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# Coccolithophores in Polar Waters: *Papposphaera sagittifera* HET and HOL Revisited

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**Abstract.** The re-examination of the lightly calcified Arctic coccolithophore species, *Papposphaera sagittifera*, has some inherent challenges due to the research history on this taxon. It is thus obvious in retrospect that the species description based on just a single specimen does not adequately account for the true identity of this taxon. Today we are aware of the existence of at least three species of *Papposphaera* that have basically the same calyx design while being differentiated based on patterns of central area calcification. In order to remedy this we emend here the description of *P. sagittifera* and provide an epitype for the species. When realizing that species pairs of *Papposphaera* and *Turrisphaera* share a life history, the new combination, *P. borealis*, was established to accommodate *P. sagittifera* and *T. borealis*. However, it turns out that '*sagittifera*' is in fact the senior epithet by a few months, which means that the correct name for the species is *P. sagittifera* with *T. borealis* added as a synonym. While the *P. sagittifera* HET and HOL morphological variability across Arctic sites clearly leaves the impression of a single, fairly well defined species, the situation is different with respect to the occurrence of *P. sagittifera* in Antarctic waters. While there are obvious similarities between *P. sagittifera* HET across the Polar Regions there are also subtle differences, and most importantly it has been found that the Antarctic *P. sagittifera* shares a life history with a species of *Turrisphaera* that is markedly different from *T. borealis*. While awaiting molecular evidence the Antarctic material is tentatively referred to as *P. sagittifera* cfr.

**Key words:** Coccolithophore, *Papposphaera*, *P. sagittifera*, *Turrisphaera*, *T. borealis*, polar regions, electron microscopy.

**Abbreviations:** HET, HOL – abbreviations used to identify the heterococcolithophore and holococcolithophore phases of a shared life cycle; TEM – transmission electron microscope; SEM – scanning electron microscope; AMERIEZ, EPOS, ANT X/3 – acronyms for Antarctic cruises (see Materials and Methods); NEW, NOW – acronyms for Arctic cruises (see Materials and Methods).

## INTRODUCTION

We are currently in the process of re-examining all members of the polar communities of lightly calcified coccolithophores, and also as part of this effort describing new species when considered relevant (see e.g. Thomsen *et al.* 2013, Thomsen and Østergaard 2014a and subsequent papers). It is obvious that some of the early species descriptions will subsequently be found to be lacking in details due to e.g. limitations either quantitatively or qualitatively in the material available, and it is similarly apprehensible that the limited knowledge by then on species diversity necessarily has led to erroneous conclusions with regard to species delineation or the circumscription of a particular genus. We are for-

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tunate enough to have access to a large collection of unpublished material of TEM images of lightly calcified coccolithophores collected during extensive bipolar field campaigns undertaken during the last decades of the former century. We are furthermore in this process fortunate enough to also be able to draw upon new Svalbard material being currently processed for scanning electron microscopical examination.

Our most recent focus is on Arctic species of *Papposphaera*. Having dealt with *P. sarion* Thomsen 1981, *P. arctica* (Manton, Sutherland and Oates 1976) Thomsen, Østergaard and Hansen 1991, *P. iugifera* Thomsen and Østergaard 2016 and *P. heldalii* Thomsen and Egge 2016 (Thomsen *et al.* 2016a, b; Thomsen and Østergaard 2016; Thomsen and Egge 2016), we will here focus on *P. sagittifera* Manton, Sutherland and McCully 1976. *Papposphaera sagittifera*, as opposed to the other four species, is also possibly present in Antarctic waters (Thomsen *et al.* 1988). We therefore present here also additional material of *P. sagittifera* cfr. from the Weddell Sea.

*Papposphaera sagittifera* was first described from Homer, Alaska, by Manton *et al.* (1976a). The species has since then been frequently observed in Arctic samples. Thomsen (1981) thus provided images of *P. sagittifera* from West Greenland that added further evidence on in particular the central area calcification of the coccoliths, which happens to be a morphological aspect that was not adequately covered in the original description. Later it was shown based on findings of combination coccospheres (Thomsen *et al.* 1991) that *P. sagittifera* shares a life cycle with *Turrisphaera borealis* Manton, Sutherland and Oates 1976. This led Thomsen *et al.* (1991) to formally establish the new combination *Papposphaera borealis* (Manton, Sutherland and Oates 1976) Thomsen, Østergaard and Hansen 1991, and also to recommend that the holococcolithophore life cycle phase was henceforth referred to as the '*Turrisphaera* phase' of the species. It has since then, and in the wake of the wealth of combination coccospheres that have subsequently been found, become a common routine to refer to the different phases by adding either 'HET' for the heterococcolithophore phase or 'HOL' for the holococcolithophore phase of a particular species (Young *et al.* 2003).

In this paper we will provide an updated and extended description of *P. sagittifera* HET and HOL, and in the process also compile an emended type description linked to a West Greenland epitype, as well as solving an issue with reference to priority among the species epithets '*sagittifera*' and '*borealis*'.

## MATERIALS AND METHODS

For the purpose of this paper we have with regard to the Southern Ocean considered material sampled from south of the Antarctic Convergence, and in the Northern Hemisphere from the Arctic Ocean and its surrounding ice-covered seas.

The Arctic material originates (Fig. 1) from the R/V 'Polarstern' ARK IX/3 North-East Water Polynya (NEW) cruise June–July 1993 and the R/V 'Pierre Radisson' North Water Polynya cruise (NOW) April–May 1998. Additional Northern Hemisphere sampling of relevance here took place at the University of Copenhagen Arctic Station (Disko Bay, West Greenland) during the summers of 1988, 1990 and 1994 and from the Rijpfjorden, Svalbard, during 2012.

The Antarctic material originates from the R/V 'Polarstern' ANT VII/3 'EPOS II' cruise (Nov. 1988 – Jan. 1989) and the R/V 'Polarstern' ANT X/3 'Herbst im Eis' cruise (April–May 1992), with both cruises occupying stations in the Weddell Sea region (Fig. 2).

The protocol for processing water samples for the TEM was 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 20 µm) water sample (0.5–1 litre) or centrifugation of prefiltered material resuspended from an initial filtration of cells on top of e.g. a 1 µm 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 or Cr prior to the examination in JEOL electron microscopes property of the Botanical Institute at the University of Copenhagen.

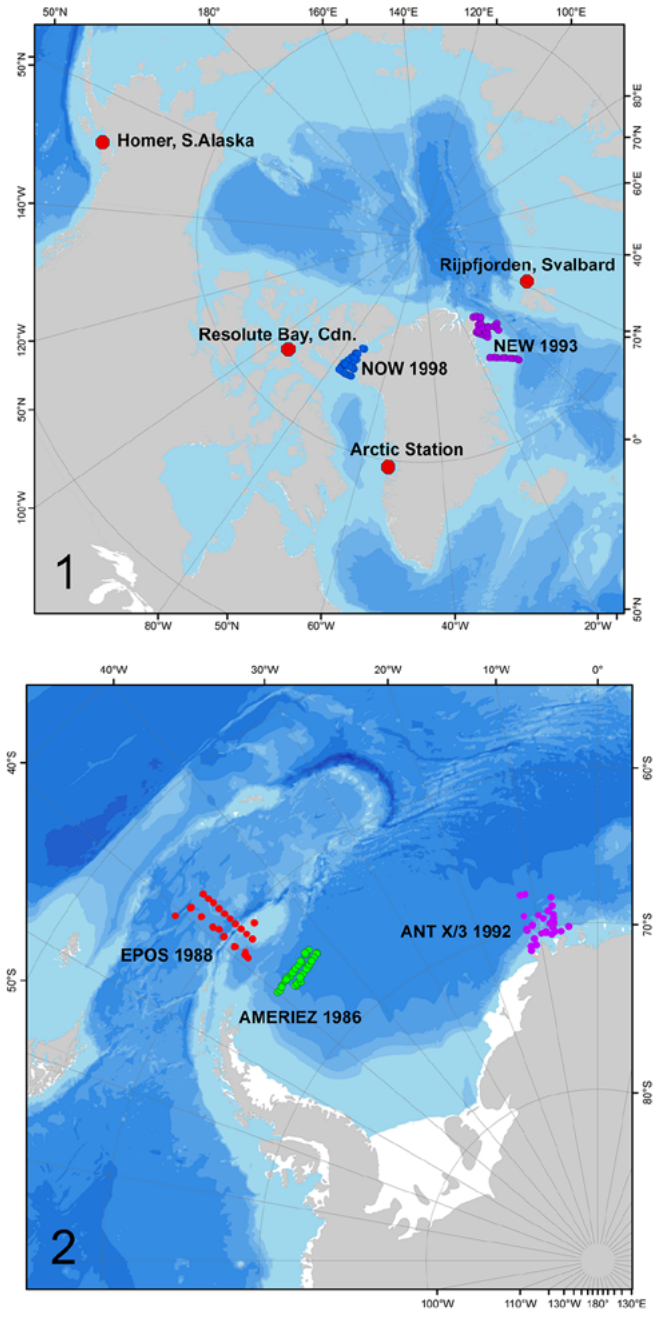
Material for the SEM was prepared by gentle filtration of a water sample on top of e.g. a 1.0 µm Nuclepore filter. The formation of salt crystals that might obstruct the visibility of cells was minimized by allowing the pumping system to almost completely dry out the filter. Filters were sputter coated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

The terminology used here largely follows Young *et al.* (1997, 2003). The calyx takes in *P. sagittifera* and related species a distinct shape which justifies the use of a special term for this. In order to simplify future descriptions of these structures we recommend that the *P. sagittifera* type of calyx is referred to as a four-winged rosette, and further that each element is referred to as a wing. In this paper we have additionally used the terms 'triangular' versus 'flaring' to easily differentiate between two types of calyces. These descriptive terms reflect the overall outline of the calyx when examined in a TEM.

## RESULTS AND INTERPRETATION

### The type material enigma

When Manton *et al.* (1976a) described *P. sagittifera* it was already referred to by the authors that the single cell available to them would suffice only for an incom-




**Figs 1–2.** Maps of collection sites. **1** – northern hemisphere; **2** – southern hemisphere. Showing also the AMERIEZ sampling grid (Thomsen *et al.* 1988).

plete species description, and that in particular the lack of detailed information on central area calcification could become an issue. The distribution and morphology of the coccolith central processes when projecting beyond the rest of the coccosphere was, however, considered adequately represented to allow for comparisons with *Pappomonas* which remained the main focus of the paper rather than the new *Papposphaera* species described.

Based on the finding of no less than 10 specimens of *P. sagittifera* from West Greenland, Thomsen (1981) provided additional information on this taxon, including observations on flagellation and central area calcification. The unifying link between the West Greenland material and the *P. sagittifera* type material from Homer, Alaska, was the quadripartite calyx that terminates the central process. In retrospect we have to conclude that this link was far from adequate. We now know that a calyx design similar to that of *P. sagittifera* is also found in *P. arctica* and *P. iugifera* (Fig. 3). In principle the single cell observed by Manton *et al.* (1976a) could be any of these forms that differ exactly in details of the lay out of the central area calcification. This nomenclatural uncertainty must obviously be solved and it seems that there are three ways to proceed.

The first option is to sample again intensively in the vicinity of the type locality. In all likelihood this will result in the finding of *P. sagittifera* cells but also, realizing the expected pan-Arctic distribution of both *P. arctica* and *P. iugifera*, these taxa would also likely turn up among the nanoflagellates sampled from the *P. sagittifera* type locality, leaving us without the option of unequivocally validating the identity of the *P. sagittifera* type material.

The second option is to re-examine the original Manton *et al.* (1976a) negatives in order to see if a meticulous handling of these could eventually unveil the central area calcification pattern, e.g. within the darker parts of the images. It turns out that the entire Manton collection of TEM negatives is maintained at the Special Collections in the Brotherton Library at Leeds University. A search in the archives (courtesy of Dr. B.S.C. Leadbeater, University of Birmingham and Dr. R. Davies, the curator of the collection) uncovered two out of the series of five negatives used for the study (Y7792.7 – Y7792.11). The negative Y7792.7 is not used in the actual publication but it represents a very low magnification of the cell. A close examination of this negative showed that there were unfortunately no isolated coccoliths in the vicinity of the cell that could otherwise have been helpful in the search for additional morphological details. The second negative found was Y7792.11 which is used for the actual publication (Manton *et al.* 1976a; loc. cit. Fig. 3). A careful examination of the negative using a magnifying lens and a light box did

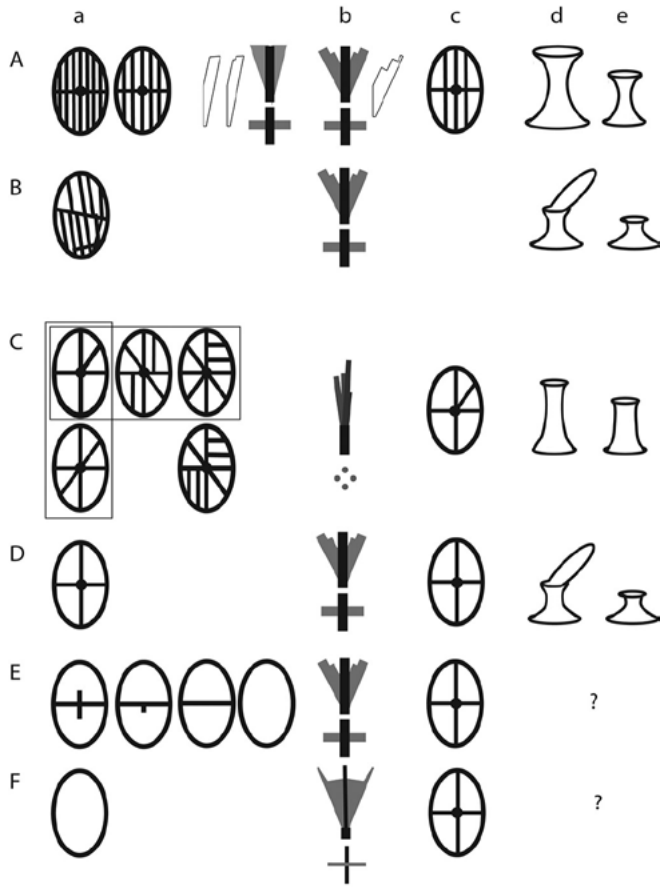



**Fig. 3.** Interpretational drawings of coccolith structure within species of *Papposphaera*. Intraspecific variation is shown where relevant. **A** – *P. sagittifera*; **B** – *P. sagittifera* cfr.; **C** – *P. sarion*; **D** – *P. arctica*; **E** – *P. iugifera*; **F** – *P. heldalii*; **a** – body coccolith central area calcification; **b** – calyx design; **c** – calicate coccolith central area calcification; **d** – holococcolith contour (flagellar pole coccolith); **e** – holococcolith contour (body coccolith). Notice the inclusion of schematic drawings of a rosette wing from each of the *P. sagittifera* types of calyces.

not reveal substantial additional information, nor was it possible to extract new details from software manipulations of a high resolution scanned copy of the negative. The conclusion is regrettably that this approach to solve the *P. sagittifera* enigma represents a dead end.

The last option and the one that we are forced to select for is in accordance with article 9.8 of the International Code of Nomenclature for algae, fungi, and plants (McNeill *et al.* 2012) to select an epitype for *P. sagittifera*. The exact wording of article 9.8 is: '*An epitype is a specimen or illustration selected to serve as an interpretative type when the holotype, lectotype, or previously designated neotype, or all original material associated with a validly published name, is demonstrably ambiguous and cannot be critically identified for purposes of the precise application of the name to a taxon*.' The most obvious way to proceed here is in alignment with the research history pertinent to *P. sagittifera* to make use of the West Greenland illustrations of the species in Thomsen 1981 (loc. cit. Figs 9, 13 (same cell)) as the selected epitype for the species. Demonstrating the presence in South Alaskan waters (Homer) of *P. sagittifera* cells with a central area calcification similar to that reported from West Greenland would be gratifying and generally supportive of the epitypification of the species. However, it must also be emphasized that even if conducting a search for *P. sagittifera* in South Alaskan waters with a negative result, this will not jeopardize the epitypification suggested here because sampling effort and conditions may easily prevent the finding of this taxon during a short-term sampling event.

**Invalid combination**

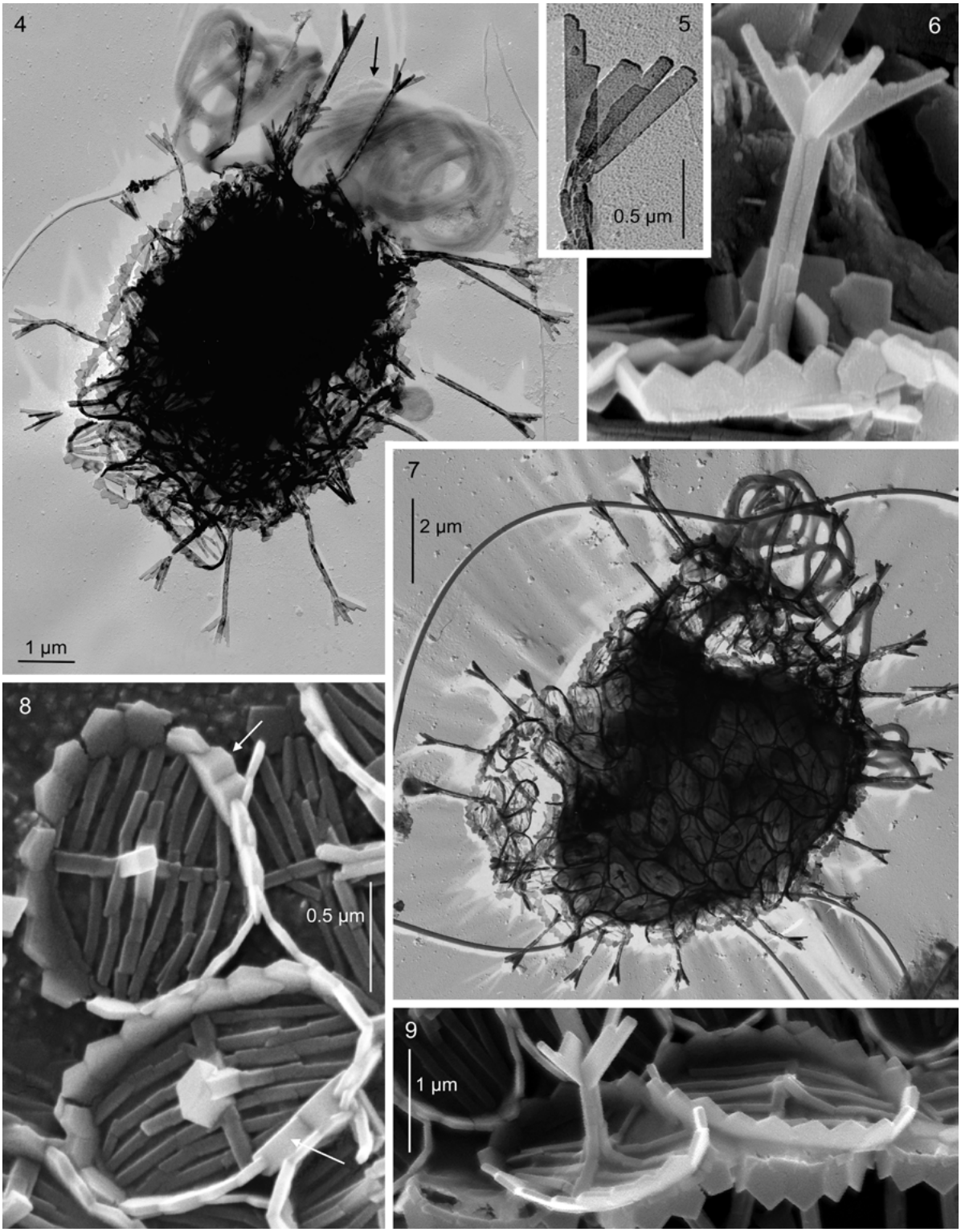
Thomsen *et al.* (1991) provided for the first time evidence for the existence of combination coccospheres of *Papposphaera* spp. with *Turrisphaera* spp. and *Pappomonas* Manton, Sutherland and Oates 1975 with *Trigonaspis* Thomsen 1980. The new combination, *Papposphaera borealis* (Manton, Sutherland and Oates 1976) Thomsen, Østergaard and Hansen 1991, was formally established by Thomsen *et al.* (1991). However, we have recently become aware of the fact that the species epithet '*sagittifera*' is in fact older (1 Sept. 1976) than '*borealis*' which dates from 15 Nov. 1976 (Manton *et al.* 1976b). The correct species name for the *P. sagittifera/T. borealis* complex must therefore be *P. sagittifera* with *T. borealis* reduced to a synonym. The proper step to remedy this situation is included as an integrated part of the emended description of *P. sagittifera* to be found below.

**New observations on *P. sagittifera* from Arctic sites**

Having now dealt with the two inescapable formal problems related to *P. sagittifera* the following paragraphs will in turn summarize all evidence available to us on the morphology of *P. sagittifera* HET and HOL from each of the Polar Regions.

***P. sagittifera* HET (Figs 4–16)**

The Arctic material from West Greenland (Fig. 7), North East Water Polynya (Figs 4–5, 13) and Svalbard (Figs 6, 8–12, 14–16) basically confirms what is al-




**Figs 4–9.** *Papposphaera sagittifera* HET (TEM / Figs 4–5, 7; SEM / Figs 6, 8–9) from the Arctic (West Greenland / Fig. 7; NEW / Figs 4–5; Svalbard / Figs 6, 8, 9). **4** – whole cell with curled up flagella and haptonema (arrow); **5** – detail of calyx; **6** – lateral view of coccolith displaying the two cycles of elements in the rim and details of the stem and the calyx; **7** – complete cell; notice the difference in length of coccolith processes from one end of the cell to the other; **8** – details of central area calcification; notice the short stub-like central proboscis and also possibly tilted pentagonal elements from the distal rim circle (arrows); **9** – detail of two coccoliths one with a short central process and the other with just a central upheaval of the arms of the crossbar.

**Table 1.** Numerical details of *P. sagittifera* HET from Arctic sites.

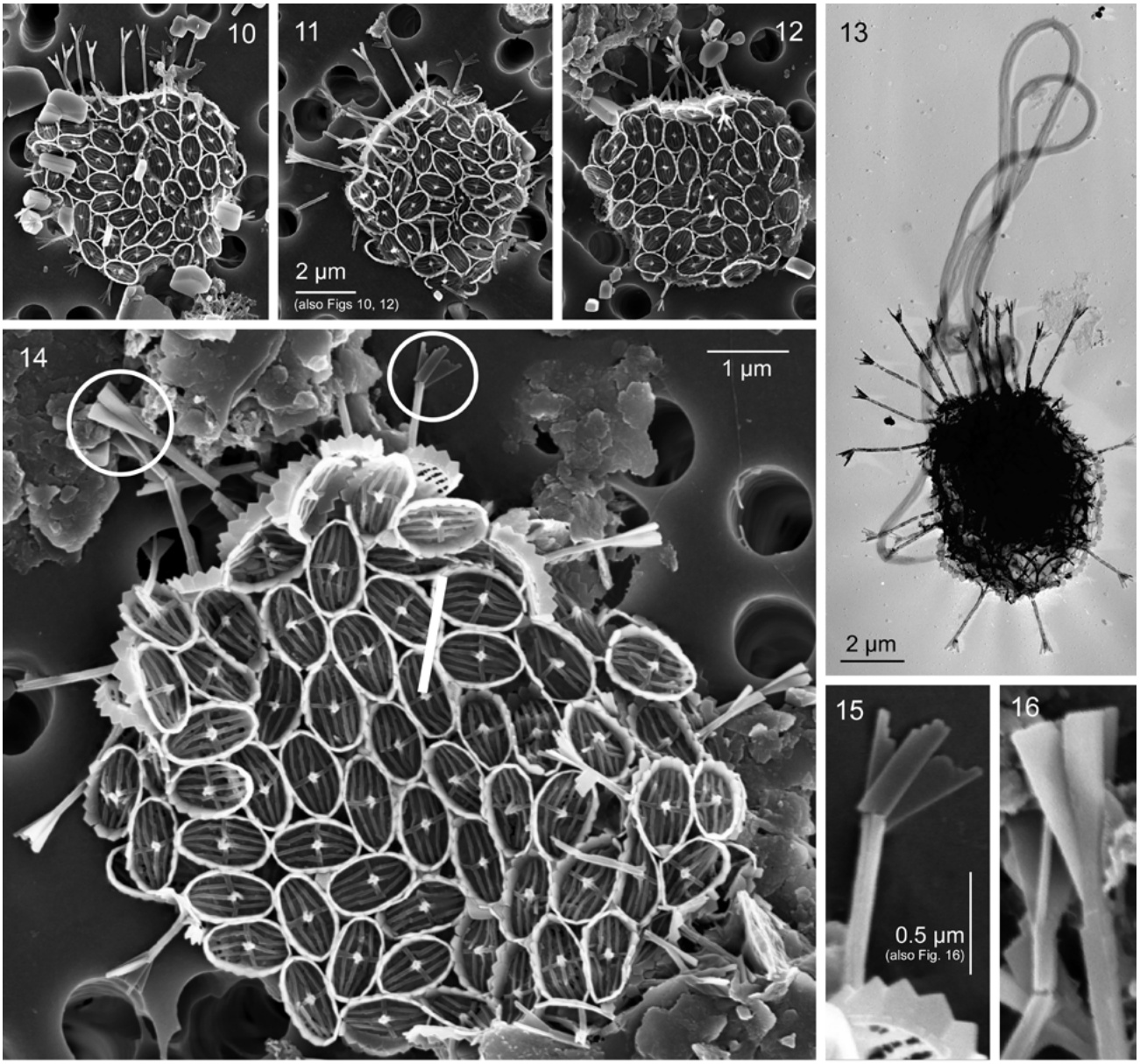
| | | | Coccolith length | | | Coccolith width | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Collection site | Reference | Cell dimensions (L × W) | Mean ± sd | min/max | n | Mean ±sd | min/max | n | Ant. Spine | Post. Spine |
| Arctic (NEW) | Fig. 4 | 6.1 × 4.2 | | 1.4–1.5 | | | 0.75–0.8 | | 2.7–3.3 | 2.3–2.4 |
| | Fig. 13 | 6.2 × 4.8 | | | | | | | 3.0–3.7 | 2.0–2.5 |
| Arctic (Disko) | Fig. 7 | 8.4 × 6.1 | 1.3 ± 0.054 | 1.20–1.39 | 14 | 0.69 ± 0.036 | 0.60–0.73 | 14 | 2.5–2.9 | 1.5–2.1 |
| Arctic (Svalbard) | Fig. 10 | 6.8 × 6.7 | 1.12 ± 0.082 | 0.95–1.31 | 31 | 0.65 ± 0.056 | 0.56–0.81 | 30 | 2.5–2.7 | 0.7–1.2 |
| | Fig. 11 | 6 × 5.7 | 1.10 ± 0.085 | 0.98–1.27 | 19 | 0.67 ± 0.072 | 0.56–0.85 | 19 | 2.6–2.8 | 1.1–1.5 |
| | Fig. 12 | 6.2 × 6.9 | 1.13 ± 0.076 | 0.10–1.35 | 30 | 0.68 ± 0.037 | 0.62–0.75 | 27 | 2.2–2.4 | 1.0 |
| | Fig. 14 | 7.7 × 6.5 | 1.11 ± 0.071 | 0.89–1.27 | 41 | 0.63 ± 0.046 | 0.54–0.71 | 38 | | |

ready known about *P. sagittifera* HET from this region (Thomsen 1981). The rationale behind illustrating three complete cells from TEM is to document the consistent presence of flagellation. The flagella and the haptonema typically curl up densely which renders measurements of these structures impossible. A haptonema is visible in e.g. Fig. 4 (arrow) where it is differentiated from the flagella by being significantly thinner. The apparent absence of flagellation in the numerous SEM illustrations of complete cells (Figs 10–12, 14) is bound to be an artefact somehow caused by the manipulation of the cells during filtration and subsequent drying. All complete cells illustrated from SEM clearly display organisms that have the typical cell polarity to be expected from a flagellated individual. Dimensional issues across the different regions are accounted for in Table 1.

Central area calcification is invariable an axial cross in combination with a number of longitudinal bars evenly spread across the coccolith. The highest number of such bars observed is 7 (Fig. 8) which creates a densely packed central area. Most coccoliths tend to have 3–5 of these longitudinal bars (see e.g. Fig. 14).

The calyx takes the shape of a four-winged rosette with the four wings positioned at right angles to each other (Figs 5–6, 9). Each wing has parallel sides. The exterior edge is straight, whereas the interior edge has a variable number of steps that gradually decrease the width of the wing towards the distal end (Fig. 3A). There seems to be a significant variability with respect to both the width of the wing and the number of steps on the interior edge. The degree to which the four-winged rosette diverges from the stem is dependent on the angle between the exterior edge and the line that proximally connects the inner and exterior edges of the wing.

*Pappsphaera sagittifera* HET is generally conceived as having monomorphic coccoliths yet with a tendency towards a varimorphic state in as much as there is variability among coccoliths in the length of the central process and with the longer ones prevailing in a circum-flagellar cluster. The SEM images (Figs 10–12, 14) clearly and consistently show that the actual pattern of variability goes beyond trivial dimensional issues. The major part of the coccoliths covering the cell surface – with the exception of the apical and antapical poles of the cell – are in fact devoid of a central process. All that is left is a short stub-like feature (Figs 8, 14) or just a low mound created by the lifting up of the arms of the axial cross where they meet centrally (Fig. 9). It is of course possible and also relevant to interpret these as being just very much reduced standard calicate coccoliths without a central appendage. However, it does anyway represent a differentiation among coccoliths that is almost equal to having dimorphic coccoliths. TEM images (e.g. Figs 4, 7, 13) are less informative with respect to illustrating the distribution of calicate and non-calicate coccoliths within a coccosphere. The electron beam does not adequately penetrate the middle part of the cell leaving the massive appearance in this area of non-calicate coccoliths difficult to resolve. The two-dimensional TEM image and a certain repositioning of individual coccoliths caused by the handling of the cells during processing, further add to the interpretational challenge with respect to accounting for the actual positioning of calicate versus non-calicate coccoliths. Disregarding these reservations the cells illustrated in Figs 4 and 13 do appear to have a few calicate coccoliths positioned in the middle part of the coccosphere. This might indicate a possible



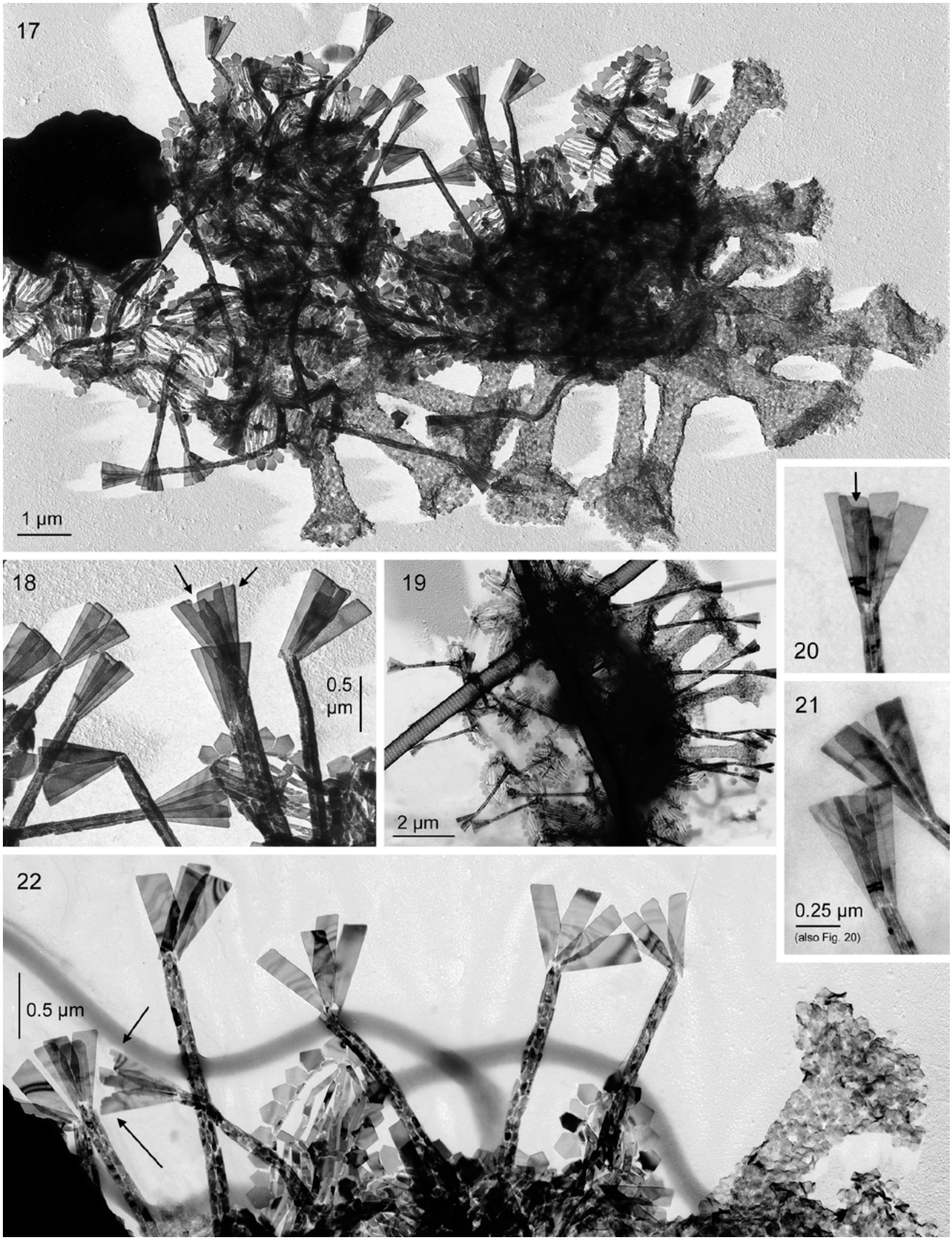

**Figs 10–16.** *Papposphaera sagittifera* HET (TEM / Fig. 13; SEM / Figs 10–12, 14–16) from the Arctic (Svalbard / Figs 10–12, 14–16; NEW / Fig. 13). **10–12** – whole cells selected to show the limited occurrence of coccoliths with central processes and the obvious irregularities in size and placement of the body coccoliths; **13** – whole cell showing flagella and haptonema; **14** – cell selected to illustrate the variability in coccolith size, orientation and central area calcification; notice the presence of two types of calyces (encircled); **15** – calyx of the standard 'flaring' *P. sagittifera* HET type (enlargement from Fig. 14); **16** – 'triangular' calyx (enlargement from Fig. 14) similar to those found in *P. sagittifera* HET when forming part of a combination coccosphere.

regional or seasonal variability among specimens of *P. sagittifera* that we do not at present have material to possibly account for.

### *P. sagittifera* combination coccospheres (Figs 17–22)

Two new occurrences of combination coccospheres from West Greenland are shown in Figs 17, 19. The cell shown in Fig. 17 is particularly appealing because here the two groups of coccoliths have become partly separated to clearly show the morphological characteristics of each of them.

With reference to the combination coccospheres of *P. sagittifera* and *T. borealis* there is one issue of particular concern. It has become apparent that in each of


17
1 µm
18
0.5 µm
19
2 µm
20
21
0.25 µm
(also Fig. 20)
22
0.5 µm

the combination coccospheres illustrated by Thomsen *et al.* (1991) and also in the two new images reproduced here, the *P. sagittifera* HET calyx is in fact different from what is typically found in *P. sagittifera* HET when not forming part of a combination coccosphere. In the *P. sagittifera* combination coccosphere the four-winged rosette comprises wings that are fairly narrow, hardly ever stepwise elaborated on the inside, and with an angle proximally between the exterior edge of the wing and the connecting line between the two edges of the wing that leads to a much less flaring rosette in comparison with that generally found in *P. sagittifera* HET (Fig. 3A). The line distally connecting the exterior and interior edges of the wing deviates a few degrees from being perpendicular (Figs 18, 20). In Fig. 22 the wings of the individual rosette have been pushed apart only to confirm the description of the individual wing provided above. A stepwise elaboration of the distal inner edge of the individual wing is occasionally observed (Figs 18, 20, 22; arrows). The design of the individual wing and the reduced overall flaring of the rosette cause the individual rosette to appear with an overall triangular outline when viewed in a TEM (Figs 18, 20–21). The very same features as described above are evident when consulting Thomsen *et al.* 1991 (loc. cit. Figs 2–4, 9).

A search among images of *P. sagittifera* HET has fortunately unveiled rare occurrences of this particular type of calyx scattered among others with the traditional stepwise and flared rosette design. First and foremost it should be mentioned that in the illustrations accompanying the type description (Manton *et al.* 1976a, loc. cit. Fig. 3) there is in fact a single calyx among more than 15 'normal' ones that fairly closely resembles those that occur in combination coccospheres. However, this feature was not specifically commented on in the type description. Also the SEM image of a Svalbard cell (Fig. 14 with enlargements in Figs 15–16) displays at least one calyx that resembles those that occur in combination coccospheres (Fig. 16) in between more typical *P. sagittifera* HET rosettes (Fig. 15). It must also be noticed that there is typically a size difference between the two types of rosettes. The 'triangular' rosette (Fig. 16) is thus close to 1 µm in length, whereas the 'flaring' rosette (Fig. 15) is only 0.6 µm in length which is typical for this kind of rosette (ca. 0.65 µm in e.g. Fig. 13). The size range among rosettes in Fig. 17 is 0.8–0.9 µm which equals measurements from previously published combination coccospheres (Thomsen *et al.* 1991; mean value 0.75 ± 0.09 µm / range: 0.65–1.00 µm / n = 12).

We have at present no explanation to offer with respect to these observations. Based in particular on the few findings referred to above of a combination coccosphere rosette type among 'normal' ones in genuine heterococcolithophore stages, but also on the fact that we have never observed a heterococcolithophore with a *P. sagittifera* central area calcification pattern and exclusively 'triangular' rosettes, we strongly believe that the heterococcolithophore forming part of combination coccospheres, such as those illustrated here in Figs 17, 19, is in fact *P. sagittifera sensu stricto*. Somehow the explanation behind these anomalies must be an integrated part of events taking place during a phase transition. However, it is very obvious that we are lacking in understanding the phenomenon of phase shift to the extent that this remains unexplainable for the time being. In our minds it makes most sense to think of combination coccospheres with these differing rosette features as representing stages in the haploid to diploid phase shift and that the 'triangular' rosette is thus a *de novo* feature of a 'new-born' diploid individual.

The holococcoliths forming part of a combination coccosphere appear to be closely similar to those described from *Turrisphaera borealis* (Manton *et al.* 1976b). Holococcoliths in Fig. 17 measure 3.0–3.5 µm in length and ca. 0.7 µm in width at the most narrow part of the tower. There are 5–6 plates of crystallites spanning the width of the tower and they each have an edge-to-edge distance of ca. 0.09 µm (type material: 0.15 µm).

◀◀

**Figs 17–22.** *Papposphaera sagittifera* combination coccospheres (TEM whole mounts from West Greenland). **17** – separated halves of a combination coccosphere; **18** – detail of calyces from Fig. 17 showing the regular triangular outline; the arrow points to a rosette wing that has a distal stepwise notching; **19** – combination coccosphere with the two halves united; **20–21** – detail of the calyces from Fig. 19; the arrow (Fig. 20) points to a rosette wing with a distal step-wise notching; **22** – the four rosette wings are spread out in a fan-like manner clearly showing the shape of the individual wing: the arrow points to a rosette wing with a distal step-wise notching.

### *P. sagittifera* HOL (Figs 23–26)

A selection of complete cells from West Greenland and the North East Water Polynya (Figs 23–26) are illustrated to show the general appearance of the cell with flagella and haptonema. Symmetrical tower-shaped holococcoliths, flaring at both ends (apple-core shaped), radiate from the entire cell surface. A monolayer of hexagonal plates of crystallites covers the entire surface of the holococcolith often united to form larger scale patterns (e.g. Fig. 26). The similarity between cells illustrated here and those included as part of the type description (Manton *et al.* 1976b) is fairly convincing, also when it comes to dimensions there is an overall agreement.

The coccosphere diameter is in our material ca. 12 µm, and the diameter of the cell ca. 6 µm. The tower-shaped holococcoliths vary in length from roughly 1.5 to 3.5 µm. The minimum width along the tower is 0.4–0.6 µm. Manton *et al.* (1976a) found that the plates of crystallites in the type material measured 0.15 µm across. When measuring 64 randomly selected individual plates from Fig. 26 (mean value: 0.144 ± 0.018 µm / overall range: 0.09–0.17 µm) the result thus corroborates the observation by Manton *et al.* (1976b).

### Emended description of *P. sagittifera* based on Arctic site material

***Pappsphaera sagittifera* Manton, Sutherland and McCully 1976 emend. Thomsen, Østergaard and Heldal**

Syn.: *Turrisphaera borealis* Manton, Sutherland and Oates 1976

**Diagnosis:**

**Heterococcolithophore phase:** Cell oblong ca. 7 × 5 µm with two flagella and a haptonema. Coccoliths (1.1–1.3 × 0.6–0.7 µm) varimorphic to dimorphic muroliths. Circum-flagellar and antapical pole coccoliths carry a conspicuous calicate spine. The longest spines (up to 3.5 µm) are at the apical pole whereas those at the antapical pole tend to be somewhat shorter (ca. 1.5 µm). The calyx is typically constructed from four perpendicularly arranged elements forming a conspicuously flaring four-winged rosette. Each wing has parallel sides. The exterior edge is straight, whereas the interior edge has a variable number of steps that gradually decrease the width of the wing towards the distal end. In rare instances the rosette adopts a regular triangular outline where the individual wing is narrower, without a distal stepwise elaboration of the interior edge, and with a different angle between the exterior edge and the line that proximally connects the exterior and interior edges causing the rosette to be less flaring. The central area calcification comprises an axial cross that is continuous with the stem of the spine. There is additionally 5 (3–7) longitudinal bars evenly distributed across the coccolith. The rim is upright (ca. 0.25 µm in height) and slightly flaring and comprising two cycles of elements. The proximal cycle is formed by short rods (0.15 × 0.05 µm) joined end to end. The distal cycle comprises pentagonal elements that give the rim a characteristic serrate margin. The two cycles of elements are mutually and regularly shifted. Body coccoliths are similar to those described above with the exception of the central spine which is here often represented by a short knob-like structure equivalent to the proximal part of the stem. In other cases the coccolith centre is mound-shaped caused by a slight upheaval of the arms of the axial cross where they meet. Unmineralized underlayer scales not observed.

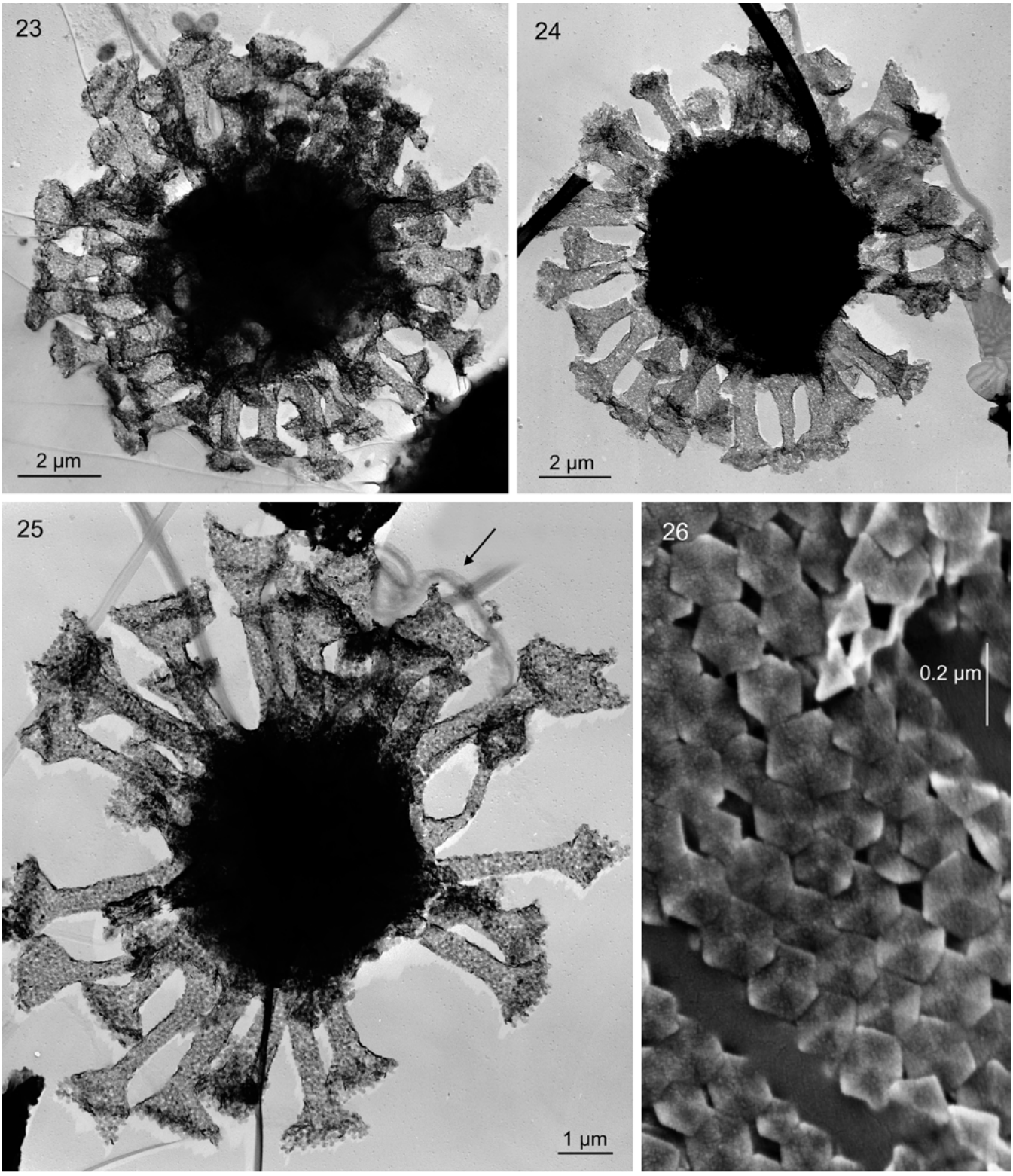
**Holotype:** Manton, Sutherland and McCully 1976a loc. cit. Figs 1–4. Material collected at Homer, South Alaska, 19 June 1975 (15 m depth / 6°C).

**Epitype:** Thomsen 1981, loc. cit. Figs 9, 13 collected August 1977 in the vicinity of the Arctic Station, Disko, West Greenland (ca. 50 m depth / ca. 2°C).

**Holococcolithophore phase:** Cell mostly spherical and ca. 6 µm in diameter. The diameter of the entire coccosphere is ca. 12 µm. Cell flagellated and with a somewhat shorter haptonema. Coccosphere formed by holococcoliths of one type, each a symmetrical and lengthy tower-shaped structure (up to 3.5 µm in length) with a distinct flaring at both ends. The organic base plate measures ca. 1.2 × 1.5 µm. Underlayer scales (0.2–0.3 µm) are present most likely occupying the gaps between the coccolith base plates. There is typically a change in overall dimensions of the individual tower from one end of the cell to the other, with the longest holococcoliths encircling the flagellar pole of the cell. The holococcoliths carry a monolayer of hexagonal plates (ca. 0.15 µm across) of crystallites arranged in reticulate patterns or in lines that spiral the towers. Each hexagonal plate may have a central perforation.

**Combination coccosphere** (*Pappsphaera sagittifera* / *Turrisphaera borealis*)

With the characteristics of the heterococcolithophore and holococcolithophore phases yet with calyx features in the heterococcoliths that differ from those typically observed in *P. sagittifera* HET. The four-




**Figs 23–26.** *Papposphaera sagittifera* HOL (TEM / Figs 23–25; SEM / Fig. 26) from the Arctic (NEW / Figs 23–25; Svalbard / Fig. 26). **23–25** – complete cells showing flagella, haptonema (Fig. 25, arrow) and large numbers of tower-shaped holococcoliths projecting in all directions; **26** – high magnification of the hexagonal crystallite plates.

winged rosette comprises wings that are fairly narrow, hardly ever stepwise elaborated on the inside, and with an angle proximally between the exterior edge of the wing and the connecting line between the two edges of the wing that leads to little flaring of the rosette. Notice that this type of calyx is also infrequently observed in *P. sagittifera* HET.

**New observations on *P. sagittifera* from Antarctic sites**

Thomsen *et al.* (1988) reported *P. sagittifera* from the Weddell Sea based on the finding of a single specimen in the AMERIEZ material. It was concluded that there was a large degree of similarity between this cell and material previously examined from the northern hemisphere and there was no reservation at that time with regard to the identity of the material. We have during subsequent sampling campaigns (EPOS, ANT X/3) found a large number of cells reminiscent of *P. sagittifera* and *T. borealis* as well as combination coccospheres in Weddell Sea samples. This material is further described below and illustrated in Figs 27–48.

***P. sagittifera* cfr. HET (Figs 27–32)**

Material from the Weddell Sea EPOS cruise seems in all major details to match the appearance of northern hemisphere *P. sagittifera*. The two complete cells (Figs 27, 30) are flagellated and both with a curled up haptonema. The coccospheres comprise the usual mixture of coccoliths with central processes clustered around the flagella and towards the antapical pole. Other coccoliths are devoid of processes and much reminiscent of *P. sagittifera* body coccoliths in terms of central area calcification (Fig. 31) and the elaboration of the rim (Fig. 32). The calyx (Figs 28–29) is a flaring four-winged rosette similar to that described from Arctic *P. sagittifera* HET. Each wing has on the interior edge a marked step-wise reduction in size.

The identity of this material is still ascertained with some element of doubt. The Antarctic material is characterized by a large degree of coccosphere cohesiveness which means that the number of coccoliths that clearly display the central area calcification is rather limited. In those that are in fact exposed, such as Fig. 31, there is an unfamiliar element of skewness in the overall design that is unknown in northern hemisphere *P. sagittifera*. The bars are obviously tilted with reference to the primary axes of the coccolith. There is also towards one end of the coccolith a 'misplacement' of elements that may at first sight appear random. However, these irregularities are in fact repeated also in the cell found earlier from the region (Thomsen *et al.* 1988, loc. cit. Figs 35–37) which indicates that this may in fact be a typical feature of *P. sagittifera* from the southern hemisphere. A third reason for our hesitation to just right away identify this form as *P. sagittifera* is the fact the combination coccosphere also deviates from the northern hemisphere established standards (see further below).

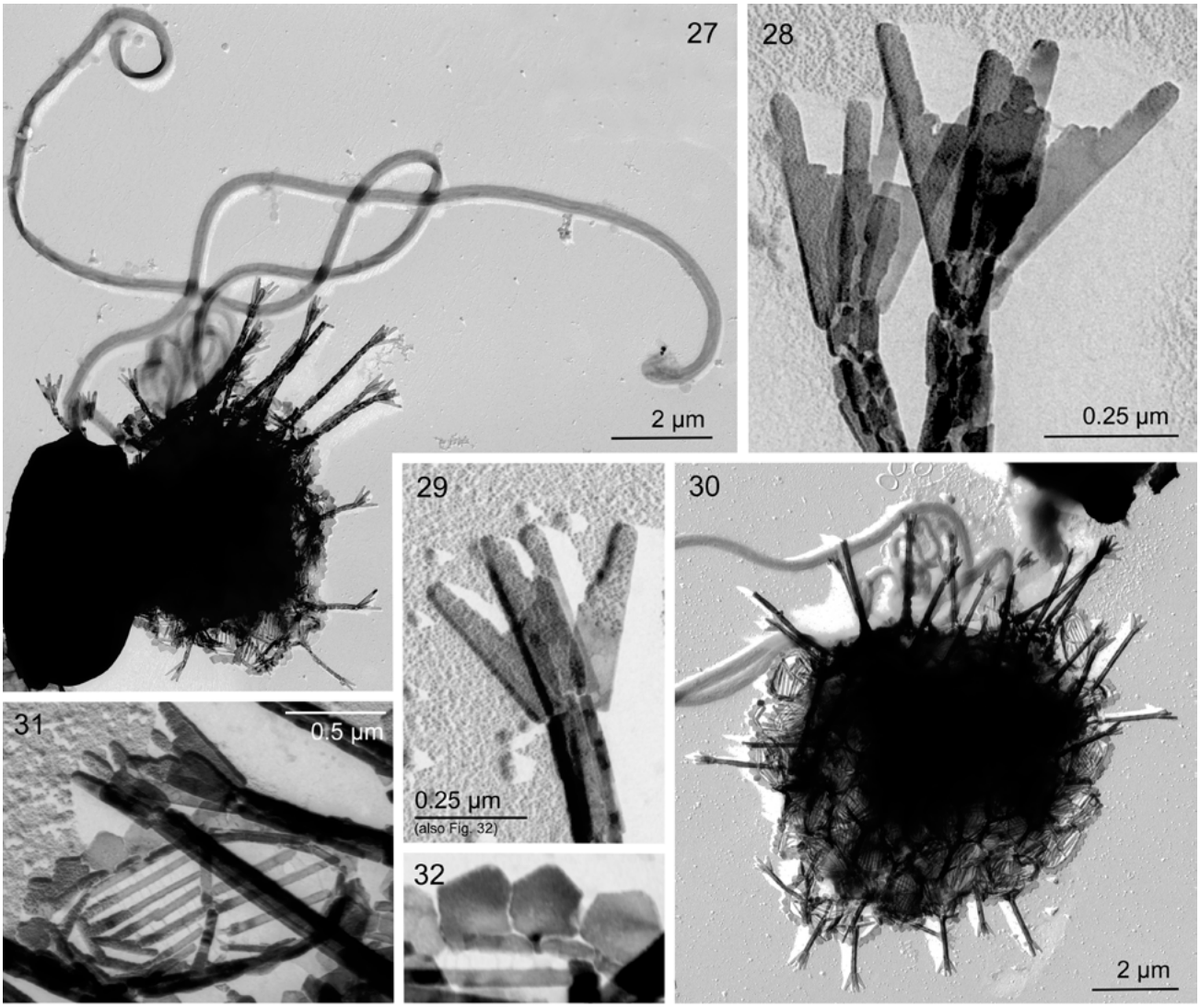
***Turrisphaera* sp. (Figs 33–37)**

Cells reminiscent of *P. sagittifera* HOL (syn. *Turrisphaera borealis*) have been regularly encountered in Antarctic samples and were expected to eventually appear as the life cycle counterpart of Antarctic *P. sagittifera* cfr. The cells illustrated here originate from the Weddell Sea (EPOS) and display the typical cell coverage of symmetrical and tower-shaped holococcoliths that are flaring at both ends. Crystallographic features (Figs 35–36) are also consistent with those of *P. sagittifera* HOL. Our main hesitation to up front identify the Antarctic material as *P. sagittifera* HOL is due to the fact that the individual holococcoliths are shorter (1.2–1.5 µm) and slightly more narrow (ca. 0.4 µm) and also accordingly have fewer hexagonal plates across the coccolith. The overall appearance of the Antarctic material is thus somewhat different from the Arctic *P. sagittifera* HOL (compare e.g. Figs 24 and 34). It should also be emphasized that we have at present no evidence from Antarctic waters of life cycle events that involves this particular form. We therefore prefer for the time being simply to refer to this material as *Turrisphaera* sp. Without molecular evidence it is hardly possible to take a different approach right now.

***P. sagittifera* cfr. combination coccosphere (Figs 38–43)**

Both the EPOS and ANT X/3 Antarctic Weddell Sea material encompassed combination coccospheres that involve *P. sagittifera* cfr. HET and a *Turrisphaera* species that is not identical to *P. sagittifera* cfr. HOL, nor the Antarctic *Turrisphaera* sp. described above.

In the heterococcolithophore component the central area calcification is rarely exposed, yet it appears to display (Fig. 41) the same aspects of asymmetry (i.e. a skewed cross bar and randomly oriented peripheral elements) described above for *P. sagittifera* cfr. HET (Fig. 31). The calyx is a flaring four-winged rosette (Figs 41–42) in which each individual wing shows a pronounced stepwise reduction in width towards the

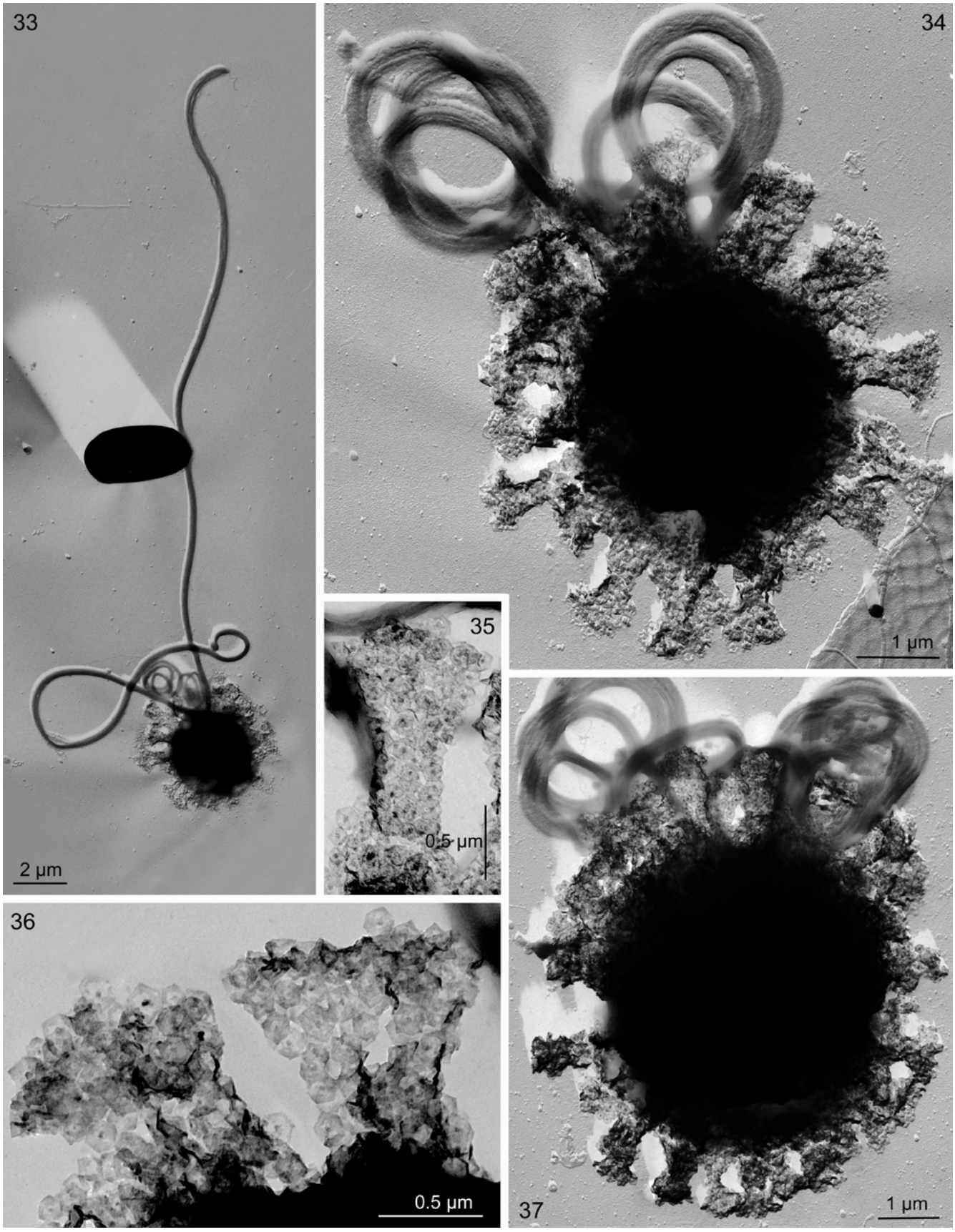


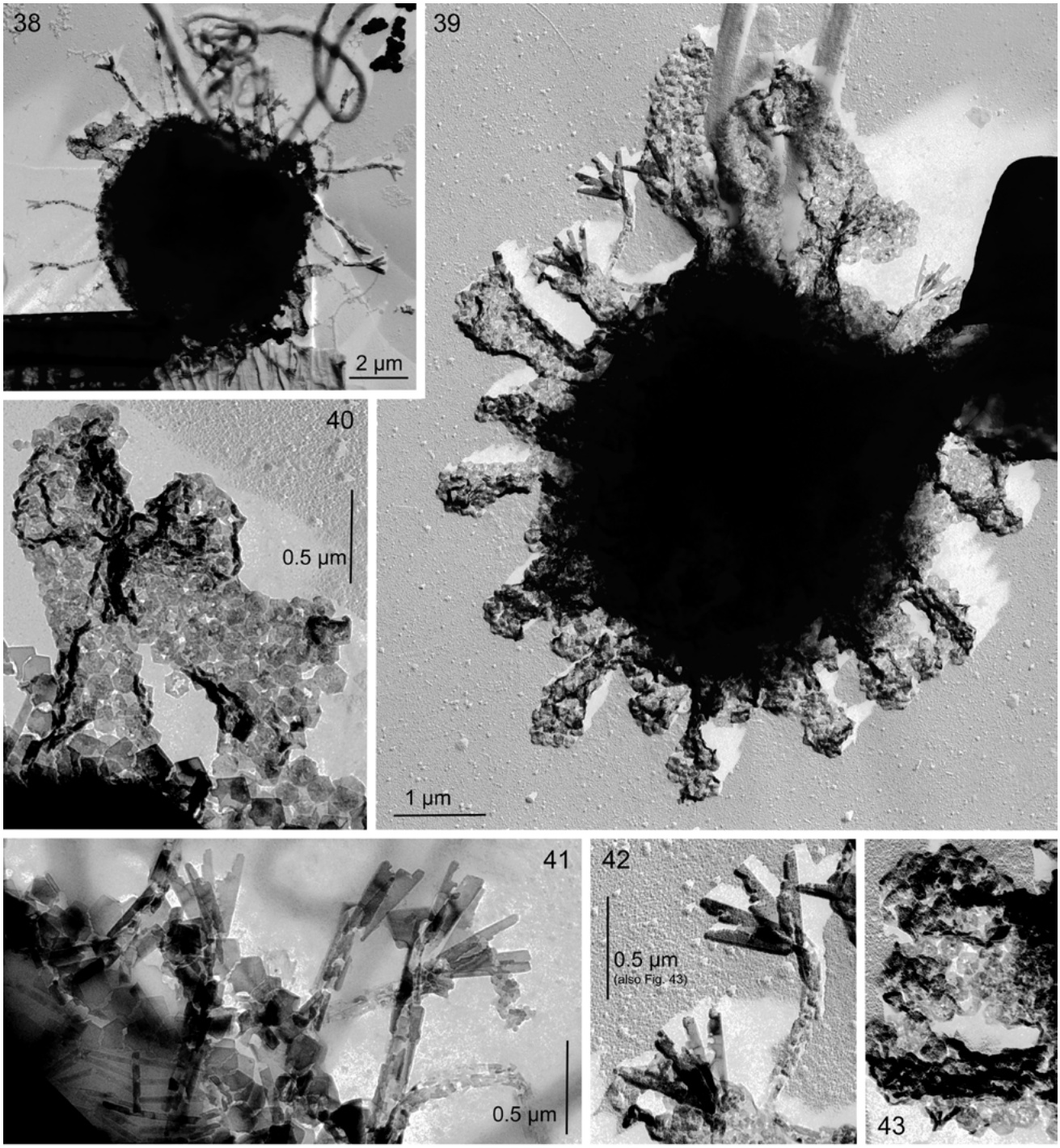

**Figs 27–32**. *Papposphaera sagittifera* cfr. HET. TEM whole mounts from the Antarctic (EPOS samples). **27** – complete cell with flagella and a curled up haptonema; **28** – high magnification of calyces (four-winged rosettes) from Fig. 27; **29** – high magnification of calyx from Fig. 30; **30** – complete cell with flagella and a curled up haptonema; **31** – detail of central area calcification from the cell in Fig. 30; **32** – details of rim structure from the cell shown in Fig. 30.

distal tip. The holococcolithophore part is characterized by a pronounced asymmetry in the flagellar pole holococcolith, and a more slender yet overall symmetrical appearance of coccoliths elsewhere in the periplast. The unilateral flaring of the circum-flagellar holococcoliths immediately distinguishes this form from both Antarctic *Turrisphaera* sp. (Figs 33–37) and Arctic *P. sagittifera* HOL (Figs 23–26).

### *P. sagittifera* cfr. HOL (Figs 44–48)

The holococcolithophore component of the combination coccospheres illustrated in Figs 38–43 was also abundantly present in the holococcolithophore phase in the EPOS and ANT X/3 samples from the Weddell Sea (Figs 44–48). The keystone feature of this species is the pronounced asymmetry in the circum-flagellar holococcoliths where one side of the distal end of the tower is drawn out into a long lip-like structure that is offset from the tower itself at an angle of up to 55 degrees. Coccoliths elsewhere are fairly narrow and symmetrical and with a pronounced distal flaring. Cell dimensions in the Antarctic specimens range between 4–6 µm. The circum-flagellar holococcoliths typically reach 3 µm in length whereas those found elsewhere tend to be


33
2 µm
34
1 µm
35
0.5 µm
36
0.5 µm
37
1 µm




**Figs 38–43.** *Papposphaera sagittifera* cfr. TEM combination coccospheres from Antarctica (EPOS / Figs 39, 42–43; ANT X/3 / Figs 38, 40–41). **38** – complete cell with flagella and haptonema; **39** – cell with just a few *P. sagittefera* cfr. HET coccoliths and '*Turrisphaera'* holococcoliths that are highly asymmetrical at the anterior pole; **40** – detail of holococcoliths from the cell shown in Fig. 38; **41** – higher magnification of anterior cell end of the cell shown in Fig. 38 to show details of the calyces and the central area calcification; **42–43** – details of coccoliths from the cell shown in Fig. 39.

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**Figs 33–37.** *Turrisphaera* sp. TEM whole mounts from the Antarctic (EPOS samples). **33** – complete cell with flagella and haptonema; **34** – whole cell at higher magnification showing the regular appearance of the tower-shaped holococcoliths; **35–36** – details of holococcoliths showing the hexagonal crystallite plates; **37** – complete cell.

ca. 1 µm long. The hexagonal plates are of standard '*Turrisphaera*' size. When measuring 37 individual plates from Fig. 48 the mean value is 0.11 ± 0.014 µm and the overall range 0.06–0.14 µm. A central hole in the hexagonal plates is a typical feature of these cells. No underlayer scales have so far been observed.

## DISCUSSION

The main outcome of revisiting Arctic material of *P. sagittifera* has been (1) to solve the taxonomical problems inherent to an insufficient quality of the type material, (2) to remedy the error introduced when first selecting a species epithet for the *P. sagittifera*/*T. borealis* consortium, (3) to verify the morphological integrity of *P. sagittifera*/*T. borealis* in Arctic waters, and (4) to provide an emended species description that covers both *P. sagittifera* HET and HOL.

There are two new observations of a more generic nature that deserve to be highlighted here. It is thus verified beyond doubt that the *P. sagittifera* coccosphere is are dimorphic. Even though it is possible to claim that the body coccoliths are in fact only very much reduced versions of the apical and antapical pole coccoliths it does not appear appropriate in this context to refer to them as varimorphic. While there is no doubt that *P. lepida*, the type species, has varimorphic coccoliths in a more strict sense, it is similarly obvious that the features here described with respect to coccolith variability in *P. sagittifera* are in fact shared by other species of *Papposphaera*, e.g. *P. arctica* (Thomsen *et al.* 2016b) and *P. iugifera* (Thomsen and Østergaard 2016). It can also be mentioned that in the recently described species, *P. heldalii* (Thomsen and Egge 2016), the coccosphere is strictly dimorphic. We do not at present consider this a critical issue with respect to the circumscription of the genus *Papposphaera* mostly because the generic type, *P. lepida*, has also been shown in all likelihood to share a life cycle with a species of *Turrisphaera* (Young *et al.* 2015). We are inclined to believe that this circumstance in fact represents a much stronger argument for a phylogenetic close affinity among these species, and that the varimorphic versus dimorphic issue is therefore likely trivial in the context of circumscribing a genus.

The second morphological feature that deserves special mentioning is the observed difference in calyx morphology between *P. sagittifera* HET when forming part or not-forming part of a combination coccosphere ('triangular' versus 'flaring'). While it is a very consistent pattern that we have no immediate explanation for, it is important to emphasize that we strongly believe that it is never the less *P. sagittifera sensu stricto* that incorporates this morphological variability. The reason for this is on the one hand the rare finding of single incidences of 'triangular' rosettes in *P. sagittifera* HET when not forming part of a combination coccosphere, and on the other hand also the fact that if the 'triangular' rosettes were indeed associated with a species that is different from *P. sagittifera sensu stricto* we would no doubt, considering the frequent occurrence of the combination coccospheres, have found heterococcolithophore phases of this form exclusively carrying the 'triangular' rosettes.

We have decided to refer to the Antarctic material presented here as *P. sagittifera* cfr. HET. There is on the one hand subtle but also likely consistent differences in e.g. central area calcification of the heterococcolithophore phase between Arctic and Antarctic material, and on the other hand also irregularities with respect to the *Turrisphaera* counterpart in the life cycle. While *T. borealis* like cells appear to be present in Antarctic waters, here referred to as *Turrisphaera* sp., it is surprisingly a much different form of *Turrisphaera* that combines with the Antarctic version of *P. sagittifera*. To make things even more complicated it must be noticed that this Antarctic holococcolithophore species is very similar to *Turrisphaera arctica* Manton, Sutherland and Oates 1976b, which is recently reported to combine with a newly described Arctic species of *Papposphaera*, i.e. *P. arctica* Thomsen, Heldal and Østergaard 2016b (see Fig. 3). The central area calcification is in *P. arctica* reduced to a simple axial cross.

In summary we have to conclude that while we can outline the *P. sagittifera* differences across the two hemispheres we have difficulties without the support from

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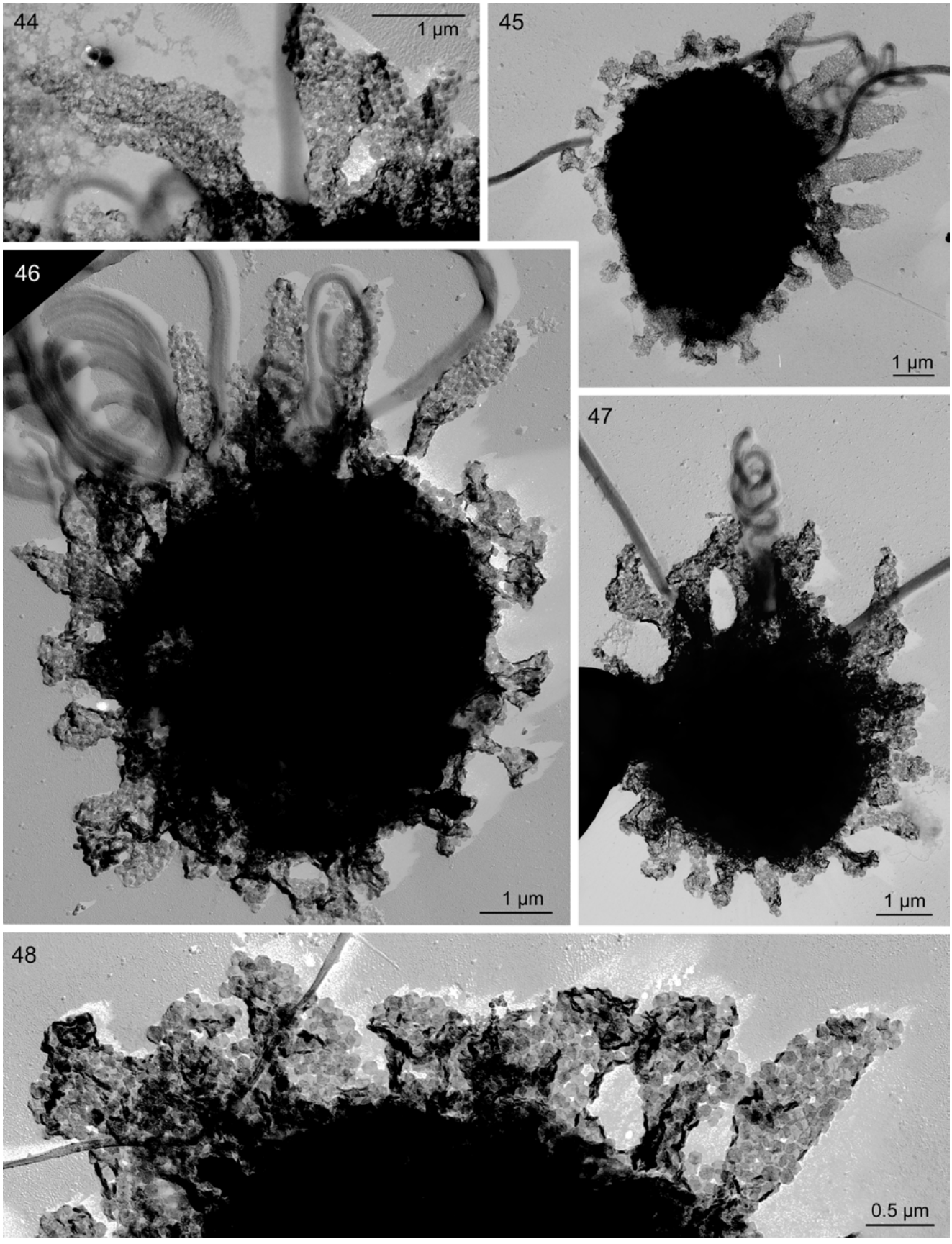
**Figs 44–48.** *Papposphaera sagittifera* cfr. HOL. TEM whole mounts from the Antarctic (EPOS / Figs 45, 47–48; ANT X/3 / Figs 44, 46). **44** – details of highly asymmetrical flagellar pole holococcoliths; **45–47** – complete cells with flagella and haptonema; **48** – holococcoliths at high magnification.


44
1 µm
45
1 µm
46
1 µm
47
1 µm
48
0.5 µm

molecular analyses of relevant material to conclude whether *P. sagittifera* has a bipolar distribution. While the differences with respect to central area calcification are certainly minor and not likely of relevance on its own in the context of circumscribing a new species, the life history issue is certainly more critical. However, due to an insufficient knowledge of natural variability among species of *Turrisphaera* we are not with any degree of certainty able to go beyond the mere description of the differences, for which reason it appears most appropriate to refer to the Antarctic taxa as *P. sagittifera* cfr. HET, *Turrisphaera* sp., and *P. sagittifera* cfr. HOL (syn. *Turrisphaera arctica* cfr.) for the actual life history counterpart of *P. sagittifera* cfr. HET.

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