Loricate choanoagellates (Acanthoecida) from warm water seas. V. Thomsenella Özdikmen (=Platyleura Thomsen)

Thomsen, Helge Abildhauge; Østergaard, Jette Buch

Published in:
European Journal of Protistology

Link to article, DOI:
[10.1016/j.ejop.2019.125633](https://doi.org/10.1016/j.ejop.2019.125633)

Publication date:
2019

Document Version
Peer reviewed version

[Link back to DTU Orbit](#)

Citation (APA):
Thomsen, H. A., & Østergaard, J. B. (2019). Loricate choanoagellates (Acanthoecida) from warm water seas. V. *Thomsenella Özdikmen* (=Platyleura Thomsen). *European Journal of Protistology*, 71, [125633].
<https://doi.org/10.1016/j.ejop.2019.125633>

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Journal Pre-proof

Loricate choanoflagellates (Acanthoeccida) from warm water seas. V.
Thomsenella Özdikmen (= *Platypleura* Thomsen)

Helge Abildhauge Thomsen, Jette Buch Østergaard

PII: S0932-4739(19)30070-7
DOI: <https://doi.org/10.1016/j.ejop.2019.125633>
Article Number: 125633
Reference: EJOP 125633

To appear in:

Received Date: 25 April 2019
Revised Date: 19 June 2019
Accepted Date: 4 July 2019

Please cite this article as: Thomsen HA, Østergaard JB, Loricate choanoflagellates (Acanthoeccida) from warm water seas. V. *Thomsenella* Özdikmen (= *Platypleura* Thomsen), *European Journal of Protistology* (2019), doi: <https://doi.org/10.1016/j.ejop.2019.125633>

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2019 Published by Elsevier.

Loricata choanoflagellates (Acanthoecida) from warm water seas. V. *Thomsenella* Özdikmen (= *Platypleura* Thomsen)

Helge Abildhauge Thomsen^{a*} and Jette Buch Østergaard^b

^aTechnical University of Denmark, National Institute of Aquatic Resources (DTU Aqua), Kemitovet, Bygning 201, DK 2800 Kgs. Lyngby, Denmark / hat@aqu.dtu.dk

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

Abstract

Loricata choanoflagellate genera that incorporate flattened costal strips in the lorica (i.e. *Calotheca*, *Stephanacantha*, *Thomsenella* (= *Platypleura*) and *Syndetophyllum*) are prevalent in warm water habitats. The genus *Thomsenella* (= *Platypleura*) thus comprises four species and three of these have an Andaman Sea type locality. Our ongoing examination of loricata choanoflagellate material from all major warm water oceans has provided us with the opportunity of revisiting species of *Thomsenella* in order to test and fortunately verify, in a morpho-specific context, the robustness of the species matrix initially defined.

Keywords: Loricata choanoflagellates; Acanthoecida; *Platypleura*; *Thomsenella*; Warm water seas

Introduction

In an attempt to advance our understanding of global diversity patterns, and also in order to update the global species catalogue of loricata choanoflagellates by introducing a number of hitherto undescribed forms, we are currently in the process of publishing an inventory of warm water species encountered in samples from all major oceans. This is the fifth paper in the series

(Thomsen and Østergaard 2019a-d) and the focus is here on species of *Platypleura* Thomsen in Thomsen and Boonruang, 1983.

The genus *Platypleura* is broadly speaking a morphological counterpart of the genus *Cosmoeca* Thomsen in Thomsen and Boonruang, 1984 (Thomsen and Boonruang 1983; Thomsen and Boonruang 1984; Thomsen and Østergaard 2019d). While species of *Cosmoeca* have loricae constructed from rod-shaped costal strips, the lorica of *Platypleura* spp. is characterized by comprising more elaborate costal strips that are flattened and often displaying pronounced three-dimensional features (e.g. a V-shaped folding of the individual costal strip along the longitudinal axis).

It has become evident (Özdikmen 2009) that the genus name *Platypleura* when used by Thomsen and Boonruang (1983) was in fact preoccupied by *Platypleura* Amyot and Serville, 1943 (Insecta: Hemiptera: Cicadidae: Cicadinae: Platypleurini). The International Code of Zoological Nomenclature (ICZN 1999 / Article 1) states that the term "animals" refers to the Metazoa and also to protistan taxa when workers treat them as animals for the purposes of nomenclature. It follows from here, because the initial description of *Platypleura* Thomsen (Thomsen and Boonruang 1983) was in fact guided by the ICZN, that the nomenclatural changes made by Özdikmen (2009) have to be adhered to.

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.

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).

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 chamber 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.

The unfortunate existence of an electron microscope specific problem causing negatives to appear horizontally flipped, was discussed in Thomsen and Østergaard (2019d). The evidence in favour of dealing with this problem as a purely technical issue is overwhelming. Scans of the affected negatives have accordingly been flipped horizontally to produce non-inverted images. In the current publication this applies to: Fig. 3a-c; Fig. 4; Fig. 5a-d; Fig. 7a, c; Fig. 9a, b; Fig. 10a, b; Fig. 11a, g-i.

Results and discussion

Thomsenella Özdikmen, 2009

The *Thomsenella* lorica comprises two well-defined chambers, 10-12 longitudinal costae (each comprising 4 costal strips) and two or three transverse costae. The distance between the two anterior transverse costae equals in most species the length of two longitudinal costal strips. The third transverse costa when present separates the anterior and posterior lorica chambers. There are abutting joints between the transverse costal strips of the anterior ring and the longitudinal costae. The anterior ring is inside the longitudinal costae. A compound pedicel (i.e. a tuft of diverging costal strips emanating from the posterior lorica end) is a typical feature of this genus. Replication is tectiform in all species as evidenced from the accumulation of supernumerary costal strips in the collar region in preparation for cell division.

The genus currently comprises four species, i.e. the generic type *T. infundibuliformis* (Leadbeater 1974), and additionally *T. acuta*, *T. cercophora* and *T. perforata* (Thomsen and Boonruang 1983). The species are distinguished based on costal strip particulars, the presence or absence of anterior short spines, the elaboration of the posterior lorica chamber, and details of the posterior pedicel.

Thomsenella infundibuliformis (Leadbeater, 1974) Özdikmen, 2009 (Figs 3-5)
(= *Platypleura infundibuliformis* (Leadbeater, 1974) Thomsen in Thomsen and Boonruang, 1983;
Parvicorbicula infundibuliformis Leadbeater, 1974)

Material identical to *T. infundibuliformis* as recorded by Thomsen and Boonruang (1983; specimens from Danish coastal waters) was found only in samples from the Gulf of California (Fig. 3a-d; Fig. 4), the equatorial Pacific Ocean (Fig. 5), and the eastern Mediterranean Sea.

Thomsenella infundibuliformis has 11 (rarely 10 or 12) longitudinal costae (4 costal strips each) and three transverse costae. All longitudinal costal strips are broad and increasing in width towards the posterior lorica end. There is a distinct midrib and the flanges on either side typically show a pattern of elongate perforations perpendicular to the long axis of the strip (Fig. 3b). The keel is most pronounced on the interiorly oriented face of the costal strip. Transverse costal strips are shorter, narrower, without conspicuous flange perforations, and with drawn out and slightly bent tips (Fig. 3c). The transverse costal strips are often folded along the densely silicified midrib giving the strip an open v-shaped transverse profile (Fig. 4) where the concave side is facing outwards. There is in undamaged specimens a compound pedicel (Figs 3a, 4). Whereas the middle transverse costa crosses exactly at the junctions between the second and third tiers of longitudinal costal strips (counted from the anterior lorica end) in *T. acuta* (Fig. 9a), *T. cercophora* (Fig. 11a) and *T. perforata* (Fig. 7c), it appears that in *T. infundibuliformis* this costa crosses anterior to the longitudinal costal strip junctions (Fig. 3a and Fig. 4; arrows; see also Thomsen and Boonruang 1983, loc. cit. Fig. 32; Espeland and Throndsen 1986, loc. cit. Fig. 27; Hara et al. 1997, loc. cit. Fig. 19; Thomsen et al. 2016, loc. cit. Fig. 8D). This feature is not convincingly detectable in any of the micrographs that accompany the first description of the species (Leadbeater 1974) and is hence also not included in the schematic drawing that serves as the holotype.

Morphometric details of cells observed in Gulf of California and eastern Mediterranean samples are shown in Table 1 together with data summarizing dimensional characteristics of our findings of this species from Danish coastal waters (see also Fig. 6a). A single specimen (Fig. 3e) was found in samples from the Sargasso Sea. While obviously closely related to *T. infundibuliformis* sensu stricto this particular specimen is almost twice the size of specimens observed elsewhere (Fig. 6a). It is

unclear what is happening here and all that can be done for the time being is just to put the observation on record.

Specimens from the equatorial Pacific Ocean are included here (Fig. 5) because of a general similarity to the Gulf of California specimen illustrated in Fig. 4. Lorica height (11.9-12.9 μm), anterior transverse costa diameter (7.7-8.4 μm) and middle transverse costa diameter (6.8-7.2 μm) for the three specimens shown in Fig. 5 all fall within the ranges that are typical for *T. infundibuliformis* sensu stricto (Fig. 6a). A small reservation has to be flagged with reference to the identification of these Pacific Ocean specimens as *T. infundibuliformis*. A proper compound pedicel has not been documented, and the positioning of the middle transverse costa (anterior to the longitudinal costal strip junctions) is not doubtlessly displayed in these specimens, yet, see encircled area in Fig. 5c. Finally, it appears that in the Pacific Ocean specimens (Fig. 5) the longitudinal costal strips are generally less broad and the flanges more parallel-sided and on the whole less pronounced. It is worth emphasizing that the Pacific Ocean specimens (Fig. 5) are in general much similar to a Bay of Algiers specimen published by Leadbeater (1974; loc. cit. Pl. IIA) as an integrated part of the first description of this species.

Distribution: Observed in samples from the Gulf of California, the equatorial Pacific Ocean, the eastern Mediterranean Sea, and the Sargasso Sea (Table 2) and additionally recorded from the Bay of Algiers (Leadbeater 1974), Danish coastal waters (Thomsen and Boonruang 1983; Thomsen et al. 2016), Kilsfjorden, Norway (Espeland and Throndsen 1986), the North Pacific Central Gyre (Hoepffner and Haas 1990; identification uncertain), Japanese and Taiwanese coastal waters (Hara et al. 1997), Darwin, Australia (Lee et al. 2003; no illustrations included leaving the identification uncertain), Pettaquamscutt River, Rhode Island, USA (Menezes 2005), New Zealand (Fig. 2;

micrograph courtesy of Dr. Ø. Moestrup), the Beagle Channel, Patagonia (Thomsen, unpublished material).

Material from the Red Sea (Thomsen 1978) has been re-examined and it is now evident that the species present in the Gulf of Elat is *T. acuta* rather than *T. infundibuliformis*.

Thomsenella perforata (Thomsen in Thomsen and Boonruang, 1983) comb. nov. (Figs 7, 8)

Bas.: *Platyleura perforata* Thomsen in Thomsen and Boonruang, 1983

Thomsenella perforata is a tiny species (Table 1 and Fig. 6d) with 11 longitudinal costae and three transverse costae (Fig. 7a, c). All longitudinal costal strips have a heavily silicified midrib and lateral flanges that are densely perforated in an open-mesh pattern perpendicular to the long axis of the strip (Fig. 7b). The costal strips increase in width when moving from the anterior to the posterior lorica end (Fig. 7c). While the anterior longitudinal costal strips are almost parallel-sided, yet with a distinct difference between the rounded anterior tip and the more attenuated posterior tip of the costal strip, costal strips further down in the lorica show a more pronounced polarity where the anterior part of the costal strip is much widened. While longitudinal costal strips appear to be flattened and largely two-dimensional, the transverse costal strips are folded along the densely silicified midrib and thus acquiring a V-shaped profile (Fig. 7a, c). Transverse costal strips appear more narrow than longitudinal costal strips and more symmetrical along the long axis of the strip. The *T. perforata* lorica often carries a simple pedicel. It is most often just a single costal strip (Fig. 7a) but may occasionally also be a line of costal strips joined end-to-end (Fig. 7d, f, h). Our current material is in good agreement with the Andaman Sea type material (Thomsen and Boonruang 1983) both with reference to lorica dimensions (Table 1) and morphological particulars.

The recognition of this taxon from LM is by and large not complicated. However, it has to be emphasized that the key-stone feature of this species, i.e. the costal strip perforations, can never be resolved from LM. When comparing LM images (e.g. Fig. 7d-n) with TEM whole mounts (Fig. 7a, c) it is obvious that there is significant congruence with respect to lorica dimensions and constructional details, and further that none of the LM images selected bear any resemblance to either *T. cercophora* (Fig. 9c-h) or *T. acuta* (Fig. 11b-f).

Thomsenella perforata falls within the exact same size range as *T. infundibuliformis* (Fig. 6a, d). When the two taxa co-occur within the same water mass it will be nearly impossible to separate these light-microscopically with any degree of certainty. Features to look for are the very broad costal strips in *T. infundibuliformis* where it is possible in clean preparations to distinguish the outline of the flanges. The presence of a compound pedicel (*T. infundibuliformis*) versus a simple pedicel (*T. perforata*; single strip or a line of strips) are additional distinguishing, however not definitive clues to the identity of a given specimen. A simple or compound pedicel is a fragile structure that appears to be easily broken in nature or during preparation. Some of the specimens illustrated in Fig. 8 may in fact be identical to the *T. infundibuliformis* variant described (Fig. 5) from the equatorial Pacific Ocean. In this variant type the longitudinal costal strips are narrower and longitudinally more symmetrical than in *T. infundibuliformis* sensu stricto.

Distribution: Observed from all geographic sites examined here (Table 2) and previously recorded from the Andaman Sea (Thomsen and Boonruang 1983), Californian central waters, USA (Thomsen et al. 1991; as *P. perforata* cfr.) the equatorial Pacific Ocean (Vørs et al. 1995), and Sydney, Australia (Tong et al. 1998). Booth (1990) also reports the species from the subarctic North Pacific Ocean. However, based on the scanning electron micrograph published (Booth 1990; loc. cit. Fig.

19) it appears more likely that this should rather be identified as *T. infundibuliformis* which is size-wise similar to *T. perforata*, characterized by more prominent costal strips than *T. perforata*, and finally previously reported from places outside the global warm-water belt (see above).

Thomsenella cercophora (Thomsen in Thomsen and Boonruang, 1983) comb. nov. (Figs 9, 10)
Bas.: *Platypleura cercophora* Thomsen in Thomsen and Boonruang, 1983

In *T. cercophora* there are three principal types of costal strips. Those forming the anterior lorica chamber are fairly narrow, yet with a distinct heavily silicified midrib (Fig. 9a, arrow) and lateral flanges. There are no anterior spines. The posterior lorica chamber is compact and formed by very broad costal strips (c. 1.5 μ m wide) with a pronounced heavily silicified midrib and a weak pattern of ornamentation at right angles to the rib. There is no transverse costa that separates the anterior and posterior lorica chambers (Fig. 9a). A delicate membrane lines the posterior lorica chamber and also surrounds the basal part of the collar tentacles (Fig. 9f, g). The compound pedicel typically comprises three to four costal strips that taper to a sharp point at the distal end. They have a well-developed midrib and a lateral flange that is larger than that found in anterior lorica chamber costal strips. The material examined here (Table 1 and Fig. 6c) is in full agreement with the Andaman Sea type material (Thomsen and Boonruang 1983). The LM recognition of this species is generally straightforward (Fig. 9c-h) based on the pronounced difference between anterior and posterior lorica chamber costal strips, the absence of anterior spines, and the compound pedicel comprising a cluster of diverging and tapering costal strips. The posterior lorica chamber encasing armour is on occasion difficult to visualize when a protoplast is still occupying the space (Fig. 9e, h).

Distribution: Observed in samples from the Andaman Sea, West Australia, the equatorial Pacific Ocean (Fig. 10) and the Eastern Mediterranean Sea (Table 2), and additionally recorded from the equatorial Pacific Ocean (Vørs et al. 1995), Shark Bay, Australia (Tong et al. 1997), Taiwan (Hara et al. 1997)

Thomsenella acuta (Thomsen in Thomsen and Boonruang, 1983) comb. nov. (Fig. 11)
 Bas.: *Platypleura acuta* Thomsen in Thomsen and Boonruang, 1983

Thomsenella acuta is much similar to *T. cercophora* in overall appearance. The distinguishing features are the short anterior spines in *T. acuta*, the existence of a transverse costa separating the two lorica chambers, and the presence of only three or four longitudinal costal strips (much similar to those from the anterior chamber) in the posterior chamber 'wall'. *Thomsenella acuta* specimens are overall slightly larger than *T. cercophora* (Table 1 and Fig. 6b, c) and have typically 11 longitudinal costae versus only 10 in *T. cercophora*.

The original description of *T. acuta* (Thomsen and Boonruang 1983) mentions that the *T. acuta* pedicel comprises just a single tapering costal strip. In our recent material, specimens of *T. acuta* invariably display a compound pedicel with typically four costal strips. When scrutinizing the illustrations accompanying the original description of this species (Thomsen and Boonruang 1983; loc. cit. Fig. 52) it is obvious that one specimen displayed actually shows a similar compound pedicel.

The anterior spines are sharp-pointed and nail-like (Fig. 11a, i). Costal strips are typically rather narrow and with a heavily silicified keel surrounded on either side by narrow laminar flanges. Costal strips often appear folded and with a V-shaped transverse section (Fig. 11i). The posteriormost longitudinal costal strips and strips from the compound pedicel have spatulate tips where they meet (Fig. 11g, h).

As evidenced from Fig. 11b-f a LM recognition of this taxon is generally trouble-free. The most important single feature to look for is the anterior spines. Even when a protoplast is present (Fig. 11b, e) it is still possible to precisely verify the structural details of the posterior lorica chamber.

Distribution: Observed in samples from all sites reported here with the exception of the Gulf of California (Table 2). Previously reported from the Andaman Sea (Thomsen and Boonruang 1983), central California waters (Thomsen et al. 1991), the equatorial Pacific Ocean (Vørs et al. 1995), the Gulf of Carpentaria, Australia (Gustav Hallegraeff, pers. comm.), New Zealand (Øjvind Moestrup, pers. comm.), the Beagle Channel, Patagonia (Thomsen, unpublished), and the Red Sea, Gulf of Elat (Thomsen 1978; Thomsen and Boonruang 1983; erroneously referred to as *P. infundibuliformis*).

Conclusions

The opportunity to revisit the genus *Thomsenella*, while exploring a large material collected from widely separated geographical sites, has in a classical morphospecies perspective confirmed both the circumscription of the genus and the separation into four individual species.

It has become evident, following meticulous analyses of a vast number of TEM and SEM images (Leadbeater 2015), that patterns of costal strip abutments are universal across a wide range of genera and species. The overlaps between adjacent strips forming the anterior transverse costa when seen from the outer surface take the following form in a clockwise direction. The left-hand end of a costal strip underlaps the right-hand end of the adjacent strip. When this junction interacts with a longitudinal costa, the result in a dried specimen is to produce a small triangle on the right-hand side of the longitudinal costal strip (when seen from the outer surface). In the

middle transverse 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. As with the anterior transverse costa, when the junction interacts with a longitudinal costa the triangle is on the right-hand side of the longitudinal costa. It should be noted that the small triangles referred to are artefactual but are, nevertheless, very consistent in location. The simple construction of the *Thomsenella* lorica results in that the generic patterns of costal strip abutments outlined above can be observed in almost all TEM micrographs published here (see e.g. Fig. 3a, c; Fig. 4; Fig. 5a, d; Fig. 7a, c; Fig. 9a; Fig. 10a; Fig. 11a, i). It should be emphasized that in particular Fig. 4 offers unique opportunities (left hand side of the lorica) to study costal strip abutments in this genus.

There is an overrepresentation of loricate choanoflagellate species with flattened and elaborate costal strips in warm water habitats (e.g. *Calotheca* sp. (Thomsen and Moestrup 1983), *Stephanacantha* spp. (Thomsen and Boonruang 1983), *Thomsenella* spp. (Thomsen and Boonruang 1983) and *Syndetophyllum* sp. (Thomsen and Moestrup 1983). It is unclear to us whether there is an environmentally induced advantage in warm water habitats to be gained from building a lorica from flattened costal strips, or whether the observed occurrence pattern is more likely to be understood in an evolutionary context. However, it should be emphasized that the majority of warm water loricate choanoflagellates thrive in these water masses while making use of a 'standard' rod-shaped costal strip when assembling a lorica, and further that a few loricate choanoflagellate species with flattened costal strips occur outside the warm water realms. The most prominent example here is *Parvicorbicula serrulata* Leadbeater, 1975 (Manton et al. 1975) which is so far distributionally confined to Arctic waters (Thomsen and Østergaard 2017). These observations collectively point to that it will be difficult to advocate that the warm water

environment somehow selects, biologically, physically or chemically, for forms with elaborate flattened costal strips.

A future application of molecular tools will be needed to confirm the established *Thomsenella* morphospecies matrix (and in this context also verify the identification of Pacific Ocean material (Fig. 5) as *T. infundibuliformis* sensu stricto), and to elucidate the relationship between the closely related genera *Thomsenella* (flattened costal strips) and *Cosmoeca* (rod-shaped costal strips), as well as evolutionary ramifications across the entire warm water contingent of species with flattened and elaborate costal strips.

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.

Acknowledgements

Crew and scientists on board the research vessels ('Dana', 'Vaedderen', 'Chakratong Tongyia', 'Malcolm Baldrige') are acknowledged for good collaborative spirit and for providing excellent facilities for collecting and processing our samples. Dr. Gunni Ærtebjerg and Steffen Mariager Pedersen are acknowledged for providing the samples from outside the Alexandria Harbour. We are grateful to Dr. Sergio Sanudo-Wilhelmy and Kurt Buck for organizing the trip to Gulf of California. Carol Kosman is warmly acknowledged for her meticulous contribution to this paper. Main funding sources, i.e. the Independent Research Fund Denmark, the Carlsberg Foundation,

Danida (Denmark's development cooperation), the Højgaard Foundation, and the Danish Centre for Marine Research are acknowledged for their generous contributions to our research. The present work was carried out as part of the Galathea3 expedition under the auspices of the Danish Expedition Foundation (Galathea3 contribution no. 128) and also as part of the Sargasso-Eel 2014 Expedition.

References

- Booth, B.C., 1990. Choanoflagellates from the subarctic North Pacific Ocean, with description of two new species. *Can. J. Zool.* 68, 2393-2402.
- Espeland, G., Throndsen, J., 1986. Flagellates from Kilsfjorden, southern Norway, with description of two new species of Choanoflagellida. *Sarsia* 71, 209-226.
- Hara, S., Sheu, J., Chen, Y.L., Takahashi, E., 1997. Choanoflagellates (Sarcomastigophora, Protozoa) from the coastal waters of Taiwan and Japan (II): Species composition and biogeography. *Zool. Stud.* 36, 98-110.
- Hoepffner, H., Haas, L.W., 1990. Electron microscopy of nanoplankton from the North Pacific central gyre. *J. Phycol.* 26, 421-439.
- ICZN, 1999. International Code of Zoological Nomenclature. Fourth Edition. The International Trust for Zoological Nomenclature, London, UK. 306 pp.
- Leadbeater, B.S.C., 1974. Ultrastructural observations on nanoplankton collected from the coast of Yugoslavia and the Bay of Algiers. *J. Mar. Biol. Assoc. U.K.* 54, 179-196.
- Leadbeater, B.S.C., 2015. The Choanoflagellates: Evolution, biology and ecology. Cambridge University Press.
- Lee, W.J., Brandt, S.M., Vørs, N., Patterson, D.J., 2003. Darwin's heterotrophic flagellates. *Ophelia* 57, 63-98.
- Manton, I., Sutherland, J., Leadbeater, B.S.C., 1975. Four new species of choanoflagellates from Arctic Canada. *Proc. R. Soc. Lond. B.* 189, 15-27.
- Menezes, S., 2005. Nanoplankton biodiversity in the Pettaquamscutt river estuary, Rhode Island, U.S.A. PhD thesis, Univ. Rhode Island, 319pp.

- Moestrup, Ø., 1979. Identification by electron microscopy of marine nanoplankton from New Zealand, including the description of four new species. *New Zeal. J. Bot.* 17, 61-95.
- Moestrup, Ø., Thomsen, H.A., 1980. Preparation of shadow-cast whole mounts. In: Gantt, E. (Ed.), *Handbook of Phycological methods*. Vol. III. pp. 385-390 (Cambridge).
- Özdikmen H., 2009. Substitute names for some unicellular animal taxa (Protozoa). *Mun. Ent. Zool.* 4, 233-256.
- Thomsen, H.A., 1978. Nanoplankton from the Gulf of Elat (= Gulf of Aquaba), with particular emphasis on the choanoflagellates. *Isr. J. Zool.* 27, 34-44.
- Thomsen, H.A., 1982. Planktonic choanoflagellates from Disko Bugt, West Greenland, with a survey of the marine nanoplankton of the area. *Meddr. Grønland, Bioscience* 8, 1-35.
- Thomsen, H.A., Boonruang, P., 1983. A microscopical study of marine collared flagellates (Choanoflagellida) from the Andaman Sea, SW Thailand: Species of *Stephanacantha* gen. nov. and *Platypleura* gen. nov. *Protistologica* 19, 193-214.
- Thomsen, H.A., Boonruang, P., 1984. A light and electron microscopical investigation of loricate choanoflagellates (Acanthoecidae, Choanoflagellida) from the Andaman Sea, SW Thailand: Species of *Cosmoeca* gen.n. *Zool. Scripta* 13, 165-181.
- Thomsen, H.A., Buck, K R., 1991. Choanoflagellate diversity with particular emphasis on the Acanthoecidae. In: Patterson, D.J., Larsen, J. (Eds.), *Free-living heterotrophic flagellates*. Clarendon Press, Oxford, pp. 259-284.
- Thomsen, H.A., Moestrup, Ø., 1983. Electron microscopical investigations on two loricate choanoflagellates (Choanoflagellida), *Calotheca alata* gen. et sp. nov. and *Syndetophyllum pulchellum* gen. et comb. nov., from Indo-Pacific localities. *Proc. R. Soc. Lond. B.* 219, 41-52.

- Thomsen, H.A., Østergaard, J.B., 2017. Acanthoecid choanoflagellates from the Atlantic Arctic region – a baseline study. *Heliyon* 3 (2017) e00345 doi: 10.1016/j.heliyon.2017. e00345
- Thomsen, H.A., Østergaard, J.B., 2019a. Loricata choanoflagellates (Acanthoecida) from warm water seas. I. *Conioeca* gen. nov. and *Nannoeca* Thomsen. *Europ. J. Protistol.* 67, 77-88.
- Thomsen, H.A., Østergaard, J.B., 2019b. Loricata choanoflagellates (Acanthoecida) from warm water seas. II. *Bicosta*, *Apheloecion*, *Campyloacantha* and *Saroeca*. *Europ. J. Protistol.* 67, 114-131.
- Thomsen, H.A., Østergaard, J.B., 2019c. Loricata choanoflagellates (Acanthoecida) from warm water seas. III. *Acanthocorbis* Hara and *Takahashi* and *Stephanoeca* Ellis. *Europ. J. Protistol.* 69, 52-69.
- Thomsen, H.A., Østergaard, J.B., 2019d. Loricata choanoflagellates (Acanthoecida) from warm water seas. IV. *Cosmoeca* Thomsen. *Europ. J. Protistol.* (submitted)
- Thomsen, H.A., Buck, K.R., Chavez, F.P., 1991. Choanoflagellates of the central California waters: Taxonomy, morphology and species assemblages. *Ophelia* 33, 131-164.
- Thomsen, H.A., Nitsche, F., Richter, D.J., 2016. Seasonal occurrence of loricate choanoflagellates in Danish inner waters. *Protist* 167, 622-638.
- Tong, S.M., 1997. Heterotrophic flagellates from the water column in Shark Bay, Western Australia. *Mar. Biol.* 128, 517-536.
- Tong, S.M., Nygaard, K., Bernard, C., Vørs, N., Patterson, D.J., 1998. Heterotrophic flagellates from the water column in Port Jackson, Sydney, Australia. *Europ. J. Protistol.* 34, 162-194.
- Vørs, N., Buck, K.R., Chavez, F.P., Eikrem, W., Hansen, L.E., Østergaard, J.B., Thomsen, H.A., 1995. Nanoplankton of the equatorial Pacific with emphasis on the heterotrophic protists. *Deep-Sea Research* 42, 585-602.

|

Journal Pre-proof

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 in Thomsen and Østergaard (2019a)).

Fig. 2. *Thomsenella infundibuliformis* TEM whole mount labelled to introduce loricate choanoflagellate terminology. Micrograph (from New Zealand) courtesy of Ø. Moestrup.

Fig. 3. *Thomsenella infundibuliformis* TEM (a-c) and LM (d, e; phase contrast) whole mounts from the Gulf of California (a-d) and the Sargasso Sea (e). **(a)** Complete cell showing basic lorica

features; the arrow points to the middle transverse costa which crosses in front of the longitudinal costal strip junctions. **(b)** Detail (from a) to show costal longitudinal costal strip shape and ornamentation. **(c)** Detail (from a) of spindle-shaped and folded transverse costal strips (reverse printing). Notice the narrow flanges and the drawn-out tips. **(d, e)** Selected light micrographs to illustrate the appearance of the species under low magnification and the pronounced size differences observed. The scale bar (e) applies to both LM images.

Fig. 4. *Thomsenella infundibuliformis* TEM whole mount from the Gulf of California, Mexico. Notice (arrow) the crossing of the middle transverse costa anterior to the junctions between longitudinal costal strips. The oval demarcates longitudinally folded transverse costal strips with the concave side facing outward and the convex side inward. Numerous supernumerary costal strips are accumulated in the collar region.

Fig. 5. *Thomsenella infundibuliformis* TEM whole mounts from the Equatorial Pacific Ocean. (a-c) Selected specimens to show the morphological variability encountered; the circle (c) highlights an

area where the middle transverse costa is clearly pushed forward relative to the junctions

between longitudinal costal strips. **(d)** Detail (from a) of costal strip morphology (reverse printing).

Fig. 6. Diagrams (a-d) illustrating the *Thomsenella* spp. site-specific relationship between lorica height (x-axis) and the anterior transverse costa diameter (y-axis). The encircled area demarcates for each species its size specific 'centre of gravity'. Notice that outlier specimens are observed in all species.

Journal Pre-proof

Fig. 7. *Thomsenella perforata* TEM (a-c) and LM whole mounts (d-n; phase contrast) from the Andaman Sea (a-c), West Australia (d-g), the Sargasso Sea (l, h, k), the Caribbean Sea (j), and the Gulf of California (l-n). **(a)** Complete cell. Notice the accumulation of supernumerary costal strips

in the collar region and the simple pedicel. **(b)** High magnification of longitudinal costal strip to show the patterning of the costal strip flanges and the tip-to-tip morphological differences. **(c)** Complete cell; notice the folding of the anterior costal strips. **(d-n)** Selection of LM micrographs to show the variability encountered across vast geographic distances. The scale bar (d) applies to all light micrographs.

Fig. 8. *Thomsenella perforata* LM whole mounts (phase contrast) from the equatorial Pacific Ocean. **(a-j)** Selection of micrographs showing the variability encountered. The scale bar (a) applies to all images.

Journal Pre-proof

Fig. 9. *Thomsenella cercophora* TEM (a, b) and LM (c-h; phase contrast) whole mounts from the Andaman Sea (a, b), West Australia (c-f), and the Pacific Ocean (g, h). **(a, b)** Complete cells documenting costal strip features; arrow (a) points to costal strip that clearly displays the midrib and flanges of anterior lorica chamber costal strips. **(c-h)** Selection of *T. cercophora* LM images to show the variability encountered; the organic membrane that surrounds the protoplast is visible (f, g). The scale bar (c) applies to all LM micrographs.

Fig. 10. *Thomsenella cercophora* TEM whole mounts from the equatorial Pacific Ocean. **(a)** Whole cell documenting lorica features of the species. **(b)** Detail of the same specimen to show the anterior-posterior lorica chamber transition from narrow to broad longitudinal costal strips at the level of the demarcation between the two lorica chambers.

Journal Pre-proof

Fig. 11 *Thomsenella acuta* TEM (a, g-i) and LM (b-f; phase contrast) whole mounts from the Andaman Sea (a, g-i), West Australia (b, c), the Caribbean Sea (d), and the Sargasso Sea (e, f). **(a)** Complete cell. **(b-f)** Selection of *T. acuta* specimens to show the consistency in general appearance across vast geographic distances. **(g)** Detail (from h) of costal strip tips at the point where the compound pedicel meets the posterior lorica chamber costal strips. **(h)** Compound pedicel costal strips. **(i)** Detail of anterior transverse and longitudinal costal strips to show the exterior position of the longitudinal costae, costal strips with a rib and lateral flanges, and the nail-shaped anterior spine. The scale bar (b) applies to all LM micrographs.

|

Journal Pre-proof

Table 1. Lorica dimensions (μm) of species of *Thomsenella*. Numbers in bracket and a smaller font are from the type descriptions (Thomsen and Boonruang 1983; *Leadbeater 1974). The over-sized *T. infundibuliformis* specimen from the Sargasso Sea (Fig. 3e) is not included in the calculations.

Taxon		Lorica height	Dist. ant.-middle transv. costa	Diam. ant. transv. costa	Diam. middle transv. costa	Diam. post. transv. costa	Post. lorica chamber height	Flagellum	Longitudinal costae
<i>Thomsenella acuta</i>	Mean	17.8	7.2	10.5 (c. 8)	8.4	3.8	6.4	13.4 (c. 12)	
	St.dev.	1.54	0.70	0.98	1.28	0.78	0.61	1.93	
	Range	13.2-19.7 (12-14)	5.8-8.7	8.6-12.6	6.6-12.2	2.7-4.9	5.4-7.1 (3.5-5)	10.7-16.8	(10)-11 (11)
	n	34	31	34	34	5	8	12	
<i>Thomsenella cercophora</i>	Mean	16.3	7.3	10.5	7.7	3.5	5.4	14.0 (12.0)	
	St.dev.	1.17	0.65	0.71	0.94	0.43	0.55	1.99	
	Range	12.1-17.7 (12-17)	5-8.1	8.3-11.7 (8-10)	5.9-10.7	3.1-3.9	4-6.1 (4-5.5)	10.9-17	10-(11) (11)
	n	40	40	40	39	4	20	9	
<i>Thomsenella perforata</i>	Mean	12.2	5.5	8.6 (c. 6.5)	6.9	3.5	4.2 (c. 3)	12.6	
	St.dev.	1.11	0.46	0.51	0.70	0.47	0.69	2.19	
	Range	11.1-15.5 (9.5-11)	4.4-6.5	7.6-9.6	5.4-8.4	2.8-4.4	3.3-4.8	7.0-16.6 (11-15)	11 (11-(12))
	n	50	50	50	49	9	4	19	
<i>Thomsenella infundibuliformis</i>	Mean	13.0		8.7	7.4	3.6			
	St.dev.	0.77		0.65	0.54	0.31			
	Range	11.8-14.8 (9-13)*		7.9-9.9	6.4-8.5	3.2-3.9			(10)-11 (10-12)*
	n	13		13	13	4			
<i>Thomsenella infundibuliformis</i> (material from Danish coastal sites)	Mean	13.0		8.4					
	St.dev.	0.87		0.74					
	Range	11.2-14.5		6.7-9.5					11
	n	10		10					

Table 2. Occurrence pattern of species discussed here and in Thomsen and Østergaard (2019a-d). New species described in Thomsen and Østergaard (2019a-d) are marked with *.

	Andaman Sea, Thailand	West Australia	Sargasso Sea	Caribbean Sea	Equatorial Pacific Ocean	Gulf of California, Mexico	Mediterranean Sea, Alexandria
<i>Acanthocorbis apoda</i>	x						
<i>A. camarensis</i>	x						
<i>A. campanula</i>	x	x	x				
<i>A. conicella</i> *		x	x			x	x
<i>A. gladiella</i> *	x	x	x	x	x		
<i>A. haurakiana</i>	x	x	x				
<i>Apheloecion articulatum</i>	x	x					x
<i>A. eapacia</i> *	x	x			x		
<i>A. pentacanthum</i>	x	x			x	x	x
<i>A. quadrispinum</i>	x	x	x	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 magna</i> *	x	x		x	x		
<i>C. natans</i>						x	
<i>C. simplex</i>	x	x	x	x	x	x	x
<i>Conioeca boonruangii</i> *	x	x		x	x		
<i>Cosmoeca ceratophora</i>	x	x	x	x	x	x	x
<i>C. norvegica</i>	x	x	x	x	x	x	x
<i>C. phuketensis</i>	x	x	x	x	x		x
<i>C. subulata</i>	x	x			x		
<i>C. ventricosa</i> (incl. form A)	x	x	x	x	x	x	x
<i>C. ventricosa</i> (form B)	x	x	x		x	x	x
<i>C. ventricosa</i> (form C)	x	x	x	x	x		
<i>Crucispina cruciformis</i>	x	x	x			x	x
<i>Nannoeca mexicana</i> *		x				x	
<i>N. minuta</i>	x	x	x	x	x	x	x
<i>N. minuta</i> (form A)	x				x		
<i>Saroeca attenuata</i>			x		(x)		
<i>S. paucicostata</i>	x	x	x			x	x
<i>Stephanoeca andamanica</i> *	x	x					
<i>S. apheles</i>	x	x					
<i>S. broomia</i> *	x	x					
<i>S. diplocostata</i> var. <i>paucicostata</i>		x					
<i>S. naja</i> *	x	x	x		x	x	
<i>S. supracostata</i>	x						
<i>Thomsenella acuta</i>	x	x	x	x	x		x
<i>T. cercophora</i>	x	x			x		x
<i>T. infundibuliformis</i>			x		x	x	x
<i>T. perforata</i>	x	x	x	x	x	x	x