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Coccolithophores in Polar Waters: *Papposphaera arctica* HET and HOL revisited

Helge A. Thomsen[1], Mikal Heldal[2] and Jette B. Østergaard[3]
[1]*National Institute of Aquatic Resources, Technical University of Denmark, Jægersborg allé 1, DK-2920 Charlottenlund, Denmark*
[2]*Department of Biology, Thormøhlensgate 53, 5020 Bergen, Norway*
[3]*Nørrebrogade 52a, 5th, 2200 Copenhagen N, Denmark*
email: hat@aqua.dtu.dk

ABSTRACT: It has been generally accepted based on the finding of combination coccospheres in field samples that *Turrisphaera arctica* and *Papposphaera sarion* are alternate life-cycle phases of a single species. However, while recently revisiting *P. sarion* it became evident that the *Turrisphaera* phase of this species is not identical with *T. arctica* but rather is an undescribed species of *Turrisphaera*. The most conspicuous diagnostic feature of *T. arctica*, an asymmetrical and tilted hypertrophy of the distal tube opening in circum-flagellar coccoliths, was hinted at in the first description of the taxon. However, focus was here on the overall similarity between T. *borealis* and *T. arctica* to the extent that the rather conspicuous difference between the two taxa was not clearly recognized by the authors of the taxon, nor by any researcher who has worked on these species since then. We present here material of *T. arctica* from various Arctic locations (West Greenland, NE Greenland, Svalbard) that completely matches the type material from Resolute Bay, Cornwallis Island. This material additionally comprised (NE Greenland) large numbers of combination coccospheres that clearly indicate that *T. arctica* shares a life history with an as yet undescribed species of *Papposphaera*. This allows us to emend the description of *T. arctica* including also its heterococcolithophore phase, and in this context making use of the combination *P. arctica* which was established decades ago, yet here with the specific comment that *P. sarion* is no longer a valid synonym of *P. arctica*.

Keywords: coccolithophore, *Papposphaera arctica*, *Papposphaera sarion*, *Turrisphaera arctica*, polar regions

INTRODUCTION

Until recently it was understood that *Papposphaera sarion* Thomsen 1981, and *Turrisphaera arctica* Manton, Sutherland and Oates 1976, were part of the same heterococcolithophore-holococcolithophore life history (Thomsen et al. 1991). However, while recently examining a large collection of Arctic material of *P. sarion,* including numerous combination coccospheres and complete coccospheres that unambiguously represented *P. sarion* HOL, it became evident (Thomsen et al. 2015) that the holococcolithophore phase was in fact not equivalent to *T. arctica* sensu Manton et al. (1976). The *Turrisphaera* counterpart of *P. sarion* is characterized by highly symmetrical and slender tower-shaped coccoliths, whereas those of *T. arctica* are characterized by e.g. a pronounced asymmetry of the distal part of the individual coccolith.

We are in the process of revisiting all genera and species described during the early phase of the exploration of polar coccolithophores (Thomsen et al. 2013, 2015; Thomsen and Østergaard 2014a,b; 2015a,b), and can in this process draw upon large collections of hitherto unpublished material from both polar regions as well as Svalbard material being currently processed for scanning electron microscopical examination.

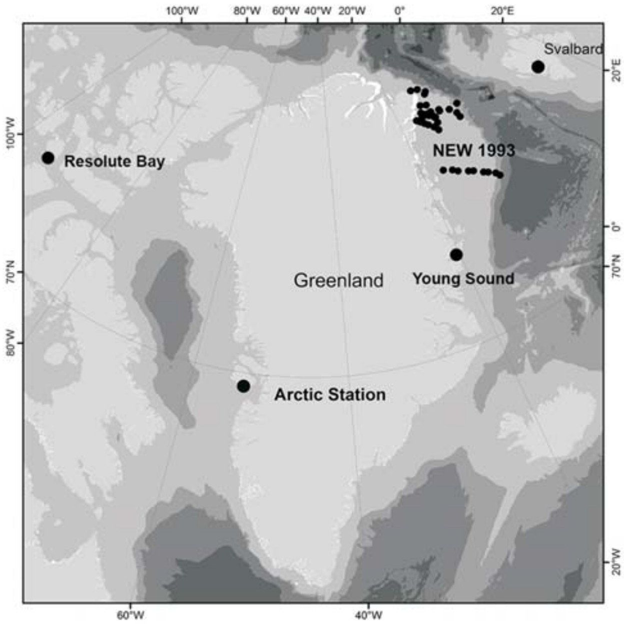
This material also includes a large number of Arctic cells that exactly match *T. arctica* sensu Manton et al. (1976). These occur both as complete coccospheres and as parts of combination coccospheres that additionally comprise heterococcoliths of an until now undescribed species of *Papposphaera*.

In this paper we will reexamine *P. arctica* (HET, HOL and combination coccospheres) and provide an emended description of the species based on extensive material from West Greenland, NE Greenland, and Svalbard.

MATERIAL AND METHODS

The Arctic material that comprised *P. arctica* HET and HOL specimens originates from the R/V 'Polarstern' ARK IX/3 North-East Water Polynya (NEW) cruise June-July 1993, and from the University of Copenhagen Arctic Station (Disko Bay, West Greenland) where sampling was conducted during the summers of 1988, 1990 and 1994. An ongoing sampling programme yielding material for a scanning electron microscope (SEM) analysis has been conducted in Svalbard waters since 2012, extending occasionally to the east coast of Greenland (Young Sound). All sampling sites are shown in text-figure 1.

The protocols for processing water samples for the transmission electron microscope (TEM) were similar on all sampling occasions (see Moestrup and Thomsen 1980). The nanoplankton community was concentrated for further processing by means of either centrifugation of a prefiltered (usually 20µm) water sample (typically 0.5-1 liter) or centrifugation of prefiltered material resuspended from an initial concentration of cells on top of e.g. a 1µm Nuclepore filter. Small droplets of cells from the resuspended final pellet of material were placed on carbon coated grids for the TEM. Cells were subsequently fixed for ca. 30 seconds in the vapour from a 1-2% solution of OsO_4. After drying the grids were carefully rinsed in distilled water in order to remove salt crystals. Grids were shadow cast with either Au/Pd



TEXT-FIGURE 1
Map showing the Northern Hemisphere sampling sites and the *T. arctica* type locality (Resolute Bay).

or Cr prior to the examination in JEOL electron microscopes property of the Botanical Institute at the Univ. of Copenhagen. Material for the SEM was prepared by gentle filtration of a water sample on top of a 1.0µm Nuclepore filter. The formation of salt crystals that might obstruct the visibility of cells was minimized by allowing the pumping system to almost completely dry out the filter. Filters were sputter coated with gold and examined on a Zeiss Supra 55VP scanning electron microscope at the Bergen University Laboratory for Electron Microscopy.

The terminology follows wherever possible Young et al. (1997, 2003).

OBSERVATIONS

Papposphaera arctica HET (= *Papposphaera* sp. nov.)
Plates 1–2

The cell is spherical to ellipsoidal with dimensions in the range of 3.5-7µm. It carries two flagella and a haptonema (Pl. 1, fig. 1). The coccosphere consists of varimorphic muroliths. All coccoliths appear to be basically identical, however, with a pronounced variability in development of the central spine dependent on the position within the coccosphere. The individual coccolith is normally elliptical (Pl. 1, figs. 3, 5; Pl. 2, fig. 1) and measures 0.6-0.8 x 1.1-1.5µm with mean values (Pl. 2, fig. 1 / n=15) corresponding to 0.69±0.045 and 1.21±0.136 respectively. Due to the limited calcification of the central area the organic base plate is often visible in TEM (Pl. 1, fig. 5) showing radiating ridges overlaying concentric fibres. The rim calcification comprises two cycles of elements. The proximal cycle is formed by linearly organized rod-shaped elements (0.09-0.14µm long and 0.04-0.05µm wide) that occur interspaced between the elements of the wall proper (Pl. 1, figs. 3, 5). The second cycle (Pl. 1, fig. 5) comprises large pentagonal elements that are 0.11-0.18µm high (mean value: 0.154±0.016; n=27) and 0.13-0.25µm wide (mean value: 0.179±0.034; n=30). They form a dense and slightly flaring wall that arises from the coccolith periphery at an angle of approx. 100 degrees (Pl. 1, fig. 3).

The central area calcification invariably consists of a cross-bar arranged roughly along the main axes of the coccolith (Pl. 1 figs 3, 5; Pl. 2, fig. 1). Coccoliths at the anterior and posterior ends of the cell carry a prominent central calicate spine which range in length from 2.0-3.3µm, with the longer ones clustering around the flagellar pole (Pl. 1 figs 1-2; Pl. 2, figs. 1-4). The stem is a continuation of the arms of the axial cross (Pl. 1, figs. 3, 5) and thus comprises four parallel rows of rods (Pl. 1, fig. 3). The calyx is formed by four diverging elements (ca. 0.4-0.5µm long and 0.1-0.15µm wide) that are irregularly stepped on the inward edge (Pl. 1 figs 3-4). Coccoliths arranged in a median band around the cell carry only a short stub like projection from the center of the axial cross (Pl. 1, figs. 3, 5; Pl. 2, fig. 1).

PLATE 1
Transmission electron microscope images of material from NE Greenland (figs 1, 5) and W Greenland (fig. 4), and scanning electron micrographs from Svalbard (figs 2-3).

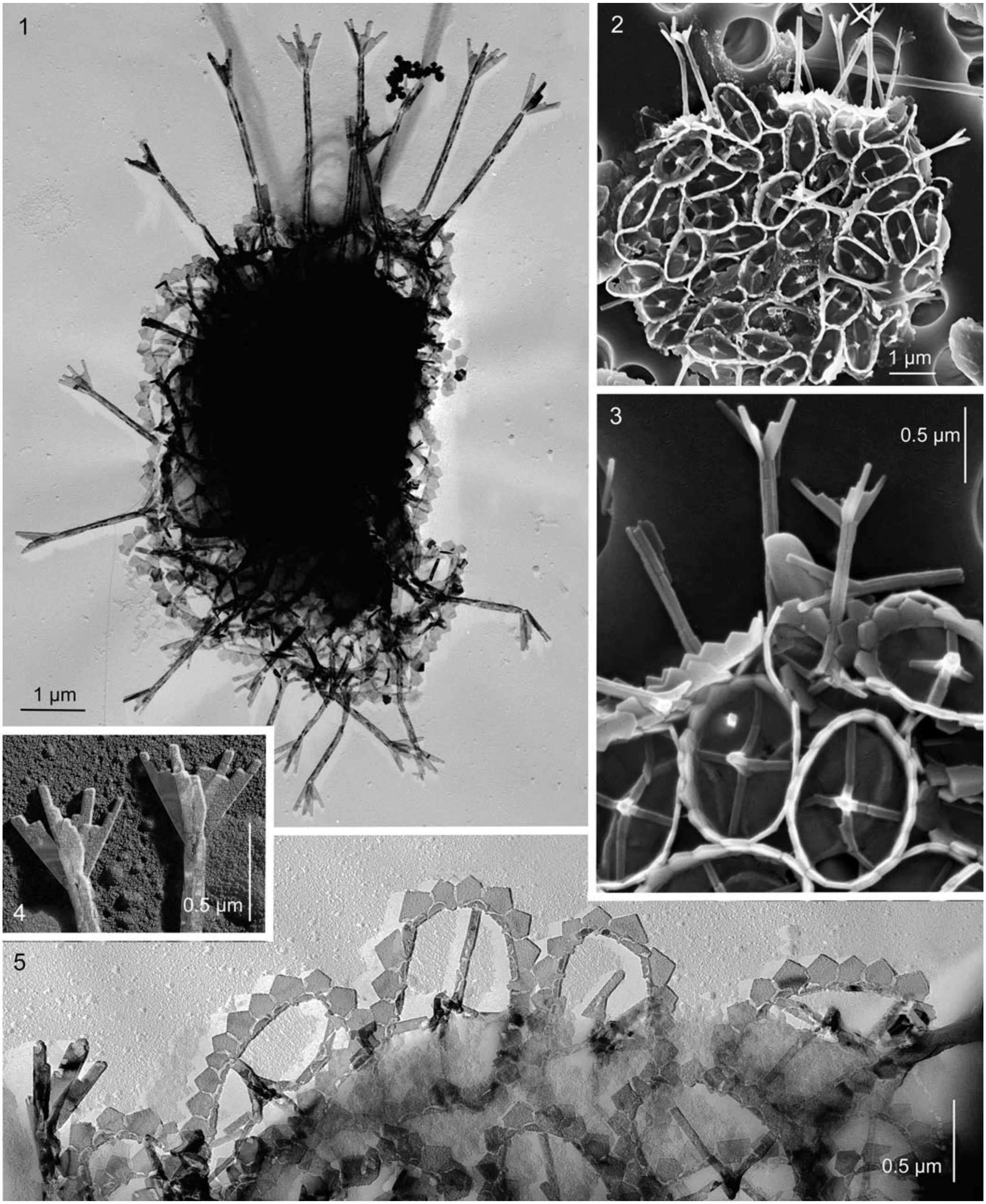
1 Complete specimen with flagella and curled up haptonema.

2 Complete cell showing the overall distribution of coccolith types. Notice that there are remains of holococcoliths in the periplast indicating that this might be a combination coccosphere.

3 Coccoliths displaying details of central area, rim calcification and the CFC spine structure.

4 Detail of calyx showing the individual elements and the stepped reduction in width of these (inward edges).

5 Scatter of coccoliths showing details of central area and rim calcification. The organic base plate pattern is evident as radiating ridges and concentric threads.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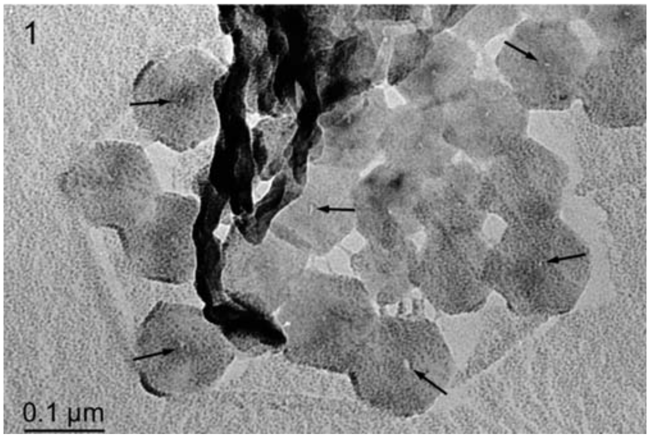

TEXT-FIGURE 2
Detail from Plate 4, figure 8 showing (arrows) the minute central pore of each heagonal crystallite group.

Papposphaera arctica combination coccospheres
Plate 3

We were fortunate enough to find numerous examples of combination coccospheres (*P.* sp. nov. / *Turrisphaera arctica*) from NE Greenland. The cells illustrated here (Pl. 3, figs. 1-3) measure 5-7 x 4-6µm and they all carry both flagella and a haptonema. Coccoliths of both types are clearly evident from all cells. Single enlarged heterococcoliths (Pl. 3, figs. 4-6) from the cells illustrated in Plate 3, figures 1-2 are identical to the cells illustrated in Plates 1-2, and so corroborate the identification of these cells as *P.* sp. nov. The calicate spines in these cells tend to be slightly longer (anterior end coccoliths: 2.7-3.7µm; posterior end coccoliths: 1.7-1.9µm) than those described above for *P. arctica* HET. Single holococcoliths (Pl. 3, figs. 7-8) from the cells depicted in Plate 3, figures 2-3 similarly corroborate the identification of this phase as *Turrisphaera arctica*. Notice that the SEM image (Pl. 1, fig. 2) of a cell from Svalbard shows traces of holococcoliths indicating that this might in fact be a combination coccosphere.

***Papposphaera arctica* HOL (=** *Turrisphaera arctica*)
Plate 4, text-figure 2

The NE Greenland material provided a rich set of complete specimens of *P. arctica* HOL. The cell is spherical to ovoid (5-6 x 4-6µm), with two flagella and a haptonema (Pl. 4, figs. 1-2).

The coccosphere comprises dimorphic tubular coccoliths with the flagellar pole coccoliths morphologically clearly distinguished from the others. The largest coccoliths cluster around the flagellar pole (2.4-3.0µm), while coccoliths elsewhere appear to be more or less uniform in size (1.2-1.5µm). The individual coccolith, irrespective of its position within the coccosphere, is proximally elliptical, ca. 0.7 x 1.2µm, and with the rim of the organic base plate commonly visible (Pl. 4, figs. 3, 8). The central area is covered by a monolayer of hexagonal crystallite groups that gradually transforms into a narrow central tubular structure (Pl. 4, figs. 3, 8). While the proximal part of the coccolith is symmetrical along the main axes of the coccolith, in apical coccoliths the distal part (uppermost half of the tube) is highly asymmetrical. One side of the tube is thus markedly extended and tilted 20-30 degrees with respect to the central and lowermost part of the tube (Pl. 4, figs. 1-3). Plate 4 figures 4-7 show individual circum-flagellar coccoliths from different angles. Body coccoliths are basically similar to the circum-flagellar coccoliths described above while lacking completely the asymmetrical termination of those. In these body coccoliths the central and narrow tube is distally slightly dilated. Whether the posterior tip of the tower is occluded or not cannot be inferred from the images available to us. The appearance of coccolith terminations such as those in Plate 4, figure 8 may also be the result of a collapse and infolding of the distal part of the tube. Antapical coccoliths are basically similar to body coccoliths (Pl. 4, fig. 1) yet in some cells (Pl. 4, figs. 2-3) with a small unilateral extension of the tube at the distal end. It is not evident that the monolayer of hexagonal crystallite plates covering the entire coccolith is anywhere organized in recognizable patterns, although there is in Plate 4, figure 8 an indication of a helical coiling pattern when examining the position of plates in the central tower. The mean distance between opposite and parallel sides of the hexagonal crystallite plate is 0.104±0.011µm (n=26) and they range in size between 0.083-0.118µm. A minute central pore is visible in the hexagonal crystallite plate (text-fig. 2). Manton *et al.* (1976) reported such central perforations in both *T. borealis* Manton, Sutherland and Oates 1976, and *T. arctica*. No unmineralized under layer scales have been observed so far.

SYSTEMATIC DESCRIPTION

An emended description of *P. arctica* is appropriate considering the fact that *P. arctica* HET represents a species of '*Papposphaera*' that has not been previously described.

Division HAPTOPHYTA Hibberd 1972
Class PRYMNESIOPHYCEAE Hibberd 1976
Order COCCOLITHALES Schwarz 1932
Family PAPPOSPHAERACEAE Jordan and Young 1990 emend Andruleit and Young 2010

Papposphaera arctica (Manton, Sutherland and Oates 1976) Thomsen, Østergaard and Hansen 1991

Basionym: *Turrisphaera arctica* Manton, Sutherland and Oates 1976, Proc. R. Soc. Lond. B 194: 189, fig. 28
Synonym: not *Papposphaera sarion* Thomsen 1981, as stated by Thomsen et al. (1991)
Type specimen: Manton, Sutherland and Oates 1976, Proc. R. Soc. Lond. B 194: 189, fig. 28.

Emended diagnosis: The species exists in two distinct life cycle forms (HET, HOL). Combination coccospheres are regularly found within the biogeographical realms of the species (the Arctic seas).

Heterococcolithophore phase: Cell spherical to ovoid (3.5-7.0µm) with two flagella and a haptonema. Coccolith (murolith) varimorphic (variability in spine development), elliptical in outline (0.6-0.8 x 1.1-1.5µm). The rim comprises two cycles, i.e. a proximal cycle of rod shaped elements and a distal palisade of pentagonal elements giving the rim a toothed upper margin. Central area calcification is sparse comprising only an axial cross. The stem of the calicate spine is formed by a continuation of the arms of the central cross. The length of the central spine varies between 2.0-3.3µm, with those at the apical end being significantly larger than those at the antapical end of the

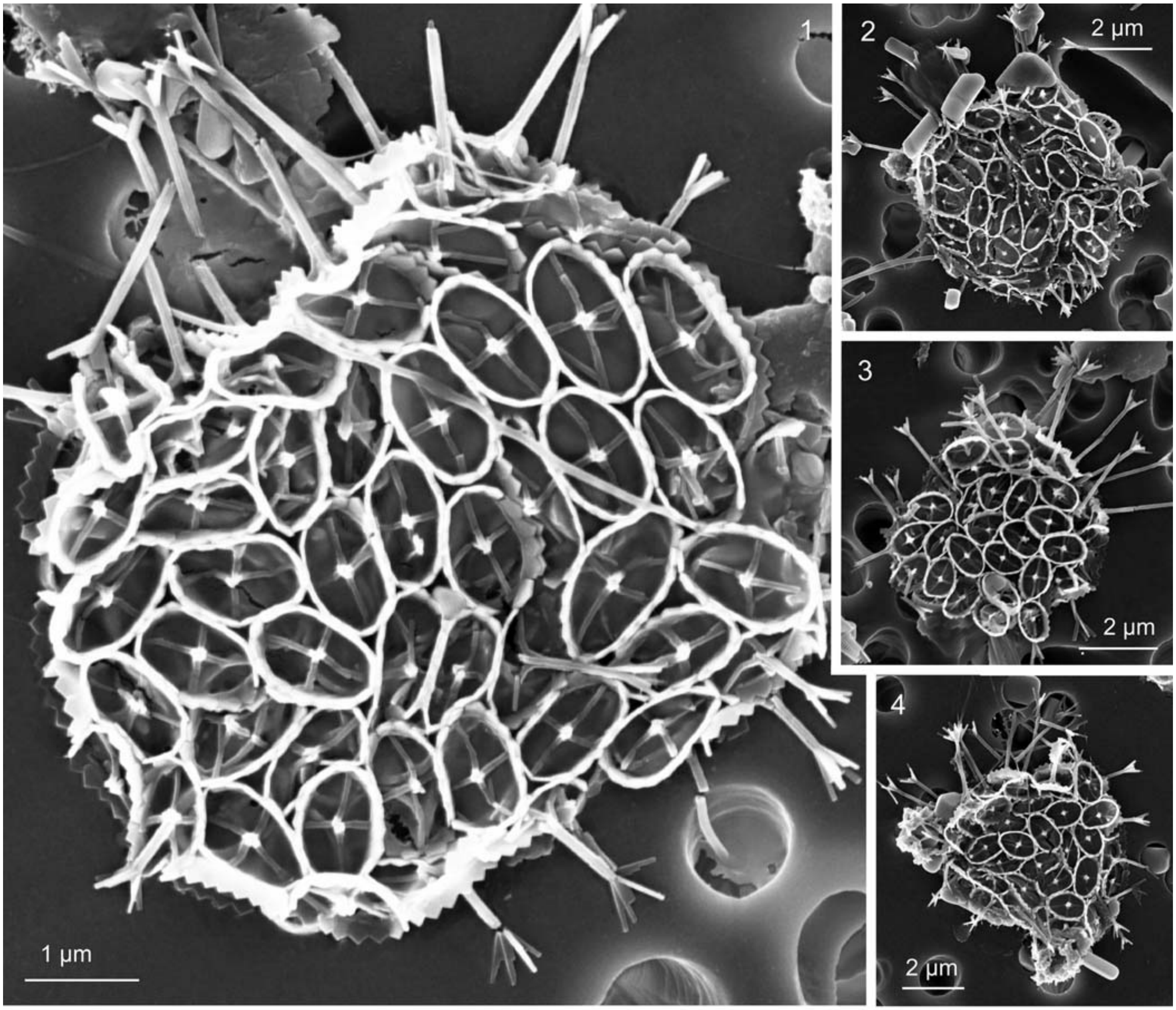

PLATE 2
Scanning electron images of material from Svalbard.

1 Notice the consistency in central area calcification among body coccoliths and the pronounced variability in size of these.

2-4 A series of complete cells showing in conjunction with figure 1 and Plate 1, figure 2, the consistent overall layout of the coccosphere, where polar coccoliths carry calicate spines while the body coccoliths are only furnished with short stub-like spines.

cell. The central spine is reduced to a short stub in body coccoliths. The calyx consists of 4 diverging elements that have a stepped inwardly oriented narrow edge. The individual element is 0.4-0.5µm long and 0.1-0.15µm wide.

Holococcolithophore phase: Cell spherical to ovoid (5-6 x 4-6µm) with two flagella and a haptonema. Coccoliths dimorphic. All coccoliths are elliptical in outline (0.7 x 1.2µm) and variations over a theme that comprises a slender tube (1.2 – 3.0µm) that is widened at both ends and much more so at the proximal end. Minute hexagonal crystallite groups (ca. 0.1µm) cover the entire surface of the coccolith. In circum-flagellar coccoliths (2.4-3.0µm) one side of the tube halfway up the coccolith is unidirectionally pulled out and tilted with reference to the main axis of the coccolith. Body coccoliths are symmetrical. Antapical coccoliths are in most respects similar to the body coccoliths although occasionally with a small distal proliferation of one side of the tube.

DISCUSSION

The most important aspect of this paper is its contribution towards solving errors introduced decades ago with respect to the life cycle affiliation of *T. arctica*. Rather than being connected with *P. sarion* as suggested by Thomsen et al. (1991) it is now evident that its heterococcolithophore life cycle partner is a hitherto undescribed species of *Papposphaera*. In a companion paper (Thomsen et al. 2015) *P. sarion* is concomitantly shown to combine with an undescribed species of *Turrisphaera*.

The stepwise and ongoing validation of *Papposphaera* species diversity in Arctic seas, and the concurrent confirmation when ever possible of critical morphological details of the *Turrisphaera* holococcolithophore life cycle counterparts, will eventually establish a taxonomic framework that can serve as a future reference tool for species identification in high latitude seas. The picture as it appears right now is that *Papposphaera* is present in Arctic seas with at least 7 species, i.e. the generic type *P. lepida* Tangen 1972, *P. sagittifera* Manton, Sutherland and McCully 1976, *P. sarion*, *P. arctica*, as well as two undescribed species and a species hitherto known only from Antarctic sites (Thomsen et al., publications pending). The *Turrisphaera* species diversity, in terms of formally described taxa recorded from Artic seas, is currently limited to 3 species, i.e. the generic type *T. borealis*, *T. arctica*, and *T. polybotrys* Thomsen 1980. Historically it has been difficult to separate species of *Turrisphaera* because of an obvious lack of prominent morphological features that easily support the setting up of a taxonomic framework, and a non-consolidated understanding of intra-specific variability. The documentation of more and more types of combination coccospheres among species of *Papposphaera* (that tends to provide more 'handles' for morphological separation) and species of *Turrisphaera* will in turn lead to a better understanding of inter- and intra-specific variability also among species of *Turrisphaera*. However, the concept of cryptic species may also very likely apply to *Turrisphaera* spp. Despite all the efforts to resolve the taxonomical problems related to the *Papposphaera*/*Turrisphaera* complex based on morphological evidence only, it is obvious that the taxonomic framework provided will need eventually to be challenged using molecular tools.

The known geographical range of *P. arctica* HET and HOL is at present the high latitude northern hemisphere, i.e. Resolute Bay, Cornwallis Island (HOL), S. Alaska, Homer (HOL), West Greenland, Disko Bay (HET, HOL), NE Greenland (HET, HOL, combination coccospheres), East Greenland, Young Sound (HET) and Svalbard (HET). *Papposphaera arctica* HOL is additionally recorded from Danish and Finnish coastal waters (Thomsen, unpublished results).

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PLATE 3

Transmission electron micrographs of combination coccospheres from NE Greenland.

1-3 Complete cells with flagella and haptonema and a coccosphere mixture of hetero- and holococcoliths.

4 Detail from Fig. 2 to corroborate the diagnostic features of *P. arctica* HET.

5 Detail from Fig. 1 to authenticate the diagnostic features of *P. arctica* HET.

6 Detail of calyx from a combination coccosphere.

7-8 Details from Figs 2, 3 to corroborate the diagnostic features of *P. arctica* HOL.

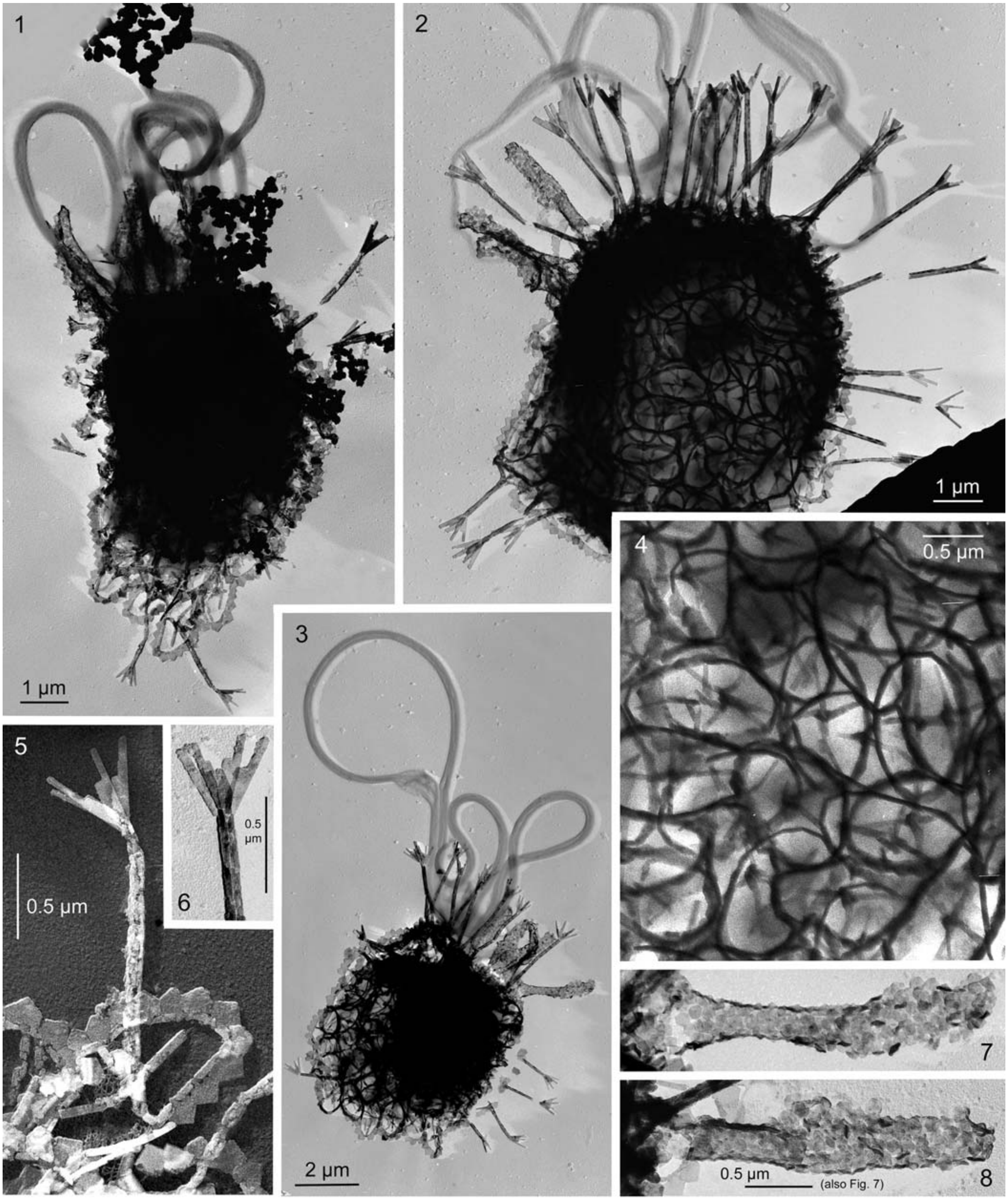
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PLATE 4

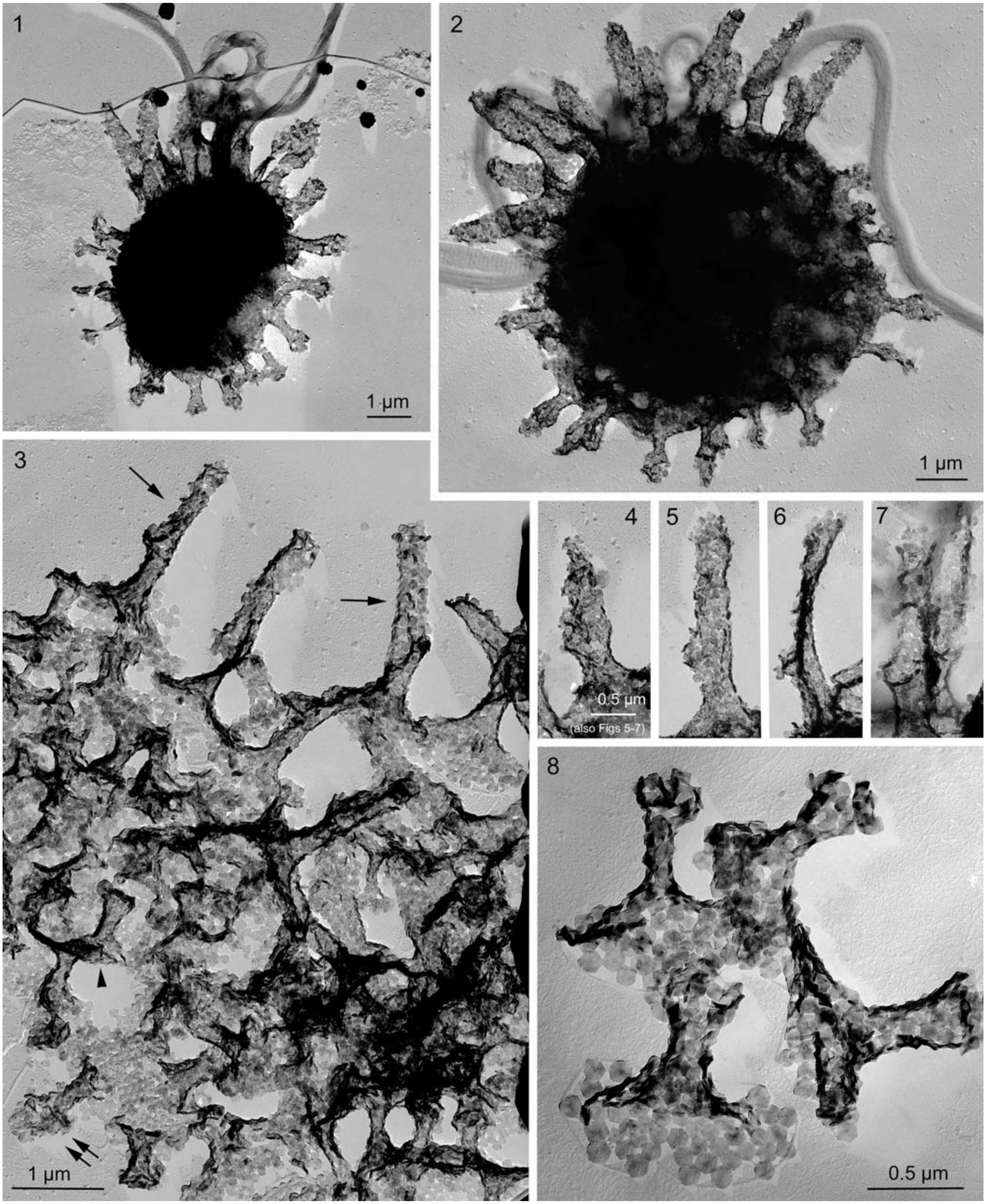
Transmission eletron micrographs of *P. arctica* HOL from NE Greenland.

1-2 Two complete specimens with flagella and haptonema. Notice the size difference between individual coccoliths in different positions within the coccosphere.

3 Complete coccosphere showing the circum-flagellar coccoliths with long distal extensions (arrows), the symmetrical and much shorter body coccoliths (arrowheads) and antapical coccoliths with a short assymetric distal spine-extension (double arrow).

4-7 Views from different angles of circum-flagellar coccoliths. The distal tongue-like spine-extension is evident in all images.

8 Scatter of symmetrical body coccoliths showing clearly the hexagonal crystallite groups that cover the entire coccolith surface, the rim of the organic base plate, and the occluded distal termination of the central spine.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