) we have only listed approximately 30 species. For comparison it can be emphasized that similarly intensely sampled regions, i.e. Danish coastal waters and Disko Bay, West Greenland, are each represented by a maximum of 45 species. It thus appears that the tropical Indian Ocean sites sampled (the Andaman Sea and West Australia) do represent unique biodiversity hot spots.

To put the regions here analysed into a proper perspective, we have assembled biodiversity data worldwide (Fig. 16A, B) and conducted a matrix analysis (Fig. 17) in the shape of a two-way clustering of localities (35) versus species (presence-absence data; the diagram includes the 50 most decisive species).

It is immediately obvious (Fig. 17), judged from the overall diagonal patterning of the matrix, that specific subsets of samples largely defined by temperature are matched by fairly clear-cut subgroups of species. The warm water sites reported on here cluster closely together (ca. 60 %

597 similarity) with maximum similarity (ca. 90%) between the Andaman Sea and the West Australian
598 collections, and match up with a cluster of 9 species that have their maximum occurrences in such
599 warm water habitats (Fig. 17 D/H; i.e. *Campyloacantha imbricata, Campyloacantha spinifera,*
600 *Cosmoeca ceratophora, Cosmoeca subulata, Pleurasiga echinocostata, Polyfibula elatensis,*
601 *Stephanacantha dichotoma, Syndetophyllum pulchellum, Thomsenella infundibuliformis*). It is
602 similarly evident that there is a well-defined square within the matrix that defines associations
603 between both Arctic and Antarctic cold-water habitats and cold-water adapted species (Fig. 17
604 B/F; i.e. *Acanthocorbis unguiculata, Bicosta antennigera, B. spinifera, Calliacantha longicaudata, C.*
605 *natans, Diaphanoeca pedicellata, Parvicorbicula quadricostata, P. socialis*). Notice that there is a
606 North Atlantic cluster of sites (Baltic Sea, Denmark, Southampton (UK) and Rhode Island (USA))
607 that is overall very similar to the cold-water community, however, with a wider range of species
608 represented. The matrix segment marked 'C' (Fig. 17) comprises species that are truly
609 cosmopolitan forms (i.e. *Bicosta minor, Cosmoeca norvegica, C. ventricosa, Calliacantha simplex,*
610 *Diaphanoeca grandis, Parvicorbicula circularis, Pleurasiga minima*). The most divergent matrix
611 element (Fig. 17 A/E) is represented by Antarctic Weddell Sea icebiota samples which display a
612 wide range of forms not encountered elsewhere. Samples from South Brazil and the North Pacific
613 Central Gyre cluster separately very likely due to limitations in the number of taxa recorded from
614 these sites (Fig. 16B). The inevitable conclusion to be extracted from the kind of analysis
615 undertaken here, is that there are consistent and well-defined temperature dependent species
616 associations with clear distributional constrictions, as well as groups of cosmopolitan species that
617 have the potential to occur everywhere.
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Conclusions

While the genus *Polyfibula* is unambiguously defined based on overall lorica constructional details and especially the facets on transverse costal strips, it applies to all remaining species examined here, that the generic allocation is negotiable and likely to change once supplemental molecular data becomes available. In order to generally move loricate choanoflagellate taxonomy ahead, it is urgently required to seek supporting molecular evidence. A cultivation approach is one obvious way to go (see e.g. Nitsche and Arndt 2008; Schiwitza et al. 2019). However, it is likely that a vast range of species, and in particular those that prefer more oceanic habitats, will be very hard and time consuming to ever establish in culture. An alternative, or perhaps rather supplemental approach, is sequencing based on the extraction of single cells from a suspension of nanoflagellates concentrated from freshly collected water samples (see e.g. Nitsche et al. 2017; Schiwitza and Nitsche 2021). While this does work adequately when dealing with large forms, that can be recognized and unambiguously identified from mixed populations at low magnification in the inverted microscope (x40-60 objective), this approach will not suffice, when it comes to small loricate forms that share basic features in lorica design and overall construction. What is needed to move fast ahead, linking specific morphotypes with their unique sequences, is an approach that allows for an initial incontestable species identification, based on e.g. an examination of natural, wet and non-coated material in an environmental scanning electron microscope, and a subsequent extraction of the specific cell for sequencing. While the identification part and the position-fixing is fairly straightforward to accomplish, it is the subsequent manipulation (extraction) of the cell that is likely to be seriously challenging. No matter the approach taken it appears that an initial accessibility to flow-sorted cells, prepared as described by e.g. Kamennaya

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et al. (2018), might be essential in optimizing the success-rate in terms of securing quality approved data linking specific morphotypes to molecular profiles.

It is evident from Fig. 16A that the loricate choanoflagellate research community has by now achieved, based on classical light and electron microscopical descriptive techniques, a fairly comprehensive global coverage of species distributions. This has in turn contributed to e.g. a basic understanding of how temperature in particular impacts on large-scale species occurrence patterns. The approach that we have taken in the current series of papers on warm water loricate choanoflagellate biodiversity, is to reduce often complex regional sampling programmes (along multiple transects covering several depths) to a single species file. While this has proved adequate with reference to the taxonomic and morphometric data analysis, and also in the context of underpinning that temperature is a decisive factor in global biodiversity patterns, it conceals, however, species associations differentiating e.g. near coastal versus offshore habitats, surface water versus fluorescence maximum layers, and also where relevant (e.g. the Andaman Sea, Thailand) possible seasonal patterns in species occurrences. Once adequately progressed the molecular tools will be keystone elements in puzzling out any such small-scale patterns in species occurrences.

Author contribution statement

Helge A. Thomsen (HAT) has conceived and designed the analysis, generated funding, and collected and processed samples. HAT is further responsible for compiling and writing the paper. Jette B. Østergaard (JBO) has been much involved in data collection and sample processing with reference to the Andaman Sea and the Pacific Ocean. Seiko Hara (SH) has contributed data from

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667

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684 References

685
686 Bérard-Therriault, L., Poulin, M., Bossé, L., 1999. Guide d'identification du phytoplancton marin de
687 l'estuaire et du Golfe du Saint-Laurent incluant également certains protozoaires. Publ. spec. sci.
688 halieut. Aquat. 128, 1-387.

Journal Pre-proofs

689 Bergesch, M., Odebrecht, C., Moestrup, Ø., 2008. Loricate choanoflagellates from the South
690 Atlantic coastal zone (~32 °S) including the description of *Diplotheca tricyclica* sp. nov. Biota
691 Neotrop 8, 111-122.
692 Booth, B.C., 1990. Choanoflagellates from the subarctic North Pacific Ocean, with description of
693 two new species. Can. J. Zool. 68, 2393-2402.
694 Buck, K., 1981. A study of choanoflagellates (Acanthoecidae) from the Weddell Sea, including a
695 description of *Diaphanoeca multiannulata* n. sp. J. Protozool. 28, 47-54.
696 Clarke, K.R., Gorley, R.N., 2015. PRIMER v7: User Manual / Tutorial. PRIMER-E: Plymouth.
697 Clarke, K.R., Gorley, R.N., Somerfierld, P.J., Warwick, R.M., 2014. Change in marine communities:
698 an approach to statistical analysis and interpretation, 3rd edition. PRIMER-E: Plymouth.
699 Daugbjerg, N., Hansen, L.E., Skovgaard, K., Østergaard, J.B., 1991. An investigation on some protist
700 plankton groups from the marine waters around Igloolik Island, NWT, Canada. In: Jørgensen, M.
701 (ed.), Arctic Biology Course 1989, Igloolik Island, NWT Canada. Publ. by Zoological Museum,
702 Univ. of Copenhagen, pp. 39-59.
703 Espeland, G., Throndsen, J., 1986. Flagellates from Kilsfjorden, southern Norway, with description
704 of two new species of Choanoflagellida. Sarsia 71, 209-226.
705 Hallegraeff, G.M., 1983. Scale-bearing and loricate nanoplankton from the East Australian Current.
706 Bot. Marina 26, 493-515.
707 Hara, S., Sheu, J., Chen, Y.L., Takahashi, E., 1997. Choanoflagellates (Sarcomastigophora, Protozoa)
708 from the coastal waters of Taiwan and Japan (II): Species composition and biogeography. Zool.
709 Stud. 36, 98-110.
710 Hoepffner, H., Haas, L.W., 1990. Electron microscopy of nanoplankton from the North Pacific
711 central gyre. J. Phycol. 26, 421-439.

712 ICZN 1999. International Code of Zoological Nomenclature, Fourth Edition: The International Trust
713 for Zoological Nomenclature, London, UK, 306 pp.
714 Ikävalko, J., 1998. Further observations on flagellates within sea ice in northern Bothnian Bay, the
715 Baltic Sea. Polar Biol. 19, 323-329.
716 Ikävalko, J., Thomsen, H.A., 1997. The Baltic Sea ice biota (March 1994): a study of the protistan
717 community. Eur. J. Protistol. 33, 229-243.
718 Kamennaya, N.A., Kennaway, G., Fuchs, B.M., Zubkov, M.V., 2018. "Pomacytosis" – Semi-
719 extracellular phagocytosis of cyanobacteria by the smallest marine algae. PLOS Biol. 16,
720 e2003502, doi: 10.1371/journal.pbio.2003502.
721 Leadbeater, B.S.C., 1973. External morphology of some marine choanoflagellates from the coast of
722 Jugoslavia. Arch. Protistenk. 115, 234-252.
723 Leadbeater, B.S.C., 1974. Ultrastructural observations on nanoplankton collected from the coast of
724 Jugoslavia and the Bay of Algiers. J. mar. biol. Ass. U.K. 54, 179-196.
725 Leadbeater, B.S.C., 2015. The Choanoflagellates: Evolution, biology and ecology. Cambridge
726 University Press.
727 Lee, W.J., Brandt, S.M., Vørs, N., Patterson, D.J., 2003. Darwin's heterotrophic flagellates. Ophelia
728 57, 63-98.
729 Manton, I., Bremer, G., 1981. Observations on lorica structure and aspects of replication in the
730 *Pleurasiga sphyrelata* Thomsen complex (= *Polyfibula* spp., gen.n.) (Choanoflagellata). Zool. Scr.
731 10, 273-291.
732 Marchant, H.J., 1985. Choanoflagellates in the Antarctic marine food chain. In: Siegfried, W.R.,
733 Condy, P.R., Laws, R.M. (Eds.), Antarctic Nutrient Cycles and Food Webs. Springer-Verlag Berlin
734 Heidelberg, pp. 271-276.

Journal Pre-proofs

735 Marchant, H.J., Perrin, R.A., 1990. Seasonal variation in abundance and species composition of
736 choanoflagellates (Acanthoecidae) at Antarctic coastal sites. Polar Biol. 10, 499-505.
737 McKenzie, C.H., Deibel, D., Thompson, R.J., MacDonald, B.A., Penney, R.W., 1997. Distribution and
738 abundance of choanoflagellates (Acanthoecidae) in the coastal cold ocean of Newfoundland,
739 Canada. Mar. Biol. 129, 407-416.
740 Menezes, S., 2005. Nanoplankton biodiversity in the Pettaquamscutt river estuary, Rhode Island,
741 U.S.A. PhD thesis, Univ. Rhode Island, 319pp.
742 Moestrup, Ø., 1979. Identification by electron microscopy of marine nanoplankton from New
743 Zealand, including the description of four new species. New Zeal. J. Bot. 17, 61-95.
744 Moestrup, Ø., Thomsen, H.A., 1980. Preparation of shadow-cast whole mounts. In: Gantt, E. (Ed.),
745 Handbook of Phycological methods. Vol. III. pp. 385-390 (Cambridge).
746 Nitsche, F., Arndt, H., 2008. A new choanoflagellate species from Taiwan: Morphological and
747 molecular biological studies of *Diplotheca elongata* nov. spec., and *D. costata.* Eur. J. Protistol.
748 44, 220-226.
749 Nitsche, F., Thomsen, H.A., Richter, D.J., 2017. Bridging the gap between morphological species
750 and molecular barcodes – exemplified by loricate choanoflagellates. Eur. J. Protistol. 57, 26-37.
751 Schiwitza, S., Nitsche, F., 2021. A needle in the haystack – mapping sequences to morphology
752 exemplified by the loricate choanoflagellate *Enibas thessalia* sp. nov. (Acanthoecida,
753 Acanthoecidae). Protist 172(1):125782 https://doi.org/10.1016/j.protis.2020.125782
754 Schiwitza, S., Arndt, H., Nitsche, F., 2019. First description of an euryoecious acanthoecid
755 choanoflagellate species, *Enibas tolerabilis* gen. et sp. nov. from a salar in the Chilean Andes
756 based on morphological and transcriptomic data. Eur. J. Protistol. 67, 106-113.

Journal Pre-proofs

757 Soto-Liebe, K., Collantes, G., Kuznar, J., 2007. New records of marine choanoflagellates off the
758 Chilean coast. Invest. Mar. Valparaíso 35, 113-120.
759 Takahashi, E., 1981. Floristic study of ice algae in the sea ice of a lagoon, Lake Saroma, Hokkaido,
760 Japan. Mem. National Inst. Polar Res. Series E: Biology and Medical Science 34, 49-56.
761 Thomsen, H.A., 1973. Studies on marine choanoflagellates I. Silicified choanoflagellates of the
762 Isefjord (Denmark). Ophelia 12, 1-26.
763 Thomsen, H.A., 1976. Studies on marine choanoflagellates II. Fine-structural observations on some
764 silicified choanoflagellates from the Isefjord (Denmark), including the description of two new
765 species. Norw. J. Bot. 23, 33-51.
766 Thomsen, H.A. 1978. Nanoplankton from the Gulf of Elat (= Gulf of Aquaba), with particular
767 emphasis on the choanoflagellates. Isr. J. Zool. 27, 34-44.
768 Thomsen, H.A., 1979. Electron microscopical observations on brackish-water nannoplankton from
769 the Tvärminne area, S.W. Coast of Finland. Acta Bot. Fenn. 110, 11-37.
770 Thomsen, H.A., 1982. Planktonic choanoflagellates from Disko Bugt, West Greenland, with a
771 survey of the marine nanoplankton of the area. Meddr. Grønland, Bioscience 8, 1-35.
772 Thomsen, H.A., Boonruang, P., 1983. A microscopical study of marine collared flagellates
773 (Choanoflagellida) from the Andaman Sea, SW Thailand: Species of *Stephanacantha* gen. nov.
774 and *Platypleura* gen. nov. Protistologica 19, 193-214.
775 Thomsen, H.A., Buck, K R., 1991. Choanoflagellate diversity with particular emphasis on the Acan-
776 thoecidae. In: Patterson, D.J., Larsen, J. (Eds.), Free-living heterotrophic flagellates. Clarendon
777 Press, Oxford, pp. 259-284.

Journal Pre-proofs

778 Thomsen, H.A., Moestrup, Ø., 1983. Electron microscopical investigations on two loricate
779 choanoflagellates (Choanoflagellida), *Calotheca alata* gen. et sp. nov. and *Syndetophyllum*
780 *pulchellum* gen. et comb. nov., from Indo-Pacific localities. Proc. R. Soc. Lond. B. 219, 41-52.
781 Thomsen, H.A., Østergaard, J.B., 2017. Acanthoecid choanoflagellates from the Atlantic Arctic
782 region – a baseline study. Heliyon 3 (2017) e00345
783 https://doi.org/10.1016/j.heliyon.2017.e00345
784 Thomsen, H.A., Østergaard, J.B., 2019a. Loricate choanoflagellates (Acanthoecida) from warm
785 water seas. I. *Conioeca* gen. nov. and *Nannoeca* Thomsen. Eur. J. Protistol. 67, 77-88.
786 Thomsen, H.A., Østergaard, J.B., 2019b. Loricate choanoflagellates (Acanthoecida) from warm
787 water seas. II. *Bicosta*, *Apheloecion*, *Campyloacantha* and *Saroeca*. Eur. J. Protistol. 67, 114-131.
788 Thomsen, H.A., Østergaard, J.B., 2019c. Loricate choanoflagellates (Acanthoecida) from warm
789 water seas. III. *Acanthocorbis* Hara and Takahashi and *Stephanoeca* Ellis. Eur. J. Protistol. 69, 52-
790 69.
791 Thomsen, H.A., Østergaard, J.B., 2019d. Loricate choanoflagellates (Acanthoecida) from warm
792 water seas. IV. *Cosmoeca* Thomsen. Eur. J. Protistol. 71, pp. 125632
793 https://doi.org/10.1016/j.ejop.2019.125632
794 Thomsen, H.A., Østergaard, J.B., 2019e. Loricate choanoflagellates (Acanthoecida) from warm
795 water seas. V. *Thomsenella* Özdikmen (= *Platypleura* Thomsen). Eur. J. Protistol. 71, pp. 125632
796 https://doi.org/10.1016/j.ejop.2019.125633
797 Thomsen, H.A., Østergaard, J.B., 2021. Loricate choanoflagellates (Acanthoecidae) from warm water
798 seas. VIII. *Crinolina* Thomsen and *Diaphanoeca* Ellis. Eur. J. Protistol. (in press)

Journal Pre-proofs

799 Thomsen, H.A., Buck, K.R., Coale, S.L., Garrison, D.L., Gowing, M.M., 1990. Loricate
800 choanoflagellates (Acanthoecidae, Choanoflagellida) from the Weddell Sea, Antarctica. Zool.
801 Scr. 19, 367-387.
802 Thomsen, H.A., Buck, K.R., Chavez, F.P., 1991. Choanoflagellates of the central California waters:
803 Taxonomy, morphology and species assemblages. Ophelia 33, 131-164.
804 Thomsen, H.A., Garrison, D.L., Kosman, C., 1997. Choanoflagellates (Acanthoecidae,
805 Choanoflagellida) from the Weddell Sea, Antarctica, taxonomy and community structure with
806 particular emphasis on the ice biota; with preliminary remarks on choanoflagellates from Arctic
807 sea ice (Northeast Water Polynya, Greenland). Arch. Protistenk. 148, 77-114.
808 Thomsen, H.A., Nitsche, F., Richter, D.J., 2016. Seasonal occurrence of loricate choanoflagellates in
809 Danish inner waters. Protist 167, 622-638.
810 Thomsen, H.A., Kamennaya, N., Zubkov, M.V., Østergaard, J.B., 2020a. Loricate choanoflagellates
811 (Acanthoecida) from warm water seas. VI. *Pleurasiga* Schiller and *Parvicorbicula* Deflandre. Eur. J.
812 Protistol. 75, pp. 125717 https://doi.org/10.1016/j.ejop.2020.125717
813 Thomsen, H.A., Kamennaya, N., Zubkov, M.V., Østergaard, J.B., 2020b. Loricate choanoflagellates
814 (Acanthoecida) from warm water seas. VII. *Calotheca* Thomsen and Moestrup, *Stephanacantha*
815 Thomsen and *Syndetophyllum* Thomsen and Moestrup. Eur. J. Protistol. 76, pp. 125728
816 https://doi.org/10.1016/j.ejop.2020.125728
817 Tong, S.M., 1997a. Choanoflagellates in Southampton Water including the description of three
818 new species. J. Mar. Biol. Ass. U.K. 77, 929-958.

Journal Pre-proofs

819 Tong, S.M., 1997b. Heterotrophic flagellates from the water column in Shark Bay, Western
820 Australia. Mar. Biol. 128, 517-536.
821 Tong, S.M., Nygaard, K., Bernard, C., Vørs, N., Patterson, D.J., 1998. Heterotrophic flagellates from
822 the water column in Port Jackson, Sydney, Australia. Eur. J. Protistol. 34, 162-194.
823 Vørs, N., 1992. Heterotrophic amoebae, flagellates and heliozoa from the Tvärminne area, Gulf of
824 Finland, in 1988-1990. Ophelia 36, 1-109.
825 Vørs, N., Buck, K.R., Chavez, F.P., Eikrem, W., Hansen, L.E., Østergaard, J.B., Thomsen, H.A., 1995.
826 Nanoplankton of the equatorial Pacific with emphasis on the heterotrophic protists. Deep-Sea
827 Research 42, 585-602.

Journal Pre-proofs

Legends

Fig. 1. Map showing the approximate sampling sites for material reported here and MODIS sea surface temperatures (2003-2011 average). A circular dot refers to a single spot sampling, while a line or square indicates that samples were collected along extended transects. For further information see the materials and methods section in Thomsen and Østergaard (2019a).

Fig. 2.a-m. TEM (a, d-g) and LM whole mounts (b, c, h-m; phase contrast) of *Polyfibula elatensis* from the Gulf of California (a-c, e), the Andaman Sea, Thailand (d, f, g), the Caribbean Sea (h), the Sargasso Sea (i), West Australia (j, l, m), and the equatorial Pacific Ocean (k). **(a, d)** Complete cells; the arrow (a) points to a single facetted costal strip from the posterior transverse costa; **(b, c, h-m)** Selection of specimens from various geographic regions; **(e-g)** Detail (reversed printing) of distinctly (e) and less distinctly (f, g) facetted transverse costal strips; the arrows (f, g) point to median thickenings of costal strips. The scale bar (b) applies to all LM images.

Fig. 3.a-j. TEM (a-e) and LM whole mounts (f-j; phase contrast) of *Parvicorbicula pedicellata* from the Andaman Sea, Thailand (a, b, d, e), the Gulf of California (c, i, j), the equatorial Pacific (f), and West Australia (g, h). **(a-c)** Complete loricae; **(d)** Detail (reversed printing) of anterior transverse costa (from (b)) showing the exterior position of the longitudinal costae; **(e)** Detail of the mid-lorica transverse costa (from (a)) to highlight the interior position of the longitudinal costae; **(f-j)** Selection of specimens from various geographic regions. The scale bar (g) applies to all LM images.

Fig. 4.a-h. Drawings to approximate scale of *Coronoeca* spp. The drawings are based on dried material as observed in either LM or TEM, hence the protoplast position and dimensions are only

853 indicative. **(a)** *C. kosmaniae*; **(b, c)** *C. conicella*; **(d)** *C. superpositus (Thailand)*; **(e)** *C. superpositus*
854 *(Denmark)*; **(f)** *C. tongiae*; **(g)** *C. patongiensis*; **(h)** *C. marchantii*. The scale bar applies to all images.
855
856 **Fig. 5.a-f.** LM (a-c; phase contrast) and TEM (d-f; reversed printing (e)) whole mounts of *Coronoeca*
857 *kosmaniae* sp. nov. from the Gulf of California, Mexico. **(a-c)** Selection of images to show the lorica
858 as appearing from different angles; **(d)** Complete lorica (type specimen); **(e, f)** Detail of
859 longitudinal costal strip swelling and crossing of transverse costa. The scale bar (a) applies to all
860 LM images.
861
862 **Fig. 6.a-d.** *Coronoeca kosmaniae* sp. nov. TEM (a) and LM (b-d; phase contrast) whole mount
863 micrographs from the Gulf of California, Mexico. **(a)** Detail of posterior lorica end to show the
864 arched and often duplicated or triplicated (arrows) costal strips that terminate the lorica posterior
865 end. Notice the only solitary longitudinal costal strip (arrowhead) and the attenuating single costal
866 strip from the pedicel; **(b-d)** Selection of specimens to show the variability in appearance under
867 the LM; notice (b) the marked accumulation of additional costal strips at the posterior lorica end,
868 and (d) the vacated membrane that normally secures the protoplast into position. The scale bar
869 (b) applies to all light micrographs.
870
871 **Fig. 7.a-l**. *Coronoeca conicella* sp. nov. TEM (a-c) and LM (d-f; DIC / g-l; phase contrast) whole
872 mounts from the Andaman Sea, Thailand (a-f) and West Australia (g-l). **(a-c)** Selected specimens to
873 show the variability encountered including specimens with extended (a) and short pedicel (c). **(d-f)**
874 Andaman Sea images of long (d) and short (e, f) pedicellate forms. **(g-l)** The variety encountered in
875 samples from West Australia. The scale bar (g) applies to all LM images.

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877 **Fig. 8.a-k.** *Coronoeca conicella* sp. nov. TEM (a, b) and LM (c-i; phase contrast / j, k; DIC) whole
878 mounts from the Gulf of California, Mexico (a-e), the Sargasso Sea (f), the Caribbean Sea (g), the
879 equatorial Pacific Ocean (h, i), and the eastern Mediterranean Sea (j, k). **(a)** Complete lorica
880 without protoplast; notice five longitudinal costae; **(b)** High magnification (reverse printing) to
881 show anterior projections and connections between longitudinal and transverse costal elements;
882 **(c-k)** Specimens from various sources selected to show the morphological variability encountered;
883 notice (e-f) five longitudinal costae. The scale bar (e) applies to all light micrographs.

884

885 **Fig. 9.a, b.** *Coronoeca conicella* sp. nov. TEM whole mounts from the Seto Inland Sea, Japan. **(a)**
886 Complete cell. Notice that the lorica has five longitudinal costae; **(b)** Dividing cell that verifies
887 tectiform division in this species.

888

889 **Fig. 10.** Diagrams (A) to show the relationship between lorica height and length of pedicel in *C.*
890 *conicella*, and site-specific variability, and (B) the site-specific occurrence of loricae with five rather
891 than four longitudinal costae.

892

893 **Fig. 11.a-c.** *Coronoeca superpositus* TEM (a, b) and LM (c; phase contrast) whole mounts from the
894 Andaman Sea, Thailand. **(a, b)** Complete loricae (a; reverse printing) both with eight longitudinal
895 costae and a similar number of projections; the interior location of the mid-lorica transverse costa
896 is evident (a); **(c)** Empty lorica.

897

898 **Fig. 12.a-g**. *Coronoeca superpositus* TEM (a-c) and LM (d-g; phase contrast) whole mounts of
899 specimens from Danish coastal waters (Kulhuse) selected to supplement the limited image gallery
900 available of this taxon. **(a, b)** Well preserved loricae with four and six anterior projections. **(c)**
901 Sharply pointed anterior projection and detail of the connection between transverse and
902 longitudinal costal strips (reverse printing). **(d-g)** LM images showing the variability typically
903 encountered. The scale bar (d) applies to all light micrographs.
904
905 **Fig. 13.a-k**. *Coronoeca marchantii* sp. nov. TEM (a), SEM (b) and LM (c-k; phase contrast) whole
906 mounts from the Andaman Sea, Thailand (a-b), West Australia (c-e; g, h), the Sargasso Sea (f), the
907 Caribbean Sea (i), the equatorial Pacific Ocean (j), and the Gulf of California, Mexico (k). **(a)** Type
908 specimen; notice the membrane that secures the protoplast in position; **(b)** Empty lorica which
909 clearly shows the interior location of all three transverse costae; **(c-e, j, k)** Selected specimens to
910 show the variability encountered across all regions sampled; **(f-i)** Deviant forms where the anterior
911 transverse costa is pushed forward. The arrows point to the overlap between anterior longitudinal
912 costal strips. The scale bar (e) applies to all LM images.
913
914 **Fig. 14.a-h.** *Coronoeca tongiae* sp. nov. (a-g) and *C. patongiensis* sp. nov. (h, i) TEM (a, b, d-f, i) and
915 LM (c, g, h; phase contrast) whole mounts from the Andaman Sea, Thailand (a, b, d, i), West
916 Australia (c, h), the eastern Mediterranean Sea, Alexandria (e), the Seto Inland Sea, Japan (f), and
917 the Gulf of California, Mexico (g). **(a)** Type specimen (reverse printing). **(c, g, h)** Images selected to
918 demonstrate that species specific details can be resolved using LM; **(b, d-f)** Specimens selected to
919 document the consistency in lorica construction; **(i)** Type specimen (reverse printing). The scale
920 bar (h) also applies to (c).

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922 **Fig. 15.a-h.** TEM (a, b, g, h) and LM (c-f; phase contrast) whole mounts of miscellaneous species
923 from the equatorial Pacific Ocean (a, b), the eastern Mediterranean Sea, Alexandria (c, d, h), the
924 Sargasso Sea (e, f), and the Andaman Sea, Thailand (g). **(a)** Choano sp. 1; **(b)** Chono sp. 2; **(c, d, h)**
925 *Stephanoeca urnula*; **(e, f)** *Savillea* sp.; **(g)** Choano sp. 3. The scale bar (f) applies to all LM images.

926

927 **Fig. 16. (A)** Map showing the approximate geographical positions of material that is part of the
928 analysis presented in Fig. 17. Data sources: **(1)** NEW, N.E. Greenland; Thomsen and Østergaard
929 2017; **(2)** NOW, N.W. Greenland; Thomsen and Østergaard 2017; **(3)** Disko Bay, West Greenland;
930 Thomsen and Østergaard 2017; **(4)** Igloolik, Canada; Daugbjerg et al. 1991; **(5)** Baltic Sea; Thomsen
931 1979, Vørs 1992, Ikävalko and Thomsen 1997, Ikävalko 1998; **(6)** Denmark; Thomsen et al. 2016;
932 **(7)** Kilsfjorden, Norway; Espeland and Throndsen 1986; **(8)** Southampton, UK; Tong 1997a; **(9)**
933 Newfoundland, Canada; McKenzie et al. 1997; **(10)** Gulf of St. Lawrence, Canada; Bérard-Therriault
934 et al. 1999; **(11)** Rhode Island, USA; Menezes 2005; **(12)** Sargasso sea; Thomsen and Østergaard
935 2019a and subsequent papers; **(13)** Algiers; Leadbeater 1974; **(14)** Croatia and Montenegro;
936 Leadbeater 1973, 1974; **(15)** Mediterranean Sea, Alexandria; Thomsen and Østergaard 2019a and
937 subsequent papers; **(16)** Caribbean Sea; Thomsen and Østergaard 2019a and subsequent papers;
938 **(17)** South Brazil; Bergesh et al. 2008; **(18)** Beagle Channel, Patagonia; Thomsen, unpublished; **(19)**
939 Weddell Sea, Antarctica; Thomsen et al. 1990, Thomsen et al. 1997; **(20)** Prydz Bay, Antarctica;
940 Marchant 1985, Marchant and Perrin 1990; **(21)** Andaman Sea, Thailand; Thomsen and Østergaard
941 2019a and subsequent papers; **(22)** Darwin, Australia; Lee et al. 2003; **(23)** West Australia;
942 Thomsen and Østergaard 2019a and subsequent papers; **(24)** Shark Bay, Australia; Tong 1997b;
943 **(25)** Sydney, Australia; Tong et al. 1998; **(26)** New Zealand; Moestrup 1979; Moestrup,

944 unpublished; **(27)** Taiwan; Hara et al. 1997; Hara unpublished; **(28**) Japan; Hara et al. 1997; Hara,
945 unpublished; **(29)** Subarctic North Pacific; Booth 1990; **(30)** California, USA; Thomsen et al. 1991;
946 **(31)** Gulf of California, Mexico; Thomsen and Østergaard 2019a and subsequent papers; **(32)** North
947 Pacific Central Gyre; Hoepffner and Haas 1990; **(33)** Equatorial Pacific Ocean; Thomsen and
948 Østergaard 2019a and subsequent papers. **(B)** Number of species observed at each locality.
949
950 **Fig. 17.** Shade matrix plot (Primer 7) showing relationships among clusters of samples (35) and
951 species (50 most important taxa only / presence-absence data). Both samples and species are
952 hierarchically clustered independently. Clusters are further identified by letters (species: A-D;
953 localities: E-H). See text for further explanation.
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	Lorica height (excl. pedicel)	Diam. ant. transv. costa	Diam. 2nd transv. costa	Diam. 3rd transv. costa	Diam. 4th transv. costa	# longitudinal costae	# long. costal strips (incl. projections / excl. pedicel)	Projecting spines	Pedicel	Flagellum	n
Coronoeca *conicella*	16.4 ±2.5	11.1 ±1.7	6.8 ±1.4			4-(5)	4	4-(5)	12.5 ±6.7	8.1 ±1.0	63
Coronoeca kosmaniae	25.5 ±1.2	15.5 ±0.6	15.2 ±0.7			7	4	7	7.9 ±0.4		26
Coronoeca marchantii	14.2 ±1.4	8.9 ±0.8	6.6 ±0.5	2.9 ±0.4		(7)-8	4	(7)-8	3.9 ±0.6	6.6 ±0.4	27
Coronoeca patongiensis	15.2/16.5	12.4/15.9	8.0/10.2	3.3/		10	4	10			2
Coronoeca patongiensis (Davis, pers. comm. / Sargasso Sea)	18.5	16.1	10.3	4.6		11	4	11		15.2	1
Coronoeca superpositus (Thail.)	13.7	10.0	7.3			8	4	7-8	4.2	7.9	2
Coronoeca superpositus (Denm.)	18.8 ±1.9	13.6 ±1.1	9.5 ±0.7			10-(11)	4	4-8	10.7 ±2.6	11.9 ±1.7	19
Coronoeca tongiae	14.6 ±0.9	12.0 ±1.1	9.9 ±0.5	7.4 ±0.8	3.1 ±0.5	9-10	4	9-10			7
Coronoeca tongiae (Leadbeater 1974; loc. cit. Pl. 2E)	13.5	11.0	10.4	8.4	3.4	9	4	9			1
Parvicorbicula *pedicellata*	13.7 ±1.1	7.7 ±0.6	6.1 ±0.6	2.9 ±0.3		7	4	7	3.6 ±0.5	5.7 ±1.8	15
Polyfibula *elatensis*	18.0 ±1.7	9.8 ±1.4	9.0 ±1.0	3.9 ±0.4		8	4	8	9.6 ±6.0	12.3 ±2.0	26

Table 1. Summary of lorica morphometric features.

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Table 2. Occurrence pattern of species discussed here and in Thomsen and Østergaard (2019a-e, 2021) and Thomsen et al. (2020a, b; 2021). New species described are marked with *.

	Andaman Sea, Thailand		West Australia	Sargasso Sea	Caribbean Sea	Equatorial Pacific Ocean	Gulf of California, Mexico	Mediterranean Sea, Alexandria	Reference
Acanthocorbis *apoda*	x								Thomsen and Østergaard 2019c
A. camarensis	x								Thomsen and Østergaard 2019c
A. campanula	x	x							Thomsen and Østergaard 2019c
*A. conicella**		x					x		Thomsen and Østergaard 2019c
*A. gladiella**	x	x	x	x		x			Thomsen and Østergaard 2019c
A. haurakiana	x	x	x						Thomsen and Østergaard 2019c
Apheloecion *articulatum*	x	x						x	Thomsen and Østergaard 2019b
*A. eqpacia**	x	x				x			Thomsen and Østergaard 2019b
A. pentacanthum	x	x				x	x	x	Thomsen and Østergaard 2019b
A. quadrispinum	x	x	x	x		x		x	Thomsen and Østergaard 2019b
Bicosta *minor* (form A)	x	x		x		x			Thomsen and Østergaard 2019b
B. spinifera							x		Thomsen and Østergaard 2019b
Calliacantha *magna**	x	x		x		x			Thomsen and Østergaard 2019b
C. natans							x		Thomsen and Østergaard 2019b
C. simplex	x	x	x	x		x	x	x	Thomsen and Østergaard 2019b
Calotheca *alata*	x	x							Thomsen et al. 2020b
Campyloacantha *imbricata*	x	x	x	x		x			Thomsen and Østergaard 2019b
C. spinifera	x	x	x				x	x	Thomsen and Østergaard 2019b
Conioeca *boonruangii**	x	x		x		x			Thomsen and Østergaard 2019a
Coronoeca *conicella**	x	x	x	x		x	x	x	This paper
*C. kosmaniae**							x		This paper
*C. marchantii**	x	x	x	x		x	x		This paper
*C. patongiensis**	x	x							This paper
C. superpositus	x								This paper
*C, tongiae**	x	x					x	x	This paper
Cosmoeca *ceratophora*	x	x	x	x		x	x	x	Thomsen and Østergaard 2019d
C. norvegica	x	x	x	x		x	x	x	Thomsen and Østergaard 2019d
C. phuketensis	x	x	x	x		x		x	Thomsen and Østergaard 2019d
C. subulata	x	x				x			Thomsen and Østergaard 2019d

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C. ventricosa (incl. form A)	x	x	x	x	x	x	x	Thomsen and Østergaard 2019d
C. ventricosa (form B)	x	x	x		x	x	x	Thomsen and Østergaard 2019d
C. ventricosa (form C)	x	x	x	x	x			Thomsen and Østergaard 2019d
***Crinolina** aperta*	x	x	x	x	x		x	Thomsen and Østergaard 2021a
C. isefiordensis	x	x				x		Thomsen and Østergaard 2021a
***Crucispina** cruciformis*	x	x	x			x	x	Thomsen and Østergaard 2019b
***Diaphanoeca** cylindrica*	x	x						Thomsen and Østergaard 2021
D. grandis	x	x				x	x	Thomsen and Østergaard 2021
D. multiannulata		x						Thomsen and Østergaard 2021
D. pedicellata		x			x	x		Thomsen and Østergaard 2021
D. pedicellata cfr.		x	x		x		x	Thomsen and Østergaard 2021
*D. pseudoundulata**	x	x			x	x		Thomsen and Østergaard 2021
*D. sargassoensis**			x					Thomsen and Østergaard 2021
D. spiralifurca	x	x						Thomsen and Østergaard 2021
*D. throndsenii**	x	x	x			x		Thomsen and Østergaard 2021
***Nannoeca** mexicana**		x				x		Thomsen and Østergaard 2019a
N. minuta	x	x	x	x	x	x	x	Thomsen and Østergaard 2019a
N. minuta (form A)	x				x			Thomsen and Østergaard 2019a
***Parvicorbicula** circularis*		x	x	x		x	x	Thomsen et al. 2020a
P. pedicellata	x	x			x	x		This paper
P. socialis		x						Thomsen et al. 2020a
***Pleurasiga** echinocostata*	x	x	x	x	x	x	x	Thomsen et al. 2020a
P. echinocostata form A	x	x						Thomsen et al. 2020a
P. echinocostata form B	x				x			Thomsen et al. 2020a
P. minima	x	x	x	x	x	x		Thomsen et al. 2020a
*P. minutissima**	x	x	x	x	x	x	x	Thomsen et al. 2020a
*P. quadrangiella**	x	x			x			Thomsen et al. 2020a
P. reynoldsii		x	x		x	x		Thomsen et al. 2020a
P. tricaudata						x		Thomsen et al. 2020a
***Polyfibula** elatensis*	*x*	*x*	*x*	*x*	*x*	*x*		This paper
***Saroeca** attenuata*			x		(x)			Thomsen and Østergaard 2019b
S. paucicostata	x	x	x			x	x	Thomsen and Østergaard 2019b
***Savillea** sp.*			x					This paper
***Stephanacantha** campaniformis*					x	x		Thomsen et al. 2020b
S. dichotoma	x	x	x	x	x	x	x	Thomsen et al. 2020b
S. formosa	x	x	x	x	x	x	x	Thomsen et al. 2020b
*S. oceanica**		x	x					Thomsen et al. 2020b
S. parvula	x	x	x	x	x			Thomsen et al. 2020b
S. zigzag	x	x	x	x	x	x	x	Thomsen et al. 2020b
***Stephanoeca** andemanica**	x	x						Thomsen and Østergaard 2019c
S. apheles	x	x						Thomsen and Østergaard 2019c
*S. broomia**	x	x						Thomsen and Østergaard 2019c
S. diplocostata var. paucicostata		x						Thomsen and Østergaard 2019c
*S. naja**	x	x	x		x	x		Thomsen and Østergaard 2019c
S. supracostata	x							Thomsen and Østergaard 2019c
S. urnula							x	This paper
***Syndetophyllum** pulchellum*	x	x				x	x	Thomsen et al. 2020b
***Thomsenella** acuta*	x	x	x	x	x		x	Thomsen and Østergaard 2019e
T. cercophora	x	x			x		x	Thomsen and Østergaard 2019e

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T. infundibuliformis			x		x	x	x	Thomsen and Østergaard 2019e
T. perforata	x	x	x	x	x	x	x	Thomsen and Østergaard 2019e

	Anterior transverse costa	2nd transverse costa	3rd transverse costa	4th transverse costa	Anterior costal strip joins
Coronoeca *conicella*	(I)	I			T
Coronoeca kosmaniae	(I)	I			T
Coronoeca marchantii	I	I	I		E
Coronoeca patongiensis	O	I	I		E
Coronoeca superpositus (Thail.)	(I)	I			T
Coronoeca superpositus (Denm.)	(I)	I			T
Coronoeca tongiae	I	I	I	I	T
Parvicorbicula *pedicellata*	I	O	I		T
Polyfibula *elatensis*	I	I	I		T

Table 3. Summary of lorica features. I: Transverse costa inside longitudinal costae'; (I): Transverse costa outside anterior tip of the penultimate longitudinal costal strip, but inside the anterior projections; O: Transverse costa outside longitudinal costae; T: T-joins anteriorly; E: E-joins anteriorly

Helge A. Thomsen (HAT) has conceived and designed the analysis, generated funding, and collected and processed samples. HAT is further responsible for compiling and writing the paper. Jette B. Østergaard (JBO) has been much involved in data collection and sample

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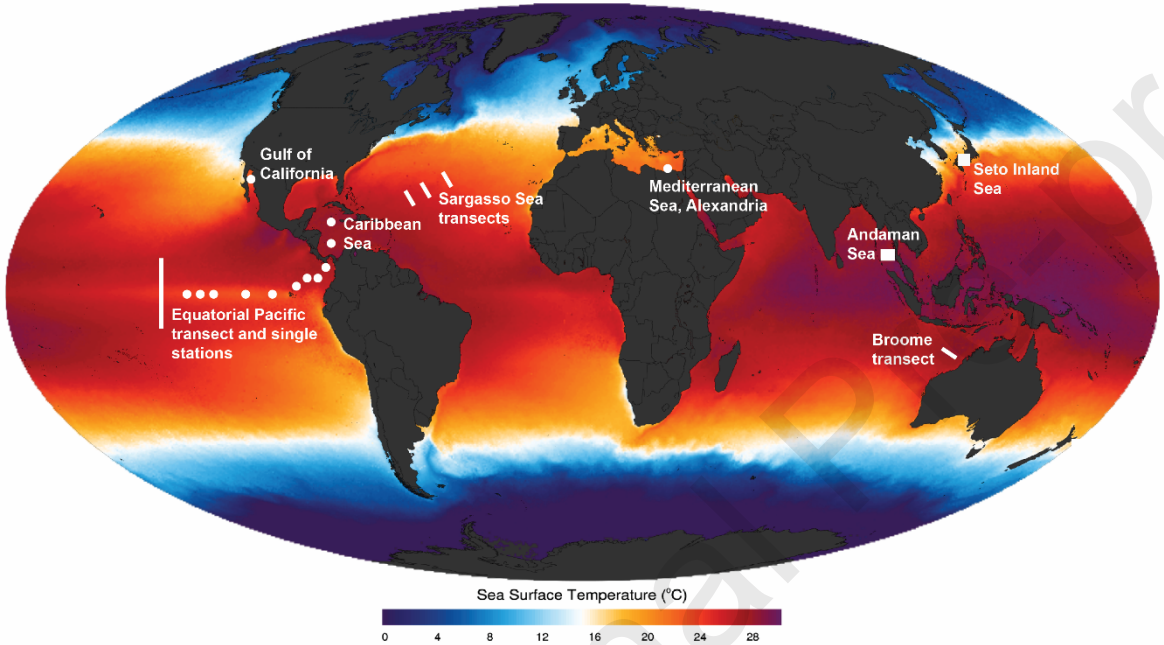
processing with reference to the Andaman Sea and the Pacific Ocean. Seiko Hara (SH) has contributed data from the Seto Inland Sea, Japan, and has been instrumental in finalizing the later versions of the manuscript.



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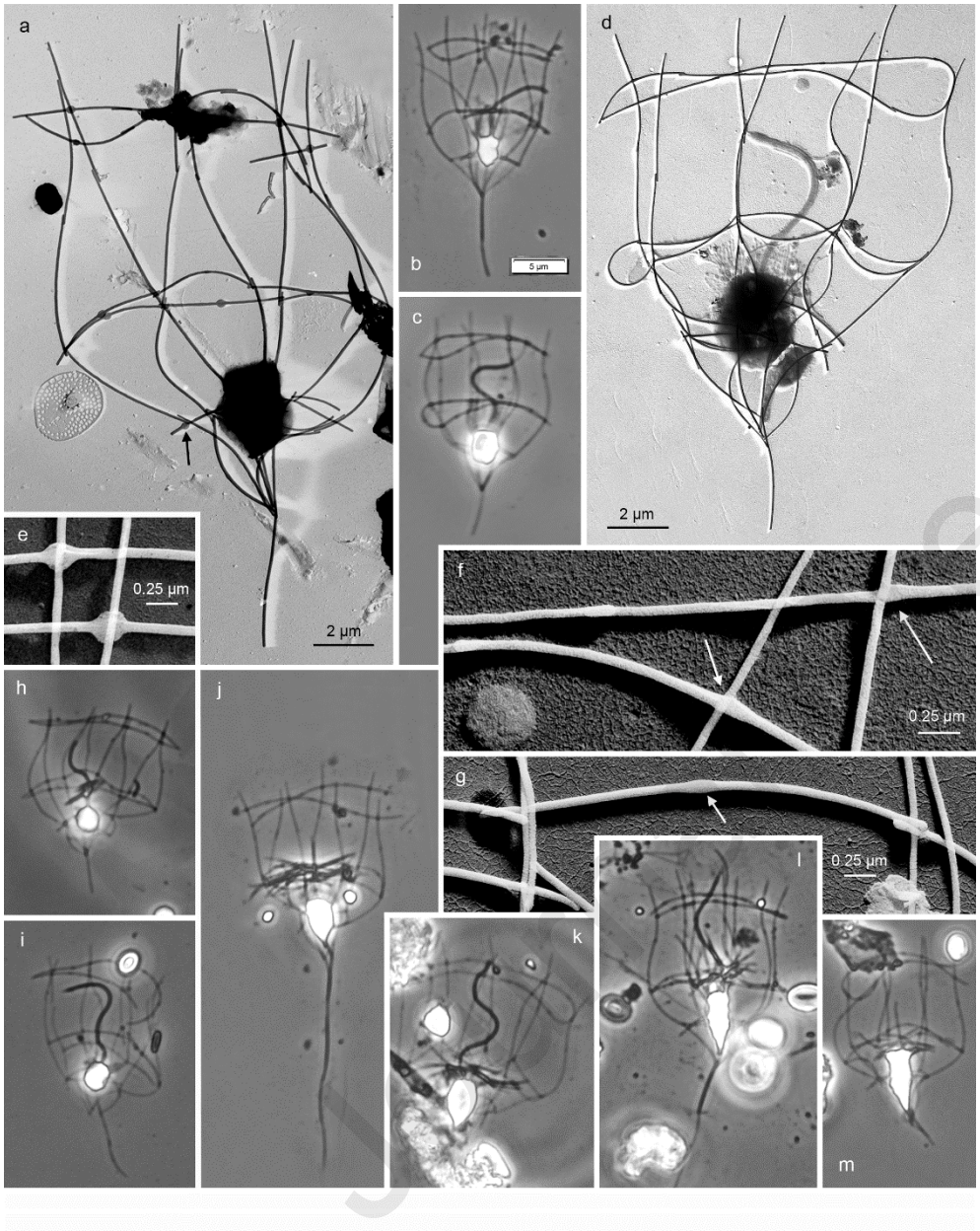

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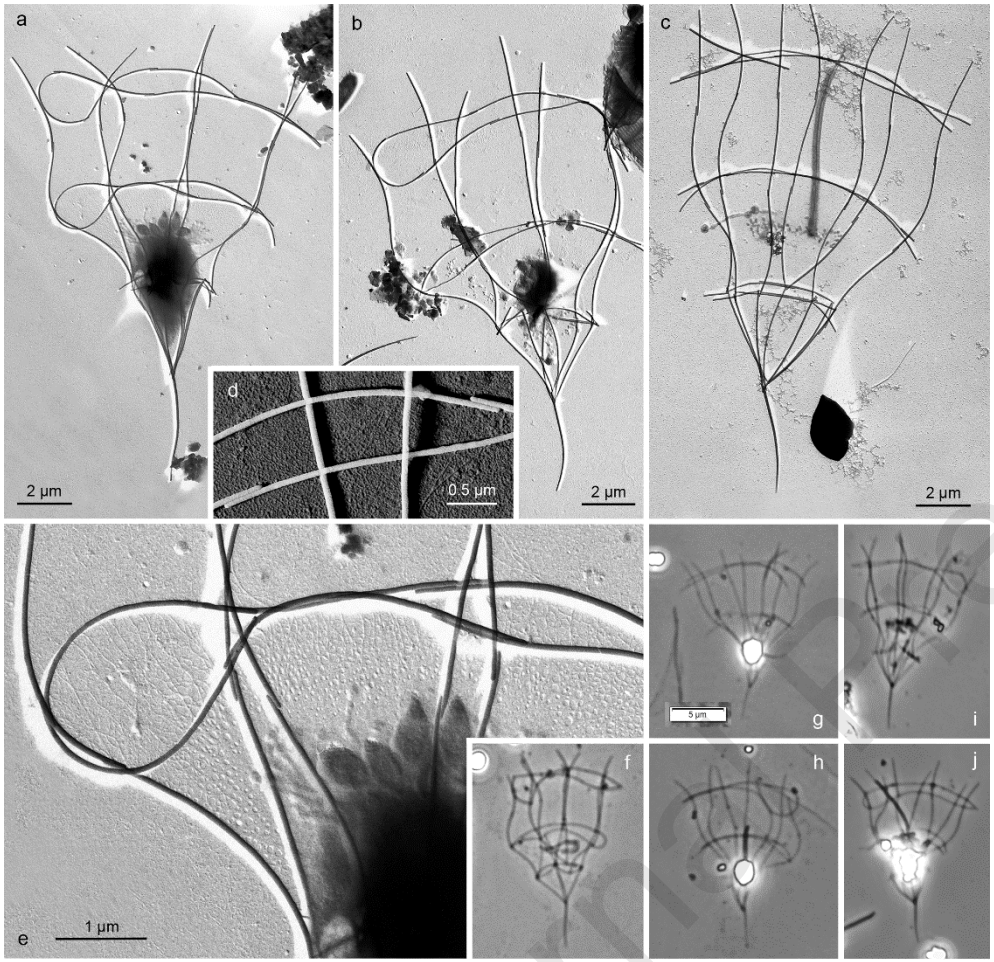

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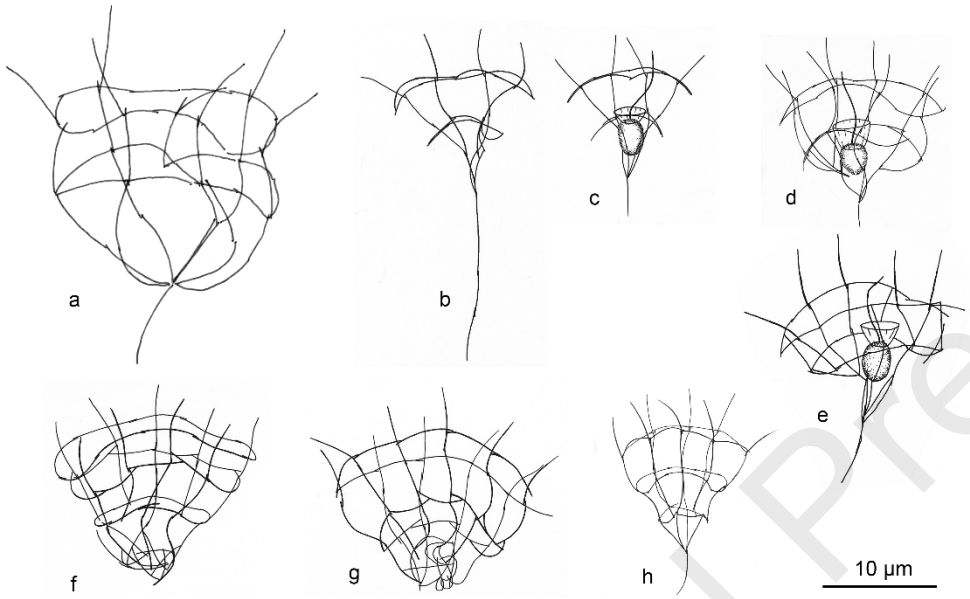

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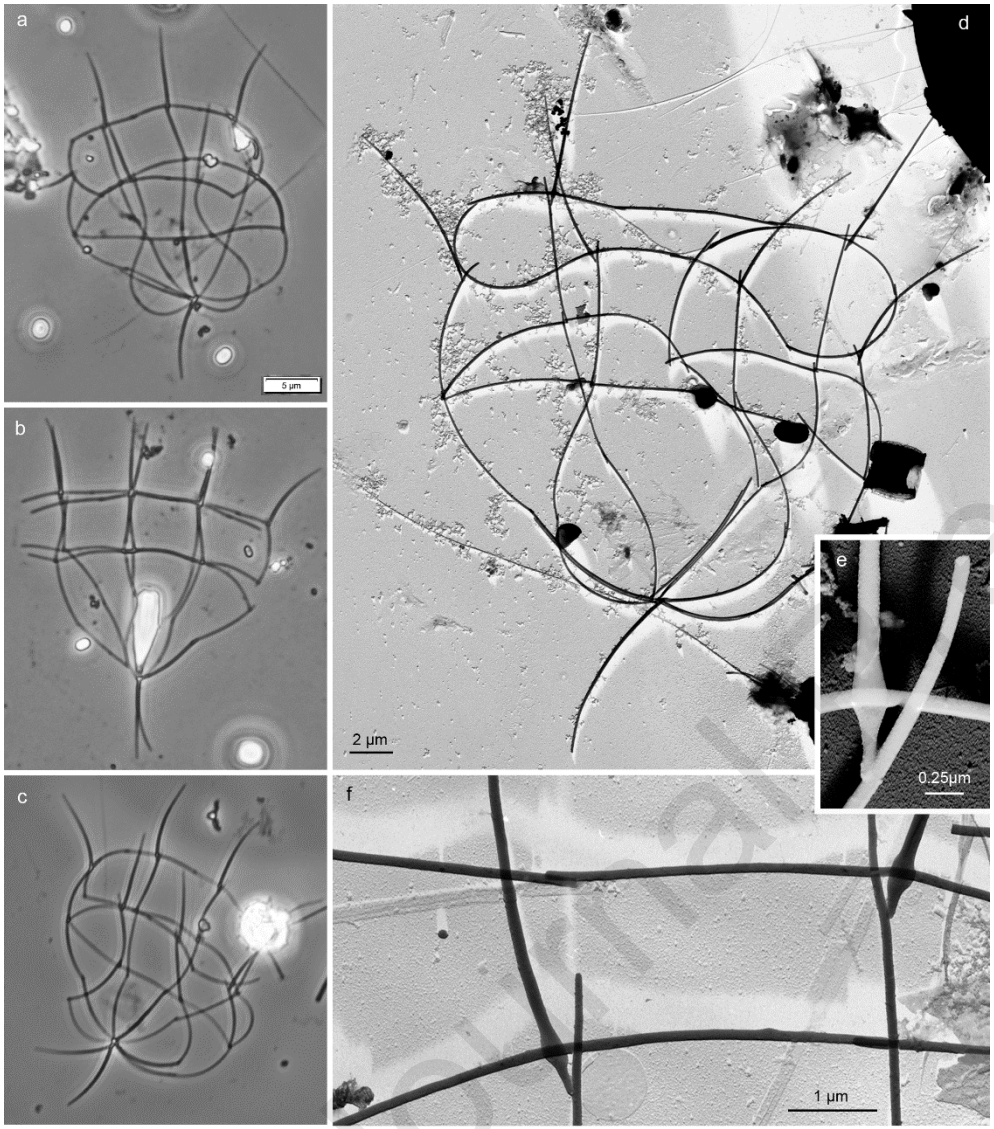

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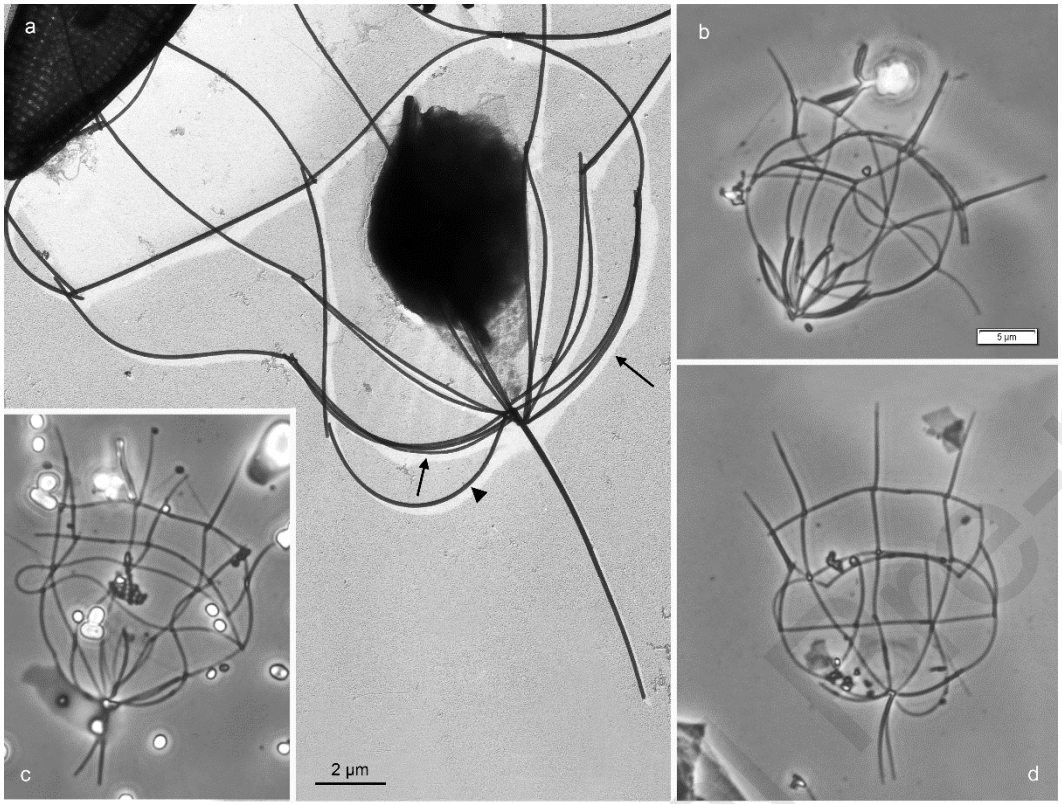

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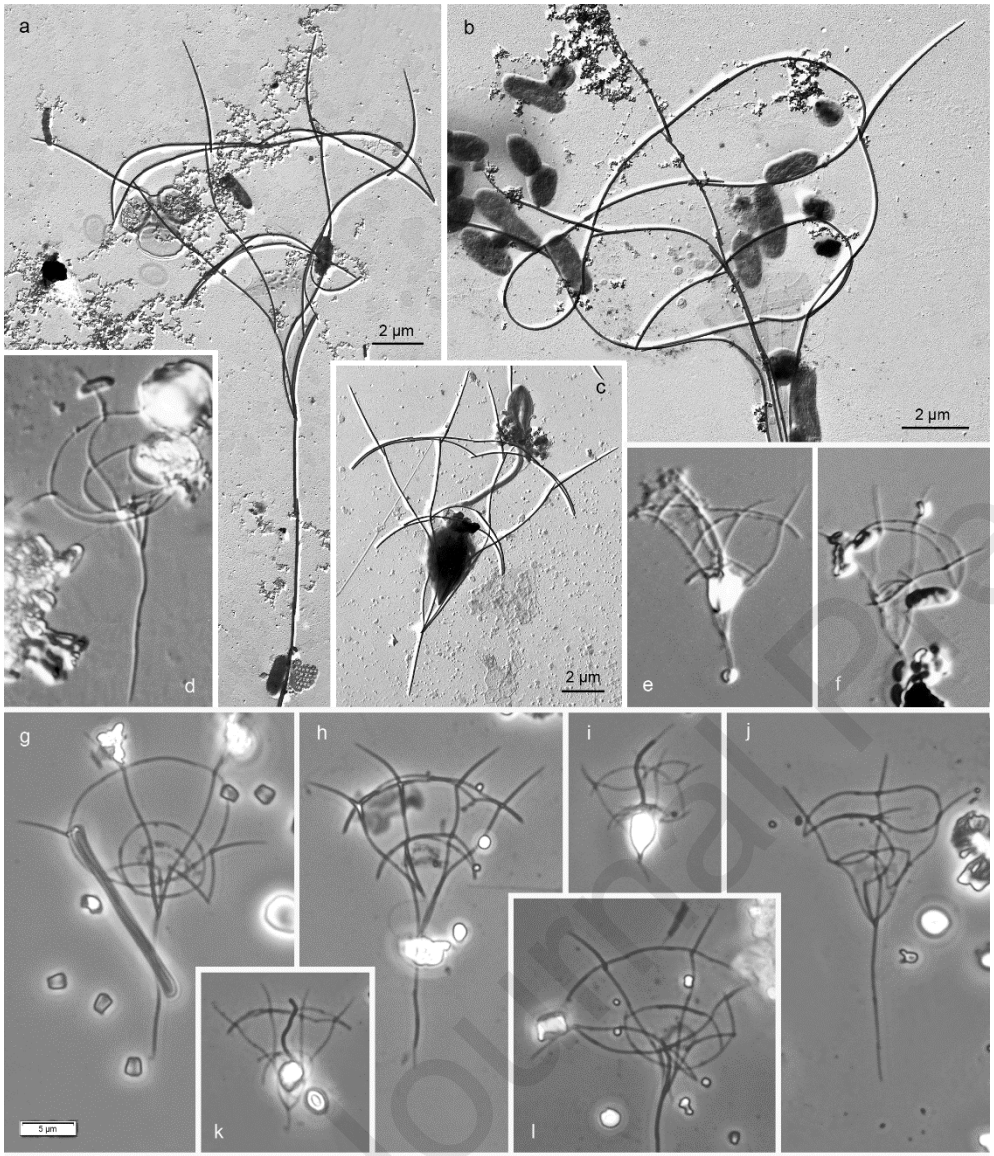

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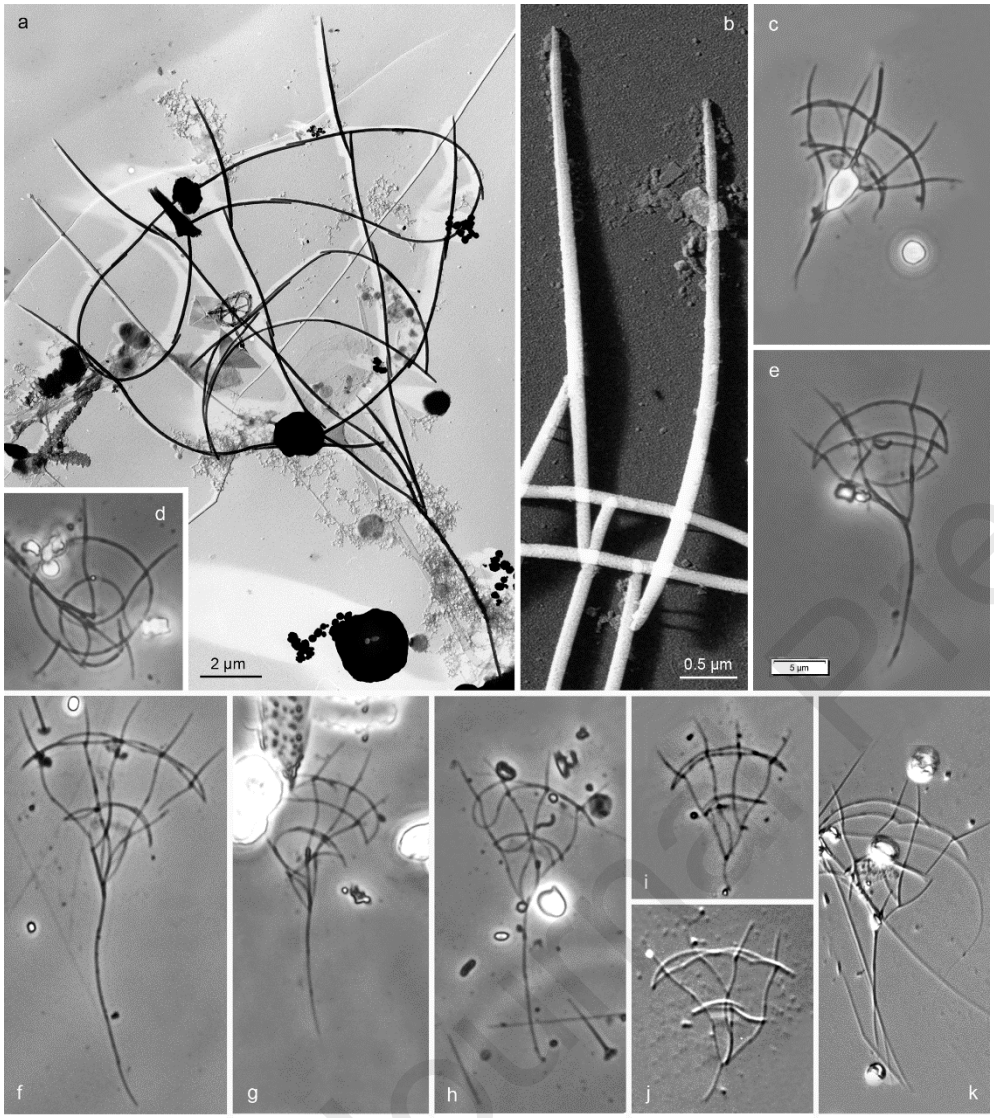

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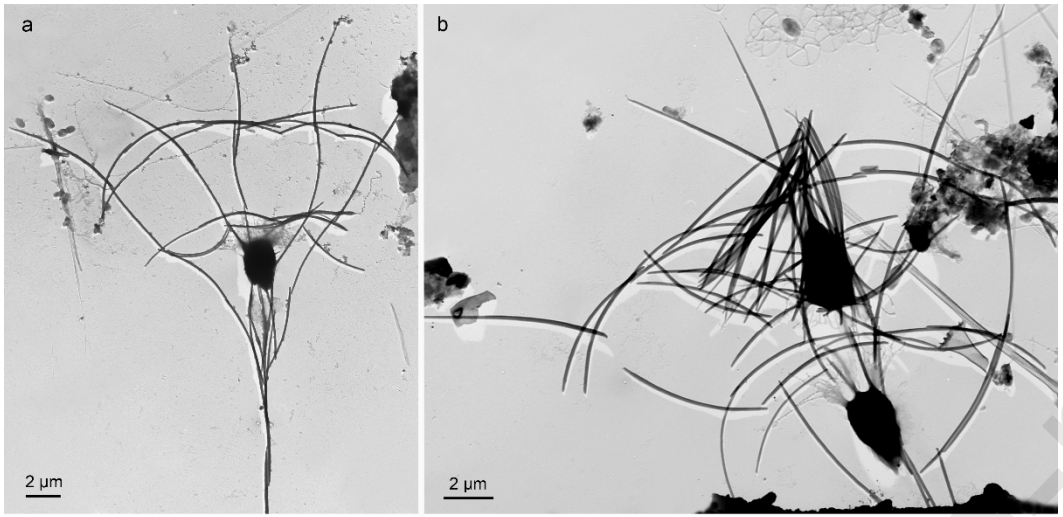

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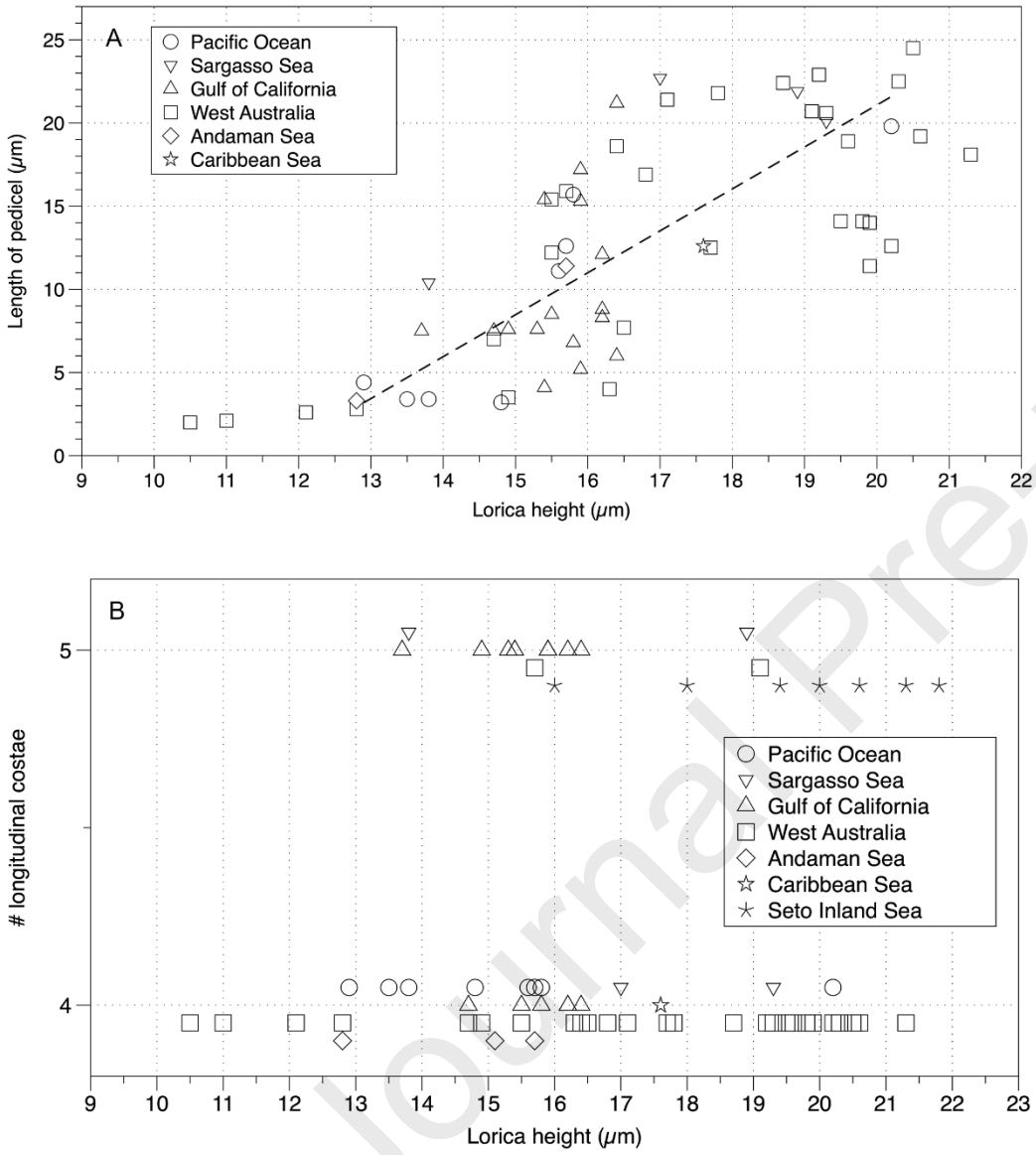

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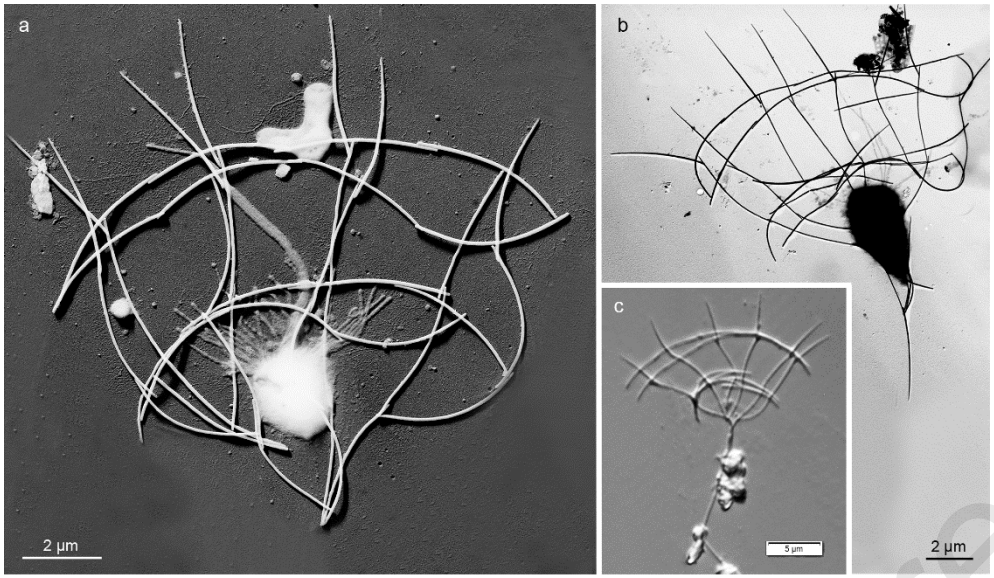

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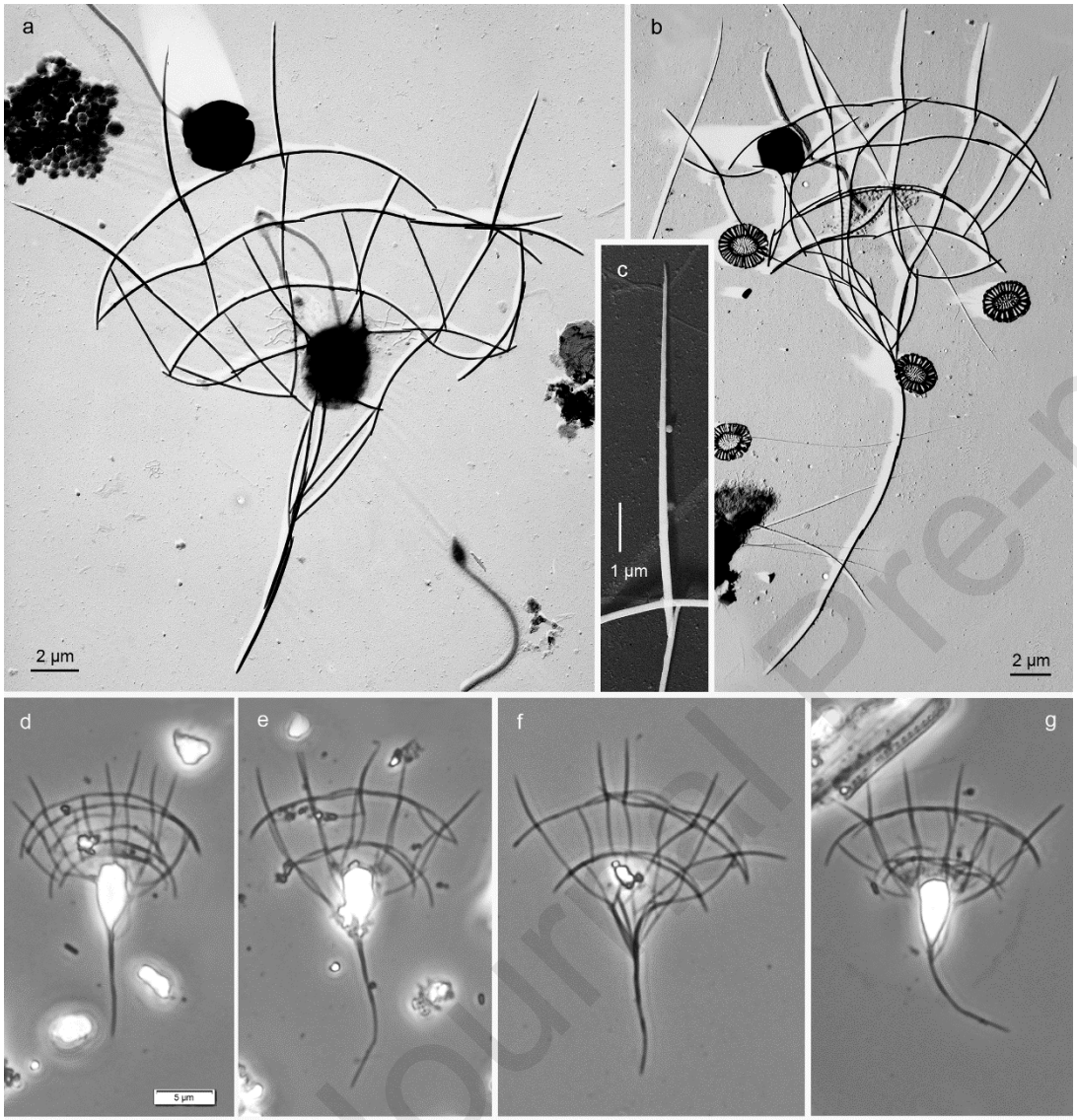

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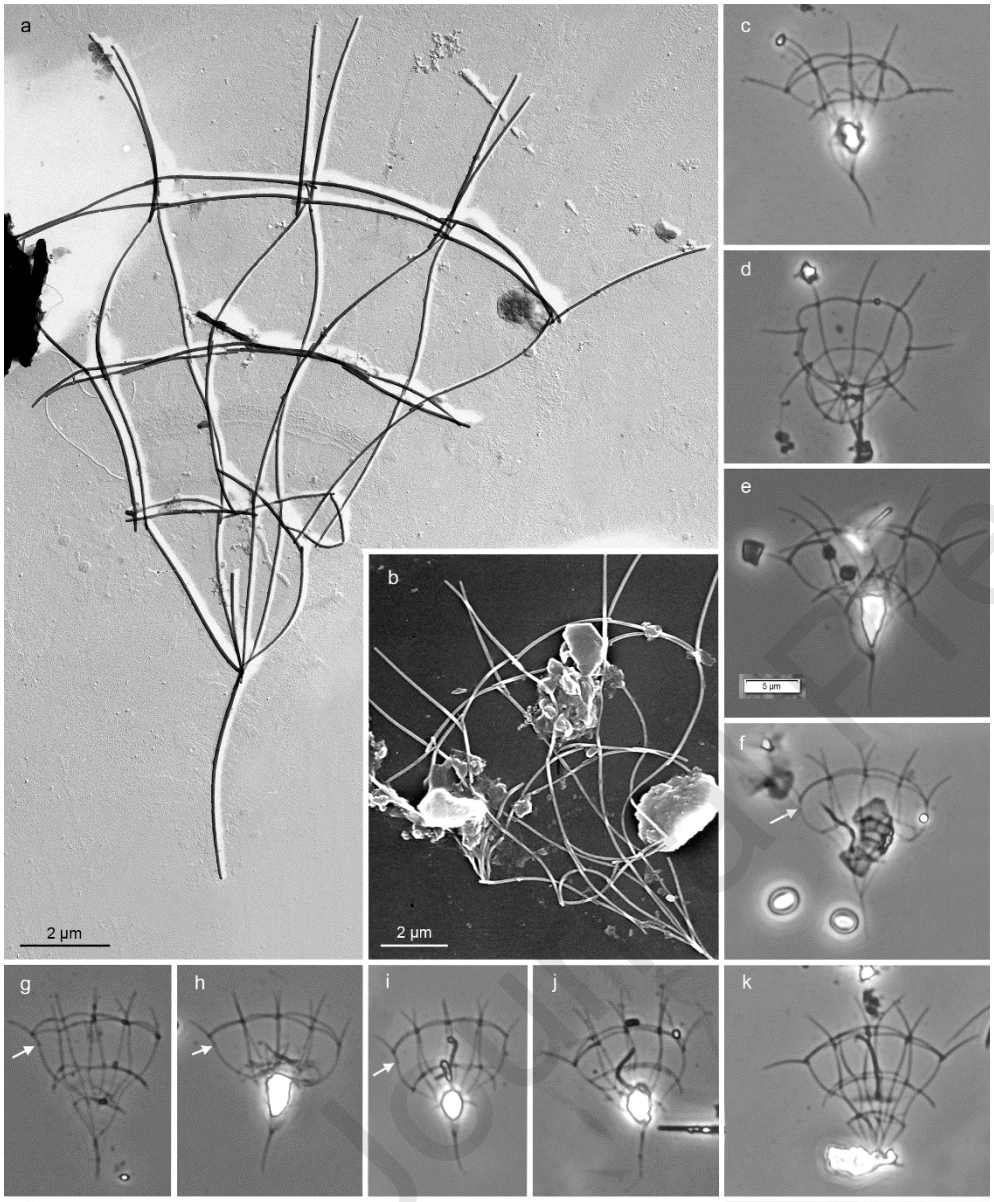

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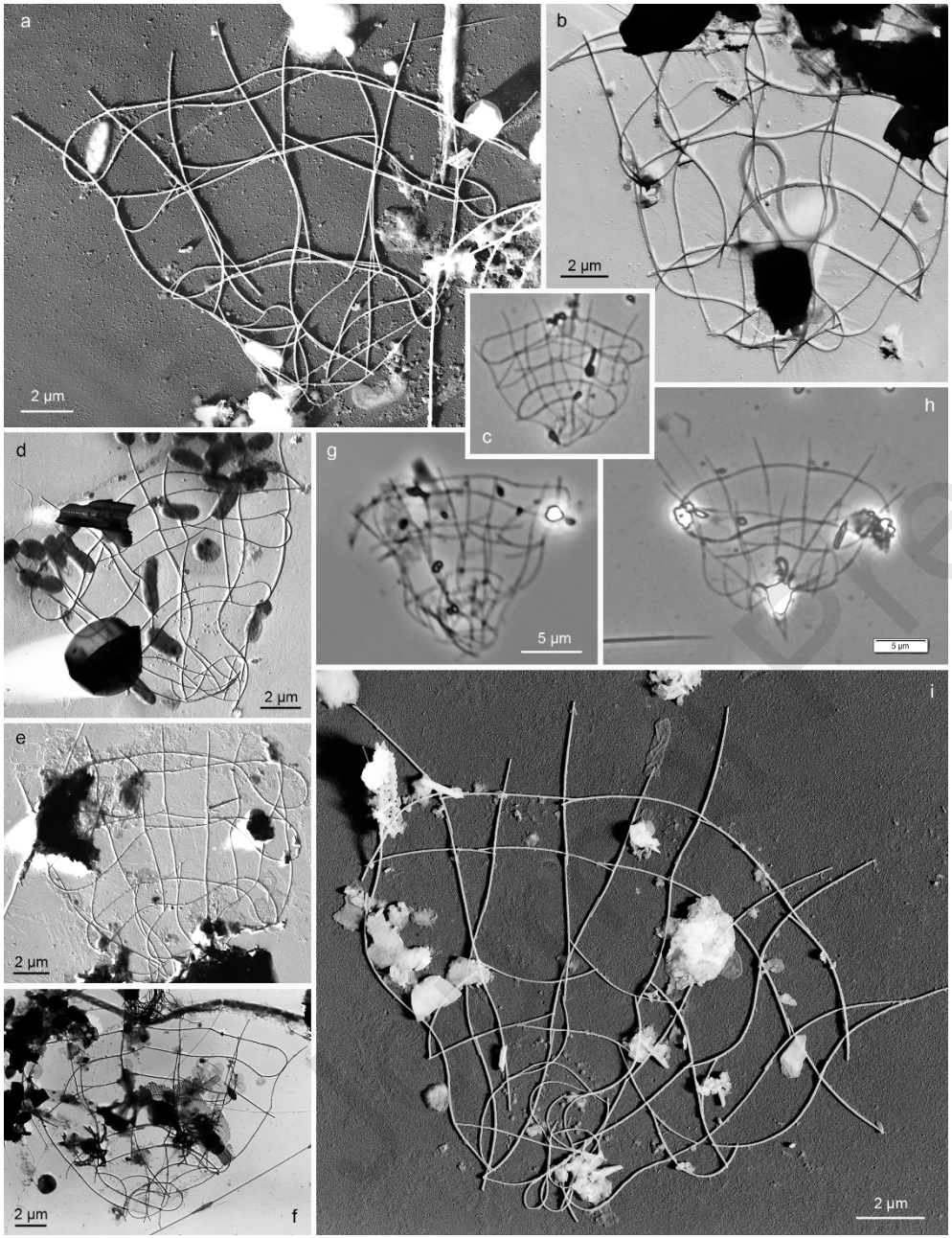

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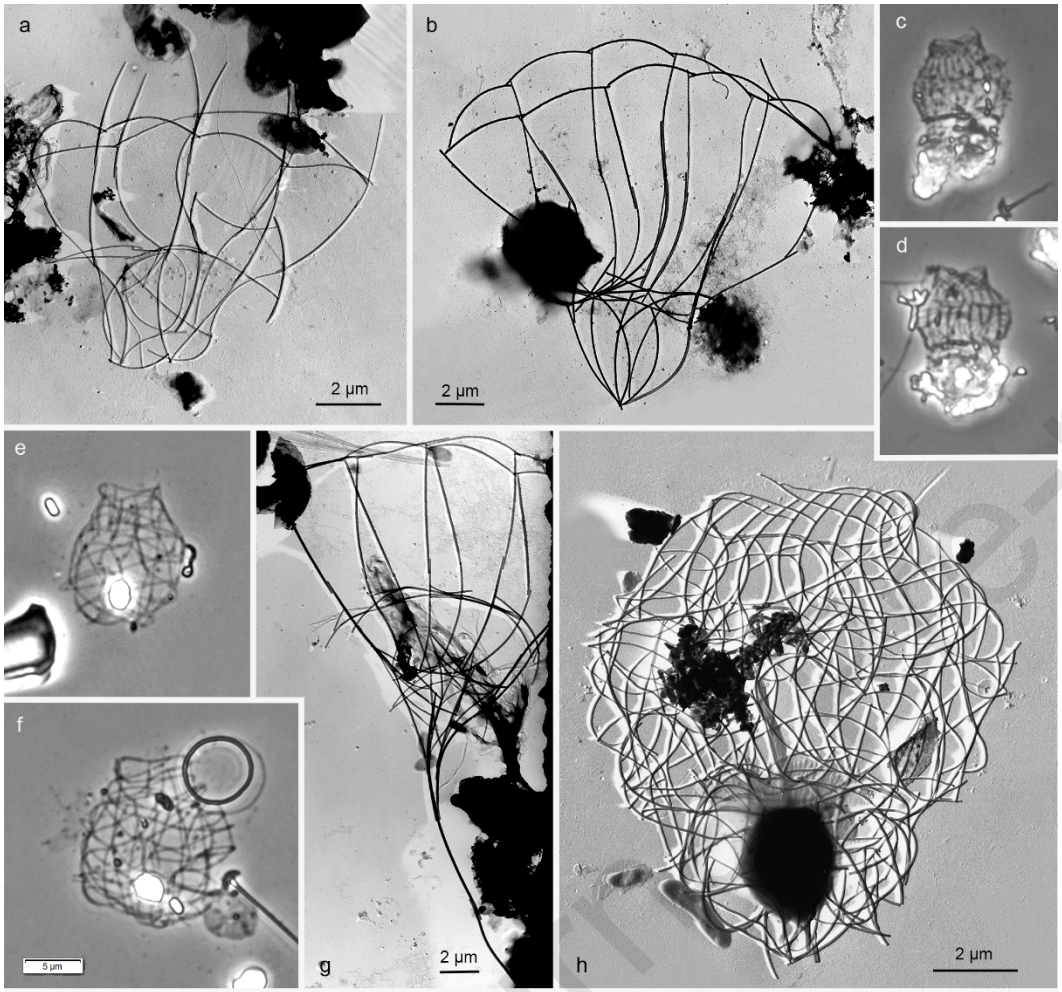

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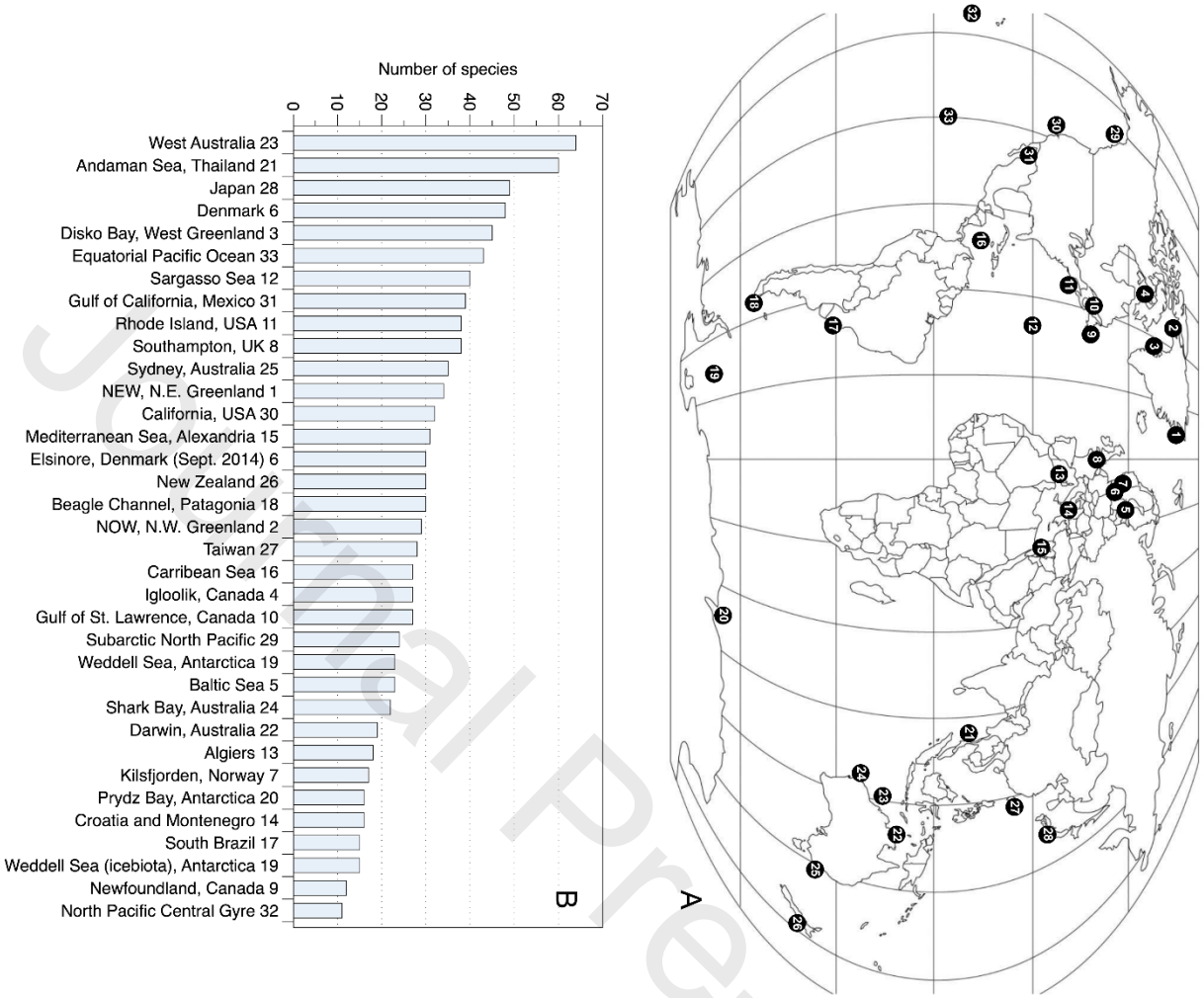

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