Loricate choanoflagellates (Acanthoecida) from warm water seas. VI. *Pleurasiga* Schiller and *Parvicorbicula* Deflandre

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Abstract

The loricate choanoflagellate genera *Pleurasiga* and *Parvicorbicula* are taxonomically ambiguous. *Pleurasiga* because of the uncertainty that relates to the true identity of the type species, and *Parvicorbicula* because too many newly described species over time have been dumped here in lack of better options. While all species currently allocated to the genus *Pleurasiga* (with the exception of the type species) are observed in our samples from the global warm water belt, the genus *Parvicorbicula* is represented by just a few and mostly infrequently recorded taxa. Two new species, viz. *Pl. quadrangiella* sp. nov. and *Pl. minutissima* sp. nov., are described here. While the former is closely related to *Pl. echinocostata*, the latter is reminiscent of *Pl. minima*. Core species of *Pleurasiga* and *Parvicorbicula* deviate from the vast majority of loricate choanoflagellates in having both the anterior and the mid-lorica transverse costae located exterior to the longitudinal costae. In *Pl. quadrangiella* there is no mid-lorica transverse costa but rather a small posterior transverse costa located inside the longitudinal costae. In *Pl. minutissima* the mid-lorica transverse costa has extensive costal strip overlaps which reveal patterns of costal strip junctions that deviate from the norm.

Keywords: Acanthoecida; Loricate choanoflagellates; *Pleurasiga*; *Parvicorbicula*; Warm water seas

Introduction

In an ongoing effort (Thomsen and Østergaard 2019a-e) to provide a first comprehensive overview of warm water loricate choanoflagellate diversity, based on a traditional microscopical approach, we here deal with species of *Pleurasiga* Schiller, 1925, and *Parvicorbicula* Deflandre, 1960. Both of these genera are taxonomically problematic, yet in markedly different ways. While the true identity of the *Pleurasiga* type species (*Pl. orculaeformis* Schiller, 1925) remains an enigma (Thomsen and Østergaard 2019a, d), the *Parvicorbicula* type species (*Pa. socialis* (Meunier, 1910) Deflandre, 1960) is well known (Manton et al. 1976). However, *Parvicorbicula* has over time become flooded with new species added that have seriously blurred the circumscription of the genus. The morphometric approach taken here will not resolve the taxonomical issues, but hopefully through a critical update on some of the previously described species, and also an addition of species new to science, pave the road for a future more definitive investigation, that can build on the species matrix presented here, while also using whatever sampling technique and molecular tool that might be available.

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 morphological details highlighted below we have added material from Danish waters (Fig. 5a; the Sound, collected Sept. 2014 / Fig. 15a, b; western Kattegat, collected 18 March 1976; 0m, 19 PSU, 0.7°C), New Zealand (Fig. 13a; courtesy of Ø. Moestrup, Univ. of Copenhagen), South Atlantic Ocean (Fig. 13b; collected 26 Oct. 2017; 42° 7.743’ S, 30° 25.023’ W; 20m, 34.5 PSU, 10.8°C / Fig. 14e; collected 26 Oct. 2017; 42° 1.283' S, 30° 4.167' W, 20m, 34.5 PSU 10.8° C) and South Pacific Ocean (Fig. 13c; collected 13 Jan. 2016; 36° 21.729' S, 132° 40.493' W; 30m, 34.6 PSU, 16.3°C).

The general protocol for processing water samples for the light microscope (LM) and transmission electron microscope (TEM) 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).

SEM images of cells from the Sound (Fig. 5a) originate from Au-coated coverslip preparations that were prepared in accordance with the routine described by Moestrup and Thomsen (1980) and examined in a FEI Quanta 200 ESEM FEG (property of DTU Cen).

SEM micrographs of specimens from the South Atlantic and South Pacific oceans (Fig. 13b, c, 14e) originate from Au/Pd-coated polycarbonate filters with flow-sorted cells prepared as described by Kamennaya et al. (2018) and imaged with the high-resolution SEM UltraPlus instrument (Zeiss Gemini) at the Imaging and Analysis Centre of the Natural History Museum in London, UK.

The material examined here is dried, which means that the natural 3-D structures have collapsed to become 2-D structures leading to 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 be measured directly but only calculated from measurements of the circumference. This approach has been taken in the species descriptions below.

Efforts are made to make use of a concise terminology when describing lorica features and we follow the 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). Some essential terms are explained in Fig. 2. Notice that while the term ‘mid-lorica transverse costa’ refers to the costa that separates longitudinal costal strips two and three (Fig. 2), the term ‘posterior transverse costa’ is used when referring to a transverse costa (viz. *Pl. quadrangiella*) that separates longitudinal costal strips one and two. Subsamples of species that from a morphological point of view form a fairly well-defined cluster, typically in association with the type species (viz. *Parvicorbicula*) but also occasionally otherwise (viz. *Pleurasiga*) are for the sake of simplicity referred to as a ‘core’ group of species.

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-reversed images. In the current publication this applies to: Fig. 4a, q; Fig. 5c; Fig. 11a, b, i, q; Fig. 12a-c; Fig. 14h.

Results

Pleurasiga Schiller, 1925

The genus *Pleurasiga* currently comprises only four mutually closely related species (Fig. 3b, d-f) in addition to the enigmatic and only light microscopically studied type species *Pl. orculaeformis* Schiller, 1925 (Fig. 3a). Efforts to recover material from the Adriatic Sea that convincingly mirrors the Schiller type material (Schiller 1925) have been unsuccessful (Leadbeater 1973). However, as previously discussed (Thomsen and Østergaard 2019a, d) there is a striking similarity between *Pl. orculaeformis* and species of *Polyfibula* Manton in Manton and Bremer, 1981, and also to *Campanoeca dilatata* Throndsen, 1974, a species which was described based on light microscopy only (Throndsen 1974). Species of *Pleurasiga* are thus clearly in a standby position to be permanently separated from *Pl. orculaeformis,* and positionally redefined within the loricate choanoflagellate morphospecies matrix.

The *Pleurasiga* lorica is of modest dimensions and constructed from ca. 30 costal strips typically arranged to form two transverse costae of approximately the same size, and seven longitudinal costae. One transverse costa closes the lorica anteriorly, while the second transverse costa (mid-lorica transverse costa) is shifted downwards corresponding to the length of one longitudinal costal strip. It is a characteristic feature of *Pl. minima*, *Pl. reynoldsii*, and *Pl. tricaudata*, that the transverse costae are nearly of the same size, giving the anterior lorica a quasi-cylindrical appearance. The transverse costae are exterior relative to the longitudinal costae. There are 'T-joints' anteriorly and '4-point' abutting joints at the level of the mid-lorica transverse costa. In species of *Pleurasiga* the costal strips comprising the longitudinal costae overlap each other from the posterior forwards. This is in general agreement with standard lorica features as outlined by Leadbeater (2015). *Pleurasiga echinocostata* is considered a core member of the genus

(Leadbeater 2015), despite the fact that it has only a single anteriorly located transverse costa which causes the lorica to be conical in outline. At present only two species, viz. *Pl. minima* and *Pl. reynoldsii* have been sequenced (Nitsche et al. 2017). Not surprisingly they were found to cluster close together. Species of *Pleurasiga* typically possess a flagellum that reaches far out of the lorica. This indicates that the flagellum does not merely serve the purpose of generating flow fields that aid the filtration of food items in a passively floating organism, but rather that species of *Pleurasiga* are true pelagic and actively swimming organisms.

Pleurasiga echinocostata Espeland in Espeland and Throndsen, 1986 (Figs. 3f, 4, 5)

The *P. echinocostata* lorica is constructed from approximately 28 costal strips organized as seven longitudinal costae and a single anteriorly positioned transverse costa (Fig. 4a) where the anterior longitudinal costal strips attach to the inside of costal strips from the transverse ring. The exact number of posterior longitudinal costal strips appears to be variable. The lorica illustrated in Fig. 4a has five costal strips while there are only four in Fig. 5q. An examination of the light micrographs (Fig. 4b-p) also hints at that the number of longitudinal costal strips converging at the posterior lorica end is typically less than seven. However, it remains a possibility that the reduced number of posterior longitudinal costal strips is simply caused by loss of strips during preparation or elsewhere. The conical lorica measures 7.5-10.0 µm in length, while the anterior diameter is 5.0-8.3 µm (Espeland and Throndsen 1986). The warm water specimens examined here (Fig. 4a-l) are slightly larger than those comprising the Norwegian type material (i.e. lorica height: 10.5 ± 0.64 µm, range: 9.4-11.7 µm; transverse costa diameter: 8.5 ± 0.52 µm, range: 7.6-9.4 µm; n = 28). However, with reference to morphological details there is complete agreement between the type material and specimens examined here (e.g. Fig. 4a). More specifically this includes features such as (1) the occurrence of unilateral spines on anterior transverse costal strips, (2) the flattened and slightly forked tip of each anterior longitudinal costa (Fig. 5a, b), which provides structural strength to the positioning of the anterior transverse costal strip ('T'-junctions), and (3) the overlap between the middle and posterior longitudinal costal strips (Fig. 4a, q), bringing about anchor points for the organic membrane that envelopes the protoplast.

In material examined here (Fig. 4a, 5a, b), the spine is always at the right-hand end of a transverse costal strip when viewing the lorica from the outside. This appears to be a shared feature across material sampled from many parts of the world (Leadbeater 1973, loc. cit. Pl. 16e (referred to as *Pleurasiga reynoldsii* aff.); Espeland and Throndsen 1986, loc. cit. Fig. 32; Booth 1990, loc. cit. Fig. 20; Hoepfner and Haas 1990, loc. cit. Fig. 41; Thomsen et al. 1991, loc. cit. Fig. 35; Hara et al. 1997, loc. cit. Fig. 20; Leadbeater 2015, loc. cit. Fig. 4.64; Thomsen et al. 2016, loc. cit. Fig. 8B). The actual shape of the unilateral costal tip elaboration is that of an asymmetrical fork (Fig. 5a, b) where the subterminal upwardly pointing part of the bifurcation is sharply pointed and more or less perpendicular to the main axis of the costal strip. The other part of the fork, representing the termination of the costal strip, is short and with a rounded tip, and deviates only slightly (25-35°) from the overall curvature of the costal strip. The left-hand end of the transverse costal strip (when viewed from the outside) is obliquely cut off (Fig. 5b) to produce a surface area that snugly fits along the lower terminal fork of the adjacent costal strip.

The region-specific size variability is negligible as appears from Fig. 6 (circular symbols).

Pleurasiga echinocostata is easily recognized from LM (Fig. 4b-l). Even the existence of anterior spines is evident from some micrographs (e.g. Fig. 4d, e).

While examining material in particular from West Australia, an aberrant form of *P. echinocostata* (henceforth referred to as form A) was recognized (Fig. 3g; 4m-p). This form is larger (Fig. 6; square symbols) and has a barrel-shaped, rather than a strictly conical lorica. There are still seven longitudinal costae and a characteristic overlap between the middle and posterior longitudinal costal strips. There is a significant morphological and dimensional similarity between the West Australian material (Fig. 4m-p) and a single specimen observed in samples from the Andaman Sea (Fig. 4q; Fig. 6). Notice that the Andaman Sea specimen (Fig. 4q) has unilateral spines similar to *P. echinocostata* sensu stricto, and that these are also at the right-hand end of the transverse costal strip when viewed from the outside of the lorica.

A spine-less variety (henceforth referred to as form B) of *P. echinocostata* (Fig. 3h; Fig. 5c-e) was observed in samples from the Andaman Sea and the equatorial Pacific Ocean. These specimens all fall within the size ranges (lorica height: 9.5-11.2 µm; anterior diameter: 7.8-8.2 µm) that are typical for *P. echinocostata* sensu stricto (Fig. 6). In addition to the absence of anterior spines, these deviant specimens also lack the characteristic major overlap between the middle and the posterior longitudinal costal strips, that in *P. echinocostata* sensu stricto produces distinct anchor points to the organic membrane encasing the protoplast. Hara et al. (1997) illustrates (loc. cit. Fig. 21) and comments on the presence in the same Taiwanese water sample of *P. echinocostata* sensu stricto and the spine-less form.

Apart from drawing attention to the two morphologically deviant forms (here labelled form A and B respectively) of *P. echinocostata,* it is premature to taxonomically separating them from *P. echinocostata* sensu stricto. Molecular tools and evidence will obviously be needed to support a more confirmative decision with reference to the phylogenetic relationship between these morphotypes. From a purely morphological point of view it appears likely that the form B, which differs from *P. echinocostata* sensu stricto with reference to two major characteristics, i.e. the lack of anterior spines and the absence of a major costal strip overlap in the middle part of the lorica, will eventually be singled out as a separate taxon. It is in our opinion more unclear whether the size difference and variability in overall lorica shape, as noticed between *P. echinocostata* sensu stricto and form A, will be sufficient to similarly support the description of a new taxon.

Distribution: *Pleurasiga echinocostata* sensu stricto has previously been recorded from the Adriatic Sea (Leadbeater 1973; as *Pleurasiga reynoldsii* aff); Kilsfjorden, Norway (Espeland and Throndsen 1986); the North Pacific Central Gyre (Hoepfner and Haas 1990); the Subarctic North Pacific (Booth 1990); central Californian waters (Thomsen et al. 1991); the equatorial Pacific Ocean (Vørs et al. 1995); Japanese and Taiwanese coastal waters (Hara et al. 1997); Sydney Harbour, Australia (Tong et al. 1998); north-west of South Georgia, Southern Ocean (Leakey et al. 2002); Danish coastal waters (Thomsen et al. 2016); and the Beagle Channel, S. America (Thomsen, unpublished). The findings reported here are summarized in Table 1. Despite extensive collection work reported from both polar regions (e.g. Thomsen and Østergaard 2017; Thomsen et al. 1997) it is worth pointing out that *Pl. echinocostata* has so far not been recorded at latitudes >60° in either hemisphere.

Pleurasiga quadrangiella sp. nov. (Figs. 3i, j, 7, 8)

Diagnosis: Conical lorica (9-10 µm) comprising seven longitudinal costae and two transverse costae, i.e. an anterior ring (seven costal strips; diam. 7.5-8.5 µm) where 'T-junctions' unite transverse and longitudinal costal strips, and a posterior transverse costa (four costal strips; diam. 3-4 µm) located at the level of the junctions between the middle and posterior longitudinal costal strips. Anterior transverse costal strips are without unilateral spines. The anterior tip of a longitudinal costa is flattened and slightly biforked. The protoplast is located posteriorly and secured by a membrane suspended from the free posterior tips of the middle layer longitudinal costal strips. The flagellum is conspicuous and reaches far outside the lorica.

Holotype: The specimen illustrated in Fig. 7a of the present work is fixed as holotype (ICZN 1999, Article 73.1.4).

Type locality: Surface water sample collected 14 Sept. 1981 from the pier at the Phuket Marine Biological Center (PMBC), Andaman Sea, Thailand (28°C; 35 PSU).

Etymology: The species-group name is chosen to emphasize the small quadrangular posterior transverse costa; from ‘quadrangulus’ and ‘-ella’ diminutive.

The main distinguishing feature between *Pl. echinocostata* and *Pl. quadrangiella* is the presence of a posterior transverse costa in the latter.

The posterior transverse costa is in *Pl. quadrangiella* inside the longitudinal costae. This is particularly evident from Fig. 7c, 8a, b. In Fig. 8b an arrow points to costal strip junctions where the longitudinal element is evidently exterior to the transverse costal strips. The arrowhead (Fig. 8b) points to a transverse costal strip that is sandwiched between longitudinal costae. The possibility that the posterior transverse costa is nothing but randomly re-positioned longitudinal costal strips is highly unlikely, considering the regularity in appearance of the costa (Fig. 7c, d), and the fact that the transverse costal strips forming this costa are morphologically differing in e.g. thickness from the neighbouring longitudinal costal strips. There is no evidence supporting that the internal posterior transverse costa of *Pl. quadrangiella* should be homologous with the external mid-lorica transverse costa of other species of *Pleurasiga*. Apart from being internal and external to the longitudinal costae respectively, it can be added that in *Pl. quadrangiella* the transverse costa is at the base of the middle longitudinal costal strip, whereas in other species of *Pleurasiga* the transverse costa is at the level between the upper and middle longitudinal costal strip. A further difference is that while in other species of *Pleurasiga* the mid-lorica transverse costa has the same number of costal strips as the anterior transverse costa, it applies to *Pl. quadrangiella* that the posterior transverse costa in this species comprises fewer costal strips.

Posterior longitudinal costal strips are markedly reduced in number in *Pl. quadrangiella.* The lorica illustrated in Fig. 7c has two posterior longitudinal costal strips, while there are three longitudinal costal strips adjoining posteriorly in Fig. 7a. This becomes obvious only when the image is digitally manipulated. In the lorica depicted in Fig. 8a, b there is only a single posterior longitudinal costal strip discernable. In some of the light micrographs (Fig. 7d, g, h) there are no posterior longitudinal costal strips visible. It thus appears that a noticeable reduction in the number of posterior longitudinal costal strips is an innate lorica feature in *Pl. quadrangiella* in parallel with observations discussed above with reference to *Pl. echinocostata*.

Recognition of this taxon in the light microscope is easy when examining an empty lorica (Fig. 7d) and possible in most cases also when a protoplast is present (Fig. 7e-i).

In addition to the Andaman Sea, Thailand, this species is also observed in samples from the equatorial Pacific Ocean, and West Australia (Table 1).

Pleurasiga minima Throndsen, 1970 (Figs. 3b, 9, 16)

The *Pl. minima* lorica invariably comprises seven longitudinal costae each consisting of three costal strips. Overlaps between longitudinal costal strip one and two produce distinct anchoring points (Fig. 9a) for the membrane that envelopes the posteriorly positioned protoplast and part of the collar. All seven longitudinal costae adjoin posteriorly in an undamaged lorica (see e.g. Fig. 9a, e, f). There are two transverse costae separated by the tier of anterior longitudinal costal strips. In the anterior ring the longitudinal costae attach midway along each of the transverse costal strips, forming ‘T-joints’ (Fig. 9a). Costal strips from the mid-lorica transverse costa join with longitudinal costal strips in ‘4-point’ abutting joints mostly with short overlaps. Both transverse costae are exterior relative to the longitudinal costae (Fig. 9a).

The lorica height is in our material 15.6 ± 1.4 µm (range: 12.1-19.8 µm; n = 68). The diameter of the anterior transverse costa is 12.3 ± 0.8 µm (range: 9.8-15.3 µm; n = 68), and thus marginally smaller than the mid-lorica transverse costa (12.7 ± 1.1 µm; range: 9.5-16.6 µm; n = 68). There are no obvious regional size differences across the specimens examined (Fig. 10a) except for the occurrence of two unusually large specimens from West Australia. The flagellum protrudes far beyond the lorica (Fig. 9c).

The anterior tips of longitudinal costae have bifurcations (Fig. 9j) that greatly enlarges the contact area between adjoining costal strips. Anterior transverse costal strips often have unilateral spines (Fig. 9j) much similar to those described above from *Pl. echinocostata* (Fig. 5a, b). The spine is, as was the case also with reference to *Pl. echinocostata*, located at the right-hand end of a costal strip when viewed from the outside of the lorica (Fig. 9a, j). A literature search confirms the generality of this lorica feature (see e.g. Leadbeater 1973, loc. cit. Pl. 16b (identified as *Pl. reynoldsii* aff.); Booth 1990, loc. cit. Fig. 21 (identified as *Pl*. cf. *minima*); Tong 1997a, loc. cit. Fig. 5a, d). It is unclear whether the presence (Fig. 9a) or absence (Fig. 16b) of spines on anterior transverse costal strips in specimens of *Pl. minima* is in fact signaling that we are dealing with two separate taxa rather than e.g. temperature induced morphotypes within a single species. Cells with spines are most frequently reported from warm water habitats and virtually absent in *Pl. minima* specimens from high latitude regions (i.e. > 60° North and South).

The recognition of this taxon from light microscopy is straightforward (Fig. 9b-i, k-p).

Distribution: *Pleurasiga minima* has a genuine cosmopolitan distribution being reported in most loricate choanoflagellate surveys from all biogeographical provinces of the oceans, perhaps with the exception of brackish water sites, such as the innermost parts of the Baltic Sea with salinities below 10 PSU (Thomsen, unpublished results).

Pleurasiga minutissima sp. nov. (Figs. 3c, 11-13)

Diagnosis: Lorica 10.6 ± 1.3 µm long (range: 8.2-13.0 µm; n = 67) comprising seven longitudinal and two transverse costae. One costa forms an anterior ring (diam. 8.9 ± 0.85 µm; range: 7.3-10.6 µm; n = 67) where transverse and longitudinal costal strips form 'T-joints'. The mid-lorica transverse costa is located at the junctions between the anterior and middle longitudinal costal strips. This ring is smaller (6.9 ± 0.85 µm; range: 5.2-8.8 µm; n = 67) and with pronounced overlaps between neighbouring strips. Both transverse costae are located exterior to the longitudinal costae. The anterior tip of a longitudinal costa is flattened and slightly biforked. Anterior transverse costal strips are furnished with unilateral spines. The protoplast is located posteriorly and surrounded by a membranous sheet. The flagellum is 2-3 times longer than the lorica.

Holotype: The specimen illustrated in Fig. 11a of the present work is fixed as holotype (ICZN 1999, Article 73.1.4).

Type locality: Water sample (15m depth) collected 24 March 1996 at #31 (9.14.31° N / 97.21.12° W), Andaman Sea, Thailand. The maximum depth at the sampling site is 200 m.

Etymology: The species-group name chosen highlights the fact that this species is similar to *Pl. minima,* yet significantly smaller and with a reduced mid-lorica transverse costa diameter.

Pleurasiga minutissima is on a superficial view much similar to *Pl. minima.* The two species share basic morphometric features such as the presence of seven longitudinal costae (each costa comprising three costal strips), and two transverse costae (seven costal strips each) forming an anterior ring and a second ring located posteriorly at a distance of one longitudinal costal strip. Transverse costae are in both species exterior to the longitudinal costae. There are in both species 'T-joints' anteriorly and variations on the '4-point' abutting overlap theme posteriorly. Flattened and biforked anterior tips on longitudinal costae and unilateral spines on anterior transverse costal strips (right hand end of the strip when viewed from the outside) are further shared features.

The most conspicuous differences between the two species refer to (1) lorica height (Fig. 10c) where *Pl. minutissima* is distinctly smaller (10.6 µm in *Pl. minutissima* vs. 15.6 µm in *Pl. minima*) and (2) transverse costae diameter (Fig. 10d). Those of *Pl. minutissima* are generally smaller (8.9 µm vs. 10.3 µm) and also displaying a noticeable mutual size difference, with the mid-lorica transverse costa being significantly smaller (6.9 µm). In *Pl. minima* the two costae are almost of the same size, yet with the mid-lorica transverse costa being marginally larger. The *Pl. minima* lorica chamber is thus almost parallel sided, whereas cone-shaped in *Pl. minutissima*. There are no obvious regional size differences across the specimens examined (Fig. 10b).

The *Pl. minutissima* lorica is sometimes terminated by a posterior spine in the shape of a single costal strip that protrudes fully or partially beyond the point where the longitudinal costae adjoin (Fig. 11n-p, 13b). Whereas *Pl. minima* is characterized by a posterior amalgamation of all seven longitudinal costae there appears to be in *Pl. minutissima* a certain reduction in the number of posterior longitudinal costal strips (Fig. 11a: 4; Fig. 11b: 5).

It has been verified from all TEM and SEM micrographs available to us, that the mid-lorica transverse costa is exteriorly located relative to the longitudinal costae. It is similarly evident in undamaged specimens that the flattened and biforked anterior tips of the longitudinal costae attach to the inside of anterior transverse costal strips.

Patterns of costal strip junctions have been intensively discussed by Thomsen and Østergaard (2019d) referring back to observations summarized by Leadbeater (2015) which lead to the formulation of rules of lorica assembly of likely universal validity across major parts of the loricate choanoflagellate species matrix. The lorica assembly model as outlined by Leadbeater (2015) is as follows when focusing in particular on the mid-lorica transverse costa. In this costa, when seen from the outer surface and in a clockwise direction the left-hand end of a costal strip overlaps the right-hand end of the adjacent costal strip. When the junction interacts with a longitudinal costa the triangle is on the right-hand side of the longitudinal costa. When the transverse costae are seen from the inner surface of the lorica the respective triangles are located on the left-hand side of a longitudinal costa. It should be noted that the small triangles referred to are artefactual but are, nevertheless, very consistent in location.

However, when scrutinizing images of *Pl. minutissima* as depicted here (in particular Fig. 11a, b, 12a, 13a-c) it is evident, when seen from the outer surface and in a clockwise direction, that the left-hand end of a costal strip underlaps the right-hand end of the adjacent strip, and further that the ‘triangles’ are located to the left of a longitudinal costa. This deviation in terms of symmetry is unexpected and obviously in need of further confirmation. When carefully examining some of the light micrographs of this species (Fig. 11k, l, n; encircled) it is possible to observe costal strip patterns that support the evidence extracted from e.g. Fig. 11a. The most convincing of these is Fig. 11n where it appears completely obvious that the costal strip junction encircled is located in the proximal part of the lorica. The fortunate finding of cells of *Pl. minutissima* examined in a scanning electron microscope (Fig. 13b, c) convincingly corroborates the fact that there is in *Pl. minutissima* a reversal of the costal strip pattern with reference to the mid-lorica transverse costa. While the arrowhead (Fig. 13b) points to a junction in the proximal part of the lorica and thus seen from the outside, the arrows (Fig. 13b) similarly point to junctions seen from the inside of the lorica. We have added (Fig. 13c) a South Pacific specimen of *Pl. minutissima* that occurs immediately next to a specimen of *Cosmoeca ventricosa* form B (Thomsen and Østergaard 2019d). The *Pl. minutissima* specimen displays (Fig. 13c; circles) the exact same variant costal strip pattern as described above, while the *C. ventricosa* specimen (Fig. 13c; squares) adheres to the principles as detailed by Leadbeater (2015). Further evidence can finally be extracted from a New Zealand image (Fig. 13a; courtesy of Dr. Øjvind Moestrup) where costal strip junctions, when observed from the outside, are encircled using a heavy line, while junctions as observed from the inside of the lorica are encircled using a thin line (Fig. 13a). In both cases the patterns are identical to those described above.

It is important to emphasize that the mere identification of the new species from any kind of microscopy is not dependent on that the mid-lorica transverse costal strip pattern is fully resolved. Overall size differences between this species and *Pl. minima* in combination with the much reduced mid-lorica transverse costa diameter in *Pl. minutissima* are sufficient diagnostic features.

It is obvious from any TEM and SEM image of *Pl. minutissima* that this species shares with *Pl. echinocostata*, and *Pl. minima* the fact that the spine is placed at the right-hand end of a transverse costal strip when viewed from outside the lorica.

Pleurasiga minutissima is widespread across the global warm water belt (Table 1) and additionally observed in samples from the Pettaquamscutt river estuary, Rhode Island, USA

(Menezes 2005; loc. cit. Pl. VIII A identified as *Pl. minima*), New Zealand (Fig. 13a), as well as the South Atlantic (Fig. 13b) and South Pacific Oceans (Fig. 13c).

Pleurasiga reynoldsii Throndsen, 1970 (Fig. 3d, 14a-e)

This species was infrequently observed in samples from the warm water habitats visited. The specimens collected are marginally smaller than those from the Norwegian type material (Throndsen 1970). The lorica height in our material ranges from 19.5 to 22.0 µm (type material: 23 µm). It is a characteristic feature of *P. reynoldsii* that the two transverse costae are almost of equal size, but also that the mid-lorica transverse costa is consistently larger than the anterior transverse costa (18.0-19.5 versus 14.5-16.0 µm in our material). Throndsen (1970) simply states that the maximum diameter is 23 µm. *Pleurasiga reynoldsii* is distinguished from *P. minima* based on overall size, the differently sized transverse costae in *P. reynoldsii*, and the amalgamation into pairs of six out of seven longitudinal costae at the posterior lorica end.

Distribution: *Pleurasiga reynoldsii* has a cosmopolitan distribution being previously observed in samples from Bear Island and Nordåsvatnet, Norway (Throndsen 1970, 1974), Resolute Bay, Canada and Disko Bay, Greenland (Manton et al. 1976), Western Baltic Sea, Denmark (Thomsen 1976; Thomsen et al. 2016), Kaikoura, New Zealand (Moestrup 1979), Prydz Bay and Weddell Sea, Antarctica (Marchant 1985; Thomsen and Larsen 1992), central Californian waters, USA (Thomsen et al. 1991), Igloolik, Canada (Daugbjerg and Vørs 1994), Southampton, UK (Tong 1997b), Darwin, Shark Bay and Sydney Harbour, Australia (Lee et al. 2003; Tong 1997a; Tong et al. 1998), St. Lawrence, Canada (Bérard-Therriault et al. 1999), Bering Sea (Sukhanova 2001), NEW, NE Greenland (Thomsen and Østergaard 2017), and Beagle Channel, Patagonia (Thomsen, unpublished results). Here reported from the Gulf of California, the equatorial Pacific Ocean, West Australia and the Sargasso Sea (Table 1).

Pleurasiga tricaudata Booth, 1990 (Fig. 3e, 14e-h)

Pleurasiga tricaudata is similar to *Pl. minima* in all basic morphometric features. The distinguishing feature is the compound pedicel that consists of typically three costal strips that flare out like a tripod (Fig. 14e, h). The species is easily recognized from LM (Fig. 14f, g). In *Pl. tricaudata* the anterior tip of middle layer longitudinal costal strips is also bifurcated (Fig. 14e, h). This also occasionally applies to posterior longitudinal costal strips (Fig. 14h; arrow). Both the anterior and the mid-lorica transverse costa are exterior relative to the longitudinal costae (Fig. 14e). There are '4-point' abutting joints at the level of the mid-lorica transverse costae. However, these are slightly more elaborate than just abutting which reveals that the joint pattern is similar to that described above for *Pl. minutissima*. The left-hand end of a transverse costal strip, when viewed in a clockwise direction, clearly underlaps the neighboring strip (Fig. 14e; arrows). The posterior longitudinal costal strips are tapering from the posterior towards the anterior end (Fig. 14e). The costal strips forming the compound pedicel are exactly similar to these, yet turned upside down. It is tempting to speculate that the cell, when assembling its lorica, has at its disposal a total of seven posterior longitudinal costal strips, which in this species equals the common denominater for lorica components. The seven costal strips are typically distributed with two or three costal strips entering the compound pedicel, and the remaining four or five costal strips forming the posterior lorica chamber. While the distribution was 3:4 in the type material (Booth 1990), we have encountered mostly specimens with a 2:5 distribution (Fig. 14e, h). Notice that in Fig. 14e one posterior longitudinal costal strip is doubled. No anterior unilateral spines have so far been reported for *Pl. tricaudata*.

Distribution: Previously recorded from the subarctic Pacific Ocean (Booth 1990), central Californian waters, USA (Thomsen et al. 1991), Sydney Harbour, Australia (Tong et al. 1998), St. Lawrence, Canada (Bérard-Therriault et al. 1999), New Zealand (Moestrup, unpublished results), and Beagle Channel, Patagonia (Thomsen, unpublished results). Here we report findings of *Pl. tricaudata* from the Gulf of California (Table 1).

Parvicorbicula Deflandre, 1960

Within the genus *Parvicorbicula* (replacement name for *Corbicula* Meunier, 1910), the situation is quite the opposite of what applies to *Pleurasiga*. We are here confronted with a well-defined type species, *P. socialis* (Meunier, 1910) Deflandre, 1960 (Fig. 3k), that despite being first described using light microscopy only (Meunier 1910), has been convincingly redefined using electron microscopical techniques (e.g. Buck 1981; Manton et al. 1976; Thomsen 1973). It is additionally possible to select a handful of species of *Parvicorbicula* (i.e. *P. quadricostata* Throndsen, 1970 (Fig. 3l); *P. circularis* Thomsen, 1976 (Fig. 3m); *P. corynocostata* Thomsen, Garrison and Kosman, 1997 (Fig. 3n), and possibly also *P. manubriata* Tong, 1997 (Fig. 3o)) that, from a morphological point of view, form a fairly well-defined cluster (core group of species) in association with the type species. In these species there are 4-10 longitudinal costae and two transverse costae, one of which closes the lorica anteriorly.

The genus additionally comprises (Fig. 3p-x) eight species (i.e. *Pa. pedicellata* Leadbeater, 1973 (Fig. 3p); *Pa. serrulata* Leadbeater in Manton et al., 1975 (Fig. 3q); *Pa. pedunculata* Leadbeater, 1980 (Fig. 3r); *Pa. ongulensis* Takahashi, 1981 (Fig. 3s); *Pa. superpositus* Booth, 1990 (Fig. 3t); *Pa. zigzag* Thomsen in Thomsen et al., 1991 (Fig. 3u); *Pa. pachycostata* Thomsen in Thomsen et al., 1997 (Fig. 3v), and *Pa. aculeatus* Tong, 1997 (Fig. 3x)) that have been placed here in the lack of better choices and also in consideration of not establishing too many monotypic genera while in the process of describing basic loricate choanoflagellate diversity. A redefinition of the genus *Parvicorbicula* focusing in particular on the core group of species will thus leave us with a large contingent of species that will have to be moved elsewhere. Molecular data only exist for *Pa. pedunculata* (Nitsche et al. 2011) and show that this species is phylogenetically far removed from both *Pl. minima* and *Pl. reynoldsii* (Nitsche et al. 2017). It currently clusters with species of *Acanthocorbis* and *Stephanoeca* which is not completely unexpected based on elements of similarity in certain lorica features.

Parvicorbicula zigzag is the only *Parvicorbicula* species that is abundantly present in warm water habitats. This species deviates markedly from the core species of *Parvicorbicula*. It is, however, from a lorica constructional point of view almost identical to *Stephanacantha parvula* Thomsen in Thomsen and Boonruang, 1983. Despite the fact that *Stephanacantha* as presently circumscribed (Thomsen and Boonruang 1983) comprises only species with flattened and elaborate costal strips, it is likely that in a phylogenetic perspective lorica constructional details will be more decisive than the actual elaboration of the individual costal strip. A formal transfer of *Pa. zigzag* to the genus *Stephanacantha* is planned for the next following publication in this series.

Two further species, viz. *Pa. pedicellata* (Fig. 3p) and *Pa. superpositus* (Fig. 3t), were commonly observed but are better dealt with in a later publication that focuses on taxa with free anterior spines.

Parvicorbicula socialis (Meunier, 1910) Deflandre, 1960 (Fig. 3k, 15a)

This is the type species of the choanoflagellate genus *Corbicula* Meunier, 1910. However, due to the fact that the genus name was preoccupied (*Corbicula* Mergerle, 1811), Deflandre (1960) later provided a substitute name (*Parvicorbicula* Deflandre, 1960) for the loricate choanoflagellate taxon.

The funnel-shaped lorica comprises 10 longitudinal costae and two transverse costae. Only solitary specimens were observed.

Distribution: Very frequently reported at latitudes >40° North and South. *Parvicorbicula socialis* becomes a community dominant species at high latitudes in both hemispheres and is often forming huge colonies (Escalera et al. 2019). The number of previous recordings from warm water habitats are extremely sparse comprising only Lyons, Mediterranean Sea (Pavillard 1917), the Cape Town region, S. Africa (Manton et al. 1976), and the Red Sea (Thomsen 1978). Here we report the species from West Australia only (Table 1).

Parvicorbicula circularis Thomsen, 1976 (Fig. 3m, 15b-e)

Parvicorbicula circularis is closely related to *Pa. quadricostata* Throndsen, 1970. However, in this species the mid-lorica transverse costa is square and comprising only four costal strips (Throndsen 1970). In *Pa. circularis* the mid-lorica transverse costa is circular comprising 6-8 transverse costal strips (Thomsen 1976; Thomsen et al. 1990). The specimens reported on here all possess two equally large transverse costae (Fig. 15b-e).

Distribution: Previously recorded from Danish coastal waters (Thomsen 1976; Thomsen et al. 2016): the Baltic Sea proper (Thomsen 1979); Lützow-Holm Bay, Prydz Bay, Davis, King George Island, Weddell Sea, Antarctica (Buck and Garrison 1988; Chen 1994; Marchant 1985; Marchant and Perrin 1990; Takahashi 1981;Thomsen et al. 1991); the Subarctic North Pacific (Booth 1990); central Californian waters (Thomsen et al. 1991); Disko Bay, North East Water, Greenland (Thomsen and Østergaard 2017; Thomsen et al. 1995); the equatorial Pacific Ocean (Vørs et al. 1995); Southampton, UK (Tong 1997b); Newfoundland, Canada (McKenzie et al. 1997); Shark Bay and Sydney Harbour, Australia (Tong 1997a; Tong et al. 1998); Beagle Channel, Patagonia (Thomsen, unpublished). Here we report findings of *Pa. circularis* from five out of seven regions sampled (Table 1).

Discussion

It is evident from the above that the current taxonomy of both *Pleurasiga* and *Parvicorbicula* is confused and in need of revision. In an attempt to circumscribe morphologically, and hence also phylogenetically, well-defined core group species selected from both genera, Leadbeater (2015) listed the following taxa: *Parvicorbicula socialis*, *Pa. quadricostata*, *Pa. circularis*, *Pa. corynocostata*, *Pleurasiga minima*, *Pl. reynoldsii*, and *Pl. tricaudata* as being distinct from the majority of other tectiform species based on that they have both the anterior and, more significantly, the lower transverse costa on the outer surface of the lorica. This cluster of species shares additional lorica features, i.e. longitudinal costae composed of three costal strips, two transverse costae (an anterior ring and a second transverse costa at the join between the second and third longitudinal costal strip), a membrane enveloping the protoplast and suspended from the free posterior tips of the middle layer longitudinal costal strips, anterior ‘T-junctions’ between longitudinal and transverse costal strips, and a flattened and slightly biforked termination of the tip of the anterior longitudinal costal strips. Leadbeater (2015) further adds *Pl. echinocostata* to the core group of species listed above, despite the absence of a mid-lorica transverse costa in this species, but with reference to a great overall similarity with *Pl. minima*. Also *Pa. corynocostata* and *Pa. manubriata* are identified as potential members of this clustering despite minor differences in lorica features.

There is little doubt that the grouping of species as suggested by Leadbeater (2015), although likely with minor modifications, will eventually also be supported by molecular evidence. Moving all species of *Pleurasiga*, with the exception of the type species *Pl. orculaeformis*, to the genus *Parvicorbicula* will solve the *Pleurasiga* enigma by leaving this as a monotypic genus. However, a redefinition of the genus *Parvicorbicula* as indicated above, to accommodate only a small selection of species from both genera, will as previously pointed out, leave many additional species in jeopardy. Despite basically agreeing with the view put forward by Leadbeater (2015) we are inclined to take a conservative approach here while awaiting (1) further morphological analyses of any single species from the cluster of outskirt species of *Parvicorbicula* (to enable qualified decisions about their future positioning in the morphospecies matrix), and (2) molecular evidence that can support the choice of classification scheme.

The finding of reversed patterns of costal strip junctions in *Pl. minutissima* sp. nov*.,* when comparing with the generalized picture as outlined by Leadbeater (2015), is exceptional and unexpected. We are painfully aware of that we have in the past had to deal with mirror-image issues in part of the material collected from warm water habitats and examined using different microscopes (Thomsen and Østergaard 2019d) resulting in a decision to flip images from one particular microscope horizontally. While this produced images of e.g. *Cosmoeca* that from a constructional point of view confirmed previous observations, and thus also corroborated the general picture as outlined by Leadbeater (2015), it did at the same time produce images of *Pl. minutissima* that clearly have a reversed symmetry. Fortunately, we have been able to verify, based on SEM and TEM images from alternative sources (Fig. 13a-c), that this is in fact the general picture in this species. It is tempting to speculate that the reversed pattern is somehow connected to the fact that the mid-lorica transverse costa is exterior relative to the longitudinal costae. Other species that share this feature with *Pl. minutissima*, i.e. the external positioning of both transverse costae, have ‘4-point’ abutting joints at the level of the mid-lorica transverse costa. This means that a transverse costal strip stretches from one longitudinal costa to the next without much overlap, and without leaving many options for a detailed study of any repetitiveness in costal strip joint patterns. From a purely morphological point of view *Pl. minutissima* is much similar to *Pl. minima*. A search for images of *Pl. minima* (also from outside the warm water habitats) that would at least allow for a preliminary inter-specific comparison of costal strip patterns at the level of the mid-lorica transverse costa, has resulted in the finding of a few specimens, in which the ‘4-point’ abutting joints are slightly more elaborate than just abutting. The pattern observed is illustrated here based on material from Danish coastal waters (Fig. 16). The enlarged lorica segment (Fig. 16a) and the schematic drawing (Fig. 16c), show the lorica joint patterns as seen from both the outside and the inside of the lorica. The mid-lorica transverse costa is obviously external relative to the longitudinal costa, and the left-hand end of a transverse costal strip, when viewed in a clockwise direction, clearly underlaps the neighboring strip and also projects beyond the longitudinal costal strip indicating that this is where the artificial triangle would form in case the costal strip overlaps had been any larger. This is thus basically the same pattern as observed in *Pl. minutissima.* It is unclear to us how profound this difference in symmetry of costal strip patterns is, and also what sort of consequences this will have with reference to e.g. our understanding of lorica formation principles and hence also our morphospecies based loricate choanoflagellate classification schemes. An essential additional question that needs to be addressed is what are the possible relative benefits of a transverse costa being inside or outside the lorica chamber.

Costal strips comprising the longitudinal costae of *Pleurasiga* species overlap each other from the posterior forwards and thus confirms to the general pattern as detailed by Leadbeater (2015). The overlap between longitudinal costal strip one and two is particularly evident in most species and morphotypes (except in *Pl. echinocostata* form B) and provides points of attachment for the membranous sheath that surrounds the protoplast and part of the collar.

A final major point to address here is the finding that a right-hand positioning of the anterior spine on anterior transverse costal strips (when viewed from the outside of the lorica) appears to be a universal feature of species of *Pleurasiga* (here documented for *Pl. echinocostata*, *Pl. minima* and *Pl. minutissima*). A literature search has already confirmed the generality of this lorica feature in *Pl. echinocostata* and *Pl. minima* (see above). Manton et al. (1976; loc. cit. Figs 61, 62) documented that also in *Pl. reynoldsii* the anterior transverse costal strips may occasionally carry minute spines at the right-hand of a costal strip, when viewed from the outside of the lorica.

Author contribution statement

Helge A. Thomsen (HAT) has undertaken a major part of the sampling activities and the subsequent microscopical analyses. HAT is further responsible for compiling and writing the paper. Jette B. Østergaard (JBO) has been much involved in the Andaman Sea and the Pacific Ocean sampling. JBO has also carried out part of the transmission electron microscopical examination of these samples. Nina A. Kamennaya (NAK) and Mikhail V. Zubkov (MVZ) have provided SEM images of *Pl. minutissima* and commented on the drafts of the manuscript.

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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. *Pleurasiga minima* TEM whole mount labelled to introduce loricate choanoflagellate terminology. Micrograph from central Californian waters (RV 'Point Sur' cruise; see Thomsen et al. 1991).

Fig. 3.a-x. Drawings to approximate scale of *Pleurasiga* and *Parvicorbicula* species. **(a)** *Pleurasiga orculaeformis* (type species); **(b)** *Pl. minima*; **(c)** *Pl. minutissima* sp. nov.; **(d)** *Pl. reynoldsii*; **(e)** *Pl. tricaudata*; **(f)** *Pl. echinocostata*; **(g)** *Pl. echinocostata* form A; **(h)** *Pl. echinocostata* form B; **(i, j)** *Pl. quadrangiella* sp. nov.; **(k)** *Pa. socialis* (type species); **(l)** *Pa. quadricostata*; **(m)** *Pa. circularis*; **(n)** *Pa. corynocostata*; **(o)** *Pa. manubriata*; **(p)** *Pa. pedicellata*; **(q)** *Pa. serrulata*; **(r)** *Pa. pedunculata*; **(s)** *Pa. ongulensis*; **(t)** *Pa. superpositus*; **(u)** *Pa. zigzag*; **(v)** *Pa. pachycostata*; **(x)** *Pa. aculeatus*.

Fig. 4.a-q. *Pleurasiga echinocostata* TEM (a) and LM (b-l; phase contrast, except h (NIC)) and *P. echinocostata* form A TEM (q) and LM (m-p) whole mounts from the Gulf of California (a-c), the Sargasso Sea (d-f), the equatorial Pacific Ocean (g), the Andaman Sea (h, q), West Australia (i-k, m-p), and the Caribbean Sea (l). **(a)** Empty lorica (reversed printing) showing basic lorica features; notice the posterior membrane which is suspended by the protruding tips of longitudinal costal strips; **(b-l)** Selected light micrographs to illustrate the diversity encountered when examining

specimens from a wide range of localities under low magnification; **(m-p)** Aberrant forms (*P. echinocostata* form A) encountered in samples from West Australia; **(q)** Complete lorica of form A specimen; notice the anterior spines and the overlap between middle lorica longitudinal costal strips. The scale bar (h) applies to all LM images.

Fig. 5.a-e. *Pleurasiga echinocostata* (a, b) and *P. echinocostata* form B (c-e) SEM (a) and TEM (b-e) whole mounts from Danish coastal waters (a), the Andaman Sea, Thailand (b, d, e) and the equatorial Pacific Ocean (c). **(a)** Anterior transverse costa showing details of spines and the attachment between transverse and longitudinal costal strips; **(b)** High magnification (reversed printing) of a single transverse costal strip viewed from the outside of the lorica; **(c-e)** Complete form B loricae; notice the absence of both spines and extended costal strip overlaps in the lower mid lorica region; reversed printing (d).

Fig. 6. Graph illustrating the relationship between lorica height and the diameter of the anterior transverse costa, as well as differences between geographic regions sampled in *Pleurasiga echinocostata* sensu stricto (circular markers) and *Pl. echinocostata* form A (square markers).

Fig. 7.a-i. *Pleurasiga quadrangiella* TEM (a, c) and LM (b, d-i; phase contrast except b (NIC)) whole mounts from the Andaman Sea (a-c), West Australia (d-g), and the Equatorial Pacific Ocean (h, i). **(a)** Complete cell (holotype) with protoplast, collar and flagellum; **(b, d-i)** Selected specimens to show the morphological variability encountered; **(c)** Empty lorica showing costal strip details; notice the membrane that envelopes the protoplast; costal strips terminating the lorica posteriorly are limited in number (2). The scale bar (i) applies to all light micrographs.

Fig. 8.a-b. *Pleurasiga quadrangiella* TEM micrographs from the Andaman Sea, Thailand. **(a)** Complete cell with protoplast, collar and a well-defined posterior transverse costa; **(b)** Detail from a (reversed printing); the arrow points to costal strip junction where the transverse costal strips are unmistakably inside the longitudinal costae; the arrowhead marks a transverse costal strip located between a proximal (to the right) and a distal (to the left) longitudinal costa.

Fig. 9.a-p. *Pleurasiga minima* TEM (a), SEM (j) and LM (b-i, k-p; phase contrast except b (NIC)) whole mounts from the Andaman Sea, Thailand (a, b, j), the Sargasso Sea (c, e), the Gulf of California, Mexico (d), West Australia (f-h), the Caribbean Sea (i, k), and the equatorial Pacific Ocean (l-p). **(a)** Details of lorica; notice that the seven longitudinal costae meet posteriorly; **(b-i, k-p)** Selected specimens to show the morphological variability encountered; **(j)** Detail of anterior transverse and longitudinal costal strips viewed from the inside of the lorica. The scale bar (g) applies to all light micrographs.

Fig. 10.a-d. Diagrams illustrating **(a)** the *Pl. minima* site-specific relationship between the diameter of the anterior transverse costa (x-axis) and the mid-lorica transverse costa (y-axis); **(b)** the *Pl. minutissima* site-specific relationship between the diameter of the anterior transverse costa (x-axis) and the mid-lorica transverse costa (y-axis); **(c)** lorica height in *Pl. minima* and *Pl. minutissima*; **(d)** a comparison between *Pl. minima* and *Pl. minutissima* (diameter of anterior transverse costa versus diameter of mid-lorica transverse costa); notice the well-defined slopes of the trend lines calculated.

Fig. 11.a-q. *Pleurasiga minutissima* TEM (a, b, i, q) and LM whole mounts (c-h, j-p; phase contrast) from the Andaman Sea (a), the Gulf of California (b, h, i, m), the Sargasso Sea (c, f, g), West Australia (d, j-l, n-p), the Caribbean Sea (e), and the equatorial Pacific Ocean (q). **(a, b, i)** Complete cells showing details of lorica construction; the encircled areas (a) show costal strip junctions as seen from the outside of the lorica; arrows (b) show costal strip junctions as seen from the inside of the lorica; **(c-h, j-p)** Selected micrographs to show the morphological variability encountered; **(q)** High magnification of anterior costal strips to show the spines on transverse strips and the bifurcated termination of the longitudinal strip. Notice that one transverse costal strip quite unusually has been completely turned around so that two spines appear together. The scale bar (c) applies to all light micrographs.

Fig. 12.a-i *Pleurasiga minutissima* TEM (a-c) and LM whole mounts (d-i; phase contrast) from the equatorial Pacific Ocean. **(a-c)** Complete cells documenting costal strip features; **(d-i)** Selection of micrographs showing the variability encountered. The scale bar (i) applies to all light micrographs.

Fig. 13.a-c. *Pleurasiga minutissima* TEM (a) and SEM (b, c) micrographs from New Zealand (a; courtesy of Øjvind Moestrup) and the South Atlantic (b) and South Pacific (c) oceans; **(a)** Reverse printing of a complete cell; the arrowheads point to proximal longitudinal costae, and the arrows to distal longitudinal costae; costal strip junctions as seen from the outside (thick line) and inside of the lorica (thin line) are encircled; see text for further explanation; **(b)** Complete lorica with intact protoplast; the arrows point to costal strip junctions in the mid-lorica transverse costa viewed from the inside of the lorica; the arrowhead points to an intact costal strip junction viewed from the outside of the lorica; notice also the short posterior pedicel and the external position of

the mid-lorica transverse costa relative to the longitudinal costae; **(c)** Complete cells of *Pl. minutissima* (right) and *Cosmoeca ventricosa* form B (left); costal strip junctions in the mid-lorica transverse costa as viewed from the outside of the lorica are mirror images and encircled (*Pl. minutissima*) or framed by rectangles (*C. ventricosa* form B).

Fig. 14.a-h. *Pleurasiga reynoldsii* (a-d) and *Pl. tricaudata* (e-h), LM (a-d, f, g; phase contrast), SEM (e), and TEM (h) whole mounts from the Gulf of California (a, f-h), West Australia (b), the Sargasso Sea (c), the equatorial Pacific Ocean (d), and the South Atlantic Ocean (e); **(a-d)** Selection of *Pl. reynoldsii* LM images to show the variability encountered; notice the pronounced size variability; **(e)** Cell documenting basic costal strip and lorica features; notice the exterior positioning of both transverse costae and the costal strip junctions (arrows); **(f, g)** Light microscopical images of *Pl. tricaudata*; **(h)** Cell with protoplast documenting basic lorica features; the arrow points to a biforked tip on a posterior lorica chamber costal strip. The scale bar (c) applies to all LM micrographs.

Fig. 15. a-e. *Parvicorbicula socialis* (a) and *Pa. circularis* (b-e) LM whole mounts (phase contrast) from West Australia (a, e), the Caribbean Sea (b), and the Gulf of California (c, d). **(a)** Single specimen documenting the rare occurrence of *Pa. socialis* in warm water habitats; **(b-e)** Differently sized specimens of *Pa. circularis*. The scale bar (c) applies to all LM micrographs.

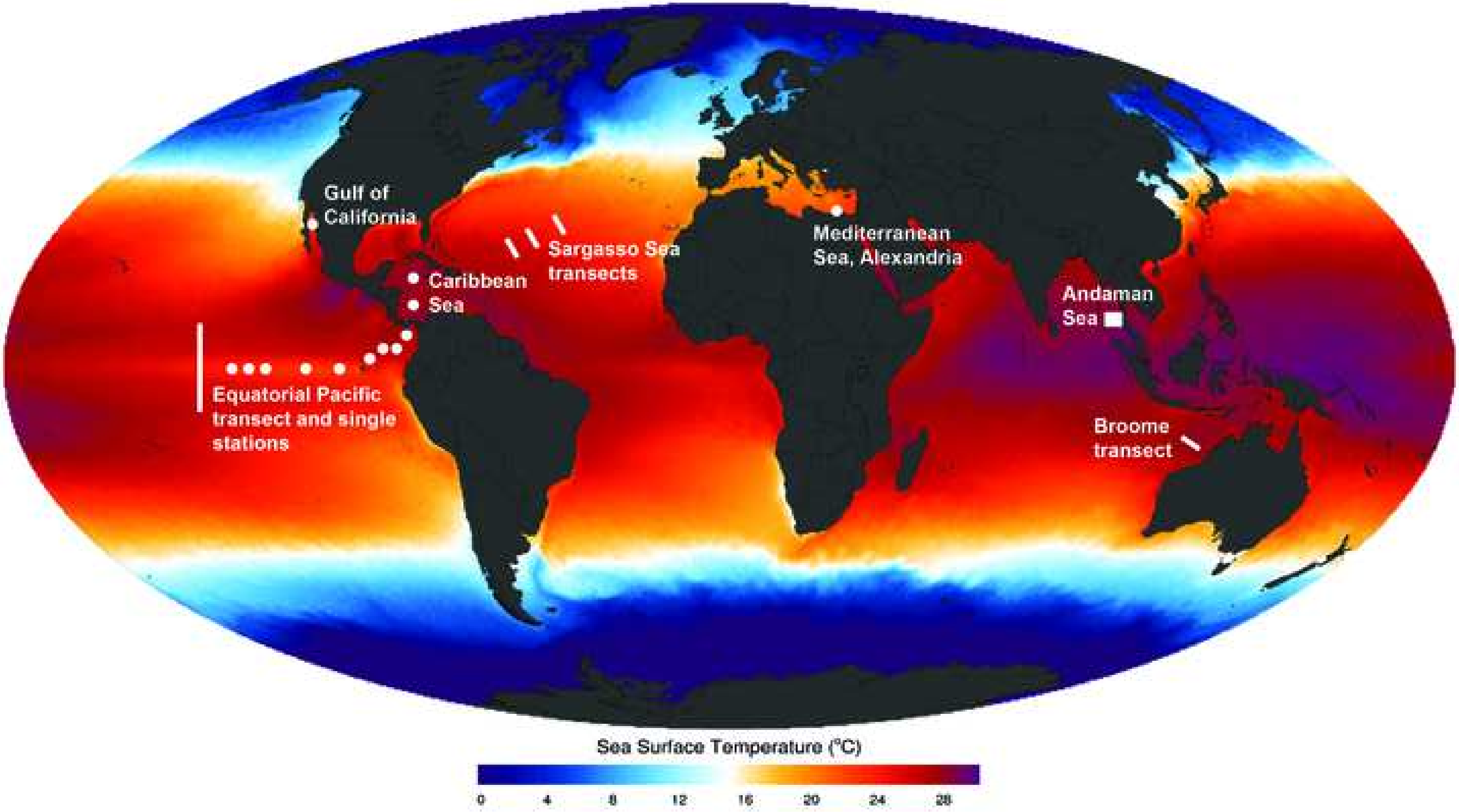
Fig. 16.a-c. *Pleurasiga minima* TEM whole mounts from Danish coastal waters; **(a)** High magnification of lorica details from **(b)** to show costal strip junctions as appearing from both the outside and the inside of the lorica; **(c)** Schematic drawings of the junctions between transverse

and longitudinal costal strips; the labelling is identical to that used by Leadbeater (2015) where (a) is the transverse costal strip that terminates at the longitudinal costa, (b) denotes the overlapping transverse costal strip, and (c, d) the longitudinal costal strips.

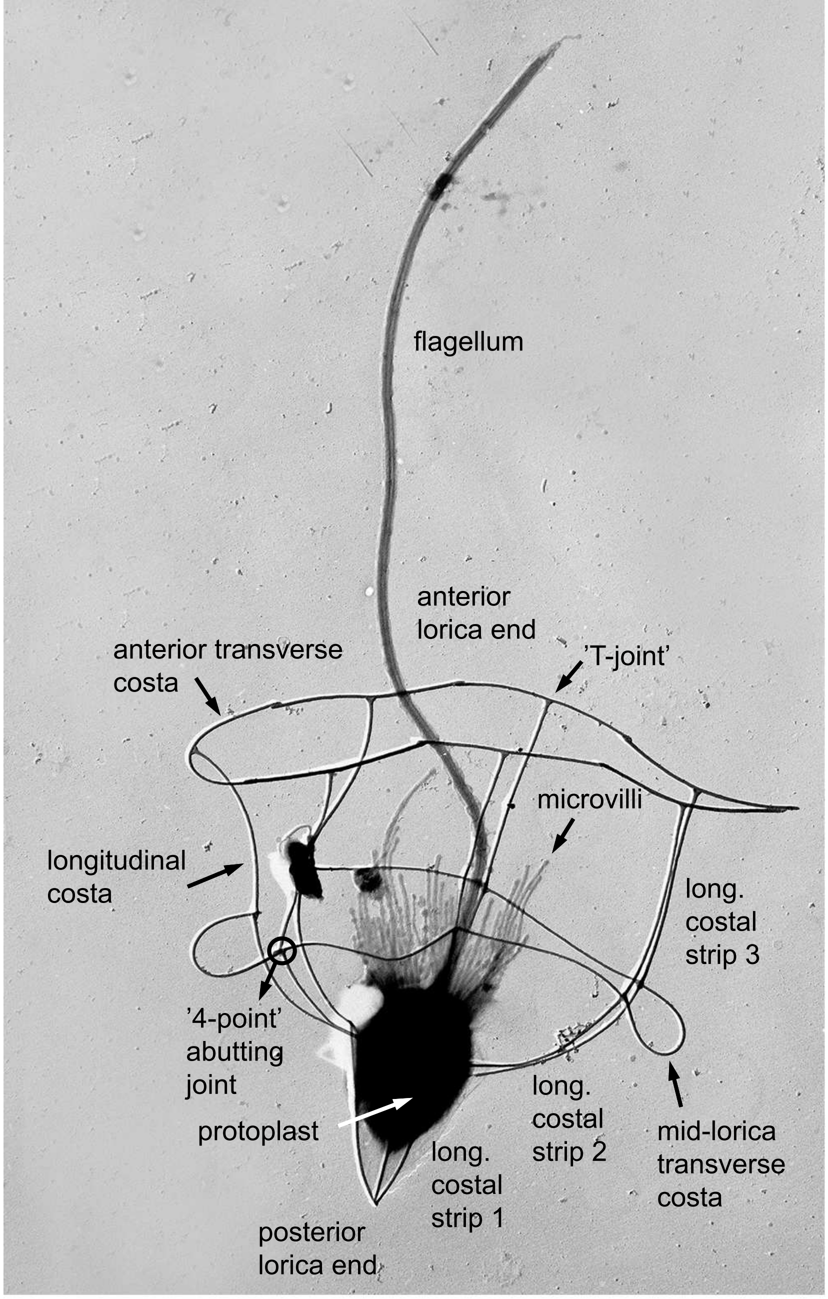
Table 1. Occurrence pattern of species discussed here and in Thomsen and Østergaard (2019a-e). New species described in Thomsen and Østergaard (2019a-e) are marked with *.

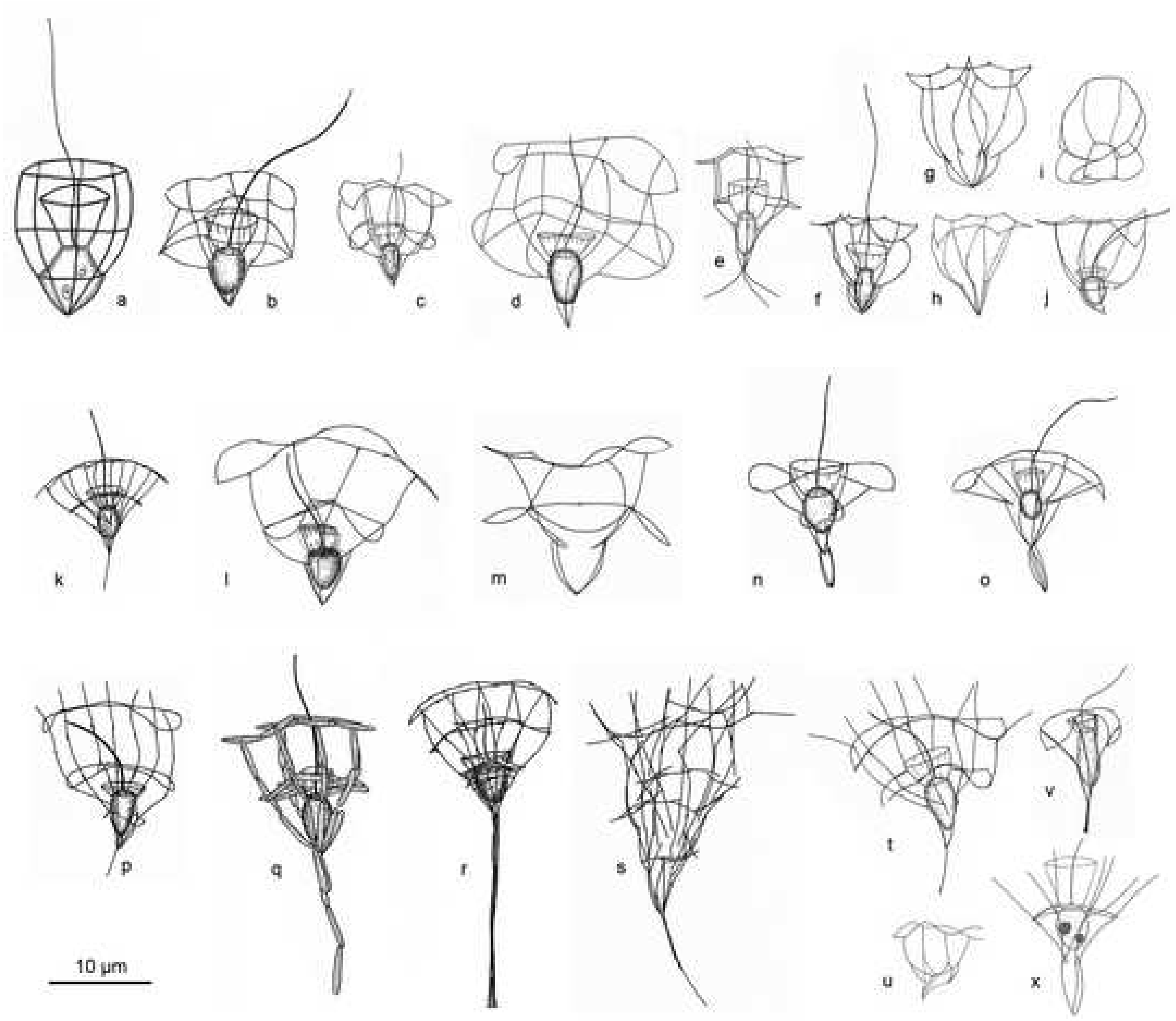
	Andaman Sea, Thailand	West Australia	Sargasso Sea	Caribbean Sea	Equatorial Pacific Ocean	Gulf of California, Mexico	Mediterranean Sea, Alexandria
***Acanthocorbis** apoda*	x						
A. camarensis	x						
A. campanula	x	x	x				
*A. conicella**		x	x			x	x
*A. gladiella**	x	x	x	x	x		
A. haurakiana	x	x	x				
***Apheloecion** articulatum*	x	x					x
*A. eqpacia**	x	x			x		
A. pentacanthum	x	x			x	x	x
A. quadrispinum	x	x	x	x	x		x
Bicosta minor (form A)	x	x		x	x		
B. spinifera						x	
***Campyloacantha** imbricata*	x	x	x	x	x		
C. spinifera	x	x	x			x	x
***Calliacantha** magna**	x	x		x	x		
C. natans						x	
C. simplex	x	x	x	x	x	x	x
***Conioeca** boonruangii**	x	x		x	x		
***Cosmoeca** ceratophora*	x	x	x	x	x	x	x
C. norvegica	x	x	x	x	x	x	x
C. phuketensis	x	x	x	x	x		x

C. subulata	x	x			x		
C. ventricosa (incl. form A)	x	x	x	x	x	x	x
C. ventricosa (form B)	x	x	x		x	x	x
C. ventricosa (form C)	x	x	x	x	x		
Crucispina *cruciformis*	x	x	x			x	x
Nannoeca *mexicana**		x				x	
N. minuta	x	x	x	x	x	x	x
N. minuta (form A)	x				x		
Parvicorbicula *circularis*		x	x	x		x	x
P. socialis		x					
Pleurasiga *echinocostata*	x	x	x	x	x	x	x
P. echinocostata form A	x	x					
P. echinocostata form B	x				x		
P. minima	x	x	x	x	x	x	
*P. minutissima**	x	x	x	x	x	x	x
*P. quadrangiella**	x	x			x		
P. reynoldsii		x	x		x	x	
P. tricaudata						x	
Saroeca *attenuata*			x		(x)		
S. paucicostata	x	x	x			x	x
Stephanoeca *andemanica**	x	x					
S. apheles	x	x					
*S. broomia**	x	x					
S. diplocostata var. paucicostata		x					
*S. naja**	x	x	x		x	x	
S. supracostata	x						
Thomsenella *acuta*	x	x	x	x	x		x
T. cercophora	x	x			x		x
T. infundibuliformis			x		x	x	x
T. perforata	x	x	x	x	x	x	x


Gulf of California
Sargasso Sea transects
Mediterranean Sea, Alexandria
Caribbean Sea
Andaman Sea
Equatorial Pacific transect and single stations
Broome transect
Sea Surface Temperature (°C)
0
4
8
12
16
20
24
28

Figure


flagellum
anterior lorica end
anterior transverse costa
'T-joint'
microvilli
longitudinal costa
long. costal strip 3
'4-point' abutting joint
protoplast
long. costal strip 2
long. costal strip 1
mid-lorica transverse costa
posterior lorica end


a
b
c
d
e
f
g
h
i
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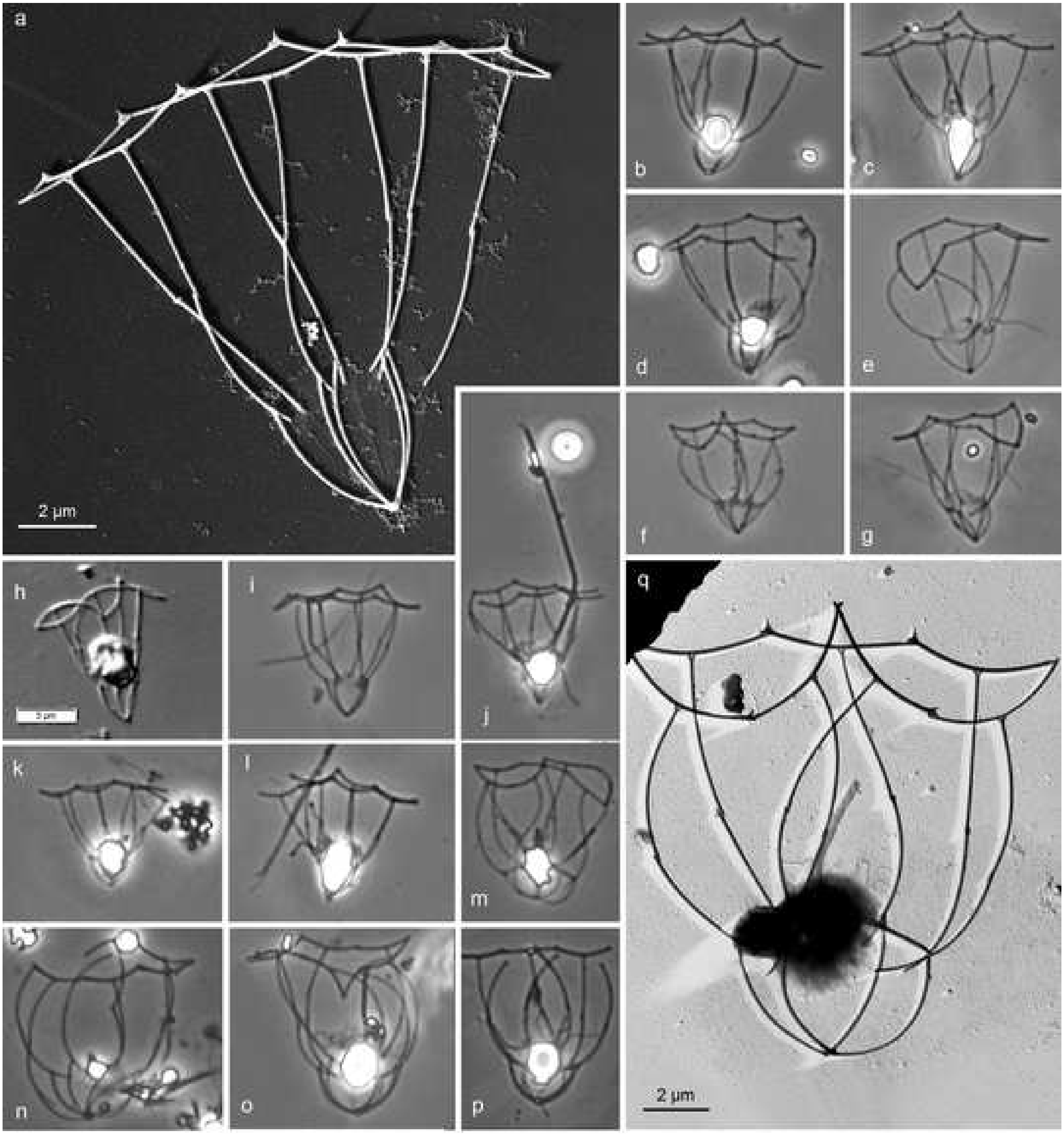
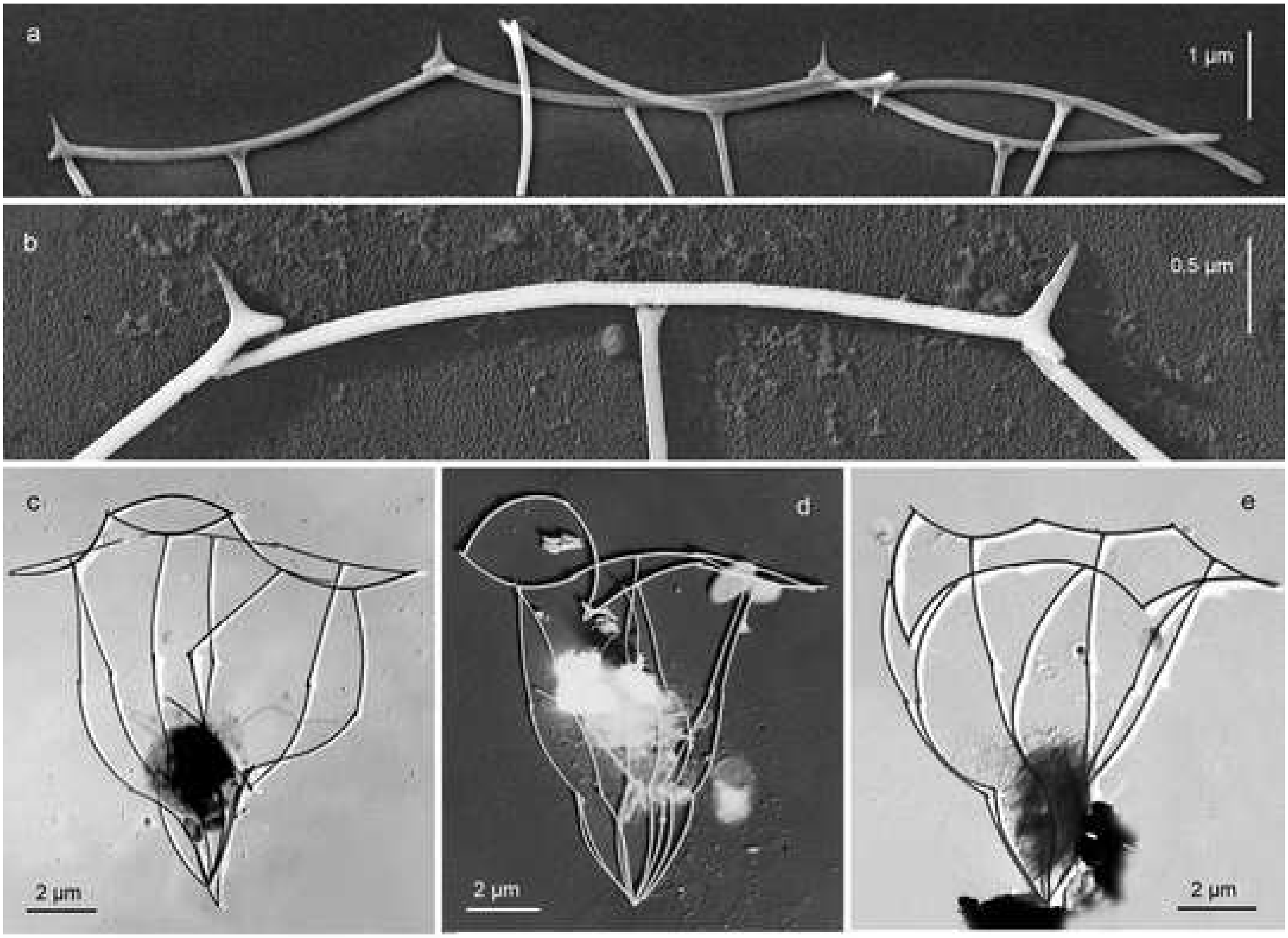
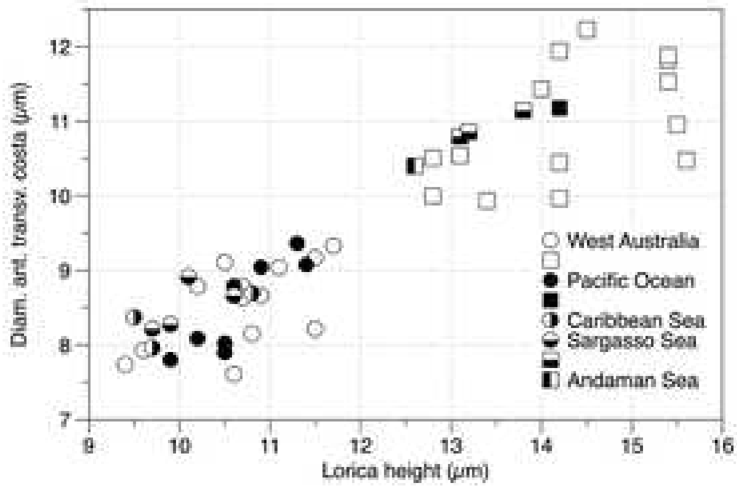
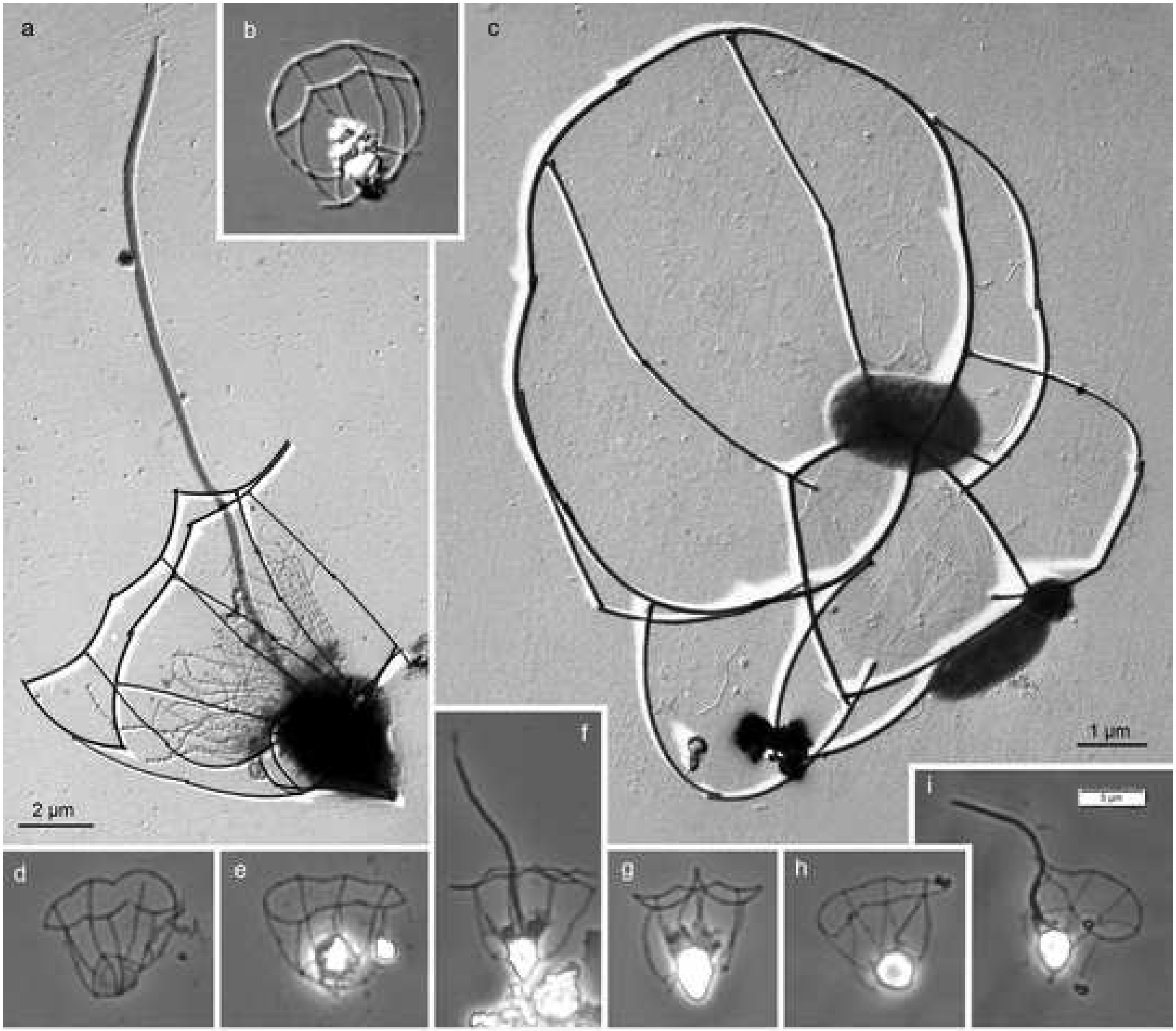
k
l
m
n
o
p
q
r
s
t
v
u
x
10 µm

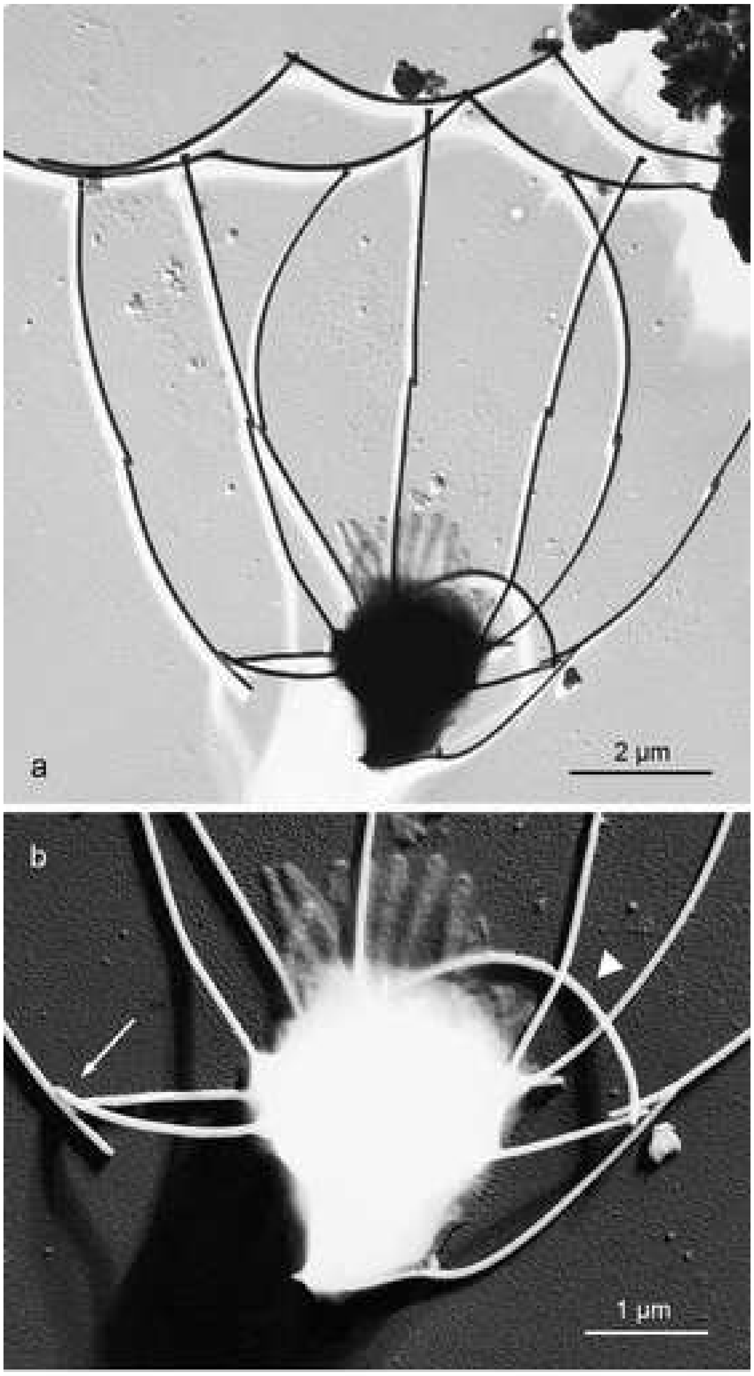

a
2 µm
b
c
d
e
f
g
h
5 µm
i
j
k
l
m
n
o
p
q
2 µm

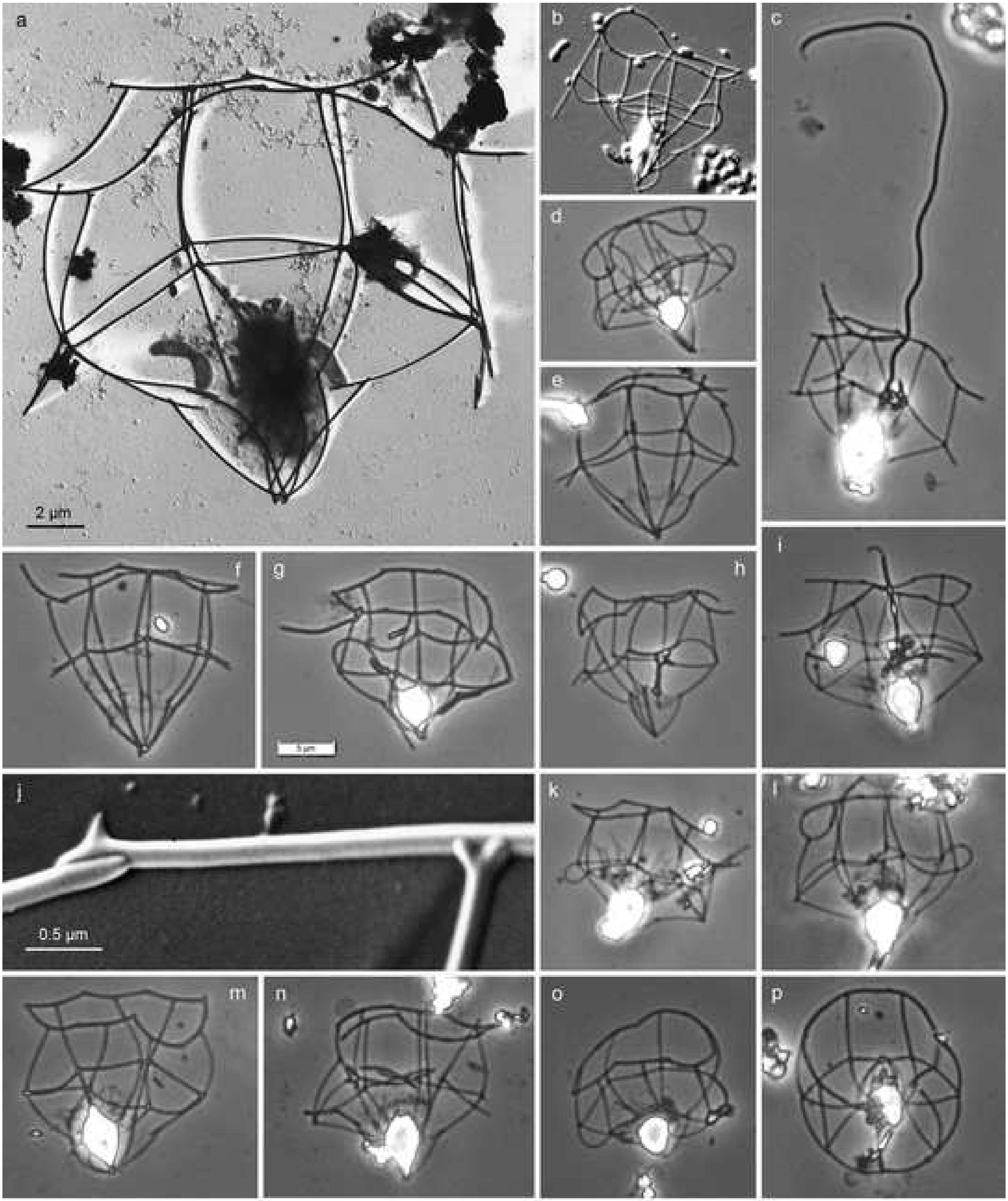
Click here to access/download;Figure;Fig_5_Pleu_echinocost_2.tif




Diam. ant. transv. costa (µm)
12
11
10
9
8
7
Lorica height (µm)
9
10
11
12
13
14
15
16
West Australia
Pacific Ocean
Caribbean Sea
Sargasso Sea
Andaman Sea


a
b
c
2 µm
1 µm
d
e
f
g
h
i


a
2 µm
b
1 µm


a
2 µm
b
c
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i
j
0.5 µm
k
l
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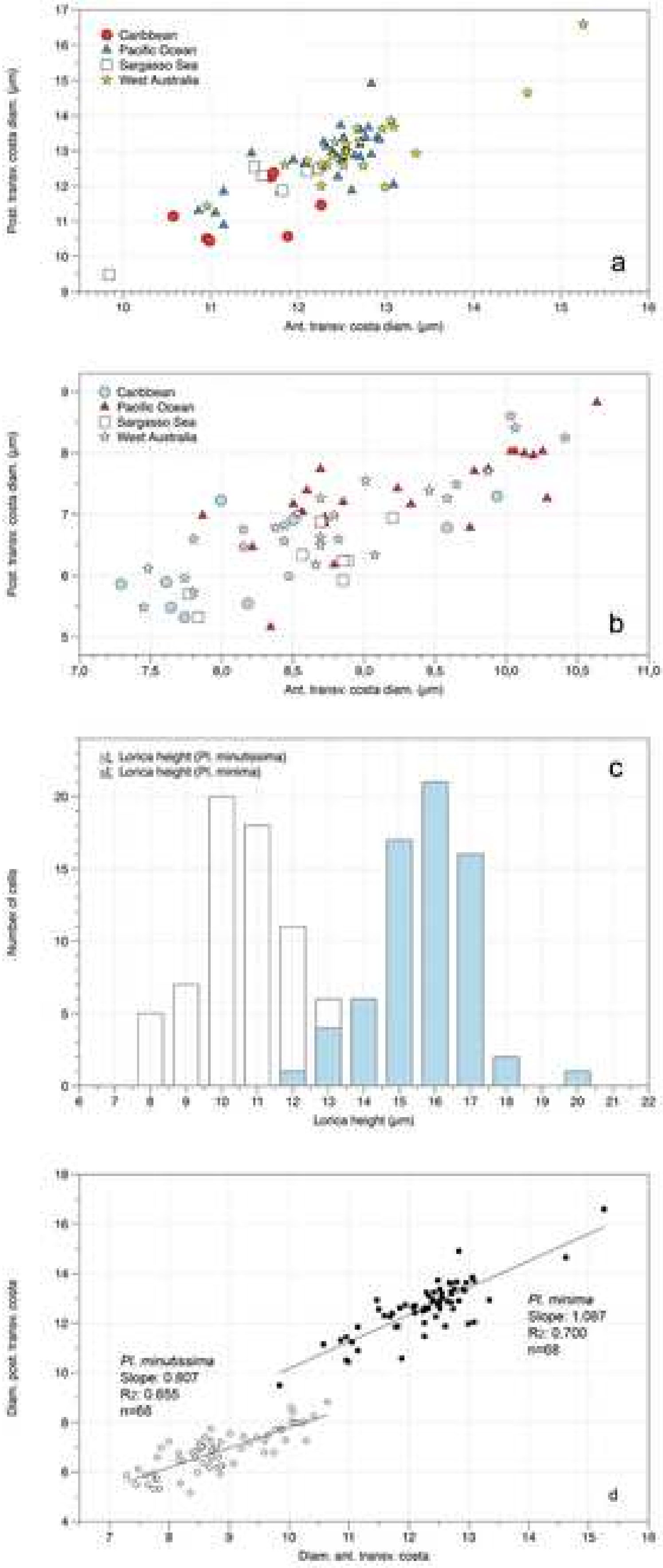
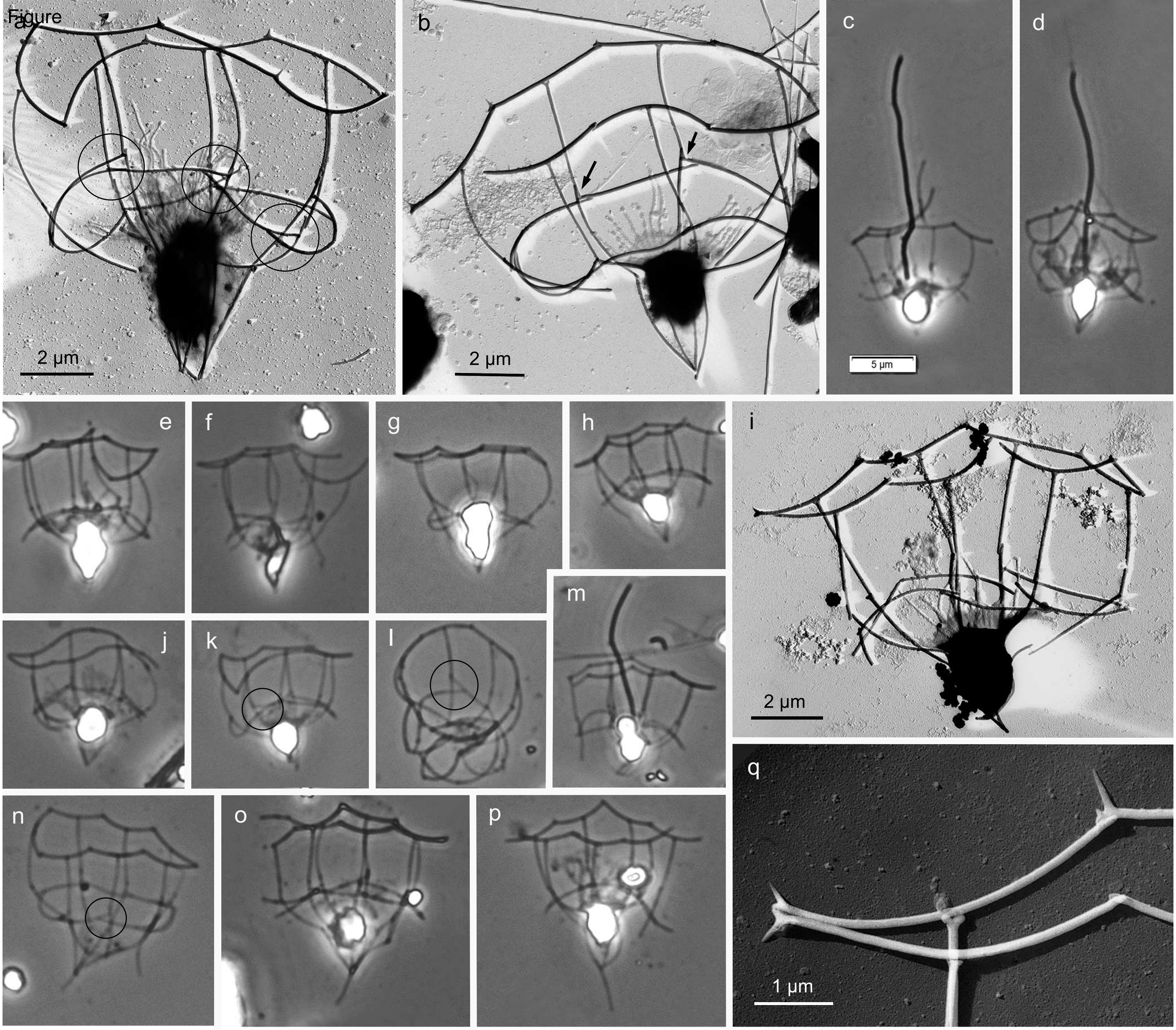
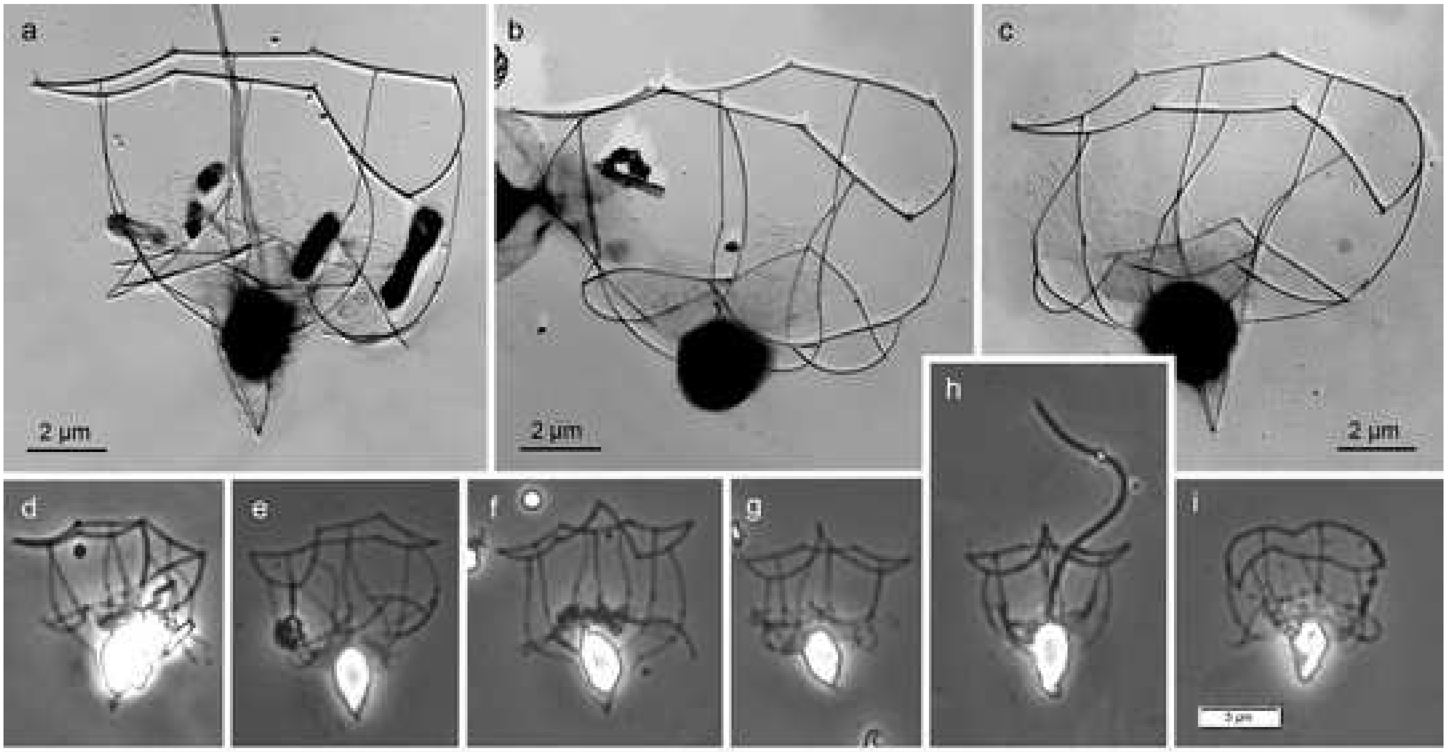

Caribbean
Pacific Ocean
Sargasso Sea
West Australia
Post. transv. costa diam. (µm)
Ant. transv. costa diam. (µm)
a
17
16
15
14
13
12
11
10
9
10
11
12
13
14
15
16
Caribbean
Pacific Ocean
Sargasso Sea
West Australia
Post. transv. costa diam. (µm)
Ant. transv. costa diam. (µm)
b
9
8
7
6
5
7,0
7,5
8,0
8,5
9,0
9,5
10,0
10,5
11,0
Lorica height (Pl. minutissima)
Lorica height (Pl. minima)
c
Number of cells
Lorica height (µm)
20
15
10
5
0
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
Pl. minutissima
Slope: 0.807
R2: 0.656
n=68
Pl. minima
Slope: 1.087
R2: 0.700
n=68
d
Diam. post. transv. costa
Diam. ant. transv. costa
18
16
14
12
10
8
6
4
7
8
9
10
11
12
13
14
15
16

Figure

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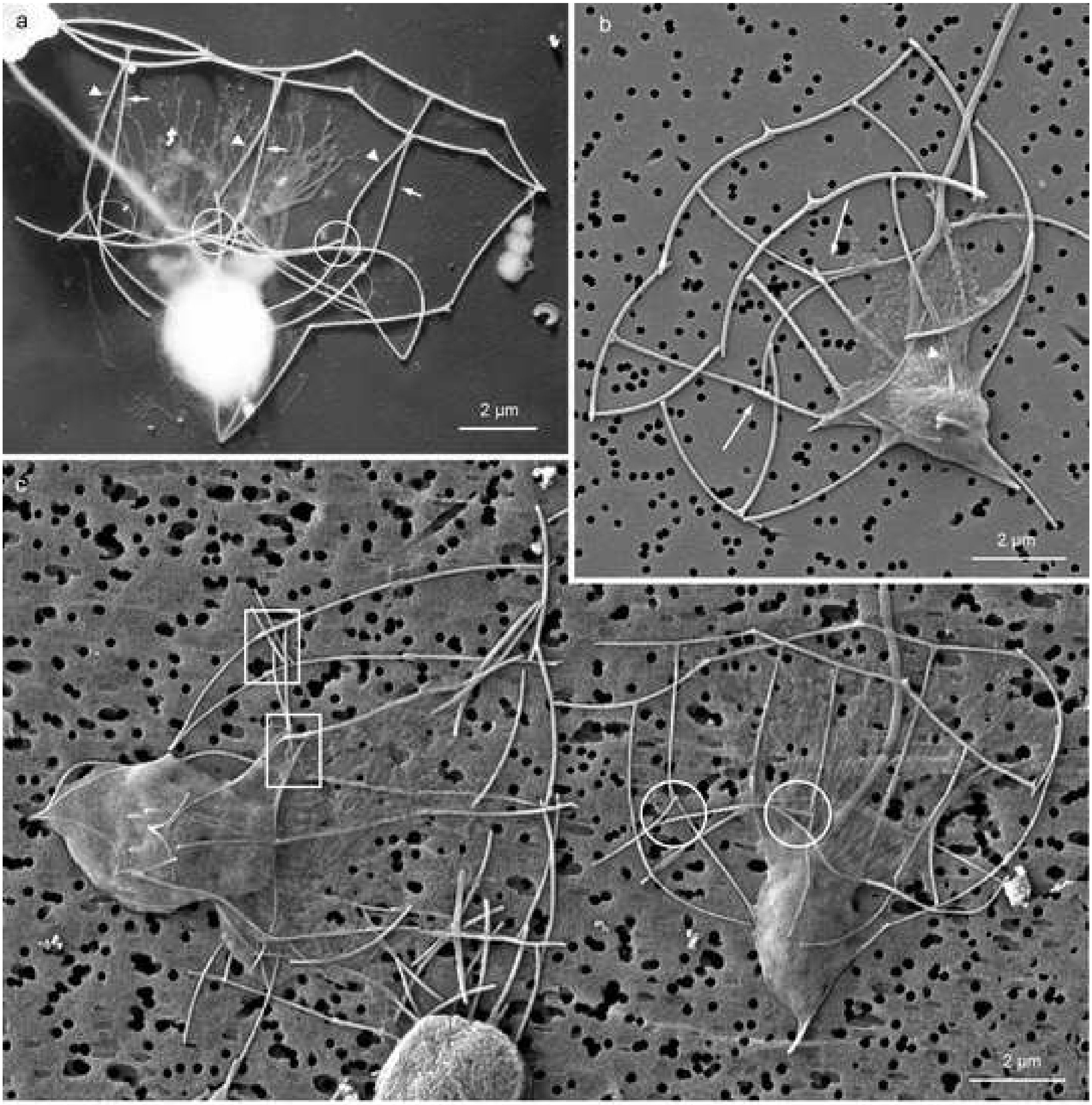
a
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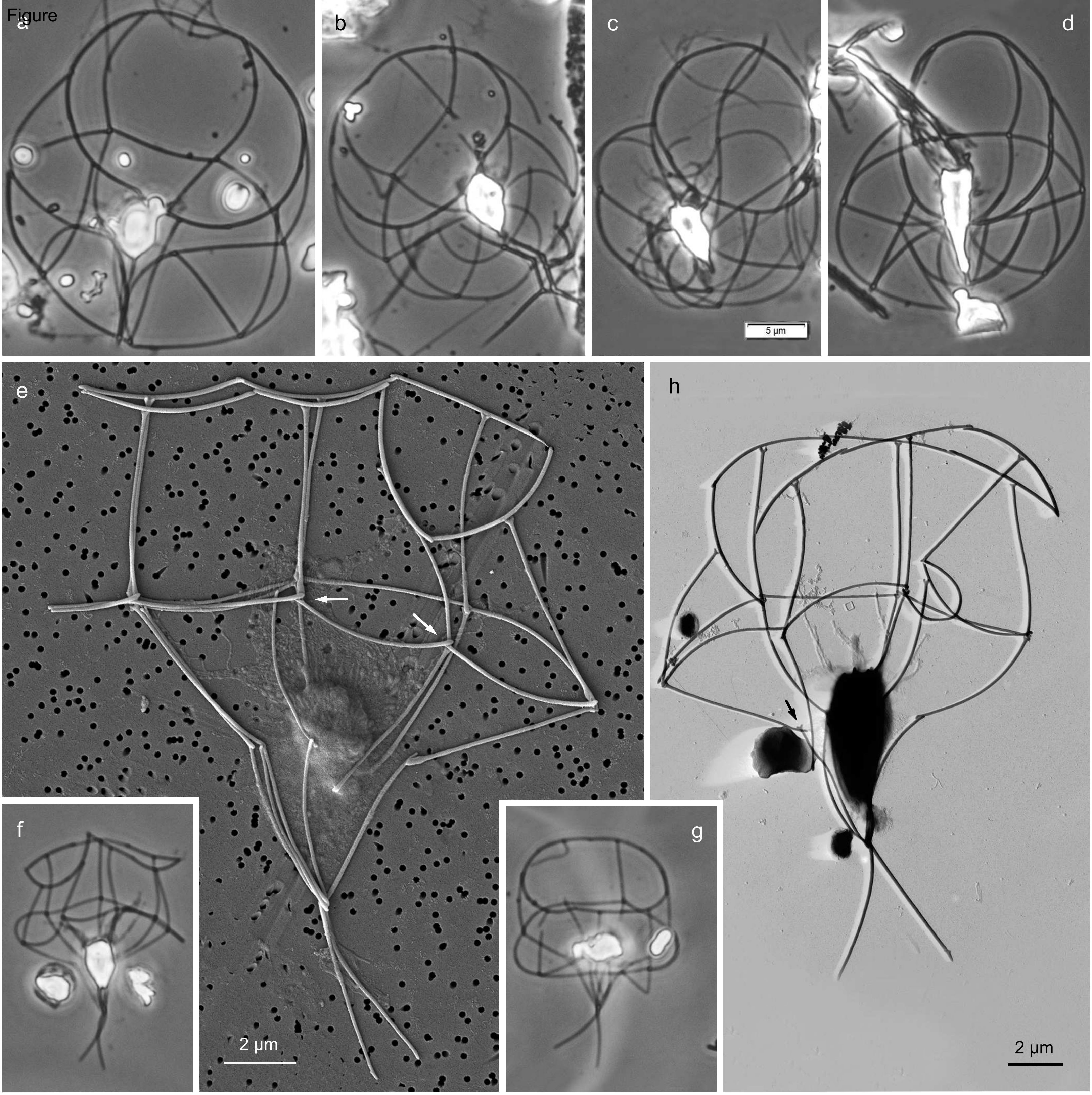
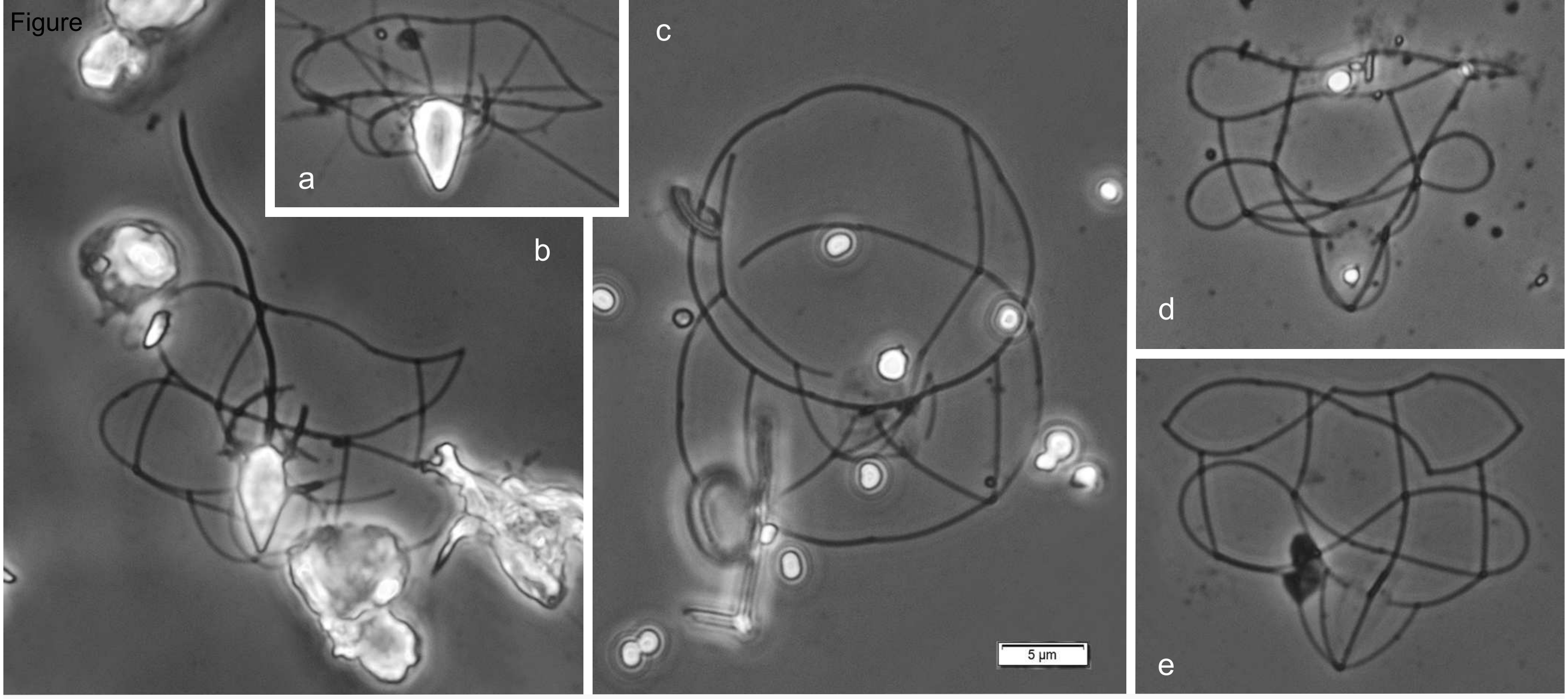
c
2 µm

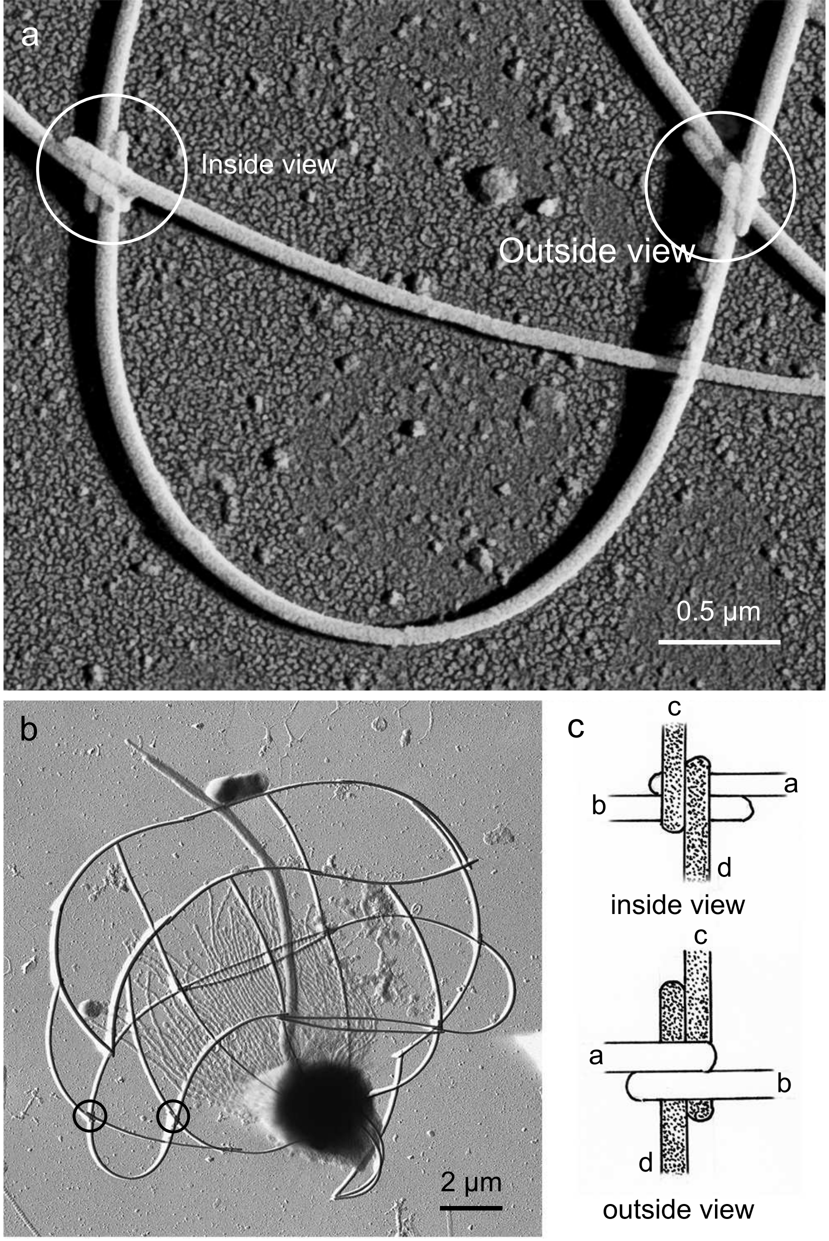
Figure



Figure



Figure


a
Inside view
Outside view
0.5 μm
b
2 μm
c
c
a
b
d
inside view
c
a
b
d
outside view