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# Observations on the morphological diversity and distribution of two siliceous nanoplankton genera, *Hyalolithus* and *Petasaria*

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**ABSTRACT:** Scale-bearing siliceous nanoplankton are occasionally encountered in surface seawater samples, but are rarely identified or illustrated. In this study, the morphological diversity of the haptophyte *Hyalolithus neolepis* and the enigmatic *Petasaria heterolepis* are investigated in scanning and transmission electron microscopes using materials from around the world. Results show that *H. neolepis* scales exhibit variation in the width of the marginal hyaline area, but intermediate specimens make separation of the two morphologies difficult. *Petasaria heterolepis* scales also show differences, in the presence of tubercle rows in the hyaline area and degree of hyaline areal coverage, but separation into discrete varieties is difficult at present. However, specimens with scales bearing a protuberance are considered to be distinct enough to warrant the erection of a new species, *Petasaria protuberans* Jordan, Malinverno, Šupraha, Thomsen et Young sp. nov.

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## INTRODUCTION

Microplankton assemblages collected from the world's oceans often include siliceous scale- or plate-bearing organisms in low abundances (e.g. Nishida 1979, LeRoi and Hallegraeff 2006).

Many of these organisms belong to algal groups like the Parmales (e.g. *Tetraparma* Booth) and Chrysophyceae (e.g. *Paraphysomonas* Stokes), or to microzooplankton groups such

TABLE 1

Detailed information on the samples associated with the photographic images shown in Plates 1-7. Coordinates have been rounded up to two decimal places, so they may be slightly different to those given in other publications. The notation n/a (not applicable) refers to samples taken close to the shore, which did not use research ships.

Region	Location	Station No.	Latitude	Longitude	Date	Cruise	Collection method	Sampling depth	Data supplied by	
Atlantic	Oslo Fjord	OF2	59.19°N	10.69°E	21-8-2013	R/V Trygve Braarud	Niskin water sampler	20 m	Supraha	
	Bergen Fjord	Raunefjorden	60.27°N	5.21°E	21-10-2009 3-11-2009 2-10-2012 8-9-2010	R/V Aurelia	Hand pump	5 m	Heldal	
	Læsø Rende, Denmark	T1359	57.33°N	11.50°W	17-3-1976	Belt project, R/V Martin Knudsen	water bottle	10 m	Thomsen	
	North Atlantic	11290	26.17°N	30.01°W	3/4-6-1986	Cruise 159, R.R.S. Discovery	water bottle	35 m	Jordan	
	Portuguese margin	89	39.57°N	9.12°W	10-3-2015	NRP Almirante Gago Coutinho	CTD rosette	5 m	Guerreiro	
	Mid-Atlantic	276-20	18°N	17°E	19-7-2006	M68-3, R/V Meteor	CTD rosette	30 m	Young	
Mediterranean	South Atlantic	CTD063 CTD089	9.97°S 32.18°S	24.99°W 29.83°W	26-10-2008 2-11-2008	AMT18, R.R.S. James Clark Ross	CTD rosette	104 m 96 m	Young	
	Eastern Adriatic Sea	AD2	43.61°N	15.88°E	13-7-2008	R/V Hydra	Niskin water sampler	60 m	Supraha	
	central Aegean Sea	T1-2	37.83°N	24.93°E	21-8-2001	01ED100/PENED, Leonidas Azapis	Hydrobios bottle	0 m	Dimiza, Triantaphyllou	
	south Aegean Sea	Rho	35.62°N	27.70°E	31-1-2007	M71-3, R/V Meteor	CTD Rosette	150 m	Dimiza, Triantaphyllou, Malinverno	
	Ionian Sea	n04 n12	35.28°N 35.62°N	21.42°E 17.23°E	22-11-1997 9-12-1997	SIN97, R/V Urania	Niskin bottle	10 m 80 m	Malinverno	
	western Mediterranean	23 69	36.62°N 37.43°N	1.10°E 0.42°E	29-9-1999 5-10-1999	MATER II, R/V Hespérides	CTD rosette	38 m 90 m	Young	
	Gulf of Mexico	off Alabama	A8	29.63°N	86.10°W	15-9-2013	R/V Bellows	CTD rosette	40 m	Cruz
	off Louisiana	76	27.88°N	93.00°W	1-5-1993	LATEX 93E	CTD rosette	39.9 m	Young	
	Tsugaru Strait	offshore Matsumae	41.40°N	140.13°E	23-6-2006	n/a	bucket	surface	Hagino	
	Sea of Japan	Tomari Port st. 1	35.52°N	133.98°E	6-2011	n/a	bucket	surface	Hagino	
	Pacific	Seto Inland Sea, Japan	Seto-29	34.14°N	132.90°E	29-4-2008	R/V Bosei-Mar	seawater supply	surface	Jordan
		Kuroshio, south of Japan	Bosei-11	32.50°N	138.19°E	27-4-2008	n/a	water bottle	surface	Thomsen
south of Japan		S12 S13	20.84°N 19.84°N	131.74°E 131.18°E	21-12-1996 22-12-1996	KH96-5, R/V Hakuho-Mar	seawater supply	surface	Jordan	
Gulf of California		Bahia de los Angeles	29.02°N	113.52°W	7, 8-1-1990	n/a	water bottle	surface	Thomsen	
off Point Sur, California		#1	36.02°N	122.00°W	25-9-1989	R/V Point Sur	water bottle	surface	Thomsen	
Equatorial Pacific		#11	36.18°N	122.67°W	26-9-1989	R/V Malcolm Baldrige	water bottle	75 m	Thomsen	
off W. Australian coast		S92	35.20°S	110.00°E	30-4-1992	KH96-5, R/V Hakuho-Mar	seawater supply	surface	Jordan	
Southern Ocean	Weddell Sea	#142 #182	56.55°S 57.02°S	50.70°W 48.92°W	26-11-1988 27-12-1988	EPOS, Polarstern	water bottle	10 m 10 m + 20 m	Thomsen	
	Sub-Antarctic Zone	#8	52.74°S	144.98°W	20-2-2007	R/V Astrolabe	seawater supply	surface	Eriksen	
		SOTS	47°S	142°E	8-2011 to 7-2012	Mooring array	McLane RAS sampler	35 m		

as the Thaumatomastigidae (e.g. *Thaumatomastix* Lauterborn), filose or testate amoebae (e.g. *Pinaciophora* Greeff and *Paulinella* Lauterborn, respectively). Others remain enigmatic (e.g. *Meringosphaera* Lohmann).

In 1979, Moestrup described *Petasaria heterolepis* from a marine coastal location about 1 km off Kaikoura (New Zealand) based on transmission electron microscope (TEM) images and also showed a similar specimen from the Red Sea (Gulf of Elat), which had been found by Helge Thomsen. Moestrup (1979) placed his new species in the *Incertae Sedis*, but was unsure whether the Kaikoura and Gulf of Elat specimens belonged to the same species or were different, with scales of the latter possessing rows of tubercles on the periphery and more perforations. One of his specimens possessed an appendage, which was interpreted as a single flagellum (Moestrup 1979, fig.62), however, others have wondered whether this might be a haptoneuma (Yoshida et al. 2006).

In the same year, Nishida (1979) illustrated several siliceous scaly marine organisms using the scanning electron microscope (SEM), but did not describe them as new taxa or assign them to any known groups. One of these was *P. heterolepis*, which was collected from south of Japan (his pl.22, fig. 4), while the two that dominated in the North Pacific (his pl.1, figs 3-4) are now known to be species of Parmales (see Konno et al. 2007). Another two from south of Japan remained enigmatic (his pl.22, figs 1-2) until Yoshida et al. (2006) named similar forms as the siliceous haptophyte, *Hyalolithus neolepis*.

Here we present *H. neolepis* and *P. heterolepis* from samples collected by us over the last four decades in the hope that the ecology and morphologic diversity of these taxa can be better understood. As a result of these observations we herein describe a new species, *Petasaria protuberans* sp. nov.

## MATERIAL AND METHODS

The samples used in this study were collected from around the world at different times of the year and in different years, and

from various water depths between 0-200m, using a number of collection methods (Table 1). The preparation and observation methods were also quite varied.

The Oslo Fjord and Eastern Adriatic samples were obtained using a 5 litre Niskin water sampler, and filtered onto a Whatman Cyclopore filter (25 mm diameter, 0.8µm porosity), with a Whatman cellulose filter (of the same size and porosity) underneath to ensure even distribution. The filter was then rinsed with bottled water (pH 7.54) and dried in an oven at 50°C. A portion of the filter was mounted onto a metal stub, sputter coated with gold, and observed in a Zeiss Supra 35-VP SEM.

The Bergen Fjord samples were collected by hand pump and prepared by gently filtering 150-600 ml of seawater onto a Nuclepore filter (25 mm diameter, 0.6-1.0µm porosity). Salt crystal formation was minimalised by almost drying out the filter using the pumping system. A subsample of the filter was mounted onto an SEM stub, sputter coated with gold/palladium and observed in a Zeiss Supra 55 VP SEM at the Bergen University Laboratory of Electron Microscopy.

Nannoplankton in the Gulf of California, Denmark and Southern Ocean samples were prefiltered through a 20µm mesh and concentrated by gravity filtration on top of a Millipore filter (47 mm diameter, 2.0µm porosity). Organisms on the filter were re-suspended in a small volume of water and further concentrated by centrifugation. Whole mounts on formvar grids were prepared from pelleted material according to the method of Moestrup and Thomsen (1980), shadow cast with chromium at a low angle and examined in a JEM-100SX TEM at the Botanical Institute of the University of Copenhagen.

The North Atlantic water sample (1.57 litres) was filtered onto a Nuclepore polycarbonate filter (47 mm diameter, 0.4µm porosity), air-dried and then stored in a plastic filter case (Millipore Petrislide). A subsample was coated with gold-palladium and observed in a Philips XL30 FEG SEM.

TABLE 2

Geographic distribution of the ‘morphotypes’ of *Hyalolithus neolepis* (wide and narrow hyaline margins), *Petasaria heterolepis* (small and large openings) and *P. protuberans* (short, intermediate and long protuberances) found in this study.

Region	Location	<i>Hyalolithus neolepis</i>		<i>Petasaria heterolepis</i>		<i>Petasaria protuberans</i>			Data supplied by
		Narrow hyaline margin	Wide hyaline margin	Small openings	Large openings	Short	Intermediate	Long	
Atlantic	Oslo Fjord			○					Supraha Young & Heldal Thomsen Thomsen Young, Couappel & Jordan Guereiro Young Young
	Bergen Fjord			○					
	Danish waters			○					
	Bergen area			○					
	North Atlantic	○							
	Portuguese margin	○							
Mediterranean	Mid-Atlantic	○			○		○		Supraha Dimiza, Triantaphyllou, Malinverno Malinverno Young & Geisen
	South Atlantic	○			○				
	Eastern Adriatic Sea					○			
	Aegean Sea	○							
Gulf of Mexico	Ionian Sea	○						○	Cruz Young & Pariente
	western Mediterranean			○					
Pacific	off Alabama	○							Hagino Abe & Jordan Young & Geisen Abe & Jordan Fujita & Jordan Nishida Nishida Thomsen Hernández-Becerril & Pasten-Miranda Thomsen
	off Louisiana	○							
	Sea of Japan		○						
	Seto Inland Sea, Japan		○		○				
	off Miyake Jima	○							
	Kuroshio, south of Japan	○							
	south of Japan	○							
	Western Pacific	○							
	Philippine Sea	○						○	
	Pacific	○							
Indian	Gulf of California	○							Abe, Tanimoto & Jordan Nishida Shramik Thomsen
	Bahia de los Angeles, GoC		○					○	
	off W. Australian coast	○							
Southern Ocean	eastern Indian Ocean	○						○	Thomsen
	southern Indian Ocean	○							
Southern Ocean	Weddell Sea			○					Eriksen, Thomas & Harding
	Sub-Antarctic Zone		○		○				

The central Portuguese margin seawater samples (ca. 2 litres) were collected using a Neil Brown MKIIC CTD profiler equipped with an Aquatracka nephelometer, a Seapoint fluorometer and a rosette sampler (12 x 8 litre Niskin bottles). Samples were filtered onto acetate filters (47mm diameter, 0.45µm porosity) using a low pressure vacuum system. The filters were rinsed with tap water to remove salt and oven dried at 40°C for 24 h. A randomly chosen section of each filter was fixed with colloidal silver on an SEM stub, sputter coated with gold-palladium to a maximum thickness of 20 nm, and observed using a Hitachi S-3500N SEM, operated at 5 kV.

On various cruises to the Mid- and South Atlantic, western Mediterranean and Gulf of Mexico, as well as sampling off coastal Japan, 0.5 to 2.0 litres of seawater were vacuum filtered onto polycarbonate or cellulose filters (47mm diameter, 0.2-0.8µm porosity), and pieces of dried filter were sputter-coated with gold-palladium and examined in a Philips XL30 FEG SEM.

For each sampling station in the Aegean Sea, 2 litres of seawater were vacuum filtered onto Whatman cellulose nitrate filters (47mm diameter, 0.45µm porosity). Salt was removed by washing the filters with about 2 ml of mineral water. The filters were air-dried and stored in plastic Petri dishes. A piece of each filter approximately 8x8 mm<sup>2</sup> was attached to a copper electron microscope stub using double-sided adhesive tape and coated with gold. The filters were examined in a JEOL JSM 6360 SEM at the Department of Historical Geology and Paleontology, University of Athens.

For the Ionian Sea samples, four litres of seawater were collected with a Niskin bottle and filtered onto a cellulose acetate filter (47 mm diameter, 0.45µm porosity), which was then oven-dried and stored in a plastic petri dish. A subsample was cut out and fixed onto an aluminium stub with double-sided graphite tape, coated with gold and observed in a Cambridge Stereoscan SEM at the University of Milano.

The Gulf of Mexico water sample (1 litre) was collected with a Niskin water bottle rosette attached to a Conductivity Temperature Depth (C.T.D.) rig. The water was then filtered through a Nuclepore cellulose filter (44mm diameter, 0.6µm porosity) us-

ing a vacuum pump. The filter was placed in a petri dish, oven-dried at a low temperature for 4-6 hours to remove excess moisture, and stored in a sealed bag. A piece of the filter was mounted onto an aluminium stub using double-sided carbon tape, sputter-coated with gold-palladium, and observed in a JEOL JXA-840A SEM at the Florida Geological Survey warehouse in Tallahassee, Florida.

Sea surface water samples (10 litres) from Tsugaru Strait and Tomari Port, Tottori, Japan (Table 1) were collected by bucket. Samples were pre-filtered through a 50µm mesh plankton net (Sefar Inc. DIN-110) in order to remove large plankton. Pre-filtered samples were concentrated using a piece of 1µm mesh-size plankton net (Sefar Inc. NY1-HD) place on a sieve. *Hyalolithus* cells were isolated from the concentrated seawater sample using a capillary under an inverted light microscope (Olympus CKX41), and then maintained in MNK medium (Noël et al. 2004) at 20°C in an 18:6 light:dark regime. About 10 ml of cell suspension of each strain was sampled during the exponential growth phase and filtered onto a Millipore filter (47 mm diameter, 0.45µm porosity). Small pieces of dried filter were mounted onto SEM stubs and gold-coated using an ion sputter coater (Sanyu SC701 MKII), and then examined in a JEOL JSM 7001F SEM.

During the R/V *Hakuho-Maru* and R/V *Bosei-Maru* cruises, surface water samples (2-4 litres) were collected using the ship-board seawater supply for research use and filtered onto Millipore HA-type polycarbonate or nitrocellulose filters (47 mm diameter, 0.45µm porosity), air-dried and then stored in Millipore plastic petrislides. Subsamples of the filters were mounted onto aluminium stubs, coated with platinum/palladium and observed in a JEOL JSM-6510LV SEM.

The Sub-Antarctic Zone samples (500 ml) were obtained with a McLane RAS sampler, attached to the permanent Southern Ocean Time Series (SOTS) 47S mooring array for periods of up to one year, with each representing a ~9-day collection interval. The samples were preserved in-situ with glutaraldehyde. Later, 100 ml subsamples were filtered onto a Nuclepore membrane filter (13mm diameter, 0.8µm porosity), rinsed with 2 mL of Milli-Q, and air-dried. A portion of the filter was mounted onto a 12 mm stub, sputter-coated with tungsten, and observed in a



JEOL JSM-6701F Field Emission-SEM. Other samples from this region were collected onboard *L'Astrolabe*, according to McCartney et al. (2014, see supplement).

## TAXONOMIC SECTION

*Hyalolithus neolepis* Yoshida et al. 2006

Plate 1, figures 1-6; Plate 2, figures 1-8; Plate 3, figures 1-6; Plate 4, figures 1-7

*Basionym*: *Hyalolithus neolepis* YOSHIDA et al. 2006, p. 214-215, figs. 1-4, 6-13.

*Synonymy*: Genus and Species indeterminate – NISHIDA 1979, pl. 22, figs. 1a-b, 2a-b.

*Pontosphaera discopora* Schiller – JORDAN et al. 1984, pl. 2, fig. 8.

*Prymnesium neolepis* (Yoshida et al.) EDVARDESEN, EIKREM and PROBERT 2011, p. 223 – PATIL et al. 2014, text-fig. 2A-F.

*References*: *Hyalolithus neolepis* Yoshida et al. – DE VARGAS et al. 2007, figs. 7C-D – JORDAN 2011, fig. 3.

*Observations*: Yoshida et al. (2006) described two cell types; motile cells with two long equal flagella, a non-coiling haptonema, and a layer of organic scales (short spine scales), and non-motile cells with two short flagella, a non-coiling haptonema, and two scale types (siliceous hat-shaped scales and organic scales with radial and concentric patterning). The discovery of a haptophyte bearing siliceous scales was unexpected, and resulted in the recognition of a new type of haptophyte scale (hence, *hyalinus* Gr. = glass, *lithos* Gr. = stone, and *neo* Gr. = novel or new, *lepis* Gr. = scale). Here we will only discuss the siliceous scales, as the other scale types were not seen in our study.

Generally, the scale case (periplast) consists of about 60-100 hat-shaped scales (Pl. 1, figs. 1-6), which vary in shape (circular to strongly elliptical) and size (compare Pl. 2, figs. 3 and 6). In distal view, the scale has an imperforate recurved rim, and a hyaline margin surrounding a perforated centrally raised area, which often appears to be a double mound with a slightly concave centre (see scales on left-hand side of Pl. 1, fig. 3). The perforations in the central area are very numerous and very small.

In proximal view, the imperforate rim and margin and the perforated central area can be clearly seen (Pl. 2, fig. 6). In rare specimens there appears to be a swollen structure near the centre, which is also perforated (Pl. 2, fig. 8). This structure was not reported in the original description by Yoshida et al. (2006).

The width of the hyaline margin also varies, as does the size and number of the perforations (compare Pl. 1, fig. 4 with Pl. 3, fig. 2). The forms with the wider margins also exhibit a range of scale shapes and sizes (Pl. 3, fig. 5), and sometimes scales with seemingly abnormal swollen central structures are seen in distal view (Pl. 3, fig. 6). Further examples of aberrant scales were seen in cultured material (Pl. 4, figs. 1b-c), as well as scales with a central depression on the distal side (Pl. 4, fig. 4) and a swollen structure on the proximal side (Pl. 4, fig. 5). At first, these latter scales were not seen in our natural samples so we assumed that they were artifacts produced in the culture, but after many hours of searching we finally found them in two samples; from the Seto Inland Sea (Pl. 4, figs. 6-7) and off western Australia (Pl. 2, fig. 8).

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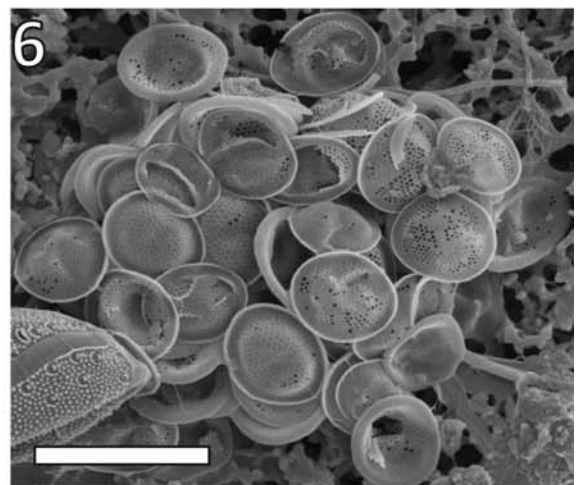
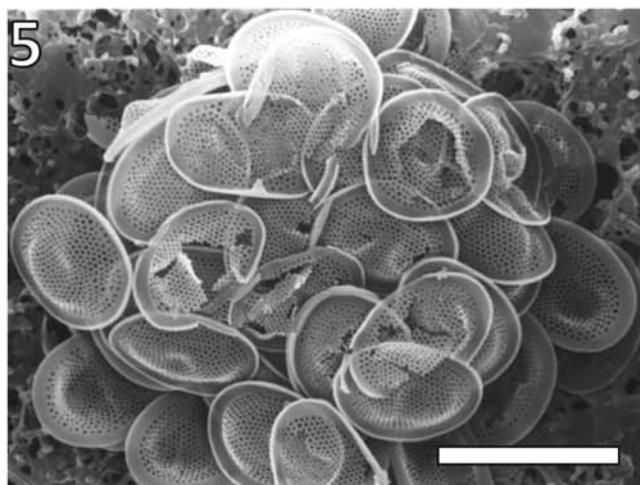
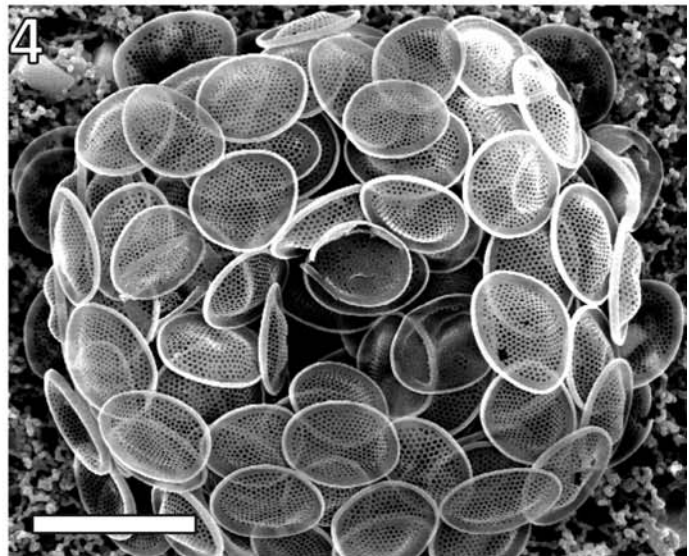
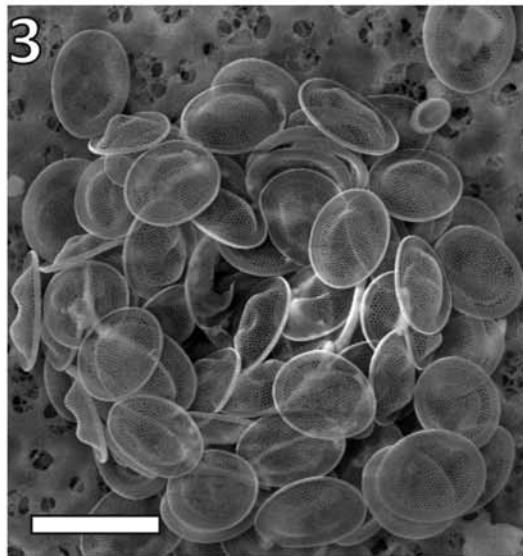
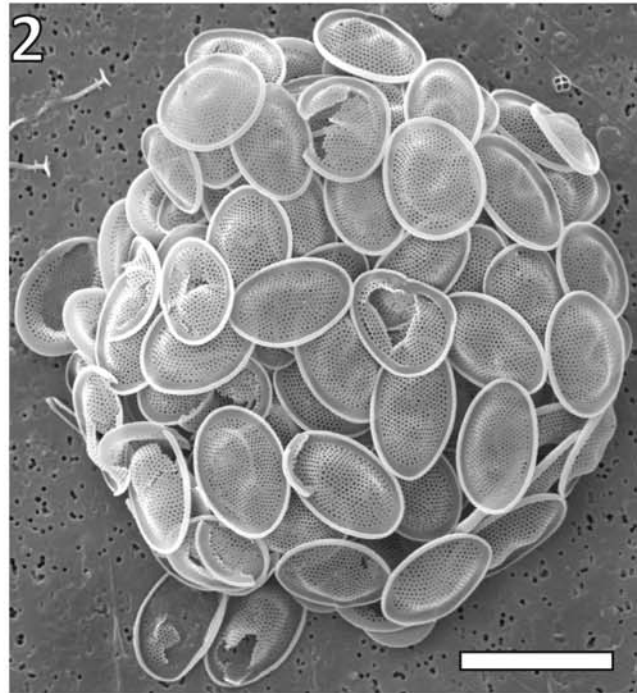
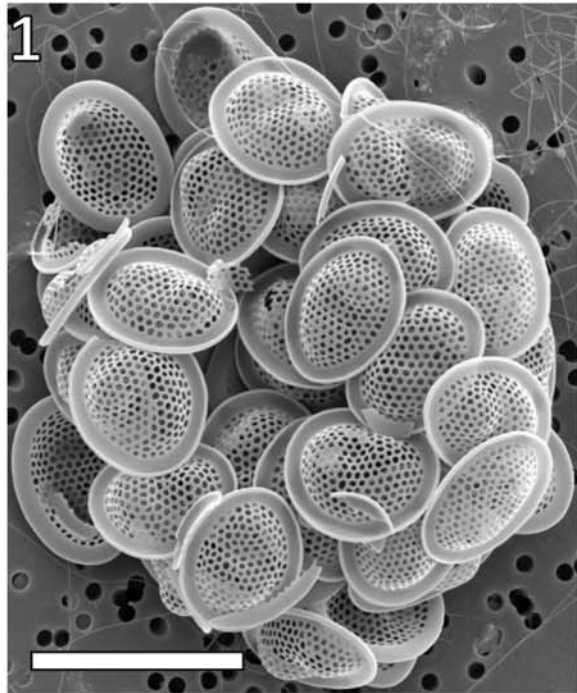
## PLATE 1

*Hyalolithus neolepis* Yoshida et al.

Figures 1-6 are SEM images. The scale bars are 10µm.

Station number, culture strain or sampling date given in brackets (see Table 1).

- |   |  |
|---|--|
| 1 Mid-Atlantic (276-20). Collapsed cell.  | 4 Central Aegean Sea (T1-2). Collapsed cell. |
| 2 Ionian Sea (n04). Collapsed cell.       | 5 North Atlantic (11290). Collapsed cell.    |
| 3 South Aegean Sea (Rho). Collapsed cell. | 6 Gulf of Mexico (76). Collapsed cell.       |



*Type locality:* Originally collected from waters (150-1950m) overlying the Shiribeshi Seamount (43.58°N, 139.55°E) off the coast of Hokkaido (Yoshida et al. 2006).

*Biogeography:* In this study, specimens with narrow hyaline margins have been found in all the major oceans and at a wide range of latitudes (40°N-35°S). However, specimens have been recorded in the Southern Ocean, as far as 65°S (Patil et al., 2014). In this study, specimens with wider hyaline margins had a more patchy distribution, being found in the Gulf of Mexico, off Japan and in the Southern Ocean (see Table 2).

*Remarks:* The finding of rare scales, with a distal depression corresponding to a proximal swollen structure, is of great interest because similar scales belonging to fossil species of *Hyalolithus* have recently been found in Eocene and Miocene outcrop materials (Abe et al., in press, Abe et al., unpubl. obs.). The swollen structure on *Hyalolithus* scales appears to be a continuous part of the central area and not a separate structure, as seen in the proximal tube of *Syracosphaera pulchra* Lohmann exothecal coccoliths. In *H. neolepis* the scales bearing the swollen structure are fewer in number than those lacking the structure. Given our knowledge of haptophyte scale cases, it is possible that scales bearing the swollen structure might be limited to the circumflagellar area without 'body' scales underlying them, or they might be exothecal scales which only cover the 'body' scales around the flagella (i.e., pseudodithecatism). Although the specimens with narrow hyaline margins were separated in the plates from those with wide hyaline margins, we have no concrete evidence that they represent distinct taxa, and in fact their morphologies tend to intergrade.

*Nomenclatural taxonomy:* Yoshida et al. (2006) described *Hyalolithus* as a separate genus on the grounds that it was morphologically very different to any other known haptophyte, with the production of siliceous scales being a unique feature. However, based on molecular genetic evidence combined with supporting cytological data, Edvardsen et al. (2011) subsequently transferred the species into *Prymnesium*, since *H. neolepis* was placed within the *Prymnesium* clade. And yet, the morphological differentiation of *H. neolepis* indicates that it strongly diverged from other members of the clade and this is supported by the discovery of Middle Eocene fossils indicating divergence of more than 40 million years ago. For these reasons and in the interests of practical taxonomy we recommend the continued use of the genus *Hyalolithus*.

*Petasaria heterolepis* Moestrup 1979

Plate 5, figures 1-9; Plate 6, figures 1-6; Plate 7, figures 1-4

*Basionym:* *Petasaria heterolepis* MOESTRUP 1979, p. 86, figs. 58, 61-66.

*Synonym:* Genus and Species indeterminate – NISHIDA 1979, pl. 22, fig. 4.

*References:* *Petasaria heterolepis* Moestrup – PATTERSON and ZÖLFFEL 1991, p. 452, fig. 26.6a – PREISIG 1994, p. 39, fig. 27 – SCOTT and MARCHANT 2005, p. 468, figs. 15.9a-d – LEROI and HALLEGRAEFF 2006, p. 223, fig. 10 – PATIL et al. 2015, p.173-174, text-figs. 2A-D.

*Observations:* Moestrup (1979) illustrated two cells, 4-6µm in diameter, both bearing a 6-7µm-long appendage that lacked hairs. Although originally assumed to be a single flagellum, it closely resembles the haptonema of *Hyalolithus neolepis*

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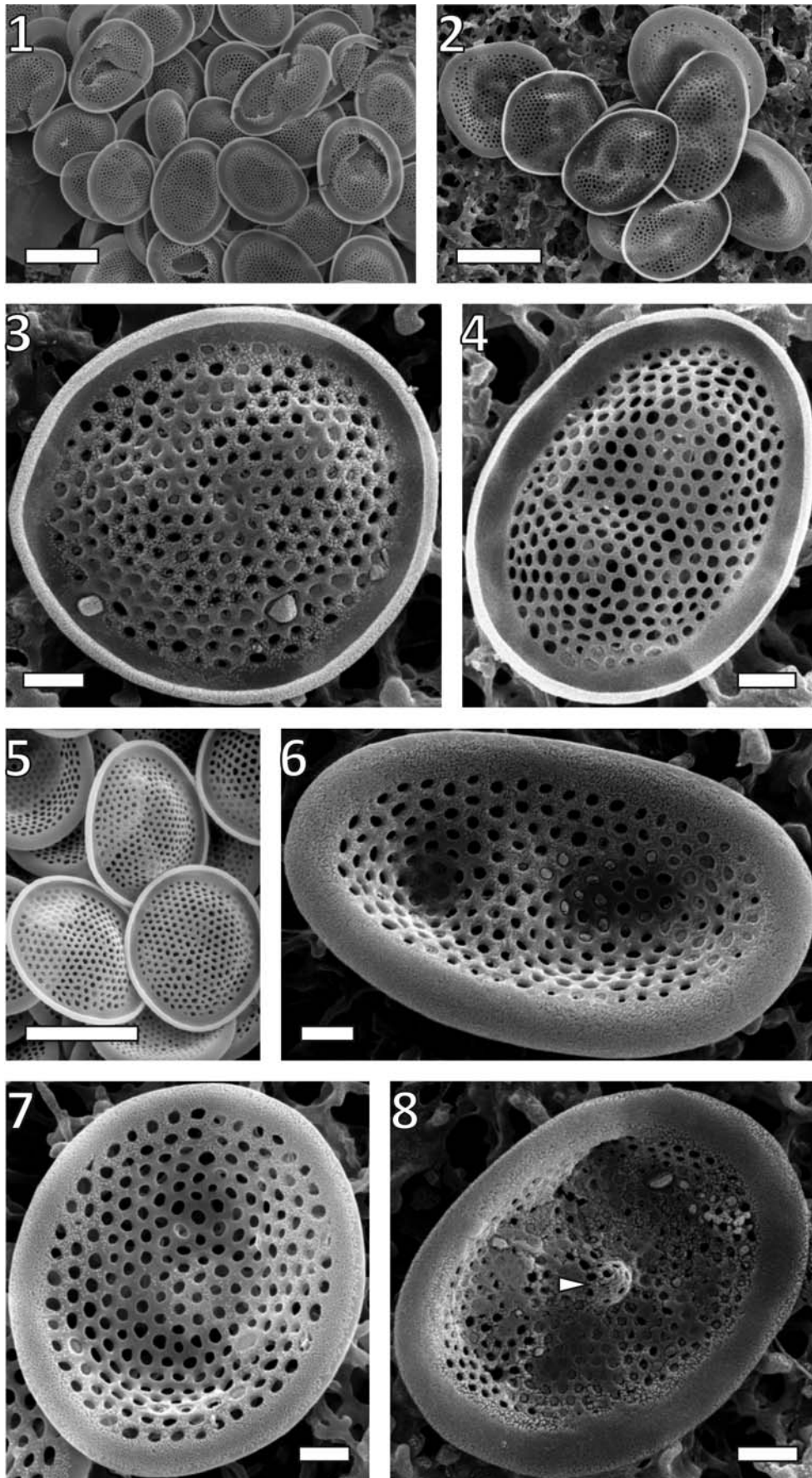
## PLATE 2

*Hyalolithus neolepis* Yoshida et al.

Figures 1-8 are SEM images. The scale bars are 5µm (figs. 1, 2 and 5) or 1µm (figs. 3, 4 and 6-8).

- |  |  |
|--|--|
| 1 Off Portugal (89). Scales in distal view.                  | 2,3,6,8 Off Western Australia (S92). 2 Scales in distal and proximal views. 3 Scale in distal view. 6, 8 Scale in proximal view. 8 Note swollen structure (arrowhead). |
| 4 Kuroshio, south of Japan (Bosei-11). Scale in distal view. |  |
| 5 North Atlantic (11290). Scales in distal view.             | 7 South of Japan (S13). Scale in proximal view.  |







(Yoshida et al. 2006). Here we show two further examples of this appendage on Antarctic specimens from the Weddell Sea (Pl. 6, figs 3-4). Note that the appendage sometimes has a bulbous end (Moestrup 1979, fig. 62; our Pl.6, fig. 3). Moestrup (1979) and LeRoi and Hallegraeff (2006) illustrated cobweb-like organic scales (similar to those of prasinophytes) together with the siliceous scales of *Petasaria*, suggesting either *Petasaria* is a prasinophyte or that the organic scales were unrelated to *Petasaria* (Moestrup 1979, Yoshida et al. 2006). Here we show two examples of similar 'cobweb-like' scales (Pl. 6, figs. 5a-b, 6). However, they appear to resemble one of the haptophyte designs, notably the plate scales of *Chrysochromulina pyramidosa* Thomsen, which also has radiating lines superimposed on equidistant concentric circles (see Thomsen 1977, LeRoi and Hallegraeff 2004). When some of our SEM images are enlarged (but not visible in the plates), faint outlines of the organic scales can be seen.

The cells are covered in numerous hat-shaped scales (*petasus* L. = broad-brimmed hat; *-aria* L. suffix = related to or connected with) of similar size and shape, which X-ray analyses confirmed are siliceous, but with a small peak of Ca (Moestrup 1979; Patil et al. 2015). In the original description, the scale dimensions were given as 1.7 x 1.9µm to 2.9 x 3.2µm (Moestrup 1979), which are corroborated here with our new data. In distal view, one end of the scale (called the 'base' in Moestrup 1979) is circular to oval-triangular and has a raised area, part of which is hyaline. The hyaline area may be extensive, almost covering the entire scale (Pl. 5, fig. 2), or as a band along the periphery (Pl. 7, fig. 2) or as a small patch (Pl. 7, fig. 5). It may also be as-

sociated with rows of tubercles (Pl. 7, fig. 3). The rest of the scale is characterised by perforations of variable size and shape (but with the largest ones near the periphery – see Pl. 6, fig. 1), and the rim is not raised. Slightly off-centre, within the perforated area, is a circular to oval crater-like depression (Pl. 5, fig. 6). In proximal view, a raised structure (corresponding to the depression on the distal side, and of similar shape) is characterised by a number of short projections that are directed towards the hyaline area (Pl. 5, fig. 6).

*Type locality:* Originally found in seawater from Kaikoura (42.42°S, 173.70°E), New Zealand (Moestrup 1979).

*Biogeography:* Although described as having a worldwide distribution (Moestrup pers. comm. in Preisig 1994), there are few papers illustrating this species – in fact, many of the references above merely reprinted the original figures of Moestrup (1979). In this study, specimens have been recorded from Danish waters, temperate North Atlantic, the Gulf of Mexico, subtropical Pacific, and the Southern Ocean. In addition, Nishida (1979) illustrated a single specimen from 30°N in the western Pacific, while LeRoi and Hallegraeff (2006) reported specimens from southern Tasmanian waters. It has also been found in the Gulf of Elat (Thomsen in Moestrup 1979).

*Morphotypes:* Some specimens show differences in the extent of the hyaline area and presence/absence of tubercle rows on the distal side, and the size of the raised structure on the proximal side (e.g. compare Pl. 5, fig. 6 with Pl. 7, fig. 2). Whether these represent morphotypes is unclear. However, the scales with pro-

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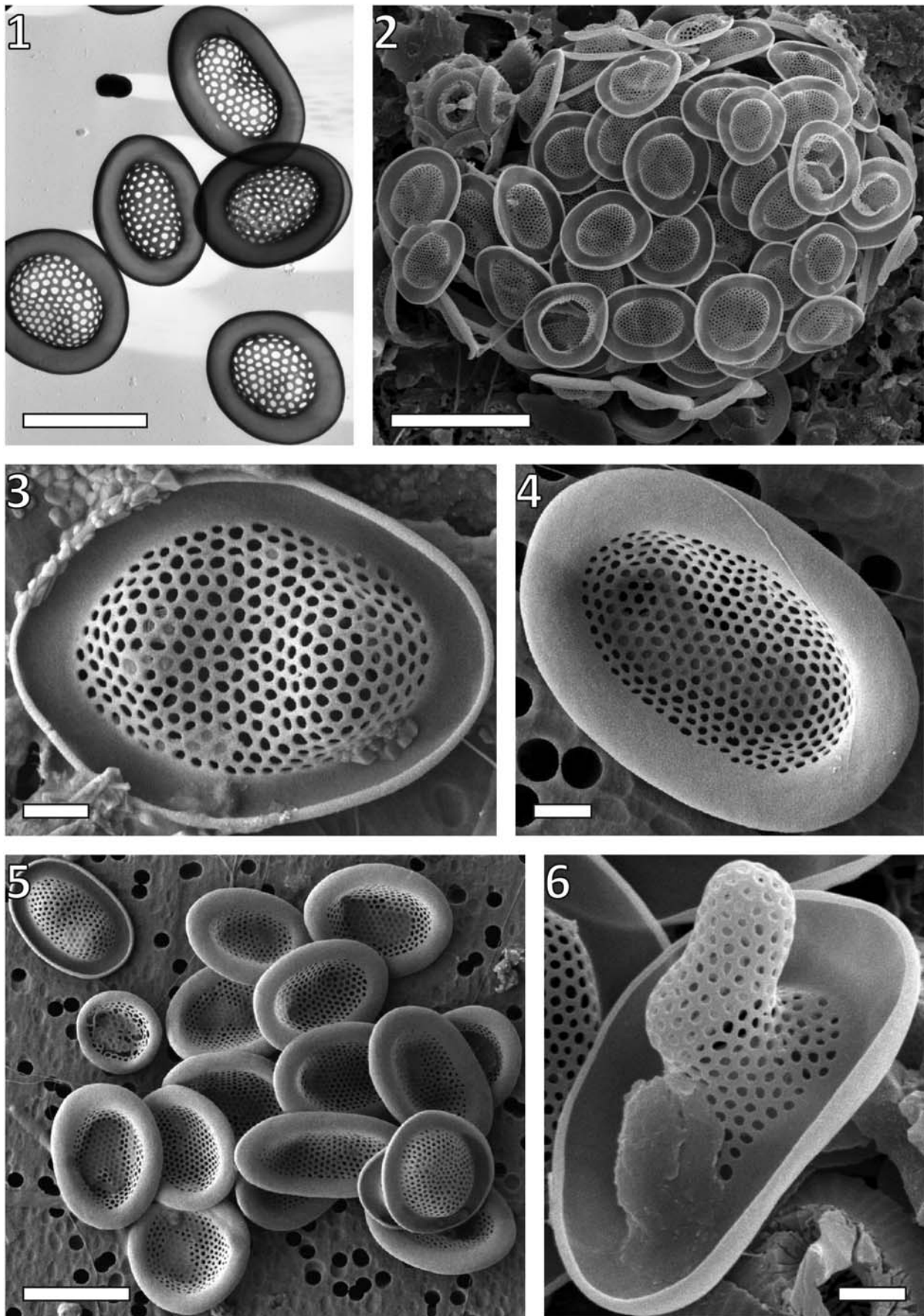
### PLATE 3

*Hyalolithus neolepis* Yoshida et al.

Figure 1 is a TEM image, figures 2-6 are SEM images. The scale bars are 10µm (fig. 2), 5µm (figs. 1 and 5), or 1µm (figs. 3, 4 and 6).

- 1 Bahia de los Angeles, Gulf of California. Scales in distal view.
- 2 Seto Inland Sea (Seto-29). Collapsed cell.

- 3-5 Sub-Antarctic Zone (SOTS). 3 Scale in distal view. 4 Scale in proximal view. 5 Scales in distal and proximal views. Note scale size and shape variability.
- 6 Seto Inland Sea (Seto-29). Scale in distal view, with unusual central structure.



tubercles (Pl. 7, figs. 5-8) are considered herein to be distinct enough to be a separate species (see below).

*Remarks:* Moestrup (1979) placed this species in the *Incertae Sedis*, because the “two completely different scale categories are not similar to any other known types of scales” (hence, *hetero-* L. prefix = different, *lepis* L. (from Gr.) = scale). Since then, no one has been able to culture or sequence it, and thus it has remained enigmatic. However, with the discovery of *Hyalolithus* (Yoshida et al. 2006), *Petasaria* may be a putative siliceous haptophyte. Despite the huge size difference (see Pl. 5, fig. 3), they do share some basic characters (e.g. siliceous hat-like scales, with a distinct rim, hyaline and perforated areas). Although rarely encountered in *Hyalolithus* but always present in *Petasaria*, the scales of both species may possess a distal depression and a corresponding proximal structure – though admittedly, the structure itself is not so similar.

*Petasaria protuberans* Jordan, Malinverno, Šupraha, Thomsen et Young sp. nov.  
Plate 7, figures 5-8

*Description:* Cell shape unknown, only collapsed cells observed. Cell cover composed of 20-30 circular to oval siliceous scales, 2.7-3.1 µm x 2.5-2.9 µm. Distal surface of scale perforated, except for a centrally raised hyaline patch and deep rectangular or circular groove. All scales with a short to long protuberance (0.3-4.0 µm x 0.3-1.3 µm) extending from rim of hyaline area, terminating in three to five short finger-like structures. Proximal surface with raised hyaline structure (corresponding to groove on distal side, and of similar shape)

possessing short projections directed towards hyaline area. Rim slightly curved towards proximal side, more prominent near hyaline area and along protuberance.

*Etymology:* *protuberans* (L.) = protuberant; in reference to the extension (protuberance) of the scale rim.

*Holotype:* Specimen seen in Plate 7, figure 6.

*Holotype material:* Cruise AMT18, station CTD 089-09, 96 m.

*Type locality:* 32.18 °S, 29.83 °W (South Atlantic). Collected 2/11/2008.

*Repository:* Filter, SEM stub and digital image are held in the Natural History Museum, London, U.K.

*Known biogeography:* Bahia de los Angeles (Gulf of California), Ionian and Adriatic Seas (Mediterranean) and South Atlantic.

*Remarks:* In the original diagnosis of *Petasaria heterolepis* (Moestrup 1979) no mention was made of forms with protuberances. Our specimens of *P. protuberans* show scales bearing a short (Pl. 7, fig. 5), intermediate (Pl. 7, fig. 6) or long (Pl. 7, figs. 7-8) protuberance. The distal groove and corresponding proximal raised structure is rectangular on those specimens with short to intermediate protuberances, whilst the groove/depression on specimens with longer protuberances is circular to oval, as in *P. heterolepis*. Here we have made no distinction between these two types of groove/depression and have assigned all pro-

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#### PLATE 4

*Hyalolithus neolepis* Yoshida et al.

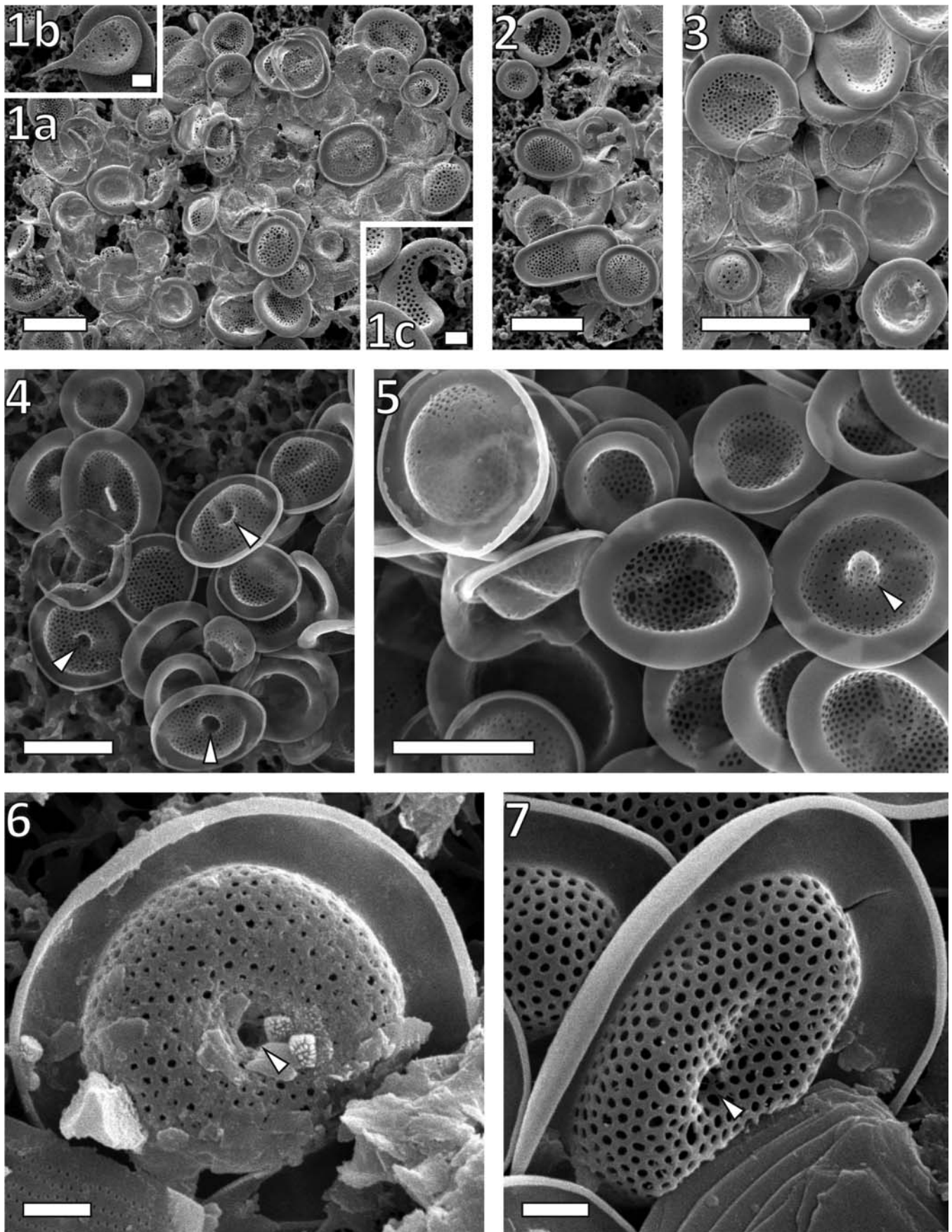
Figures 1-7 are SEM images. The scale bars are 5 µm (figs. 1a and 2-5) or 1 µm (figs. 1b, 1c, 6 and 7).

- 1-3 Tomari Port, Tottori, Japan (St.1, non-motile culture strain TMR-5). 1 Collapsed cell (a), with aberrant forms in insets (b, c). Note scale size and shape variability. 2, 3 Collapsed cells. Note presence of thinly silicified scales.
- 4,5 Tsugaru Strait (non-motile culture strain MM-4). Collapsed cells. Note scales with central depression (ar-

rowheads) in distal view (Fig. 4) and swollen structure (arrowhead) in proximal view (Fig. 5).

- 6,7 Seto Inland Sea (Seto-29). Scale in distal view, with central depression (arrowhead).





tubercle-bearing specimens to *P. protuberans*. Whether the difference is meaningful taxonomically is not known at present.

## CONCLUSIONS

In this study, specimens of *Hyalolithus* and *Petasaria* have been investigated in samples collected from all over the world. Although morphological differences exist in *H. neolepis* specimens, notably in the width of the hyaline area, there was insufficient evidence to create separate taxa. Nonetheless, this study revealed for the first time the presence of a swollen structure on the proximal surface of some scales, which corresponds to a depression on the distal side. Similarly, *P. heterolepis* scales exhibited high morphological diversity (e.g. perforation size, presence/absence of tubercle rows), but this was insufficient to form new taxa. However, forms bearing a short to long protuberance were deemed distinct enough to warrant their description as a new species, *P. protuberans*. Despite similarities between *Hyalolithus* and *Petasaria* it is still not known whether or not the latter is a *bona fide* haptophyte. This will only be confirmed if sectioned material of *Petasaria* reveals that its ‘haptonema’ is a true haptonema or its DNA is sequenced from cultured material.

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Marcus Geisen, Lisa Harding, Mette Hordnes, Susumu Konno, Norberto Pasten-Miranda, Shinichi Saito, Tomas Sorlie, Maiko Tanimoto, Isobel Thomas, and Tom Trull. Syed A. Jafar provided valuable assistance in recognition of *H. neolepis* and *P. heterolepis* from the southern Indian Ocean, while Lluís Cros and Jose-Manuel Fortuño helped to identify specimens in samples from the Central Portuguese margin. Rick van den Enden, Electron Microscope Unit, Australian Antarctic Division, is thanked for providing facilities and expert assistance. We are also grateful to those people who contributed to the discussion of this subject during two recent workshops; at COCCOLITHOPHORES 2014 (Crete) and at INA15 (Bohol). The constructive comments by M. Cachão and G. Hallegraeff during the review process are greatly appreciated. The research of MH was funded by the Norwegian Research Council project MicroPolar (NRC no. 225956/E10). RE would like to acknowledge the Southern Ocean Times Series, a facility of the Australian Integrated Marine Observing System, supported by ACE, CRC, CSIRO, BOM and the MNF; SOTS data are available via their portal [www.imos.org.au](http://www.imos.org.au).

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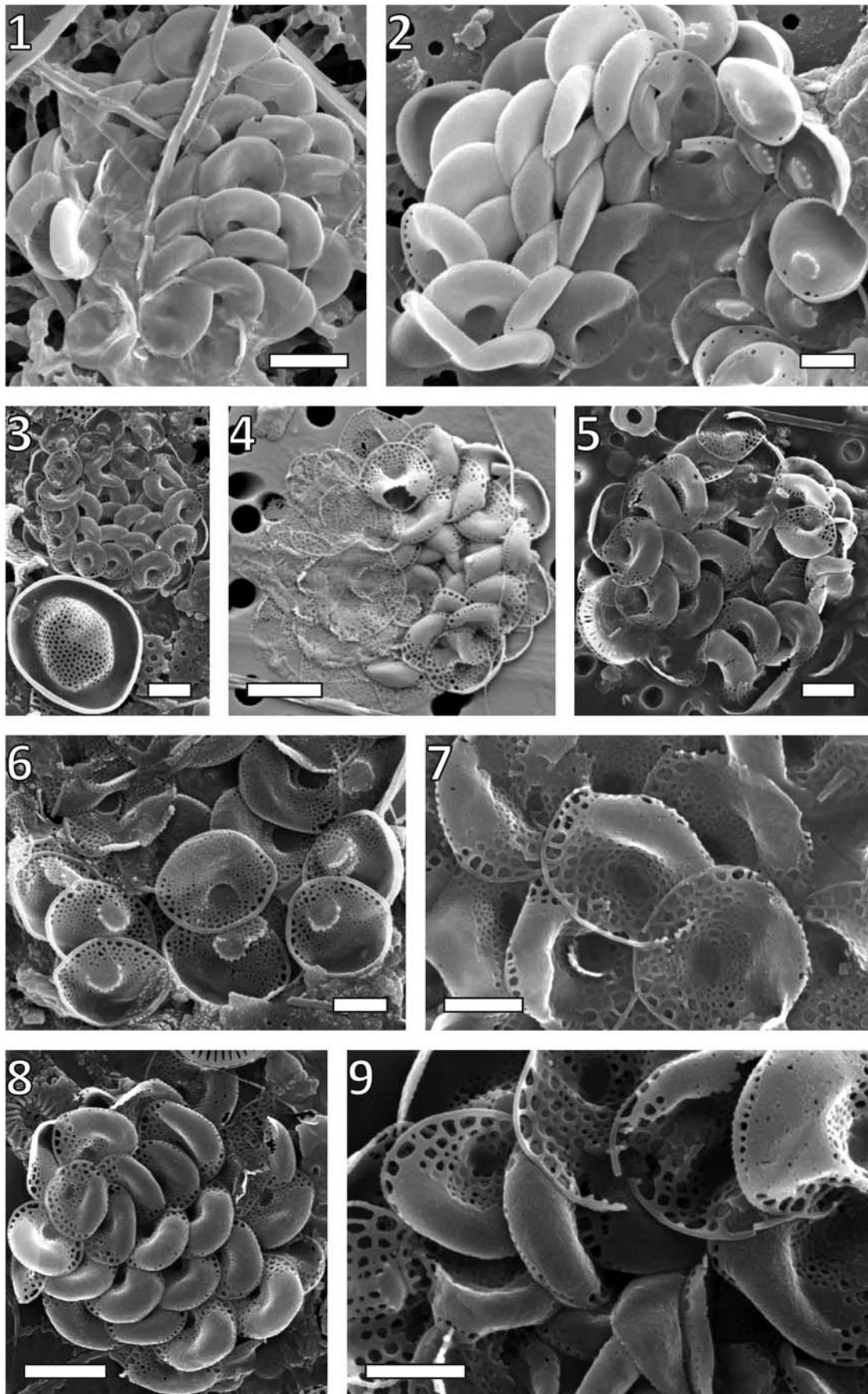
## PLATE 5

### *Petasaria heterolepis* Moestrup

Figures 1-9 are SEM images. The scale bars are 2µm (figs. 1, 3-5 and 8) or 1µm (figs. 2, 6, 7 and 9).

- |  |   |
|--|---|
| 1 Western Mediterranean (23). Collapsed cell. Scales with large hyaline area.  | 6 Seto Inland Sea (Seto-29). Scales in distal and proximal views.         |
| 2 Western Mediterranean (69). Collapsed cell. Scales with large hyaline area.  | 7 Bergen Fjord (21-10-2009). High magnification of scales in distal view. |
| 3 Seto Inland Sea (Seto-29). Collapsed cell. Note size difference between the <i>Petasaria</i> collapsed cell and individual scale of <i>Hyalolithus</i> . | 8 Bergen Fjord (8-9-2010). Collapsed cell.                                |
| 4 Oslo Fjord (OF2). Collapsed cell.  | 9 Bergen Fjord (3-11-2009). High magnification of scales in distal view.  |
| 5 Bergen Fjord (2-10-2012). Collapsed cell. Note scales with wide hyaline area and small openings.   |   |







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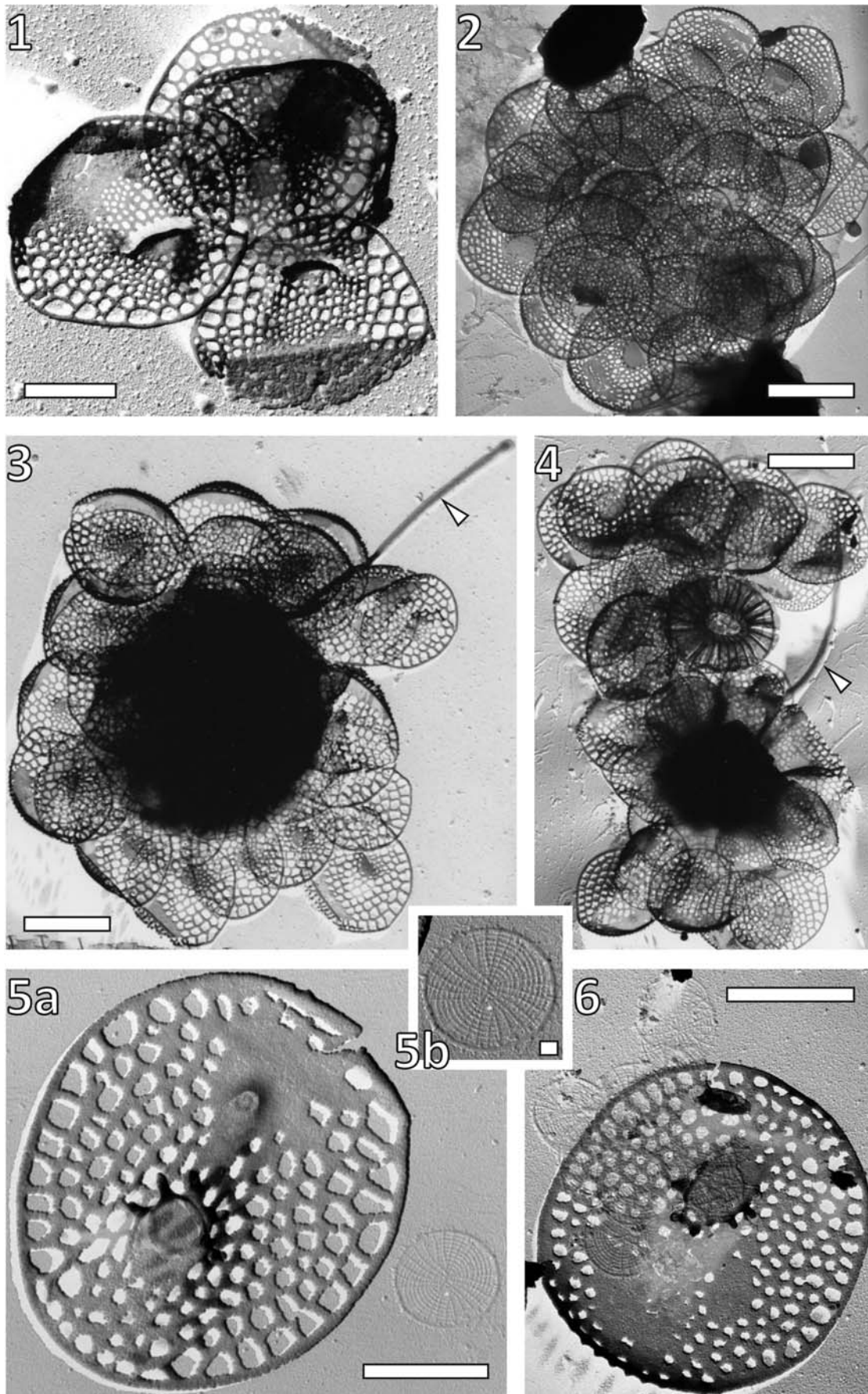
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## PLATE 6

### *Petasaria heterolepis* Moestrup

Figures 1-6 are TEM images. The scale bars are 2µm (figs. 2-4), 1µm (fig. 1), 0.5µm (figs. 5a and 6) or 0.1µm (fig. 5b).

- 1 Læsø Rende, Denmark (T1359). Scales with numerous small openings.
- 2 Off Point Sur, California (#1). Collapsed cell. Scale with numerous small openings.
- 3 Weddell Sea (#182). Collapsed cell, with haptonema (?) (arrowhead).
- 4 Weddell Sea (#142). Collapsed cell, with haptonema (?) (arrowhead).
- 5 Equatorial Pacific (#78). Scale in proximal view, associated with a ‘cobweb-like’ unmineralised scale (a). Same scale at slightly higher magnification and more contrast (b).
- 6 Bahia de los Angeles, Gulf of California. Scale in proximal view, associated with unmineralised scales.



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## PLATE 7

### *Petasaria heterolepis* Moestrup

Figures 1-4 are SEM images. The scale bars are 2µm (figs. 1 and 3-4) or 1µm (fig. 2).

- |  |  |
|--|--|
| 1-2 Sub-Antarctic Zone (SOTS). Collapsed cell. Scales with large openings. | 4 South Atlantic (CTD063). Collapsed cell. Note tubercle rows in hyaline area. |
| 3 Sub-Antarctic Zone (#8). Note tubercle rows in hyaline area.             |  |

### *Petasaria protuberans* Jordan et al. sp. nov.

Figures 5-7 are SEM images, fig. 8 is a TEM image. The scale bars are 2µm (figs. 5-7) or 0.5µm (fig. 8).

- |   |   |
|---|---|
| 5 Eastern Adriatic Sea (AD2). Collapsed cell. Note scales with short handle-like extension.               | 7 Ionian Sea (n12). Collapsed cell. Scales with pan handle-like extensions.                         |
| 6 South Atlantic (CTD089). Collapsed cell. Note scales with intermediate handle-like extension. Holotype. | 8 Bahia de los Angeles, Gulf of California. Scale in proximal view, with pan handle-like extension. |



