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Loricata choanoflagellates (Acanthoecidae) from warm water seas – a baseline study. I. *Conioeca* gen. nov. and *Nannoeca* Thomsen

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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 loricata choanoflagellates (Acanthoecidae). Loricata choanoflagellates are heterotrophic, nano-sized protists that are ubiquitous in marine and brackish water habitats. They dwell in a lorica formed by silicified costal strips organized in species specific patterns. The single anteriorly directed flagellum is surrounded by a collar formed by microvilli which together constitute the feeding apparatus. Key stone benefits from this warm water survey, which covers all three major oceans, is an improved understanding of global biogeographical patterns, and a further consolidation of the morphospecies matrix, that constitutes a highly essential reference framework for the current efforts to provide barcodes for as many species of loricata choanoflagellates as possible, based on e.g. single cell pipetting techniques. We describe here *Conioeca* gen. et sp. nov., which is so far distributionally confined to warm water habitats, and elaborate on the morphological variability encountered within the *N. minuta* complex. This leads to both the circumscription of a new *N. minuta* form A as well as the description of *N. mexicana* sp. nov.

Keywords: Loricata choanoflagellates; *Conioeca boonruangii* gen. et sp. nov.; *Nannoeca minuta*; *Nannoeca mexicana* sp. nov.

Introduction

Choanoflagellates are ubiquitous unicellular or colonial organisms that are present in all aquatic habitats, where they often contribute significantly to microbial loop processes as grazers of bacteria-sized microorganisms. The protoplast is 3-5 μm long and carries a single anterior flagellum surrounded by a collar formed by numerous microvilli. Recent molecular phylogenetic analyses have proved that the choanoflagellates is an ancestral sister group to the metazoa (e.g. Snell et al., 2001; Carr et al., 2008). A thorough description of major aspects of choanoflagellate biology, ecology and evolution was recently provided by Leadbeater (2015).

There is at present consensus with respect to distributing choanoflagellate species among two distinct clades (Nitsche et al., 2011). The order Craspedida encompasses choanoflagellates with organic coverings only, whereas the Acanthoecida (the target group of this and subsequent contributions) accommodates forms with siliceous loricae (loricate choanoflagellates) formed by meshworks of costal strips and ranging in size from 5-100 μm . The loricate choanoflagellates are further subdivided into two families, i.e. the Acanthoecidae and the Stephanoecidae, with nudiform versus tectiform division (Leadbeater, 2015). In tectiform species a complete building set of costal strips is produced prior to division of the protoplast and temporarily placed in the collar region. Costal strip production occurs in the Acanthoecidae once the progeny is released from the parental lorica (nudiform division).

The elaboration of the lorica is in a classical taxonomic framework used as the basis for species and genera delineation. Thomsen and Buck (1991) lists characters used as taxonomic criteria, e.g. lorica form and dimensions, numerical aspects and arrangement of costae, costal strip morphology, spines and projections, pedicels, anterior lorica end costal strip joint patterns etc. Also provided in this review chapter are drawings of all loricate choanoflagellate genera known at that time.

Loricate choanoflagellates are almost exclusively found in marine environments and the number of species described is at present approx. 120. When adding known undescribed forms (Thomsen, unpublished results) it appears realistic that the maximum number of taxa is close to 150. The preliminary Tara Oceans

survey (de Vargas et al., 2015) indicates that approx. 200 choanoflagellate OTU's (Operational Taxonomic Units) occur within the open ocean realms. This number also includes craspedid forms making the estimate above quite realistic.

While it is obvious that transmission (TEM) or scanning electron microscopy (SEM) of whole mounts of cells is the by far best technique when it comes to resolving lorica features (and the preferred tool when describing new taxa during recent decades), it is possible with a high level of certainty to also analyse critical lorica features in e.g. air mounted material observed using a light microscope (LM) equipped with phase or Nomarski contrast oil-immersion lenses.

Our goals are here to provide an account of warm water species diversity and community analyses as well as surveying the morphological variability among individual species primarily based on LM, but with TEM micrographs added when available to underpin the LM images and assist the interpretation of features as observed in the LM. This will contribute to an improved understanding of e.g. biogeographical issues (seasonal and spatial variability and their causes) of importance in e.g. a climate change context, and also provide for the geographical region covered, the best possible morphotype reference framework for future DNA fingerprinting of selected loricate choanoflagellate taxa.

The material examined from warm water regions around the world represents decades of collection work and microscopy and comprises close to 80 species including 15 species that are new to science. It is our ambition to illustrate and discuss both 'old' and new species as far as circumstances call for, in a series of forthcoming papers. Considering the vast geographical area sampled each taxon will be richly illustrated to first of all document the extent of morphological congruity across ocean basins, but also to provide a snapshot of multiple and differently positioned specimens, that constitutes the best possible reference patchwork for future LM based identification efforts. Once the basic and chiefly descriptive work has been completed, the final paper will examine biodiversity patterns across the range of stations sampled and also contrast the warm water fauna with that of other climatic zones using a similar approach to that of Thomsen and Østergaard (2017a).

Thomsen and Østergaard (2017b) provided circumstantial evidence that loricate choanoflagellates may take part in complex life-cycles that involve the possibility that a single ‘species’ can actually occur with completely different types of loricae that are currently allocated to separate genera. The switch from one phase to the other is hypothetically associated to environmental triggers under the assumption that a specific lorica type enables the species to better cope with the prevailing environmental settings. Once the existence of such complex life-cycles is confirmed and data starts accumulating with respect to life-cycle linkages across the loricate choanoflagellate species matrix, it will of course have major impacts on the loricate choanoflagellate species concept and basic nomenclature. However, this is a process that may take decades to accomplish although the rapid development of molecular tools may significantly facilitate the process. In the meantime and based on the assumption that a specific lorica type is selected for by a specific set of environmental parameters, it makes very good sense to continue accumulating data on the occurrence and variability of loricate choanoflagellate ‘species’ (i.e. morphotypes), and also to continue naming new species whenever deemed necessary. A continuously updated species matrix will not only improve our possibilities to ultimately more clearly spot the possible emerging life-cycle patterns linking species pair-wise across the matrix, but also significantly improve the morphological reference database for future molecular work on these organisms.

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 appears from Fig. 1.

Samples from the **Andaman Sea** were collected initially during a September 1981 survey of mostly surface samples from the vicinity of the Phuket Island, using the R/V ‘Pramong’ from the PMBC (Phuket Marine Biological Center). For further details see e.g. Thomsen and Boonruang (1983a). A subset of this material has

been published in a series of papers by Thomsen and Boonruang (1983a,b; 1984) and Thomsen and Moestrup (1983). Additional sampling covering a much larger Andaman Sea geographical area and organized in three transects (100-200 km) perpendicular to the coastline and reaching across the continental shelf took place during the period 1995-2000 (see e.g. Nielsen et al., 2004 for further details). This sampling programme was carried out as part of a Thai-Danish collaborative marine science initiative utilizing the Thai research vessel R/V 'Chakratong Tongyia'. The main source of material for this study is a cruise conducted during February 1997 comprising samples from the surface and the deep fluorescence maximum layer from stations distributed widely across the three transects.

A Danish circumglobal research expedition 'Galathea 3' took place during 2006-2007 utilizing a naval surveillance vessel 'Vaedderen'. During November 2006 the vessel operated along **West Australia** from Broome to Perth. Surface samples as well as samples from the deep fluorescence maximum layer utilized for this study originate from a 180 nm long NW-directed transect across the shelf outside Broome (119-122°E and 16-18°S).

Eastern Mediterranean samples (surface water and 1% light depth) were collected February 1983 at stations 1 and 3 nm north of the Alexandria Harbour, Egypt.

Sargasso Sea samples were collected April 9-20, 2014, at transects and single stations located within an area delimited by 59.30-37.40°W and 25.40-31.00°N. The samples processed onboard the R/V 'Dana' were from surface waters and the deep fluorescence maximum layer. For further details on cruise details and map of stations and transects see e.g. Ayala and Munk (2018).

Samples were collected on the NOAA spring 1992 cruise to the **equatorial Pacific** during the EqPac Process Study as part of the Equatorial Pacific Ocean Climate Study/Joint Global Ocean Flux Studies (EPOCS/JGOFS of NSF/NOAA (Murray et al., 1994). The material reported on here was collected from the R/V 'Malcolm Baldrige' during leg 3 from Papeete (Tahiti) to Balboa (Panama) during April to May 1992. The ship occupied stations along the 140° meridian from 10°N to 10°S. Samples processed for LM and TEM originated from surface waters and the deep chlorophyll maximum layer. Additional sampling was conducted along the

equator and also at stations north of the equator while steaming towards Balboa. Nanoplankton biodiversity data from leg 1 of the same cruise (Balboa to Hilo, Hawaii / Feb. to March 1992) where equatorial stations were sampled along the 110°W and 125°W meridians, have been published by Vørs et al. (1995).

Caribbean Sea surface samples were collected (May 1992) at two sites (12°N/80°W and 17°N/82°W) during the return voyage of the R/V 'Malcolm Baldrige' from the EqPac Process Study (see above).

Surface water samples from the **Gulf of California** were collected 7-8 January 1990 in the vicinity of Bahia de los Ángeles (28.58°N / 113.31°W) and aided by local fishing boats.

The general protocol for processing water samples for the light microscope (LM) and transmission electron microscope (TEM) was according to Moestrup and Thomsen (1980). The sample volume was 1-2 litres. Wherever possible the samples were kept at ambient temperature until further processed.

The nanoplankton community was typically concentrated for further processing by means of centrifugation of prefiltered material resuspended from an initial filtration of cells on top of e.g. a 1 µm Nuclepore filter (range of sizes used is 1-3 µm). The volume of water processed was around 0.5 litres, however, site specifically adjusted as deemed necessary in order to produce a workable suspension of cells. Small droplets of cells from the resuspended final pellet of material were placed on carbon coated grids for the TEM or on coverslips for the LM. Cells were subsequently fixed for ca. 30 seconds in the vapour from a 1-2% solution of OsO₄. After drying the grids were carefully rinsed in distilled water in order to remove salt crystals. Grids were shadow cast with either Au/Pd or Cr prior to the examination in JEOL electron microscopes property of the Botanical Institute at the Univ. of Copenhagen. Rinsed coverslips intended for LM were air-mounted upside down, using e.g. nail polish or double sticky tape, on a slide to render possible the use of oil-immersion lenses. The microscopes used were a Leitz Dialux 20 equipped with interference contrast optics and a Wild MPS 55 photo automat, an Olympus BH-2 equipped with phase contrast and an Olympus UC30 digital camera, and a Carl Zeiss Axio Imager M2 with a 63x oil immersion lens, and a Zeiss AxioCam digital camera. The public domain software ImageJ ver. 1.52i was used for all measurements of cells.

The terminology applied largely follows Thomsen and Buck (1991).

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 distinct lorica features are bundled together. While this approach is straightforward when it comes to core groups of taxa representing e.g. multispecies genera such as *Cosmoeca* spp., *Platypleura* spp., and *Stephanacantha* spp., it is less obvious when addressing e.g. monospecific genera. In this paper we will deal with *Conioeca* gen. nov. and *Nannoeca* Thomsen, 1988, two outlier genera that based on morphological objectives are not easily bundled with other known loricate choanoflagellate taxa. 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 obviously phylogenetically related.

The logic behind treating *Conioeca* and *Nannoeca* in the same paper is not that we necessarily expect these genera to be phylogenetically related, but that they after all share certain morphological features, i.e. two transverse costae of which one closes the lorica chamber anteriorly, and more importantly the fact that none of these genera appear to have a fixed and predefined pattern of costal strip joints at the anterior lorica end.

Conioeca boonruangii gen. et sp. nov. (Figs 2-4, 8d)

Diagnosis: Medium-sized marine loricate flagellate with a protoplast (ca. 4 x 10 µm) posteriorly located in a funnel-shaped lorica. Lorica height excl. posterior pedicel is 23-29 µm. The diameter at the anterior lorica opening is 18-24 µm. The diameter at the level of the posterior transverse costa is 6-7 µm. The lorica comprises 7-9 longitudinal costae, two transverse costae, and four types of costal strips. Each longitudinal costa encompasses 2-3 costal strips which are 9-11.5 µm long. Some amalgamation of longitudinal costae occurs at the posterior lorica end. The anterior transverse costa comprises ca. 14 costal strips which are 5-6 µm long. The joints between these and the anterior tips of longitudinal costae (weakly bifurcate; Fig. 2b)

appear to be random. The posterior transverse costa is located at the level of the joints between the anterior pairs of longitudinal costal strips. The transverse costal strips are here much shorter (3.5-4.5 μm) than those found elsewhere and overlap neighboring costal strips significantly (Fig. 2d). The lorica is terminated by a pedicel (2-4 slightly overlapping costal strips; each 6-8 μm long). This species has tectiform division as evidenced from the occasional presence of bundles of costal strips in the collar region (e.g. Figs 2c, 3a, 4c).

Type specimen: Fig. 2a from the Andaman Sea, Phuket Island, Thailand collected 10 September 1981 from 20 meters depth at an off-shore station SW of the southernmost tip of the Phuket Island at a depth of 160 meters.

Etymology: The generic name is derived from 'conion' (Greek) meaning cone-shaped, and 'oikos' (Greek) meaning house, i.e. the cone-shaped house. The species epithet is chosen to acknowledge the contribution by Mrs. Pensri Boonruang to our nanoplankton research at the Phuket Marine Biological Center (PMBC).

Attempts to convincingly allocate the present material to any existing loricate choanoflagellate genus were unsatisfactory. *Conioeca boonruangii* was, while work was in progress, tentatively maintained within a cluster of species of *Parvicorbicula* Deflandre, 1960, mostly because of a superficial resemblance to the *Parvicorbicula* type species, *P. socialis* (Meunier, 1910) Deflandre, 1960 (Fig. 8f) and also to *P. manubriata* Tong, 1997. However, points of resemblance are limited to an overall funnel-shaped appearance of the lorica, a simple lorica construction comprising up to 10 longitudinal costae and 1-2 transverse costae, and finally the absence of anterior spines. *Conioeca boonruangii* differs from the species above by being significantly larger and also by having no less than four well-defined types of costal strips integrated in its lorica design. The perfectly defined anterior T-joints in the generic type, *Parvicorbicula socialis*, is also a feature that is not reproduced in *C. boonruangii*. The genus *Parvicorbicula* is fortunately well-defined with reference to the type species (Manton et al., 1976). However, the genus has subsequently become cluttered due to the allocation of too many taxa that are only remotely similar to the *Parvicorbicula* core species. It is in this context, and while awaiting supporting molecular evidence, considered more appropriate to describe the material here

examined within a genus of its own, rather than adding further to the obvious heterogeneity of the genus *Parvicorbicula*. The genus *Conioeca* may further come in handy when dealing with a couple of undescribed species from Baltic brackish water (Hällfors and Thomsen, unpublished results).

Conioeca boonruangii is widely distributed across the equatorial warm water belt (Table 1). It is a large and morphologically highly distinct species that has not, so far at least, been observed outside the equatorial warm water belt. There is no indication of any basin specific morphological variability in the material examined (see e.g. Fig. 4).

Nannoeca Thomsen, 1988

The monospecific genus was established by Thomsen (1988) to accommodate *Pleurasiga minima* Thronksen, 1970 var. *minuta* Leadbeater, 1972 (= *Pleurasiga minuta* (Leadbeater, 1972) Leadbeater, 1976 in Manton et al., 1976). The genus *Pleurasiga* Schiller, 1925 is badly circumscribed because uncertainties exist with reference to the true nature of the type species *Pleurasiga orculaeformis* Schiller, 1925. Although efforts have been made (Leadbeater 1973) to sample again the *P. orculaeformis* type locality (the Adriatic Sea), it has not so far resulted in the finding of material that convincingly resembles the Schiller (1925) drawing (Fig. 8i). In consequence of this efforts have been made to solve the *Pleurasiga* enigma by gradually removing aberrant species and groups of species that were formerly part of the genus *Pleurasiga* to alternative genera (Thronksen, 1974; Manton and Bremer, 1981; Thomsen and Boonruang 1984; Thomsen 1988), leaving behind a small group of morphologically fairly congruent and well-defined species (i.e. *P. minima* Thronksen, 1970 (Fig. 8e); *P. reynoldsii* Thronksen, 1970; *P. echinocostata* Espeland, 1986 in Espeland and Thronksen 1986; *P. tricaudata* Booth, 1990). It should be mentioned in passing that the *Pleurasiga* issue, i.e. the quest for the true identity of *P. orculaeformis*, has further ramifications. In retrospect it thus becomes more and more obvious that the closest match of *P. orculaeformis* is in fact among species presently allocated to the genus *Polyfibula* Manton and Bremer, 1981 (Fig. 8g). It should be noted here that a further complication exists with reference to the *Campanoeca* Thronksen, 1984 type species *C. dilatata* Thronksen, 1984 (Fig. 8h)

which is only known from LM but obviously intimately related to the *Polyfibula* species cluster, and hence also *Pleurasiga orculaeformis*. Despite the level of complexity with reference to properly circumscribing the genus *Pleurasiga*, and the fact that *Nannoeca minuta* has previously been part of this, it is our firm belief that the extraction of this taxon to a genus of its own is still a relevant approach and further that this has no influence on how to solve the *Pleurasiga* riddle briefly introduced above.

Nannoeca minuta (Leadbeater, 1972) Thomsen, 1988 (Figs 5-6, 8a-b)

The lorica is tiny, 3.5-6 μm , and composed from 6-10 longitudinal costae and two transverse costae. One transverse costa is anteriorly positioned in the conical lorica while the second transverse costa is located slightly above the junctions between the two tiers of longitudinal costal strips. There is no recurrent and consistent pattern in anterior lorica end connections between longitudinal and transverse costal strips. A certain reduction occurs posteriorly in the number of longitudinal costal strips. Thomsen (1988) further reports that with a few exceptions all cells examined carry short anterior spines formed by the longitudinal costae protruding above the anterior transverse costa. Also the type species (Leadbeater, 1972; l.c. Fig. 12) had longitudinal costae projecting slightly above the anterior transverse costa.

The material examined here (Fig. 5) is in general accordance with the circumscription of this species as provided by Thomsen (1988) and briefly outlined above. Two specimens (Fig. 5e, i) are mirror images of material previously published (Leadbeater, 1972; Thomsen, 1988), whereas others (Fig. 5a-c, f-g) are slightly deviant from the core type due to the consistent absence of anterior spines. The occasional presence of slightly flattened and dentate anterior transverse costal strips was noticed and illustrated by Thomsen (1988; l.c. Fig. 10) in material from Thailand. However, in more recent Andaman Sea material (Fig. 5 a-d) this is a more consistent feature that here further applies to both transverse and longitudinal costal strips. The general picture seems to be that the individual costal strip has a regular concave face, whereas the convex edge is irregular and dentate (see the enlarged transverse costal strips in Fig. 5d). Spineless cells from the Equatorial Pacific are illustrated in Fig. 5f-g. In these specimens the costal strips are narrow and rod-like

similar to what is observed in e.g. the type specimen and Fig. 5e,i. It should be emphasized that ‘normal’ spiny *N. minuta* cells have previously been reported from the equatorial Pacific (Vørs et., 1995; l.c. Fig. 4). For the time being it appears appropriate to deal with these morphological dissimilarities as infraspecific variation in *N. minuta*. At the same time, it will, however, be expedient in a simple way to be able to refer to any of the two morphotypes described above. It is therefore recommended that the spine-less form, which may or may not have flattened and dentate costal strips, is henceforward referred to as *N. minuta* (form A). Notice that it is not possible with any reasonable degree of certainty to light microscopically recognize the ‘form A’ from *N. minuta* sensu stricto.

One additional deviant lorica type occurred in material from the Gulf of California (Fig. 5h). In this specimen the anterior lorica chamber encompasses no less than 13 longitudinal costal strips. With the possible exception of this latter specimen the observations presented here only adds to our understanding of the overall morphological plasticity of this taxon.

The LM identification of this species is of course hampered by the minuteness of the lorica (Fig. 6). However, the conical shape of the lorica and the presence of two transverse costae can still be verified from the majority of the specimens illustrated in Fig. 6a-u. A prominent flagellum protruding far above the lorica and ending in a conspicuous hair-point (see e.g. Fig. 6l, q) is a keystone feature of this species that is often instrumental in the initial recognition of this taxon in any sample analyzed. The lorica mean height in specimens illustrated as part of Fig. 6 is $5.1 \pm 0.47 \mu\text{m}$, while the anterior transverse diameter (calculated on the assumption that the specimens measured are completely flattened and 2-dimensional) is $4 \pm 0.53 \mu\text{m}$, and the flagellar length $8.1 \pm 1.5 \mu\text{m}$.

Nannoeca minuta is widely distributed in a global context and observed at temperatures ranging from 1-27° C and at salinities from 10-41 (Thomsen 1988). It may be relevant to mention that *N. minuta* has not so far been found in circum-Antarctic samples, nor in samples from extreme low salinity areas such as the innermost parts of the Baltic Sea. The northernmost finding of the species is Disko Bay, West Greenland (Thomsen and Østergaard, 2017a). *Nannoeca minuta* was found at all warm water sites reported here. (Table

1). The recognition of *N. minuta* form A is dependent on the access to TEM images. It has therefore so far been recorded from two sites only (Table 1).

Nannoeca mexicana sp. nov. (Fig. 7, 8c)

Recently, while examining material from e.g. West Australia (Fig. 7f-k) slightly larger (lorica height: 6-8 μm) and pedicellated specimens without anteriorly projecting longitudinal costal strips, but with an otherwise identical lorica configuration, have co-occurred with more typical forms of *N. minuta*. Such specimens were also previously encountered in Mexican waters (Fig. 7a-e) and are here referred to as *N. mexicana* and formally described as a new species.

Diagnosis: Identical to *Nannoeca minuta* in basic lorica features, i.e. up to 10 longitudinal costae and two transverse costae but deviating in terms of lorica height (5.9-8.0 μm / mean value (Fig. 7b-k): $7.3 \pm 0.7 \mu\text{m}$), lorica anterior diameter (4.8-6.7 μm / mean value (Fig. 7b-k): $5.6 \pm 0.6 \mu\text{m}$), the apparently consistent absence of tiny anteriorly projecting spines, and the presence of a simple pedicel which is 1-3 μm long (mean value (Fig. 7b-k): $2.2 \pm 0.6 \mu\text{m}$).

Type specimen: Fig. 7a observed in a surface water sample (ca. 18°C / 35 PSU) from a near coastal site at Bahia de los Ángeles, Gulf of California, Mexico collected 7 January 1990.

Ethymology: The species epithet 'mexicana' is chosen to acknowledge the geographic origin of the material first examined of this organism.

The morphological differences between *N. minuta* and *N. mexicana* justifies at least for the time being the description of the latter as an independent species. Other options considered were to establish this new morphotype as a *N. minuta* subspecies, or alternatively make further use the *Cosmoeca ventricosa* concept (Thomsen and Boonruang, 1984) of identifying deviant specimens as form A, B etc. *Nannoeca minuta* is a frequently recorded species. It applies to most loricate choanoflagellate species that the tolerance limits with

respect to infraspecific variability are still inadequately defined and only slowly clarified in parallel with the examination of material of any given species from a variety of geographic sites and abiotic regimes. Until molecular tools can be applied to support the taxonomic decision process, the degree of variability to be accepted within a given species largely reflects the traditions established during the unravelling of loricate choanoflagellate diversity as evolving during the last 50 years, and thus rests on subjective evidence as presented and evaluated by the observer. In the case of *N. mexicana* it is the co-existence at multiple sites of this larger and pedicellated form with typical *N. minuta* specimens, and the absence of forms that morphologically clearly bridges the gap between these morphotypes that prompts the description of a new species.

Light microscopical recognition of *N. mexicana* is possible but not definitive in all cases. The majority of the cells illustrated here (Fig. 7b-k) clearly distinguishes themselves from *N. minuta* (Fig. 6a-u) based on lorica dimensions and the presence of a posterior pedicel. However, two specimens, i.e. Fig. 7d and Fig. 7g, are included in the *N. mexicana* mosaic of LM images with some hesitation. While these specimens are admittedly larger than the average *N. minuta* cell (compare with Fig. 6a-u) they lack a pedicel and also differ slightly in overall appearance of the lorica.

Nannoeca mexicana is so far found at only two of the warm water sites examined (Table 1).

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 the transmission electron microscopical examination of these samples.

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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. *Conioeca boonruangii* gen. et sp. nov. TEM whole mounts from the Andaman Sea, Thailand. **(a)** Complete cell (type specimen) with protoplast and collar microvilli; **(b)** Empty lorica to show details of the

lorica chamber. Notice the membrane that supports the protoplast (arrow); **(c)** Anterior lorica chamber; **(d)** Detail of costal strip configuration of posterior transverse costa.

Fig. 3. *Conioeca boonruangii* gen. et sp. nov. TEM whole mounts from the equatorial Pacific Ocean. **(a)** Complete cell; **(b)** Detail of anterior lorica chamber.

Fig. 4. *Conioeca boonruangii* gen. et sp. nov. LM images of cells from West Australia **(a-c)**, Equatorial Pacific Ocean **(d-f)** and the Caribbean Sea **(g)**.

The scale bar (a) applies to all micrographs.

Fig. 5. *Nannoeca minuta* TEM whole mounts from the Andaman Sea, Thailand **(a-e)**, the Equatorial Pacific Ocean **(f-g)** and **(h-i)** the Gulf of California, Mexico. **(a-c)** Specimens (form A) without protruding tips (arrows) of anterior longitudinal costae; **(d)** Detail (reversed printing) of anterior transverse costal strips (from the specimen in Fig. 5c and tilted 90°). Notice the elaborate and toothed anterior rim (arrows); **(e)** Small Thailand specimen with short anterior spines; **(f-g)** Complete specimens (form A) without anterior spines; **(h)** Empty lorica (reversed printing) with many longitudinal costae and protruding spines; **(i)** Complete specimen.

The scale bar (a) applies to a-c; The scale bar (i) applies to f-i.

Fig. 6. *Nannoeca minuta* LM images of cells from the Gulf of California, Mexico **(a-g)**, the Equatorial Pacific Ocean **(h-i)**, the Sargasso Sea **(j-m)**, The Caribbean Sea **(n-p)** and West Australia **(q-u)**.

The scale bar (m) applies to all micrographs.

Fig. 7. *Nannoeca mexicana* sp. nov. TEM whole mount **(a)** and LM images **(b-k)** from the Gulf of California **(a-e)** and West Australia **(f-k)**. **(a)** Empty lorica (type illustration / reversed printing) showing the absence of anterior spines and the short pedicel; **(b-k)** Complete cells and empty loricae reminiscent of Fig. 7a. Two

specimens (d, g) deviate in being slightly smaller and without a posterior pedicel. The overall appearance of these specimens is yet more coinciding with *N. mexicana* than with *N. minuta*.

Scale bar (f) applies to all LM micrographs.

Fig. 8. Drawings to approximate scale of species and genera discussed in the text. (a) *Nannoeca minuta*; (b) *Nannoeca minuta* form A; (c) *Nannoeca mexicana*; (d) *Conioeca boonruangii*; (e) *Pleurasiga minima*; (f) *Parvicorbicula socialis*; (g) *Polyfibula sphyrelata*; (h) *Campanoeca dilatata* redrawn after Throndsen (1974); (i) *Pleurasiga orculaformis* redrawn after Schiller (1925).

Tabel 1. Occurrence pattern of species discussed here.

	Andaman Sea, Thailand	West Australia	Sargasso Sea	Caribbean Sea	Equatorial Pacific Ocean	Gulf of California, Mexico	Mediterranean Sea, Alexandria
<i>Conioeca boonruangii</i> gen. et sp. nov.	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	