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Hyalolithus tumescens sp. nov., a siliceous scale-bearing haptophyte from the middle Eocene

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Abstract: Siliceous scales resembling those of the living haptophyte *Hyalolithus neolepis* Yoshida *et al.* were discovered in middle Eocene outcrop sediments from the Kellogg Shale in California, and Chalky Mount and Springfield in Barbados. Like *H. neolepis*, the fossil scales have a marginal rim, hyaline margin and numerous openings in the central area. However, they differ in the nature of the pit-like depression on the distal surface and the corresponding swelling on the proximal surface. Such swellings are very rare in *H. neolepis* and were not part of the original description. The presence of a swelling on all fossil scales found so far is sufficient enough to warrant the erection of *H. tumescens* Abe, Tsutsui & Jordan sp. nov. These findings represent the oldest known fossil record of the Prymnesiales.

Keywords: Eocene, Haptophyta, *Hyalolithus*, Prymnesiales, siliceous microfossils

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In seawater samples, most siliceous scaly organisms belong to the Chrysophyceae, Parmales, Prasinophyceae, Heliozoa, Thaumatomastigidae or testate amoebae. On the other hand, their calcareous counterparts belong almost exclusively to the haptophytes, which possess coccoliths and/or unmineralized organic scales. Thus, it was quite unexpected when a molecular genetic analysis and ultrastructural study revealed that *Hyalolithus neolepis* Yoshida *et al.* was a siliceous scaly haptophyte (Yoshida *et al.* 2006). The cell is covered by numerous overlapping siliceous scales; thin perforated plates with two central mounds and a marginal rim on the distal side (Yoshida *et al.* 2006; Patil *et al.* 2014; Jordan *et al.* 2016). The plates appear solid, not hollow like the skeletons of silicoflagellates (Fig. 1). The species appears to have a low morphological diversity in temperate-tropical waters of both hemispheres, being found in coastal and open ocean settings (Jordan *et al.* 2016). Genetic analysis of *H. neolepis* placed it among species of *Prymnesium* Massart, leading to its transfer to the latter genus (Edvardsen *et al.* 2011). However, the *Prymnesium* clade is morphologically heterogeneous and, at present, *H. neolepis* is the only member of the Prymnesiales that has siliceous scales in the non-motile phase – although silica has been detected in the outer scale covering of non-motile cysts of *Prymnesium parvum* (Pienaar 1980; Green *et al.* 1982), and scales of *P. polylepis* are known to contain silicate (Edvardsen *et al.* 2011). However, the siliceous material is deposited only on the distal side of the organic scale and is therefore not equivalent to the completely silicified scale of *Hyalolithus*. Thus, in this paper we have retained the original name, *Hyalolithus neolepis*. Another possible siliceous haptophyte is the enigmatic *Petasaria heterolepis* Moestrup (Moestrup 1979; Patil *et al.* 2015; Jordan *et al.* 2016), but concrete evidence, such as the possession of a haptonema or molecular sequences from cultures, is still lacking.

Since the siliceous scales of the enigmatic microfossil *Macrora* Hanna (possibly related to the filose amoeba *Pinaciophora* Greeff) are preserved in Palaeogene and Neogene marine sediments, it raises the question, could siliceous haptophyte scales have a similarly long fossil record? During routine surveys of outcrop and deep-sea drilling materials of Eocene age, siliceous scales resembling *Hyalolithus* were encountered. Herein the scales are compared with those of living *Hyalolithus* and the implications of this find are discussed.

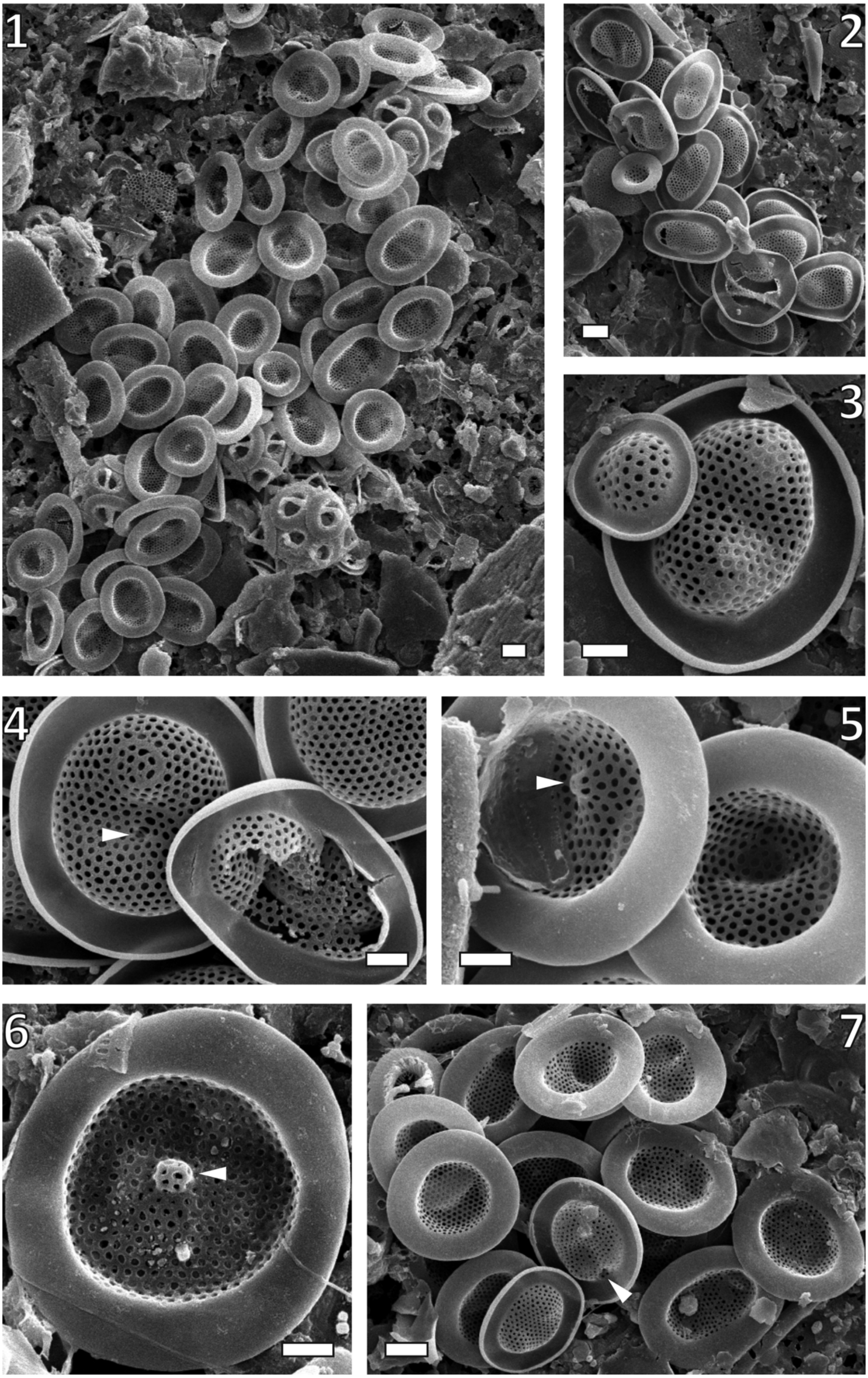
Material and methods

In this study a number of middle Eocene samples were investigated, but only those below yielded fossil *Hyalolithus*-like scales. Details on the collection and preparation of the Seto-29 sample, used in Figure 1, can be found in Abe *et al.* (2015).

- Chalky Mount, Barbados (Hustedt Diatom Collection access number AM22A) – provided by Friedel Hinz (previously at the Alfred-Wegener-Institute, Bremerhaven).
- Springfield, Barbados – provided by Frithjof Sterrenburg.
- Kellogg Shale, California (CD-11 and K5) – provided by John Barron (USGS, Menlo Park); for more details on these samples see Barron *et al.* (1984).

A small amount of raw sample was prepared as a suspension in distilled water, some of which was filtered on to a Millipore HA-type nitrocellulose filter (47 mm diameter, 0.45 µm porosity). The filter was then air-dried and stored in a plastic petrislide. A portion of the filter (6 × 6 mm) was cut out, mounted on to an aluminium scanning electron microscope (SEM) stub and coated with Pt/Pd in an Eiko IB-3 ion coater. The stubs were then observed in a JEOL JSM-6510LV SEM and digital images taken using the built-in camera system.

For light microscope (LM) permanent mounts, a portion of the suspension was pipetted on to a cover slip covered in distilled water and dried on a hot plate. Three drops of Mountmedia® (Wako Pure Chemical Industries, Ltd.; refractive index = 1.50) were pipetted on to a glass slide and the cover slip was then inverted and placed on top. The slide was then held over a hot plate until the mounting medium had stopped bubbling, after which it was removed from the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heat and left to cool down. At this time a label was added. Permanent mounts were observed on an Olympus BX40 with an oil immersion objective lens and phase contrast illumination at ×1000 magnification. Micrographs were taken with a Canon EOS Kiss X6i camera, attached to the microscope, with a type NY1S relay lens (Micronet Co., Ltd). Locations of the holotype and paratype were recorded with an England Finder (acquired from SPI Supplies, Structure Probe Inc., USA).

Systematic description

The new species is described below from the middle Eocene sediments of Barbados and California and compared with the type species, *Hyalolithus neolepis*, which has been described elsewhere (Yoshida *et al.* 2006; Patil *et al.* 2014; Jordan *et al.* 2016).

Class **Prymnesiophyceae** Hibberd, 1976
Subclass **Prymnesiophycidae** Cavalier-Smith, 1986
Order **Prymnesiales** Papenfuss, 1955
Family **Prymnesiaceae** O.C. Schmidt, 1931
Genus ***Hyalolithus*** Yoshida *et al.*, 2006

Type species *Hyalolithus neolepis* Yoshida *et al.*, 2006

Hyalolithus tumescens sp. nov.

(Figs 2–4)

Derivation of name. With reference to the swollen structure seen in proximal view; *tumescens* (L.) = swelling.
Diagnosis. A fossil species of *Hyalolithus* characterized by scales with an indentation on one side of the central mound in distal view and an off-centre swelling seen in proximal view.
Holotype. Distal view of scale (Fig. 2: 4). Slide deposited in the National Science Museum, Tokyo. England Finder Reference: R26/3.
Paratype. Distal view of scale (Fig. 2: 8). Slide (same as holotype) deposited in the National Science Museum, Tokyo. England Finder Reference: T24/3.
Material. 38 scales (21 in the SEM, 17 in the LM) from Barbados, 12 scales (7 in the SEM, 5 in the LM) from California. In total, 33 and 307 images were taken in the SEM and LM, respectively.
Type locality. Chalky Mount, Barbados.
Age. middle Eocene.
Description. Solid siliceous scales, circular to elliptical in shape. On distal side, scale with prominent marginal rim and perforated central mound, separated by narrow to wide hyaline area. Perforated area consists of about 30–150 circular to elliptical openings (pores) of variable size. One side of central mound with indentation and pit-like depression, always on long axis. On proximal side, perforated central area surrounded by hyaline margin. Swelling (corresponding to pit-like depression on distal side), perforated around the base, situated on long axis.
Dimensions. Scales 7.0–11.0 µm long, 5.5–9.4 µm wide. Openings 0.2–1.2 µm long. Swelling 0.9–2.1 µm in diameter.
Occurrence. Also found in samples from Springfield (Barbados) and Kellogg Shale (California) in this study.
Remarks. The siliceous scales of the type species *Hyalolithus neolepis*, like those of *H. tumescens* sp. nov., are solid, circular to elliptical in shape, with a prominent marginal rim and perforated central mound, separated by a narrow to wide hyaline area. However, the scales of *H. neolepis* are generally much smaller in size (2.9–7.9 µm long, 2.8–7.0 µm wide), with more (about 45–350) but smaller (0.1–0.3 µm long) openings. A small pit-like depression occurs rarely between the two central mounds on the distal side, with a corresponding centrally placed swelling (0.5–0.7 µm in diameter) on the proximal side. The swelling is perforated seemingly all over the surface, whereas that of *H. tumescens* is perforated only near the base. We chose the word ‘swelling’ because it is part of the perforated surface not a separate structure and is thus unlike the hollow imperforate tube as seen in the exothecal coccoliths of *Syracosphaera pulchra* Lohmann.

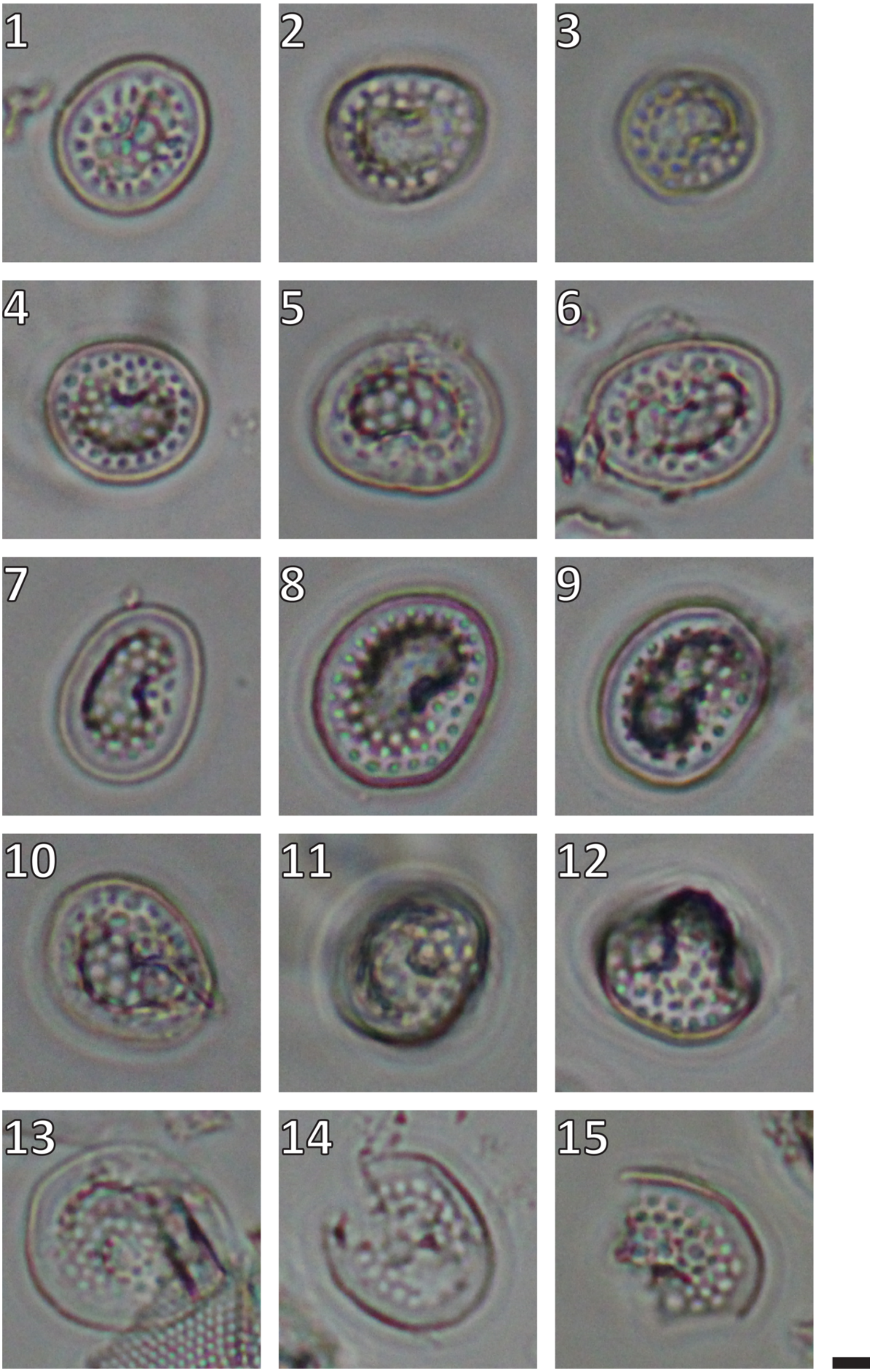
Discussion

The siliceous microfossils (notably radiolaria, diatoms and sponge spicules) in the Barbados deposits were studied over 150 years ago by Ehrenberg (1847*a*, *b*) and Greville (1861–66) and by many subsequent workers. The fossils were mostly attributed to a sedimentary layer called the Oceanic series (also Oceanic deposits or Oceanic beds), which was either calcareous, siliceous or a mixture of the two (Harrison & Jukes-Browne 1890). The radiolarian ooze-like siliceous sediments were thought to have been deposited in a deep basin, about 3600–7300 m deep, and were considered to be Pliocene or Pleistocene in age (Harrison & Jukes-Browne 1890; Jukes-Browne & Harrison 1892). Senn (1940) discussed the stratigraphic sequence and dated them using foraminifera, considering the ‘Oceanic Formation’ to be late Eocene to early Oligocene in age. An Eocene assignment was also given by Hanna & Brigger (1964) who studied the diatoms from Joe’s River, with Holmes & Brigger (1979) later refining the date to the Eocene–Oligocene boundary and giving an age of middle Eocene for the Conset and Cambridge localities based on radiolarians. Our own observations on some of the Barbados deposits (Chalky Mount, Springfield, Cambridge Estate) suggest that the assemblages belong to the Craspedodiscus oblongus Zone of the middle Eocene, since they contain *Craspedodiscus oblongus* (Greville) Grunow, *C. ellipticus* (Greville) Gombos and *Tubaformis unicornis* Gombos, characteristic middle Eocene diatoms (Gombos 1983; Fenner 1985). The absence or rarity of pennates in the samples suggests deposition in an offshore environment.

The siliceous microfossils of the Kellogg Shale in northern California have been studied since the pioneering works on radiolarians (Clark & Campbell 1942), diatoms (Kanaya 1957) and silicoflagellates (e.g. Mandra 1968). These studies quickly established a late Eocene age for the sediments, but this has since been refined to the middle Eocene (see discussion in Barron *et al.* 1984). Characteristic middle Eocene diatoms from the Kellogg Shale observed in this study include *Craspedodiscus ellipticus* and *Triceratium inconspicuum* Greville var. *trilobata* Fenner. These sediments were deposited offshore, not near the coast, as benthic diatom taxa (e.g. *Paralia* Heiberg) are scarce, while brackish and freshwater taxa (e.g. *Hantzschia* Grunow) may be modern contaminants (Barron *et al.* 1984).

The finding of *Hyalolithus tumescens* was timely and quite fortuitous, since at the time we were investigating the morphological diversity and distribution of *Hyalolithus* and *Petasaria* in living communities (Jordan *et al.* 2016) and also searching for *Macrora* species in Eocene samples. However, the discovery of fossil

Fig. 1. 1–7. *Hyalolithus neolepis* Yoshida *et al.* SEM. Seto-29, Seto Inland Sea, Japan: **1**, a collapsed cell revealing the variability in scale dimensions and shape, with most scales in proximal view; **2**, scales in distal view, with small circular scale, centre left; **3**, two scales in distal view, showing large difference in size; **4**, distal view of scale on left-hand side showing small depression (arrowhead); **5**, proximal view of scale on left-hand side showing small swelling, off centre (arrowhead); **6**, proximal view of scale showing a swelling, off centre (arrowhead); **7**, scales in distal and proximal views, with one in distal view showing off-centre indentation (arrowhead). Scale bars 1 µm (3–6) or 2 µm (1–2, 7).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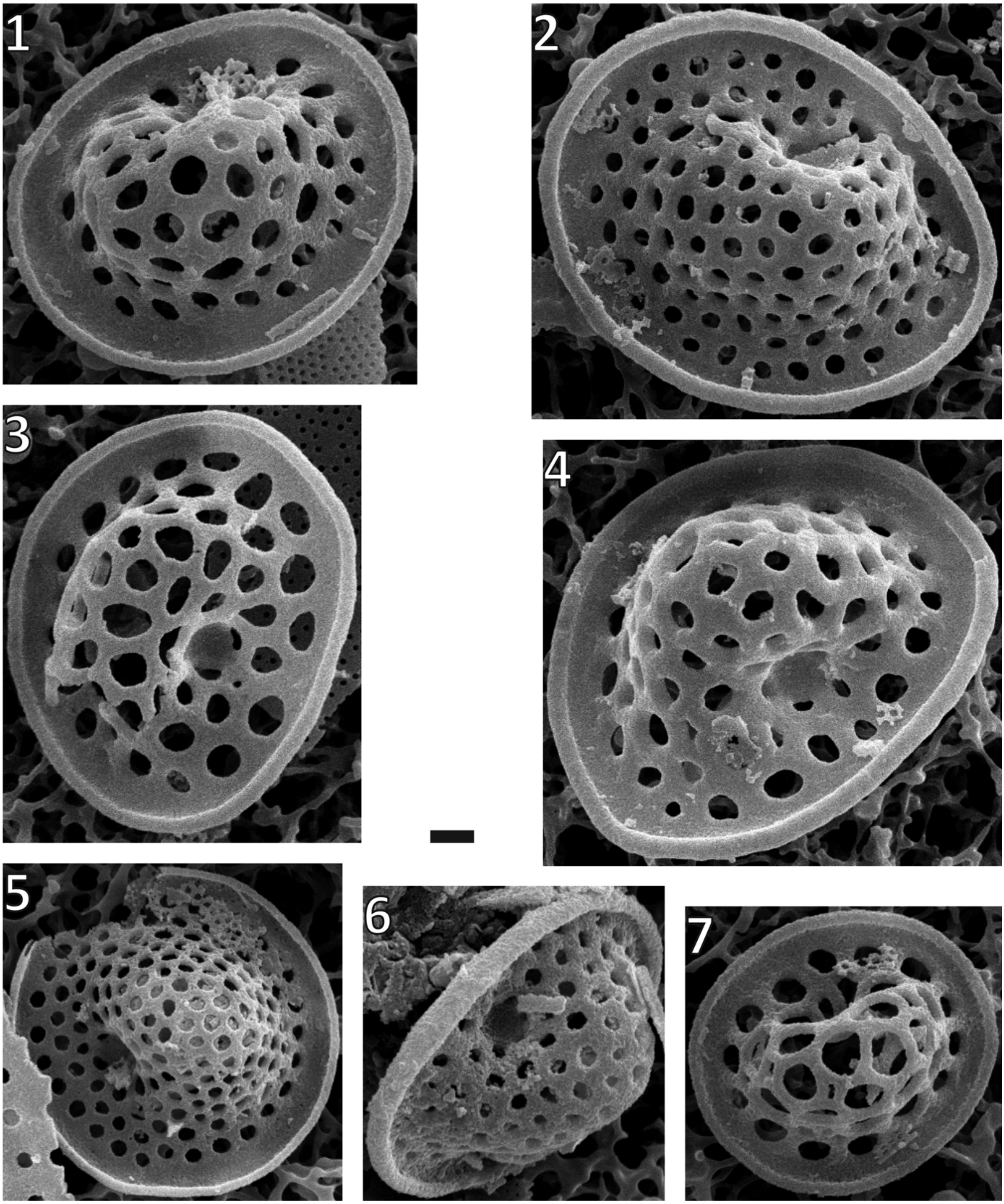
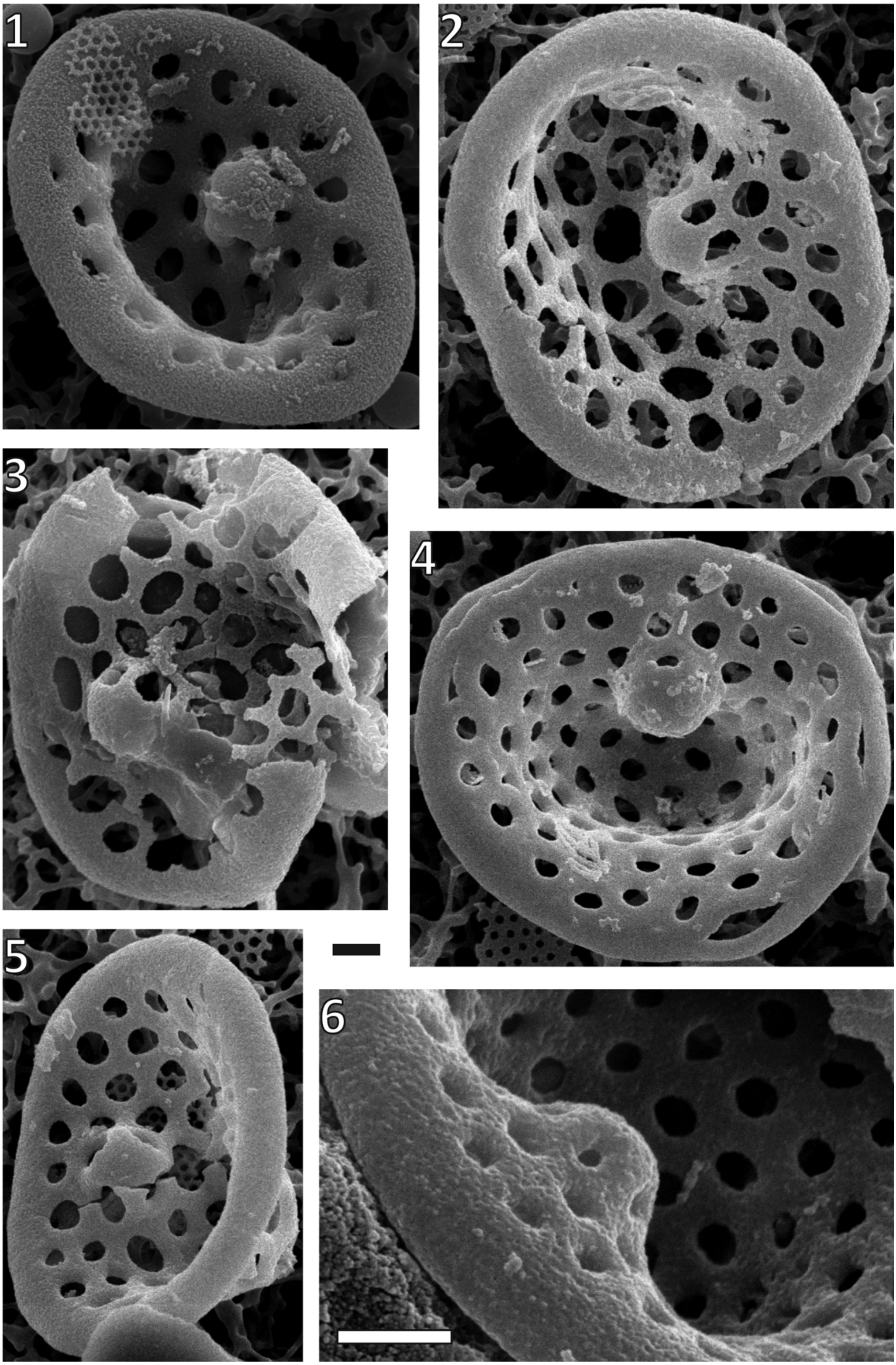


Fig. 3. 1–7. *Hyalolithus tumescens* sp. nov. SEM: **1–3, 7** (Chalky Mount, Barbados AM 22 A), **4–6** (Springfield, Barbados). 1–7, Distal view, showing indentation (1–2, 5, 7) and pit-like depression (3–4, 6) on one side of the central mound. Note variable size and number of openings, as well as the width of the marginal hyaline area. Scale bar 1 µm.

Fig. 2. 1–15. *Hyalolithus tumescens* sp. nov. LM: **1–12** (Chalky Mount, Barbados AM 22A); **13–15** (Kellogg Shale CD-11). Note nearly circular forms (1–4, 13) or elliptical forms (6–10), indentation on long axis side (4–10) and central mound (11–12). **4** is the holotype and **8** is the paratype. All scales in distal view. Scale bar 2 µm.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Hyalolithus scales raises several important questions: (1) why have they not been reported before? (2) Can we expect to find scales in younger or older deposits? (3) What conditions favour the preservation of *Hyalolithus* scales?

Given their small size, it is possible they were overlooked by previous workers using the light microscope, or simply ignored by those studying diatoms or misidentified as *Macrora*. In this study, *H. tumescens* was always found in co-existence with *Macrora barbadensis* (Deflandre) Bukry and *M. najae* Bukry, although *Macrora* is sometimes found in middle Eocene samples (e.g. Cambridge Estate, Barbados and Bet Guvrin Area, Israel) in which *Hyalolithus* is seemingly absent.

Preliminary investigations of Oligocene–Miocene sediments containing *Macrora stella* (Azpeitia) Hanna and *stella*-like forms have not yielded any *Hyalolithus*. However, one late Miocene age sample from Caltanissetta in Sicily did possess *Hyalolithus* scales and will be the subject of a separate paper. As for specimens in older sediments, it is quite possible that they exist; however, this would require a much more rigorous search of other samples using the SEM than has been carried out to date.

Neither *Macrora* nor *Hyalolithus* has been observed in middle Eocene sediments from temperate to polar regions (e.g. Norwegian Sea, Arctic Ocean or Southern Ocean), suggesting they are restricted to middle–low latitudes. The true affiliation of *Macrora* is still unknown, but our findings so far suggest it is not a haptophyte scale, but may have affinities with modern filose amoebae such as *Pinaciophora* Greeff. It is likely that further discoveries of fossil *Hyalolithus* will be rare, since tropical marine coastal sediments are poorly preserved and *Hyalolithus* never forms large populations in modern oceanographic settings.

Conclusions

The discovery of *Hyalolithus tumescens* sp. nov. in middle Eocene sediments has, for the first time, provided evidence of the fossil record of the Prymnesiales and opens up the possibility of finding other siliceous haptophyte scales in sediments of similar or different ages. Both species of *Hyalolithus* are associated with warm to temperate waters, so fossil scales may be found in tropical to temperate siliceous sediments, particularly those with good preservation. The presence of a swelling on the proximal side of the scale in both living and fossil *Hyalolithus* species suggests that this may be a common trait of the genus. Despite their overall similarity, the scales of the fossil specimens are sufficiently different from those of modern specimens to warrant their separation at the species level.

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Fig. 4. 1–6. *Hyalolithus tumescens* sp. nov. SEM: **1–2, 4–5** (Chalky Mount, Barbados AM 22 A); **3** (Kellogg Shale CD-11); **6** (Springfield, Barbados). Proximal view, showing off-centre swelling. Scale bars 1 µm (black scale bar for 1–5).