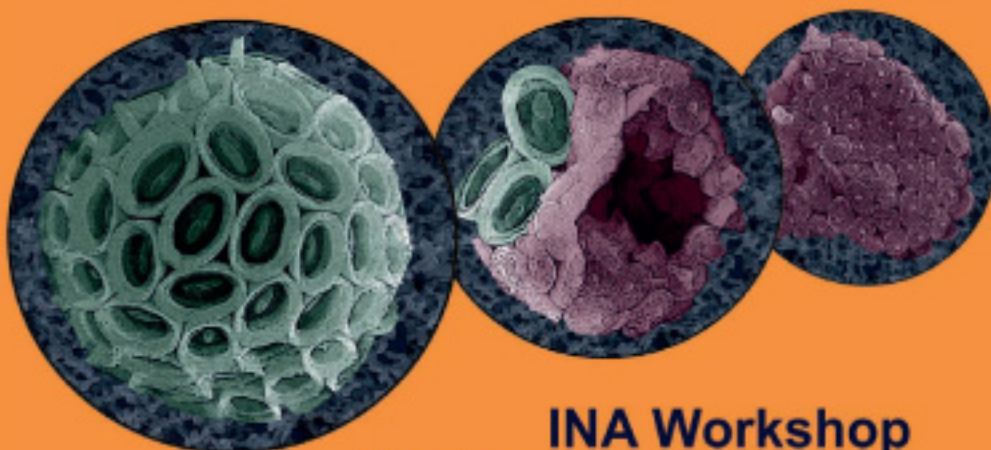


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# ***Gladiolithus adeyi* sp. nov.: a new deep photic coccolithophore species and new molecular genetic and crystallographic observations on *Gladiolithus flabellatus*.**

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**Abstract:** *Gladiolithus* is a distinctive and unusual genus of deep photic coccolithophores which has recently been shown to have a fossil record extending back to the mid Paleocene (ca 60Ma). We describe here a new species of *Gladiolithus* with long narrow tube coccoliths and demonstrate its affinity to other *Gladiolithus* species through detailed morphological and crystallographic comparison including new observations on *G. flabellatus*. We also present new molecular genetic data which suggests that *Gladiolithus* is deeply diverged from other coccolithophores and likely originated from different Mesozoic ancestors.

**Keywords:** *Gladiolithus*, coccolithophores, molecular phylogeny, deep photic

## **1. Introduction**

One of the interesting aspects of coccolithophore ecology is the existence of a distinctive deep photic assemblage occurring in oligotrophic waters beneath the deep chlorophyll maximum, in the lower part of the thermocline and below (Winter et al. 1994, Young 1994). The most characteristic species of this assemblage are *Florisphaera profunda* and *Gladiolithus flabellatus*, but a diverse range of rare taxa also occur. These include one other species of *Gladiolithus*, *G. striatus*, described by Hagino & Okada (1998) and sporadically recorded since: we have observed it in deep photic samples from the South Atlantic, the Indian Ocean and North Pacific and in Mediterranean sediments. *Gladiolithus* is less common in Quaternary sediments than *Florisphaera* but has been discovered in high abundances in the Paleogene of Tanzania as described in Bown et al. (2008, 2009). These fossil assemblages contain abundant specimens indistinguishable from modern *G. flabellatus* but also distinctly different morphotypes and three further species have been described - *G. contus*, *G. ornatus* and *G. brevis*.

The morphology of *Gladiolithus flabellatus* and *G. striatus* is illustrated here in Plate 1 and has been described in detail by Halldal & Markali (1955), Jordan & Chamberlain (1992), and Hagino & Okada (1998). All described *Gladiolithus* species are characterised by tube coccoliths formed of six elongate calcareous plates forming an approximately hexagonal tube with one end closed. Modern coccospheres of *G. flabellatus* and *G. striatus* are dimorphic also including simple disc-like coccoliths termed lepidoliths (Plate 1 Figs. E-H). Complete coccospheres are bowl-shaped, partially enclosing the cell, with a rather large opening. The lepidoliths form the base of the bowl and the tube coccoliths the rim. Collapsed coccospheres can, however, appear rather different. The tube coccoliths of both *G. flabellatus* and *G. striatus* are curved lengthwise. In *G. striatus* the inner concave surface

is smooth whilst the outer convex surface is ornamented with striations. In *G. flabellatus* ornamentation is more variable although pitting and tooth-like projections from the sutures, occur in some specimens and tend to be more strongly developed on the outer surfaces. The lepidoliths of *G. striatus* also show striations on their outer surface, whilst those of *G. flabellatus* are smooth. In both species, the lepidoliths are formed of two elements joined along a medial suture.

A research student, Tim Adey (NOC), encountered an unidentified coccolithophore in deep photic zone samples from the South Atlantic, characterised by long, spine-like coccoliths (plates 2, 3). We have now obtained high-resolution scanning electron microscope (SEM) images of a few specimens of this species and these indicate that it is a new species of *Gladiolithus*, which we name here *G. adeyi*. This discovery prompted further comparative studies of *Gladiolithus* including molecular genetic investigation of its affinities and new observations on the crystallography of the coccoliths. These results are also reported here.

## **2. Material and methods**

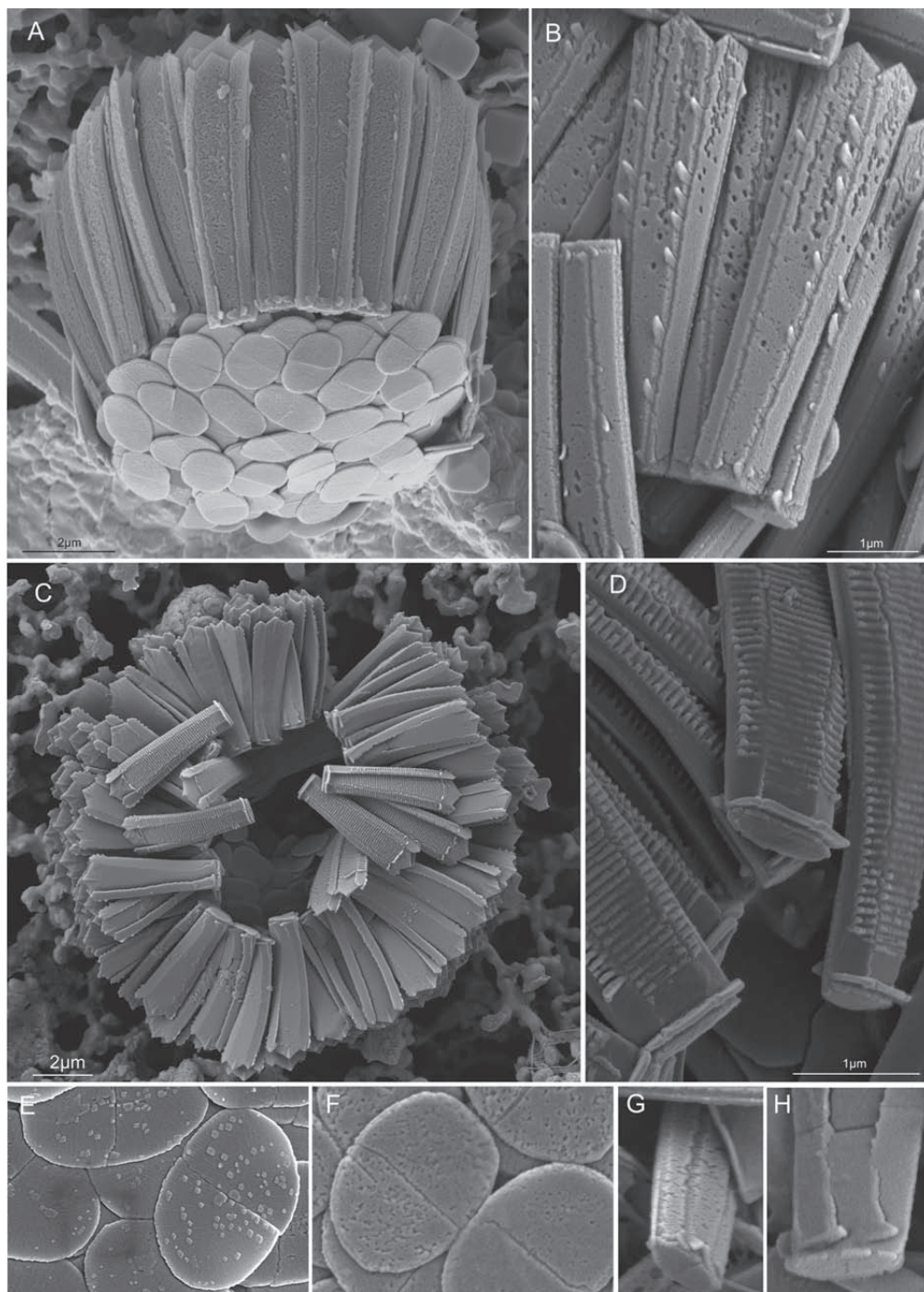
### **2.1 Plankton sampling**

We have found specimens of *G. adeyi* sp. nov. in two samples from the South Atlantic gyre deep-photoc zone, collected during Atlantic Meridional Transect cruises; respectively samples AMT14 CTD 26 120m (11.73°S, 25°W, 8/5/2004) and AMT18 CTD 75 165m. (19.12°S, 25.00°W, 29/10/2008). Two specimens were imaged at high resolution from the AMT14 sample and one from the AMT18 sample. Additional specimens have been seen in these samples in low-resolution SEM images and in the light microscope. Also, one specimen has been illustrated in the literature, as *Thorosphaera* sp. L, by Reid (1980) from the North Pacific gyre (Cruise Tasaday 11, station



# Plate 1

*Gladiolithus flabellatus* and *G. striatus*.



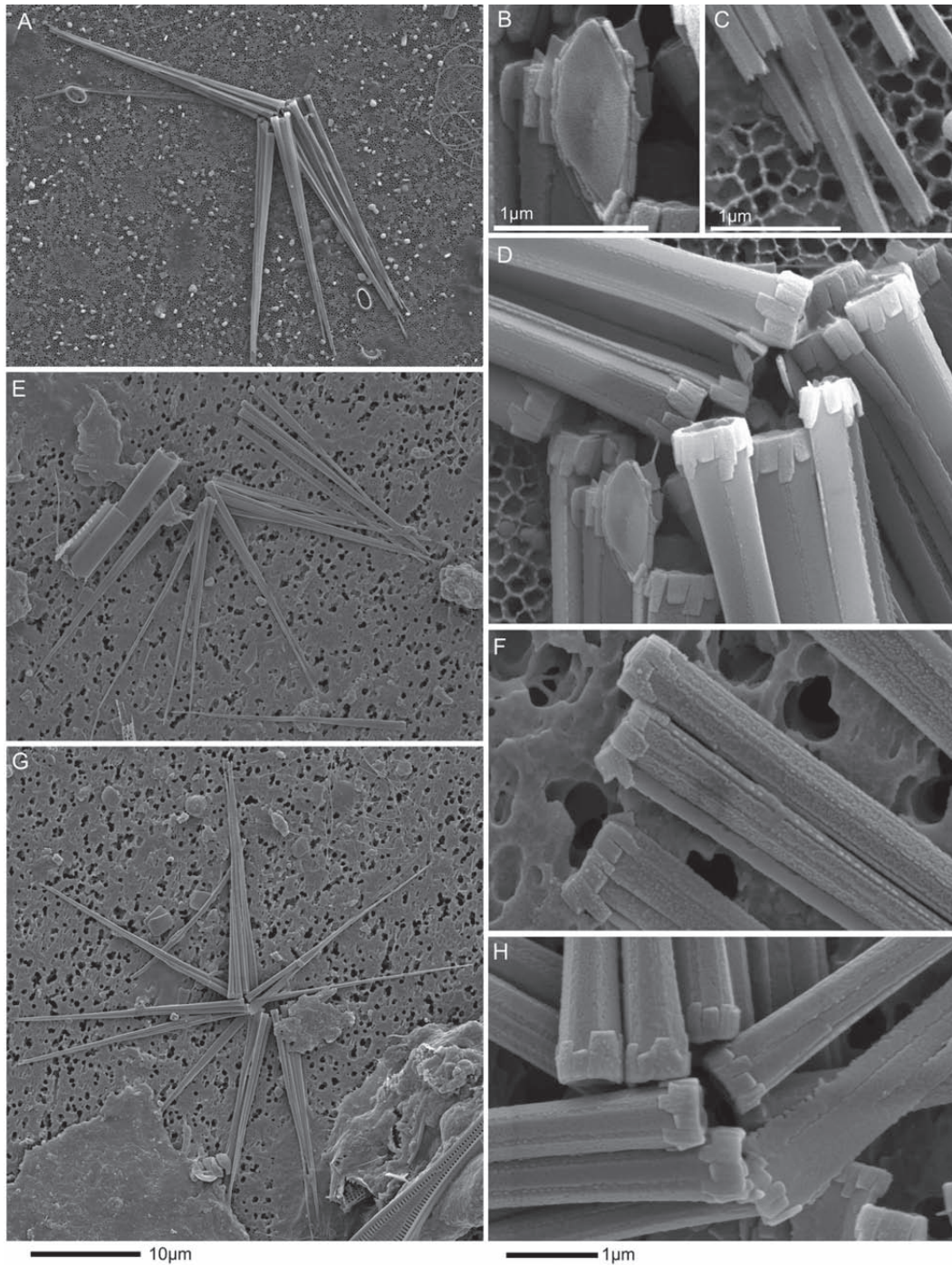
- A. *G. flabellatus*, complete specimen showing form of intact coccosphere. Image NHM219-27, HOTS 169 cruise, Hawaii May 2005.  
 B. *G. flabellatus*, group of tube coccoliths. Image NHM310-076, KH90-3 cruise, N. Pacific.  
 C. *G. striatus*, collapsed coccosphere, note that only outer surfaces of tube coccoliths show striations. Image NHM310-084, KH90-3 cruise, N. Pacific.  
 D. *G. striatus*, group of tube coccoliths. Image NHM310-082, KH90-3 cruise, N. Pacific.

- E. *G. flabellatus* lepidoliths proximal view showing baseplate scales. Image NHM304-052, AMT 18 cruise, CTD89, 108m, S. Atlantic.  
 F. *G. flabellatus* lepidoliths in distal view. Image NHM223-02, HOTS 169 cruise, Hawaii May 2005  
 G. *G. flabellatus* base of tube coccolith. Image NHM310-076, KH90-3 cruise, N. Pacific.  
 H. *G. striatus* base of tube coccolith. Image NHM310-081, KH90-3 cruise, N. Pacific.

NB The 5µm scale bar at the base of the plate indicates the scale of figs E to H.

## Plate 2

*Gladiolithus adeyi* sp. nov.



A-D Holotype specimen, from cruise AMT18, CTD station 075, 165m.

A. complete specimen, NHM322-08; B. the single lepidolith visible, proximal view with baseplate scale visible, NHM322-09; C. detail of tips of tube coccoliths, NHM322-07; D. detail of bases of tube coccoliths, NHM322-09.

E-F Specimen from cruise AMT14 CTD station 26 120m. E. complete specimen, NHM225-19; F. detail of bases of tube-coccoliths, NHM225-20.

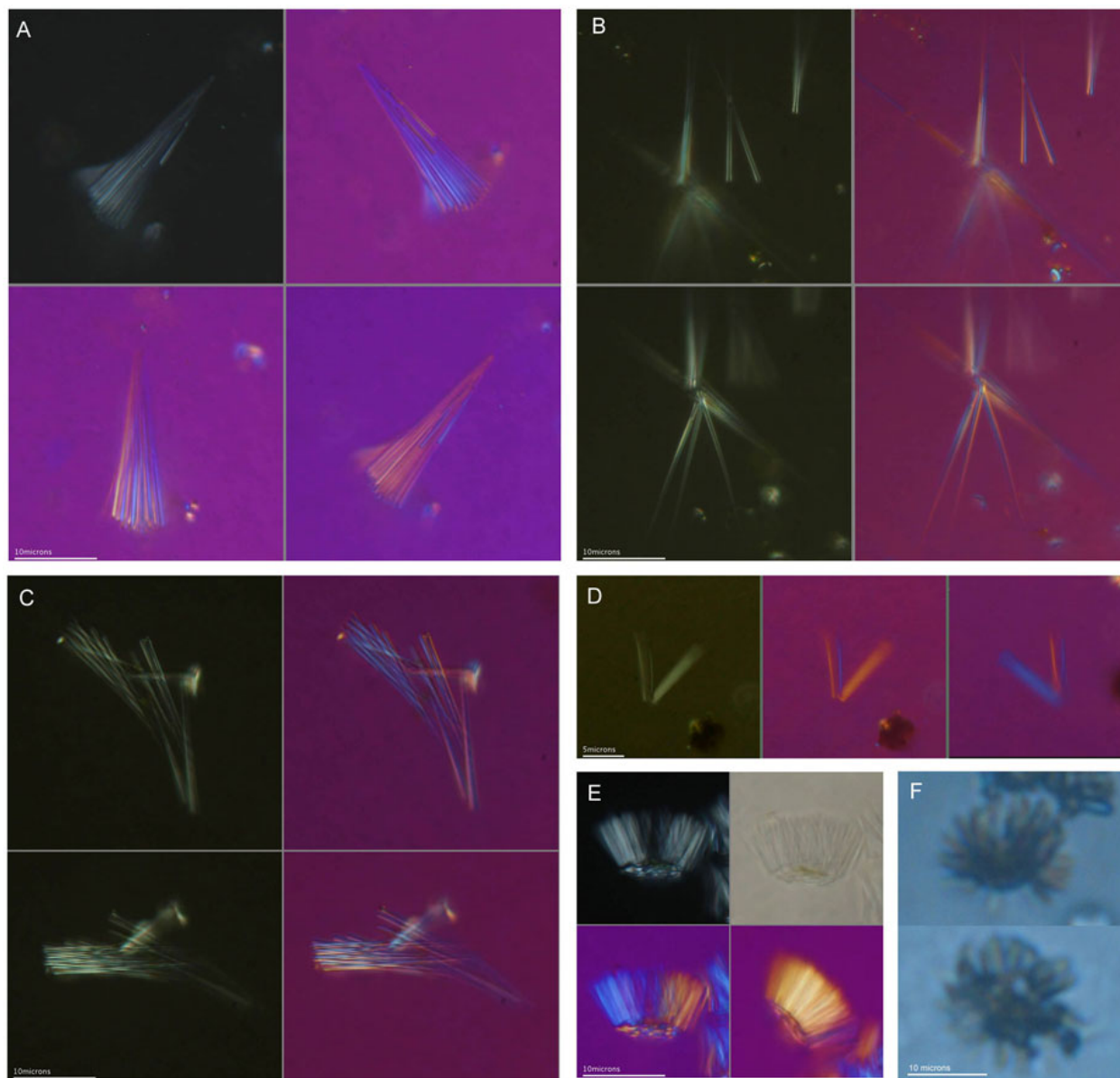
G-H Second specimen from same sample as E-F. G. complete specimen, NHM225-09; H. detail of bases of tube-coccoliths, NHM225-10.

NB The 10µm and 1µm scale bars at the bottom of the plate indicate the magnifications of images A, E & G and D, F & H respectively.



## Plate 3

Light micrographs



A-C *Gladiololithus adeyi* coccospheres in cross polars and cross polars with 1λ gypsum plate.

D. Two large isolated tube coccoliths of *G. flabellatus*.

E. *G. flabellatus* coccosphere. Chloroplasts are visible in the bright field image. Lepidoliths appear to be visible at the base of the coccosphere.

F. Light micrographs of the two *Gladiololithus* specimens from which DNA sequences were collected. Top VFsp24, bottom VFsp26. Both collected at Villefranche sur mer.

All specimens except F from sample AMT 18 CTD075 165m.

1, 28°N, 150°W, 140–160m, March 1974). Comparative material studied for other *Gladiolithus* species included a further sample from cruise AMT 18 (CTD89, 108m, 28.9°S, 26.0°W, 1st Nov 2008); two samples from Hawaiian Ocean Time Series Cruise HOT-169, May 2005 (both samples from station 2, 22.75°N, 158.0°E); one sample from cruise KH90-3 of R/V Hakuho-Maru (Station AQ11, Nov. 1990, 4.0°S, 179.0°E, 173m, type material of *G. striatus*). Samples were collected from CTD bottles, concentrated by vacuum filtration onto membrane filters and examined by electron microscopy at the Natural History Museum London. For light microscopy portions of cellulose acetate filters were mounted using Norland Optical Adhesive 74 and examined in cross polarised light.

## 2.2 Molecular genetic analysis

10L of subsurface seawater sample (100 m) were collected for floristic and molecular studies of coccolithophores using a Niskin bottle equipped to the boat *La Sagitta* of the Villefranche-sur-mer Oceanological Observatory (France) from Bay of Villefranche (43°41'N, 7°18'E) on 17th September 2007. The sample was concentrated using a 1 µm mesh-size plankton net. A total of 10 *Gladiolithus* cells (VFsp- 23–26 and 31–36) were isolated using a micropipette under an inverted LM, and then subjected to single cell PCR amplification following Takano and Horiguchi (2005). The primers used for PCR amplification, PCR conditions, procedure for sequence determination and the sequencers, were the same as those described in Hagino et al. (2009). The results were confirmed by sequencing both forward and reverse strands. Partial 18S rDNA sequences were successfully obtained from two specimens, VFsp24 and VFsp26 (Plate 3F), independently from each other.

For the phylogenetic analysis, a total of 40 18S rRNA gene sequences of the Division Haptophyta, including a sequence of the Pavlovophyceae as an out-group, were obtained from GenBank. Since 18S rDNA sequences of the specimens VFsp24 (GenBank Accession number AB983344, 1660-bp) and VFsp26 (GenBank Accession number AB983345, 1693-bp) were identical to each other in the region in which they overlapped (1660-bp), we used the sequence of VFsp24 as representative of them. The sequences were aligned together with the sequences obtained in this study using Clustal W (<http://www.genome.jp/tools/clustalw/>). Phylogenetic trees were constructed based on the Maximum Likelihood (ML) method and Bayesian inference (BI) using PAUP version 4.0b10 (Swofford, 2002) and Mr. Bayes v3.1.2 (Ronquist and Huelsenbeck, 2003), respectively.

For ML analysis, a likelihood score ( $-\ln L = 8997.8809$ ) was obtained under the GTR+I+G model with the following parameters: assumed nucleotide frequencies A = 0.2395, C = 0.2194, G = 0.2801, and T = 0.2611; substitution-rate AC = 1.0977, AG = 1.4576, AT = 0.6624, CG = 0.9323, CT = 4.4195, GT = 1; proportion of sites

assumed to be invariable = 0.6211; and rates for variable sites assumed to follow a gamma distribution with shape parameter = 0.5222, were estimated by Modeltest 3.7. The ML analysis was performed using the heuristic search option with a branch swapping algorithm Tree bisection-reconnection (TBR) with the NJ tree as a starting tree. Bootstrap analyses with 1000 replicates for the ML analysis were applied to examine the robustness and statistical reliability of the topologies (Felsenstein, 1985). For the BI analysis, the GTR+I+G model was selected by the MrModeltest. Markov chain Monte Carlo iterations were carried out to 5.5 million generations, when the average standard deviations of split frequencies were below 0.01, indicating convergence of the iterations.

The rest of the concentrated seawater sample used for the isolation of cells was filtered onto a Millipore HAWP04700 membrane. Specimens of *Gladiolithus* were identified under the SEM in order to confirm its presence and determine the species - *Gladiolithus flabellatus* was present but *G. striatus* and *G. adeyi* were not seen. Since it is not possible to distinguish *G. flabellatus* and *G. striatus* in the LM the sequences have been deposited in GENBANK as *Gladiolithus* sp., however, it is highly likely they come from *Gladiolithus flabellatus*.

## 3. Systematic taxonomy

*Gladiolithus adeyi* sp. nov. Young, Hagino & Poulton

**Synonymy:** *Thorosphaera* sp. L, Reid (1980) plate 9 figs. 3, 4, p. 170

**Diagnosis:** A species of *Gladiolithus* with long narrow tube-coccoliths tapering continuously from the base to the tip. Lepidoliths are formed of a single calcite plate with a narrow rim.

**Derivation of name:** In honour of Tim Adey, biological oceanographer, who first observed the species.

**Type specimen:** Specimens illustrated in SEMs NHM 322-05 to 322-09.

**Type material:** Plankton filter, collected at 06.35am on 29/10/2008, from 165m water-depth, at 19.12°S, 25.00°W, from CTD station 75 of cruise AMT18.

**Type repository:** Palaeontology Department, The Natural History Museum, London.

## 4. Results

### 4.1 Description of *G. adeyi*

**Coccosphere.** The four coccospheres observed in the SEM are all similar, consisting of 15 to 22 very long coccoliths (~20 µm) apparently radiating from a small central body. The specimen illustrated by Reid (1980) also has several lepidoliths - i.e. flat body coccoliths. Only one lepidolith is visible on the specimens we have imaged. It is possible that all the imaged specimens are incomplete and that the similarity in coccolith numbers on the specimens observed to date is a coincidence. Likewise the way the tube coccoliths radiate from a small central body may prove to be misleading since these are clearly collapsed

	225-9	225-19	322-06	Reid 1980
Number of tube coccoliths	22	17	17	17
Length of tube coccoliths	20.8-22.4 $\mu$ m	20.1-22.1 $\mu$ m	21.5-23.8 $\mu$ m	18.5-21 $\mu$ m
Basal width of tube coccoliths	0.7-0.8 $\mu$ m	0.8-0.9 $\mu$ m	0.7-0.8 $\mu$ m	0.7-0.9 $\mu$ m
Height of basal elements	0.34-0.47 $\mu$ m	0.33-0.52 $\mu$ m	0.36-0.55 $\mu$ m	/
Angle of inclination of basal elements	75-82°	78-83°	77-83°	/
Lepidolith length	/	/	1.2 $\mu$ m	1.1-1.2 $\mu$ m

**Table** Dimensions of observed *G. adnyi* specimens, including the one imaged by Reid 1980.

coccospheres. Most specimens seen in the light microscope were isolated tube coccoliths or radial scatters with a few coccoliths (e.g. Plate 3, Fig. B). Two coccospheres were seen, however, with all the tube-coccoliths in a cone-shaped mass (Plate 3 figs. A and C) and, by analogy with *G. flabellatus*, these may be closer to the original coccosphere form, but neither lepidoliths nor chloroplasts could be seen.

**Tube coccoliths.** These are 19-22 $\mu$ m long (see Table) tapering uniformly from a base 0.7-0.9 $\mu$ m wide to a fine point. They are hollow tubes with a flattened hexagonal cross-section formed of six laths, running the entire length of the coccolith. The sutures between the laths appear to be slightly irregular giving a beaded appearance. At the base of the coccoliths there is a low cycle of additional elements, these are clockwise imbricate, sloping at ca 80° to the base and consist of pairs of alternating short and long elements joined at the base but with a gap between them for most of their length. Each joined pair of basal elements overlies the suture between a pair of elongate laths. The base of the coccoliths appears to be covered by a calcareous plate, but it is not perfectly imaged in any of our specimens. At the apex of the coccoliths the tube is only ca. 0.2 $\mu$ m wide and all six laths are terminated at the same level leaving a small opening with minute spines extending from the sutures.

**Lepidoliths.** The specimen imaged by Reid (1980) has at least seven elliptical disk-shaped coccoliths 1.1 to 1.2  $\mu$ m long, most of which have a low rim suggesting that they are seen in distal view. We agree with her in identifying these as lepidoliths. We observed one similar coccolith (Plate 2 Fig. B). This is flattened hexagonal in shape, 1.2 $\mu$ m long and 0.5  $\mu$ m wide. It has an ornamentation of fine curved, radiating, ridges - this is almost certainly an organic baseplate scale with radiating microfibrils. Around the edge of the coccolith is a narrow rim cycle, this appears to surround a single plate, with no sign of a median suture as seen in the lepidoliths of *G. flabellatus* and *G. striatus*.

## 4.2 Crystallographic orientation of *Gladiolithus* coccoliths

The crystallographic orientation of the calcite crystals

which form coccoliths has been shown to be a conservative feature of much value for classification (e.g. Young et al. 1991, 1998). Hence we would expect all *Gladiolithus* coccoliths to have similar crystallographic orientation. There are few detailed crystallographic observations on *G. flabellatus* so we re-examined it for this comparison. On isolated *G. flabellatus* tube coccoliths (e.g. Plate 3 Fig F) the central lath is bright when the coccolith is at 45° and use of a 1 $\lambda$  plate indicates that the c-axis is parallel with the coccolith length. When the coccolith is parallel with one of the polarization directions the central lath is dark but the side laths are strongly birefringent and with a 1 $\lambda$  plate they show opposite colours (one is blue the other yellow), this is due to the c-axes being inclined about 45° from the vertical (see diagram). The lepidoliths of *G. flabellatus* can sometimes be clearly seen on complete coccospheres in side view (e.g. Plate 3 Fig E) and it is then clear that they are formed of crystal units with sub-vertical c-axes, this also means they are difficult to observe in plan view.

Observations on *G. adnyi* tube-coccoliths are slightly trickier since they are narrower and the birefringence of the side laths often dominates over that of the central lath but they show the same c-axis orientations (Plate 3 Figs A-C). Interestingly whilst isolated coccoliths typically show two parts with contrasting orientations (e.g. Plate 3 Figs. B, C), when a mass of coccoliths are aligned then a single birefringence colour dominates (Plate 3 Fig A). We have not been able to clearly identify any lepidoliths of *G. adnyi* in the light microscope.

## 4.3 Baseplate scales in *Gladiolithus*

Organic baseplate scales have not previously been described from *Gladiolithus*. Our observation of such a scale on the lepidolith of *G. adnyi* prompted us to look for examples in *G. flabellatus* and one specimen with apparent baseplate scales was found (Plate 1 Fig.E). On this specimen some lepidoliths do possess fine ridges resembling a baseplate scale, though others do not. Those with possible scales also show different curvature of the median suture to those without. This curvature of the suture is a general feature of *G. flabellatus* lepidoliths and can be used to distinguish the distal (or outer) and prox-



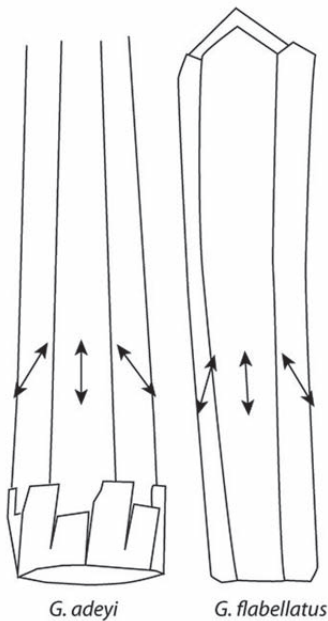


Figure 1 Drawings of *G. adehy* and *G. flabellatus* tube coccoliths. Arrows indicate the c-axis orientations as inferred from the observations with the  $\lambda$  plate.

the coccolithophore clade. This suggests that it is a true coccolithophore, but possibly only distantly related to any of the extant groups. It should be noted, however, that

imal (or inner) surface of the lepidolith. The putative scales appear on the proximal surface as would be expected for base-plate scales, so we conclude that these almost certainly are organic baseplates scales.

#### 4.4 Phylogenetic position of *Gladiolithus*

As described in the methods section partial 18S DNA sequences were obtained from two *Gladiolithus* cells collected from Villefranche-sur-mer. Comparison of these with other haptophyte sequences allows derivation of the maximum likelihood tree (Fig. 2). In this tree *Gladiolithus* falls at the base of

bootstrap values in this part of the tree are low so there is low confidence in the precise topology and indicated relationships.

#### 5. Discussion

The combination of six-sided tube coccoliths and simple body coccoliths, lepidoliths, allows us to place the new species in *Gladiolithus* with some certainty. This placement is confirmed by the identical crystallographic orientation of the tube coccoliths. It is also evidently a deep photic species like the other described extant species and the beaded sutures of the tube coccoliths is reminiscent of the ornamentation seen on *G. flabellatus*. The very elongate shape of the coccoliths is reminiscent of the fossil species *G. contus*, however in the current species the coccoliths taper uniformly from the base to the tip whilst in *G. contus* they appear to taper toward both ends. The coccoliths are clearly different to any of the other described species. So this is a new species of *Gladiolithus*.

*G. adehy* does, however, differ significantly from the other extant species. The tube-coccoliths are much longer and do not show any curvature, or differentiation of an inner smooth concave face and outer ornamented convex face. Also, the basal cycle is distinctive: there are basal elements in *G. striatus*, as described by Hagino & Okada (1998) and shown in Plate 1 Figs D and H, but they are not obviously similar, and regular basal elements are not

evident in *G. flabellatus* although small irregular elements are sometimes seen (Plate 1 figs. B and G). Finally the lepidoliths of *G. adehy* have a rim of small elements (Plate 2 fig. B), which are not present on *G. flabellatus* or *G. striatus* lepidoliths. Actually, the *G. adehy* lepidoliths resemble the baseplates of *G. flabellatus* and *G. striatus* coccoliths (compare Plate 1 figs G, H and Plate 2 fig. B). This range of rather strong differences suggests that *G. adehy* is well-separated from the other species, and possibly

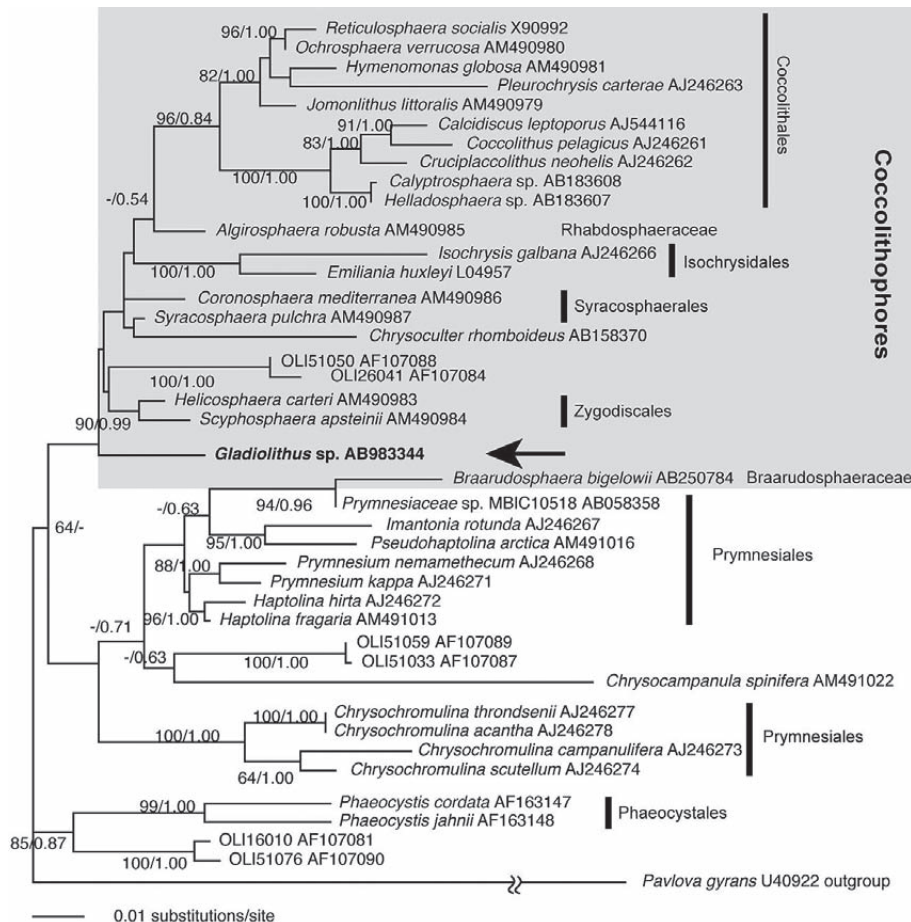


Figure 2 Maximum likelihood tree indicating possible phylogenetic relationships between the sampled haptophyte species based on the 18S ribosomal DNA gene. The numbers on each node indicate the bootstrap values from ML analysis and posterior probability of BI analysis. Higher values indicate that the node is better supported.



the differences between the extant species *G. flabelatus*, *G. striatus* and *G. adeyi* reflect the longevity of the genus, which we now know extends back to the Paleocene (Bown et al., 2009).

Our molecular phylogenetic results place the origin of *Gladiolithus* in the basal divergence of the coccolithophore clade, and this divergence is generally interpreted as corresponding to the Early Jurassic radiation of coccolithophores (e.g. Saez et al. 2004, Medlin et al. 2007). On this basis it is perhaps more likely that *Gladiolithus* had an independent origin in the Mesozoic than that it is closely related to any of the well-established Cenozoic families. This is further supported by our detailed crystallographic and structural observations, which do not suggest any extant coccolithophores as likely close ancestors. This is a rather surprising result but is supported by the recent observations of abundant *Gladiolithus* specimens in exceptionally preserved Paleogene sediments from Tanzania (Bown et al. 2009). The results suggest that it will be well worth studying the origins of *Gladiolithus* in well-preserved Palaeocene sections and attempting to isolate it into culture.

### Acknowledgements

We are grateful to fellow scientists and crew of the James Clark Ross for assistance in sampling during the Atlantic Meridional Transect cruises, and especially to Tim Adey for his initial observations. The sampling for molecular phylogenetics was facilitated by Jean-Pierre Gattuso and colleagues at Villefranche and by Colomban de Vargas. The filter sample used for SEM observation of *G. striatus* was provided by Hiromi Matsuoka. We thank Takeo Horiguchi for help with molecular phylogenetic experiments.

This is contribution number 256 of the AMT programme.

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