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Thomsen, Helge Abildhauge; Østergaard, Jette B.

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Authors: Helge Abildhauge Thomsen, Jette B. Østergaard

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Loricata choanoflagellates (Acanthoecidae) from warm water seas. II. *Bicosta*,
Apheloecion, *Campyloacantha*, *Crucispina*, *Calliacantha* and *Saroeca*

Helge Abildhauge Thomsen^{a,*}, Jette B. Østergaard^b

^aTechnical University of Denmark, National Institute of Aquatic Resources (DTU Aqua), Kemitorvet, Bygning 201, DK 2800 Kgs. Lyngby, Denmark /

^bNørrebrogade 52a 5th, 2200 Copenhagen N, Denmark

Corresponding author.

E-mail address: hat@aqua.dtu.dk (H.A. Thomsen)

Abstract

The main outcome of this and subsequent papers is to provide a baseline survey of heterotrophic protist diversity from warm water marine ecosystems, exemplified by loricate choanoflagellates (Acanthoecidae). Genera in focus here (i.e. *Bicosta*, *Apheloecion*, *Campyloacantha*, *Crucispina*, *Calliacantha* and *Saroeca*) possess anterior spines or projections and a posterior pedicel, and have 0, 1 or 2 transverse costae. Longitudinal costae are, with the exception of *Campyloacantha*, external to transverse costal elements across all genera examined here. We describe here *Apheloecion eqpacia* sp. nov. and *Calliacantha magna* sp. nov., both of which are so far distributionally confined to warm water habitats. A 'form A' of *Bicosta minor* is introduced to facilitate the distinction between *B. minor* sensu stricto and a presumed warm water adapted variant with a posterior lorica chamber twist of the longitudinal costae.

Abbreviations: TEM: Transmission Electron Microscopy; LM: Light Microscopy

Keywords: *Apheloecion eqpacia* sp. nov.; *Bicosta*; *Campyloacantha*; *Crucispina*; *Calliacantha magna* sp. nov.; Loricate choanoflagellates; *Saroeca*.

Introduction

During the last decades much emphasis has been put on piecing together a comprehensive overview of loricate (Acanthoecid) choanoflagellate species diversity at northern hemisphere middle to high latitudes (see e.g. Booth, 1990; Hara et al., 1997; McKenzie et al., 1997; Menezes, 2005; Thomsen and Østergaard, 2017; Thomsen et al., 1991, 2016; Tong, 1997a), and at southern hemisphere high latitudes investigating in particular the circum-Antarctic water masses (see e.g. Thomsen et al., 1990, 1997). Distinctively less time and effort, during the same period, have been devoted to compiling surveys of loricate choanoflagellate diversity from tropical and sub-tropical warm water sites around the globe. However, noteworthy exceptions are a series of papers that survey the Andaman Sea loricate choanoflagellate fauna based on samples collected in the vicinity of the Phuket Island (Thomsen and Boonruang, 1983a,b, 1984; Thomsen and Moestrup, 1983), and also contributions investigating Australian coastal waters (Lee et al., 2003; Tong et al., 1997b, 1998) as well as the equatorial Pacific (Vørs et al., 1995).

In order to fill in gaps in the fragmentary picture of warm water loricate choanoflagellate species diversity, we are currently in the process of analyzing unpublished data from our multi decadal extensive collection work at low level latitudes across the globe. It has become evident that the warm water adapted loricate choanoflagellate community comprises at least 80 morphospecies (including approximately 20 undescribed taxa), which is in fact more than half of all species currently described. In order to deal with this large dataset, we are currently compiling a series of papers that summarizes our findings. The first paper in the series (Thomsen et al., 2018) provides further background and perspective. The paper concluding the series will make ocean specific warm water community comparisons and also analyze the results in a global context using tools and approaches as in e.g. Thomsen and Østergaard (2017).

Our overarching perspective for this work is that once completed the papers will provide (through e.g. an extensive photo documentation of all species encountered) an effective reference tool for future light or electron microscopical identification work from anywhere across the globe's warm water belt, and further in this context that our compilation of data can come in handy when future efforts are made to link sequence data to morphospecies of loricate choanoflagellates e.g. along the lines introduced by Nitsche et al. (2017).

Material and Methods

The material that constitutes the background for this and a series of forthcoming 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 (2018) for information on each of the collection sites and sampling campaigns.

The general protocol for processing water samples for the light microscope (LM) and transmission electron microscope (TEM) was according to Moestrup and Thomsen (1980). For details on sample processing, preparational issues and microscopes used see Thomsen and Østergaard (2018).

The material examined here is dried which means that the natural 3-D structures have collapsed to become 2-D structures leading to a visual overrating of in particular the lorica width. While several structures can still be measured with confidence, e.g. lorica chamber height and the length of spines and pedicels, it applies to morphometric elements such as the diameter of the lorica, typically at the level of the transverse costa(e), that this needs to be calculated based on measurements of the circumference of these structures. This approach has been applied for the species descriptions below.

Efforts are made to make use of a concise terminology when describing lorica features. When it comes to spines and pedicels, we are applying terminology as outlined by Thomsen and Buck (1991), with slight modifications. An **anterior spine** is thus a continuation of the longitudinal costae above the transverse

costa equal to or less than the length of one costal strip, while the term **anterior projection** can be applied when the continuation of a longitudinal costa is longer than one costal strip. The term **simple pedicel** refers to a single costal strip or a line of costal strips ('**linear simple pedicel**') attached end to end terminating the lorica. An **aggregated pedicel** is constructed from linearly arranged and closely packed costal strips (as seen in e.g. species of *Calliacantha*).

Results and Discussion

The manuscripts scheduled to become part of a monographic treatment of warm water loricate choanoflagellate biodiversity are tentatively organized so that genera and species that share certain lorica features are bundled together, however, without always implying that a monophyletic relationship necessarily exists. In this context it should be emphasized that although progress has been made (see e.g. Nitsche et al., 2017), the number of species reliably sequenced is still far too small to convincingly assist the sorting of loricate choanoflagellate taxa into groups that are reliably phylogenetically related. In this paper we will deal with genera that have a fairly simple lorica construction involving anterior spines and projections, a posterior pedicel, and zero (*Bicosta* Leadbeater, 1978), one (*Apheloecion* Thomsen, 1983; *Campyloacantha* Hara and Takahashi, 1987; *Crucispina* Espeland and Throndsen, 1986; *Saroeca* Thomsen, 1979) or two transverse costae (*Calliacantha*, Leadbeater 1978; *Saroeca* Thomsen, 1979). In all genera, except for *Campyloacantha* (in which pairs of anterior transverse and longitudinal costal strips are fused together forming L-shaped configurations), the longitudinal costae are exterior to the transverse costa(e). This selection of genera partly corresponds with loricate choanoflagellate genera and species referred to the '*Bicosta/Calliacantha*' theme in Leadbeater (2015), yet with the addition of *Apheloecion*, *Campyloacantha* and *Saroeca*.

***Bicosta* Leadbeater, 1978**

The genus currently comprises three species, viz. *B. spinifera* (Thronsen, 1970)Leadbeater, 1978, *B. minor* (Reynolds, 1976)Leadbeater, 1978, and *B. antennigera* Moestrup, 1979. Manton et al. (1980) provided a thorough study of intraspecific variability across all three taxa, and also a first overview of the distributional characteristics of these taxa. While both *B. spinifera* and *B. antennigera* are largely confined to cold water environments and in particular high latitude habitats, *B. minor* was found to be less temperature sensitive. All three species are widely distributed within their specific abiotic realms. Based on material from Osaka Bay, Japan, Hara and Takahashi (1987a) presented data on morphology and seasonal occurrence patterns of *B. spinifera* and *B. minor* that largely substantiated the findings presented by Manton et al. (1980). Thomsen and Larsen (1992) and Thomsen and Østergaard (2017) address *Bicosta* morphological variability and distributional patterns within Antarctic and Arctic realms, respectively. *Bicosta* is the only genus that does not incorporate transverse costal strips in its lorica design.

***B. spinifera* (Fig. 2a,b)**

During this warm water survey, we have encountered typical *B. spinifera* specimens only in samples from the Gulf of California and at water temperatures around 18°C, which slightly increases the known temperature range of this otherwise cold-adapted taxon. The Gulf of California specimens (Fig. 2a,b) are 45 µm long (when measured along the longest longitudinal costa) and clearly display characteristic features of this species, e.g. the tapering and flexible anterior spines and posterior pedicel. The pedicel is longer than the spines and characteristically curves up at the posterior end. One anterior spine is longer than the other. The most prominent lorica chamber feature is, however, a twist of the longitudinal costae midway along the anterior lorica chamber longitudinal costal strips (Fig. 2b).

***B. minor* form A (Fig. 2 c-h)**

Typical *B. minor* cells, i.e. cells without a twist of the longitudinal costae along the lorica chamber and with a pedicel that is shorter than the anterior spines (see e.g. Manton et al. 1980, l.c. Figs. 7a, 9, 10 and Reynolds 1976, l.c. Figs 6-9) were not found during this survey. We rather found specimens (Fig. 2c-h) that morphologically match a deviant form of *B. minor* reported by Hara and Takahashi (1987a) from Osaka Bay, Japan. In these cells, which are size-wise identical to *B. minor* sensu stricto, the longitudinal costae do twist, however, the twist occurs at the posterior end of the lorica chamber. Hara and Takahashi (1987a) refrained from describing this material as a new species of *Bicosta* but decided, because of dimensional overlap with *B. minor* sensu stricto, to consider this infraspecific variation. Realizing now that this deviant type is indeed widespread within warm water realms (see Table 1) it appears relevant to at least be able to refer to this morphotype in a simple way. We therefore suggest that forms of *B. minor* with a posteriorly located twist along the longitudinal costae are hence forward referred to as *B. minor* form A. *Bicosta spinifera* was recorded from Darwin, Australia (Lee et al., 2003). However, from the description included of the cells examined (no illustrations) it is evident that these are in fact identical to the material referred to above as *B. minor* form A. Also, the *B. minor* specimen illustrated by Lee et al. (2003) is most likely *B. minor* form A rather than *B. minor* sensu stricto. The overall picture emerging is thus that the ‘two-dimensional’ and non-twisted version of *B. minor* is distributionally confined to higher latitudes, while the form prevailing in warm water habitats is *B. minor* form A. Cells examined by Tong (1997a) from Southampton Water (l.c. Figs 5F-H) and referred to as *B. spinifera* display features of *B. minor* form A as outlined above. The seasonal occurrence of these forms is not directly evident from Tong (1997a). However, it is mentioned that *Bicosta* peaked only during summertime so the generalizations above with respect to the occurrence pattern of *B. minor* form A remains valid. The overall lorica size, when measured along a longitudinal costa is in material examined here 25-36 μm . The lorica chamber measures 8-11 μm .

***Apheloecion* Thomsen, 1983 in Thomsen and Boonruang, 1983**

The genus *Apheloecion* was established based on Indian Ocean material (Thomsen and Boonruang, 1983a) to accommodate three species (*A. quadrispinum* Thomsen in Thomsen and Boonruang, 1983 (generic type species); *A. pentacanthum* Thomsen in Thomsen and Boonruang, 1983; *A. articulatum* Thomsen in Thomsen and Boonruang, 1983) with a simple single-chambered and conical lorica, comprising a single transverse costa placed inside a small number of longitudinal costae that project anteriorly as spines or projections, while posteriorly leading into a pedicel that may be either a single longitudinal costal strip or a more elaborate structure formed by several adjoining costal strips (linear simple pedicel). Three additional taxa (*A. antarctica* Thomsen, Garrison and Kosman, 1997; *A. glacialis* Thomsen, Garrison and Kosman, 1997; *A. conicoides* Thomsen, Garrison and Kosman, 1997) were added to the genus based on analyses of samples from Antarctic sea ice (Thomsen et al., 1997). Although it is convenient for the time being to maintain a flexible use of existing taxa such as *Apheloecion*, while we are still in the process of mapping basic loricate choanoflagellate morphospecies diversity, it is highly unlikely that the genus as currently circumscribed is monophyletic.

***A. pentacanthum* (Fig. 3a-e)**

The diagnostic features of this taxon are (1) 5 longitudinal costae, each comprising two costal strips (2.8-3.2 μm long), that attach to the joints between 5 costal strips (2.6-2.9 μm long) forming the transverse costa, (2) short anterior spines (approx. one third longitudinal costal strip in length; 0.8-1.4 μm long), and (3) a posterior pedicel which is very short and basically just a single longitudinal costal strip that projects beyond the posterior point of convergence. The measurements reported originate from the Mexican specimen illustrated in Fig. 3a. The lorica height of this specimen (excluding spines and pedicel) is 4.7 μm and the diameter at the level of the transverse costa approx. 4.1 μm . Notice that the longitudinal costal strips are sharp-pointed.

A light microscopical recognition of this species (Fig. 3b-e) is hampered by the minuteness of the lorica. Features to look for are a very distinct conical shape (Fig. 3d) and the presence of 5 anterior spines. The lorica height is in Fig. 3b-e 4-4.2 μm , while the flagella range from 4.5-6.0 μm , and the dried and shrunken protoplast measures 1.4-1.5 x 2.0-2.3 μm .

Apheloecion pentacanthum has previously and in addition to the Andaman Sea type locality been recorded from the coast off California (Thomsen et al., 1991), Taiwanese waters (Hara et al., 1997), Rhode Island (Menezes, 2005), the Sound between Denmark and Sweden (Thomsen et al., 2016) and the Beagle Channel (Thomsen, unpublished). During this investigation we observed *A. pentacanthum* in samples from the Andaman Sea, West Australia, Gulf of California, and the eastern Mediterranean Sea (Table 1).

A. quadrispinum (Fig. 3f-m)

The lorica of this species (Fig. 3k) is composed of 13 costal strips arranged as one transverse costa (4 costal strips / 2.9-3.4 μm long) and four longitudinal costae (costal strip length 3.5-3.8 μm). The anteriormost longitudinal costal strips project as spines that are up to almost one costal strip long. The pedicel is a single costal strip. Notice that longitudinal costal strips are distinctly pointed. Lorica chamber height (excl. spines and pedicel) is 4.2 μm and the lorica diameter at the level of the transverse costa 4 μm .

Similar difficulties, as reported above with reference to *A. pentacanthum*, exist in *A. quadrispinum* when it comes to light microscopical recognition of this species. Features to look for are the equally sized anterior spines and posterior pedicel (Fig. 3g,i) emerging from a lorica chamber shaped as an inverted pyramid (Fig. 3h). The lorica height is 3.9-4.2 μm in Fig. 3f-j,l-m, anterior spines range between 2.8-3.2 μm , and the posterior pedicel from 2.9-3.3 μm . The protoplast is 1.5-1.8 x 1.7-2.9 μm and the flagellum 8-10 μm . It is obvious from light micrographs included in Fig. 3 that the separation of *A. quadrispinum* from *A. pentacanthum* represents a serious challenge and that even the identity of some of the specimens illustrated here can be questioned. It should also be emphasized that the separation of both species of *Apheloecion* from the equally small *Nannoeca minuta* (Thomsen, 1988; Thomsen and Østergaard, 2018) can

also be problematic. When it comes to these minute forms, an unequivocal species recognition must rely on the examination of multiple specimens, and preferably empty loricae, some of which will eventually display the critical diagnostic features of the taxon.

Apheloecion quadrispinum is previously and in addition to the Andaman Sea type locality observed in samples from the subarctic North Pacific (Booth, 1990), Darwin, North Australia (Lee et al., 2003), and the Sound between Denmark and Sweden (Thomsen et al., 2016). We here report again the species from the Andaman Sea, and further from West Australia, the Sargasso Sea, the Caribbean Sea, the equatorial Pacific, and the eastern Mediterranean Sea (Table 1).

A. articulatum (Fig. 4)

Whereas the species above, with the occasional exception of the exact positioning of the transverse costa, are characterized by zero variability in lorica geometry, the situation is different with respect to *A. articulatum*, which has 4-5 longitudinal costae each comprising 2 costal strips (5-6 μm long) and 8-10 costal strips (3.9-4.3 μm long) forming part of the transverse costa. Junctions between longitudinal and transverse costal strips are more or less random. Anterior spines are up to one costal strip long and tapering towards the tip. The pedicel may be a single costal strip (Fig. 4a) or an extended line of costal strips (Fig. 4b-d) that may be up to 20 μm long. Lorica height excluding spines and pedicel is 8-10 μm and the diameter at the level of the transverse costa 7-9 μm . Flagellar length is 12-14 μm . The protoplast is fairly large and measures 2-2.5 x 3-5 μm .

The material examined here is identical to the Andaman Sea type material (Thomsen and Boonruang, 1983a) in basic lorica features and shows only minor variability with reference to e.g. dimensions of the individual lorica component. This species is easily recognized in LM dry preparations (Fig. 4b-d) simply due to its larger size. The 'zig-zag' appearance is an added feature for recognition when examining long stalked specimens.

A. articulatum is here reported again from stations close to the Andaman Sea type locality, and also from West Australia, and the eastern Mediterranean Sea (Table 1).

A. eqpacia sp. nov. (Figs 5-6)

Diagnosis: Lorica chamber (6.9 μm high) composed of 6 (7) longitudinal costae, each comprising two costal strips (4-5 μm long), and a single transverse costa, located to the inside of the longitudinal costae, and comprising up to 12 costal strips (3.5 μm long) giving a diameter of approximately 7 μm . Longitudinal costae project anteriorly as short spines. The junctions between longitudinal costae and elements of the transverse costa appear to be random. An extended pedicel, several costal strips in length and ca. 25 μm long, emanates from the posterior lorica end. The individual costal strip (3.3-3.5 μm long) is slightly curved and pointed at both ends. The direction of the costal strip curvature is opposite when comparing neighbouring costal strips giving the pedicel a meandering pattern. The protoplast measures 2 x 4 μm and the flagellum is 12.5 μm long. The division is tectiform based on the accumulation of costal strips in the collar region.

Type specimen: Fig. 5a-c from an equatorial Pacific Ocean surface sample (#72; 4°S and 140°W) collected 27 April 1992.

Etymology: The species epithet is derived from the cruise acronym (EqPac).

Apheloecion eqpacia was commonly observed in LM preparations in particular those from West Australia (Fig. 6b-g, h-k, m). Measurements of these comply well with those provided for the equatorial Pacific Ocean type material (see above): lorica height: 7.1 ± 0.33 μm (range: 6.6-7.9 μm); diameter at the level of the transverse costa: 6.5 ± 0.6 μm (range: 5.7-7.5 μm); length of pedicel: 21.2 ± 10.4 μm (range: 7.9-30.4 μm). The overall appearance of this species is further corroborated by additional TEM micrographs from the Andaman Sea (Fig. 6a) and the equatorial Pacific Ocean (Fig. 6l).

Material illustrated here in Fig. 6n-r (from West Australia and the Andaman Sea) show cells that are reminiscent of *A. eqpacia* yet without anterior spines and presumably with only a short or no posterior pedicel at all. While the spine-less appearance is unquestionable, the reduced posterior pedicel may easily be an artefact caused by the handling of the material. Notice also that the mosaic of 'genuine' *A. eqpacia* specimens from West Australia (Fig. 6b-k) does in fact include a single spine-less specimen (Fig. 6f) which has, however, a long linear pedicel that is typical of *A. eqpacia*.

***Campyloacantha* Hara and Takahashi, 1987 (Fig. 7)**

The genus comprises two species, i.e. *C. spinifera* (Leadbeater, 1973) Hara and Takahashi, 1987 and *C. imbricata* Hara and Takahashi, 1987. The generic type is *C. imbricata* and the type locality Osaka Bay, Japan. When first described from the Adriatic Sea (Leadbeater, 1973) *C. spinifera* was amended to the genus *Parvicorbicula* Deflandre, 1960. The keystone feature of the genus *Campyloacantha* is the L-shaped fusion between pairs of anterior longitudinal and transverse costal strips (Fig. 7e,k). The lorica chamber is conical and can be terminated by a pedicel (*C. imbricata*). When this genus was formally established by Hara and Takahashi (1987b) it was in practice done so that the diagnosis of the new species, *C. imbricata*, was declared to be completely identical to the generic diagnosis. This has the unintentional outcome that a species specific feature of *C. spinifera*, such as the six anterior spines, falls outside the scope of the genus.

***Campyloacantha spinifera* (Fig. 7a-j)**

This is a tiny species in which the short conical lorica chamber (excl. spines) is 5.0-5.5 μm long and has a diameter of 3.6-4.3 μm at the level of the transverse costa. There are six longitudinal costae each comprising 2-3 costal strips one of which protrudes anteriorly as a distinct and sharp-pointed spine (1.7-4.3 μm long). Some amalgamation into pairs occur among the longitudinal costae midway along the lorica chamber (Fig. 7e). The middle longitudinal costal strip is significantly shorter (Fig. 7e) than the adjoining longitudinal costal strips. The transverse costal strips measure 1.9-2.5 μm . The flagellum is 14-19 μm long.

The L-shaped fusion between pairs of longitudinal and transverse costal strips endows the lorica with a very characteristic hexagonal appearance when viewed from either end (Fig. 7a,c,g-h). Despite the overall size of this species it has an unmistakable appearance also in LM dried preparations.

Campyloacantha spinifera has previously been recorded from the Mediterranean Sea (Kastel Bay and Bay of Algiers; Leadbeater, 1973, 1974), Monterey Bay, California (Thomsen et al., 1991), Sydney Harbour and Shark Bay, Australia (Tong et al., 1997b, 1998), the equatorial Pacific (Vørs et al., 1995), the north Pacific central gyre (Hoepfner and Haas 1990), the south Atlantic coastal zone, Patos Lagoon, Brazil (Bergesch et al., 2008), and the Beagle Channel, Patagonia (Thomsen, unpublished results). The species was observed at five out of seven sites examined here (Table 1). The biogeographical imprint of *C. spinifera* is thus that of a widespread organism with a preference for warmer waters and an avoidance of high latitude habitats.

Campyloacantha imbricata (Fig. 7k-y)

The lorica chamber (excl. spines and pedicel) is elongated conical and measures $7.5 \pm 1.3 \mu\text{m}$, which is approx. 50% larger than the *C. spinifera* lorica chamber. The calculated diameter at the level of the transverse costa is $4.1\text{--}4.7 \mu\text{m}$. Material examined here inevitably displays 5 anterior spines ($6.1 \pm 1.6 \mu\text{m}$) which gives the lorica a pentagonal outline (Fig. 7q,y) when viewed from above or below. Transverse costal strip length is $2.7 \pm 0.19 \mu\text{m}$. A posterior pedicel is always present and measures $5.1 \pm 1.2 \mu\text{m}$. The flagellum is long ($12\text{--}20 \mu\text{m}$) while the protoplast measures $1.9\text{--}2.4 \times 3.3\text{--}5.4 \mu\text{m}$.

The material examined here is in accordance with the type material in all major aspects. The type material comprised only three specimens and two of these are illustrated by Hara and Takahashi (1987b; l.c. Figs 1-2). Additional TEM micrographs of *C. imbricata* are published by Vørs et al. (1995; l.c. Fig. 6) and Hara et al. (1997; l.c. Fig. 9). Norris (1984; l.c. Fig. 2) published a SEM image of a cell identified as *Parvicorbicula spinifera*.

Campyloacantha imbricata is previously recorded from Osaka Bay, Japan (Hara et al. 1987b), the equatorial Pacific (Vørs et al., 1995), Taiwanese coastal waters (Hara et al., 1997), and Darwin, Australia

(Lee et al., 2003; l.c. Fig. 1h-i; note that Fig. 1h is erroneously referred to as *Calliacantha multispina*). Here we report the species from five sites (Table 1).

The distinction between the two species of *Campyloacantha* (Hara et al., 1987b, 1997) is based on the number of anterior spines (3-5 in *C. spinifera* and 6 in *C. imbricata*), a short conical lorica chamber in *C. spinifera* versus a long conical lorica chamber in *C. imbricata*, and the presence in *C. imbricata* of a one costal strip long pedicel (a short posterior protrusion of a costal strip is occasionally observed in *C. spinifera*, however, this could easily be an artefact caused by the handling of the material). It is finally emphasized (Hara et al. 1997) that the presence of two differently sized longitudinal costal strips forming part of the lorica chamber is a feature distinguishing *C. imbricata* from *C. spinifera*. Although a distinction between two separate taxa appear immediately justified when screening the images displayed in Fig. 7, it must be emphasized that none of the distinguishing features listed above, except for lorica chamber height and the presence/absence of a pedicel, are applicable and reliable. Hara et al. (1997) reported the finding of otherwise fairly typical *C. imbricata* specimens with 6 anterior spines from Taiwanese coastal waters, and Tong et al. (1998) reported the finding of *C. spinifera* specimens in Sydney Harbour samples that had 5-6 anterior spines. However, the lack of illustrations of the Sydney material (Tong et al., 1998) renders their observations questionable, because comments on the material examined (dimensions and the presence of a pedicel) indicate that these cells are perhaps better dealt with under a *C. imbricata* heading. As discussed above *C. spinifera* in fact shares with *C. imbricata* the presence of an intermediate stratum of shorter longitudinal costal strips below the transverse costa. This feature is also evident when scrutinizing illustrations of the type material (Leadbeater 1973; l.c. Plate 15c). Realizing that the morphological features distinguishing the two taxa (size and the presence/absence of a pedicel) are not necessarily robust lorica characteristics that will survive future examination of additional material either by classical microscopical tools or molecular tools, it might be argued that the two taxa be merged into a single species. However, the consistency in our observations across vast geographical distance of a cluster of small stalk-less specimens

with a six-fold symmetry, and a second grouping of larger sized individuals invariably with a posterior pedicel and a five-fold symmetry, indicate to us that it remains relevant and practicable (also in the LM) to carry on distinguishing between two morphotypes.

The material examined here do not shed light on either how the production of the L-shaped anterior transverse/longitudinal costal strips, or the extracellular manipulation and intermittent storage of these in the collar region is handled by a *Campyloacantha* cell in preparation of cell division. The fusion between the two costal strip elements appear so solid (Fig. 7e) that it can only be the result of an intracellular silicification process.

***Crucispina* Espeland and Throndsen, 1986 (Fig. 8)**

This is a mono-specific genus comprising only *C. cruciformis* (Leadbeater, 1974) Espeland and Throndsen, 1986. The species is easy to identify also from LM (Fig. 8), because of the highly characteristic X-shaped appearance of the longitudinal costae. In our material the lorica chamber height is 3.5-4.0 μm and the calculated diameter at the level of the transverse costa 2.9-3.3 μm . The length of an entire longitudinal costa is $16.5 \pm 1.9 \mu\text{m}$. The protoplast fills completely the lorica chamber and carries a flagellum that in our material varies between 9-12 μm .

Crucispina cruciformis has previously been recorded from Bay of Algiers, the Mediterranean Sea (Leadbeater, 1974), Elat, the Red Sea (Thomsen, 1978), Kaikoura, New Zealand (Moestrup, 1979), Kilsfjorden, Norway (Espeland and Throndsen, 1986), Osaka Bay, Japan (Hara and Takahashi, 1987a), Monterey Bay, California (Thomsen et al., 1991), the Sound and inner Danish waters, Denmark (Thomsen, 1992; Thomsen et al., 2016), Southampton Water, UK (Tong 1997a), Shark Bay, W. Australia (Tong, 1997b), Sydney, Australia (Tong et al., 1998), Gulf of St. Lawrence, Canada (Bérard-Therriault et al., 1999), Darwin, N. Australia (Lee et al., 2003), and the Beagle Channel, Patagonia (Thomsen, unpublished). Here we add findings from five additional sites (Table 1). In summary it appears safe to conclude that *C. crucispina* is a

widespread organism with a preference for warm water habitats but also with the ability to thrive at the least down to 7°C (Espeland and Throndsen, 1986).

***Calliakantha* Leadbeater 1978 (Figs 9-11)**

This genus comprises forms with anterior spines (3-7) and a posterior pedicel which may be either a single longitudinal costal strip, a continuous long line of slightly overlapping costal strips, or an aggregated pedicel formed by several costal strips bundled together. There are two closely spaced transverse costae. Six species have so far been allocated to the genus, i.e. the generic type *C. natans* (Grøntved, 1956) Leadbeater, 1978; *C. longicaudata* (Leadbeater, 1975) Leadbeater, 1978; *C. simplex* Manton and Oates, 1979; *C. multispina* Manton and Oates, 1979, *C. ankyra* Thomsen, Garrison and Kosman, 1997, and *C. frigida* Thomsen, Garrison and Kosman, 1997. While the latter two species are so far distributionally confined to Antarctic sea ice the other four species thrive in the water column and are frequently found to be community dominants.

***C. natans* (Fig. 9p)**

Easily recognized in all types of preparations because of the three anterior spines and the smooth 'flow' from one spine tip to the other along a parabola shaped curve. Only observed in the Gulf of California (Mexico) during this investigation. *Calliakantha natans* is otherwise widely distributed throughout the cold-water regions of the world's oceans, see e.g. Thomsen et al. (1990) and Thomsen and Østergaard (2017). The same preference for colder water is evident also from seasonal occurrence patterns of this species from Danish coastal waters (Thomsen et al., 2016). The two species, *C. natans* and *C. simplex* occur alternately during an annual sequence, with *C. natans* dominating during late autumn and until the end of spring the subsequent year. Considering the fact that *C. natans* is not easily overlooked in e.g. a dried preparation for LM, it is troublesome to realize the marked difference between the occurrence pattern outlined above (as based on TEM and LM observations) and that which appear from a mapping using

sequence data for *C. natans* and matches against V9 OTU in the Tara Ocean data set (Nitsche et al., 2017; l.c. Fig. 5A). Using the molecular approach *C. natans* appears to be present throughout the warmer parts of both the Atlantic Ocean and the Pacific Ocean. However, it is noteworthy that the species is not recorded at any of the open water Indian Ocean stations sampled. Be aware that the Tara Ocean data set used here has a low representation of genuine cold-water habitats and that there is no quantitative element in the Tara Ocean data analysis. Presence but low abundance of the species (below detection limit of the techniques used here) might explain the discrepancies observed in the biogeographical patterns. A verification of the linkage between taxon and sequence is to be wished for in order to rule out other more trivial explanations of the observed discrepancies.

C. simplex (Fig. 9a-o,q)

This species has four longitudinal costae and anterior spines (projections) that are slightly longer than one costal strip. In the majority of specimens examined the pedicel is just a single costal strip ($5.4 \pm 1.6 \mu\text{m}$ long) but occasionally cells with a much extended pedicel (Fig. 9a,j; 22.2 and 17.4 μm respectively) comprising several costal strips occur in the samples.

It is a well-established fact that *C. simplex* at any given site sampled has a tendency to display a marked overall size variability. Whereas the size distribution tends to be bimodal when examining populations of *C. simplex* from either Antarctica (Thomsen et al., 1990) or the Arctic (Thomsen and Østergaard, 2017), the warm water material examined here shows a more evenly distributed size spectrum as is also documented by the mosaic of specimens illustrated (Fig. 9). The overall lorica size, i.e. the distance from the posterior tip of the pedicel to the anterior tip of a spine, is in our material ($n=16$) $16.2 \pm 4.0 \mu\text{m}$ with an overall range of 10.8-26.3 μm . The warm water community of *C. simplex* thus corresponds, in the context of overall lorica size, to the small sized peak (15-45 μm) of specimens from Antarctic waters (Thomsen et al., 1990; l.c. Fig. 17), and so far no co-existence of a cluster of larger specimens has been recorded. In material from Antarctica these specimens ranged between 60-70 μm (Thomsen et al., 1990; l.c. Fig. 17). Disjunct size distributions in loricate choanoflagellate species is by now fairly well documented across a number of

genera (e.g. Thomsen and Boonruang, 1984; Thomsen et al., 1990; Thomsen and Larsen, 1992; Thomsen and Østergaard, 2017). However, the reasons behind this phenomenon are not at all unravelled.

Calliacantha simplex is a genuine cosmopolitan species that can tolerate huge variability in both water temperature and salinity (Manton and Oates, 1979). In accordance with this the species is here observed frequently at all sites examined (Table 1).

C. magna sp. nov. (Fig. 10, Fig. 11a-g)

The description of a new loricate choanoflagellate species ideally calls for cultured material to improve photo documentation (LM, TEM, SEM) of species-specific morphological traits, and to enable also the application of molecular tools. Forced by circumstances, such as difficulties in culturing these organisms, it has over the last decades been a necessity, in order to unveil the diversity within this group of organisms, to base the vast majority of species descriptions on wild material processed directly for both light and electron microscopy. History shows that species descriptions based exclusively on light microscopy has the potential to clutter the taxonomical framework otherwise established. We have yet decided to make an exception to the rule and describe below a new species of *Calliacantha* based on LM dried specimens only. The rationale behind this is that this is a very large species with an unmistakable affiliation to the genus *Calliacantha*, and yet with an overall appearance that clearly sets it apart from any other described species of *Calliacantha*.

Diagnosis: The lorica has five longitudinal costae that protrude anteriorly as very robust projections. The projections are 15-21 μm long and comprise two costal strips. Junctions between costal strips are pointed out in Fig. 11f,g. There are two transverse costae (Fig. 11d,g). Anterior transverse costal strips measure 4-5 μm , and posterior transverse costal strips 3.4-4 μm . The lorica chamber height is 12-17 μm . The pedicel comprises linearly arranged costal strips and is highly variable in length (30-70 μm). The protoplast measures 4x7.5 μm (Fig. 11a) and the flagellum 27 μm (Fig. 10). Cell division is tectiform as evidenced from the presence of accumulations of costal strips in the collar region (Fig. 10; Fig. 11f).

Type specimen: Fig. 11g collected 8th September 1981 at 2 m depth in the middle of Ao Patong, Phuket Island, Thailand.

Etymology: magna (L) meaning extensive and spacious.

Calliakantha magna sp. nov. has all the key characteristics of *Calliakantha*, i.e. anterior spines/projections, a pedicel, a small number of longitudinal costae (5; 3-7 within the genus as currently circumscribed) and two transverse costae. The species described here is easily distinguished from other species of *Calliakantha* due to its large size, the long and robust anterior projections that are each made up of two costal strips, and the fact that there is consistently 5 longitudinal costae. The closest relative is *C. simplex* which also has anterior projections that exceed one costal strip in length. However, in this species there is consistently four longitudinal costae.

Calliakantha magna has so far been observed in samples from the Andaman Sea, West Australia, the equatorial Pacific Ocean, and the Caribbean Sea (Table 1).

***Saroeca* Thomsen, 1979, emend. Hara and Takahashi, 1987**

The genus *Saroeca* was first established (Thomsen, 1979) to accommodate a single species, *S. attenuata* Thomsen, 1979, found in samples from the Baltic Sea. The main features of the genus are 4-8 longitudinal costae that amalgamate towards the posterior end of the lorica chamber. The anterior longitudinal costal strips are very long and attenuated and the single transverse costa in this species crosses at a point where the longitudinal strips have their maximum width. A second species, *S. paucicostata* Hara and Takahashi, 1987, was added to the genus based on material from Osaka Bay (Hara and Takahashi, 1987b). This species has fewer longitudinal costae (3-5) and two transverse costae, where the second costa crosses at the level of the joints between the longitudinal costal strips. Hara and Takahashi (1987b) emended the genus description to also accommodate forms with two transverse costae. Following the inclusion of this second species the differences between *Saroeca* and *Calliakantha* are minimal (compare e.g. *S. paucicostata* (Fig.

12a) and *C. simplex* (Fig. 9q)). It will be interesting to see the phylogenetic picture emerging once appropriate molecular tools are applied to these taxa. We hypothesize that the genus *Saroeca* as presently circumscribed will not be monophyletic, and thus that a return to a monospecific genus comprising only *S. attenuata* is a likely outcome

S. paucicostata Hara and Takahashi, 1987 (Fig. 12a-i)

This species was commonly observed during our survey and there is good agreement in morphological and dimensional aspects with the Japanese type material. All specimens observed have four longitudinal costae. The anterior longitudinal costal strip (which reaches down to the level of the posterior transverse costa) measures $8.3 \pm 0.7 \mu\text{m}$. Lorica chamber height is $6.4 \pm 0.5 \mu\text{m}$ and the calculated diameter at the level of the anterior transverse costa $4.7 \pm 0.4 \mu\text{m}$. The distance between the two transverse costae is $2.2 \pm 0.24 \mu\text{m}$, and the length of the pedicel $7.8 \pm 1.0 \mu\text{m}$.

Saroeca paucicostata has previously been recorded from Osaka Bay, Japan (Hara and Takahashi 1987b), Taiwanese coastal waters (Hara et al., 1997), Pettaquamscutt River Estuary, Rhode Island, USA (Menezes, 2005), and Kaikoura, New Zealand (Moestrup, unpublished results). Here, we add findings of the species in samples from the Andaman Sea, West Australia, the Sargasso Sea, the Gulf of California, and the eastern Mediterranean Sea at Alexandria (Table 1).

S. attenuata Thomsen, 1979 (Fig. 12j,k)

A Sargasso Sea specimen illustrated here (Fig. 12k) is unmistakably identical to *S. attenuata*. There is a single transverse costa, seven longitudinal costae, and long attenuated and flexible anterior longitudinal costal strips. The identity of a second *S. attenuata* specimen from the equatorial Pacific (Fig. 12j) is more problematic. Critical lorica chamber details are here obscured by the presence of a protoplast. However, the slender and narrow conical appearance of the lorica (compare with e.g. Fig. 12i) indicates that there is

not a second transverse costa, and hence that this specimen is most likely correctly identified as *S. attenuata*.

Saroeca attenuata is reported repeatedly from the Baltic Sea (Ikävalko 1998; Ikävalko and Thomsen, 1997; Thomsen, 1979; Vørs, 1992) but has also been observed in samples from West Greenland (Thomsen and Østergaard, 2017; Thomsen et al., 1995) and Igloolik, Arctic Canada (Daugbjerg et al., 1991). We report here findings of the species in Sargasso Sea samples, and tentatively also from the equatorial Pacific area (Table 1).

Author contributions

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 the transmission electron microscopical examination of these samples.

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Table 1. Occurrence pattern of species discussed here (shaded entries) and in Thomsen and Østergaard (2018).

	Andaman Sea, Thailand	West Australia	Sargasso Sea	Caribbean Sea	Equatorial Pacific Ocean	Gulf of California, Mexico	Mediterranean Sea, Alexandria
<i>Apheloecion articulatum</i>	x	x					x
<i>A. pentacanthum</i>	x	x			x	x	x
<i>A. quadrispinum</i>	x	x	x	x	x		x
<i>A. eqpacia</i> sp. nov.	x	x			x		
<i>Bicosta minor</i> (form A)	x	x		x	x		
<i>B. spinifera</i>						x	
<i>Campyloacantha imbricata</i>	x	x	x	x	x		
<i>C. spinifera</i>	x	x	x			x	x
<i>Calliacantha natans</i>						x	
<i>C. simplex</i>	x	x	x	x	x	x	x
<i>C. magna</i> sp. nov.	x	x		x	x		
<i>Conioeca boonruangii</i> gen. et sp. nov.	x	x		x	x		
<i>Crucispina cruciformis</i>	x	x	x			x	x
<i>Nannoeca minuta</i>	x	x	x	x	x	x	x
<i>N. minuta</i> (form A)	x				x		
<i>N. mexicana</i> sp. nov.		x				x	
<i>Saroeca attenuata</i>			x		(x)		
<i>S. paucicostata</i>	x	x	x			x	x

Figure legends

Fig. 1. Map showing the approximate sampling sites for material reported here and MODIS sea surface temperatures (2003-2011 average). A circle 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).

Fig. 2. *Bicosta* spp. LM (phase contrast except h) and TEM (Fig. 2f-g) whole mounts from the Gulf of California, Mexico (Fig. 2a,b), West Australia (Fig. 2c), the equatorial Pacific (Fig. 2d), the Caribbean Sea (Fig. 2e), and the Andaman Sea, Thailand (Fig. 2f-h). *Bicosta spinifera* (Fig. 2a,b) and *B. minor* form A (Fig. 2c-h). **(a)** Cell complete with protoplast, collar and flagellum; **(b)** Empty lorica to show the twist of the longitudinal costae and the membrane that encases the protoplast. **(c-e)** Differently sized specimens that all display longitudinal costae that adjoin at a sharp angle posteriorly indicative of a twist in this region; **(f)** Complete specimen with a distinct twist midway along the posterior longitudinal costal strips; **(g)** Detail of lorica chamber where the twist is at the very posterior end of the lorica chamber; **(h)** Empty lorica (Nomarski) which is almost identical to the cell illustrated in (g). The scale bar (Fig. 2a) applies to all light micrographs.

Fig. 3. *Apheleocion pentacanthum* (a-e) and *A. quadrispinum* (f-m) LM and TEM (a,k) whole mounts from Mexico (a,d-e), West Australia (b-c,g-i), the Sargasso Sea (f), the Caribbean Sea (j), the Andaman Sea (k), and the equatorial Pacific Ocean (l-m). **(a)** Empty lorica. Notice the pointed spines; **(b-j, l-m)** Selection of LM images of both species; **(k)** Complete cell. Notice also here the sharp-pointed tips of all spines. The scale bar (h) applies to all LM micrographs.

Fig. 4. *Apheleocion articulatum* LM (phase contrast) and TEM (a,e) whole mounts of cells from the Andaman Sea (a), the equatorial Pacific Ocean (b,e), and West Australia (c-d). **(a)** Complete cell showing the general layout of the lorica; notice that the costal strips first accumulated in the collar region appear to

be the transverse costal strips. **(b-d)** Selection of LM images, **(e)** Specimen with little silicified costal strips.

The scale bar (a) applies to all TEM images, and (b) to all LM micrographs.

Fig. 5. *Apheloecion eqpacia* sp. nov. TEM whole mounts from the Equatorial Pacific Ocean. **(a)** Type specimen. Notice the short anterior spines and the accumulation of costal strips in the collar region. **(b)** Low magnification of the type specimen to show the entire length of the pedicel; **(c)** Detail (reversed printing) of costal strip overlap in the pedicel.

Fig. 6. *Apheloecion eqpacia* sp. nov. (a-m) and *A. eqpacia* cfr. (n-r) TEM (a,l,r) and LM (phase contrast) images of cells from the Andaman Sea (a,r), West Australia (b-k,n-q), and the Equatorial Pacific Ocean (l-m).
(a) Complete cell; **(b-k, m)** Selection of cells to show the variability encountered in West Australian waters;
(l) Well preserved protoplast in somewhat incomplete lorica; **(n-q)** Slightly aberrant forms (lack of pedicel

and anterior spines); **(r)** TEM image of lorica that is reminiscent of those illustrated in n-q. The scale bar (c) applies to all light micrographs.

Fig. 7. *Campyloacantha spinifera* (a-j) and *C. imbricata* (k-y) TEM whole mounts (a,e,k-l) and LM images (Nomarski interference contrast (x-y) and phase contrast) from the Gulf of California (a-e), the Sargasso Sea (f-g,i), West Australia (h,j,m-n,q,s-t), the Andaman Sea (k-l,x-y), the Caribbean Sea (o,p), and the equatorial Pacific Ocean (r,u-v). **(a)** Specimen showing the hexagonal symmetry; **(b-d)** Two empty loricas and a complete specimen; **(e)** Empty lorica (reversed printing) which shows costal strip morphological details and the lorica membrane; **(f-j)** Light micrographs to show variability in appearance; **(k)** Complete cell which clearly displays the L-shaped costal strip configuration; **(l)** Complete cell with five anterior spines; the pedicel is missing; **(m-y)** Morphological and dimensional variability across vast geographical distances. Scale bar (d) applies to all LM micrographs.

Fig. 8. *Crucispina cruciformis* LM (phase contrast) images from Gulf of California (a-c), West Australia (d-e), and the Sargasso Sea (f). **(a-f)** Images showing consistency in size and shape across vast distances.

Scale bar (b) applies to all images.

Fig. 9. *Calliakantha simplex* (a-o,q) and *C. natans* (p) TEM (q) and LM (phase contrast except for two (h,n) Nomarski images) from West Australia (a-g), the Andaman Sea (h,q), the equatorial Pacific Ocean (i), the Sargasso Sea (j-k,m), the Caribbean Sea (l), and the Gulf of California (n-p). **(a-o)** Images to show the size and shape variability encountered; **(p)** Empty lorica showing the distinctive *C. natans* features; **(q)** Empty lorica showing constructional details. The scale bar (a) applies to all light micrographs.

Fig. 10. *Calliakantha magna* sp. nov. LM (phase contrast) of complete specimen from the equatorial Pacific Ocean.

Fig. 11. *Calliacantha magna* sp. nov. LM images (phase contrast and (g) Nomarski) from West Australia (a-d), the Caribbean Sea (e-f) and the Andaman Sea (g). **(a-f)** Selected images to show size and shape variability across the regions sampled. Notice (d) the two transverse costae; **(g)** Type specimen. The scale bar (a) also applies to (b,e); the scale bar (c) also applies to (d,f-g).

Fig. 12. *Saroeca paucicostata* (a-i) and *S. attenuata* (j-k) TEM (a-b,e) and LM (Nomarski (c) and phase contrast) whole mounts from the Andaman Sea (a-c), West Australia (d,f), the Gulf of California (e,g-i), the equatorial Pacific Ocean (j), and the Sargasso Sea (k). **(a-b)** Empty lorica showing constructional details; **(c-d,f-i)** Variability across oceans. Notice that all specimens have invariably four longitudinal costae; **(e)** Complete cell with protoplast. Notice the long tapering anterior spines (four) and the two transverse costae; **(j-k)** Complete cell (j) and (k) empty lorica. The latter has seven longitudinal costae and only a single transverse costa. Scale bar (d) applies to all LM images.