

Supplementary information

A 15 million-year long record of phenotypic evolution in the heavily calcified coccolithophore *Helicosphaera* and its biogeochemical implications

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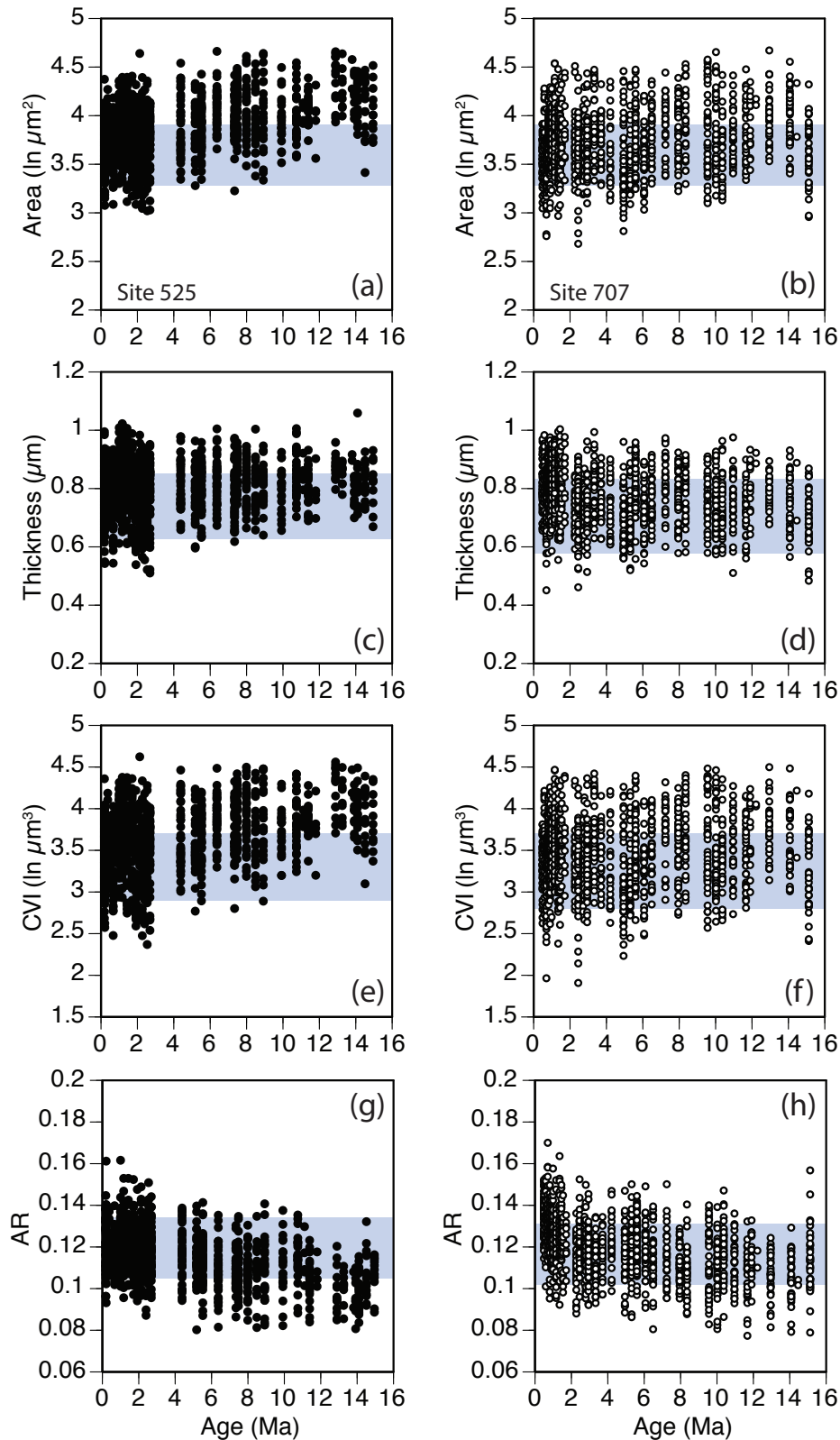


Figure S1. Evolutionary trends in *Helicosphaera carteri* over the past 15 Ma at Site 525 (black) and Site 707 (open symbols). Scatters represent measurements on individual fossil specimens of (a-b) coccolith area ($\ln \mu\text{m}^2$), (c-d) thickness (μm), (e-f) coccolith volume index (CVI, $\ln \mu\text{m}^3$) and (g-h) coccolith aspect ratio (AR, unitless). Data are shown for the fossil morphospecies *H. carteri* only, the blue shading indicates ranges measured in modern *H. carteri* strains RCC1323 (South Atlantic, left column) and RCC1334 (Mediterranean Sea, right column).

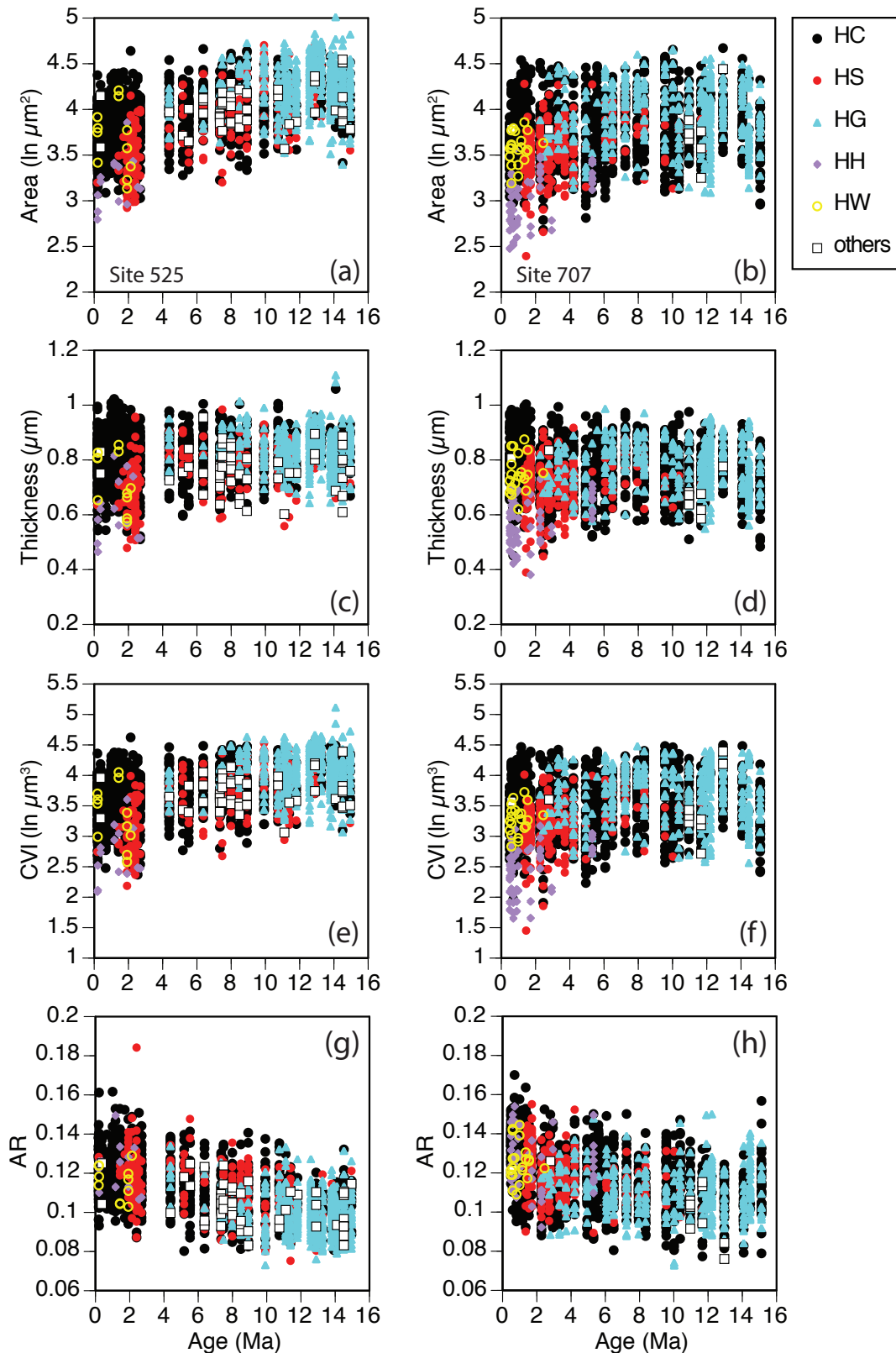


Figure S2. Evolutionary trends in all *Helicosphaera* morphospecies over the past 15 Ma at Site 525 (left column) and Site 707 (right column). Scatters represent measurements on individual fossil specimens of (a-b) coccolith area (ln μm^2), (c-d) thickness (μm), (e-f) coccolith volume index (CVI, ln μm^3) and (g-h) coccolith aspect ratio (AR, unitless). Morphospecies: HC = *H. carteri*; HS = *H. sellii*; HG = *H. granulata*; HH = *H. hyalina*; HW = *H. wallichii*; others = all other types.

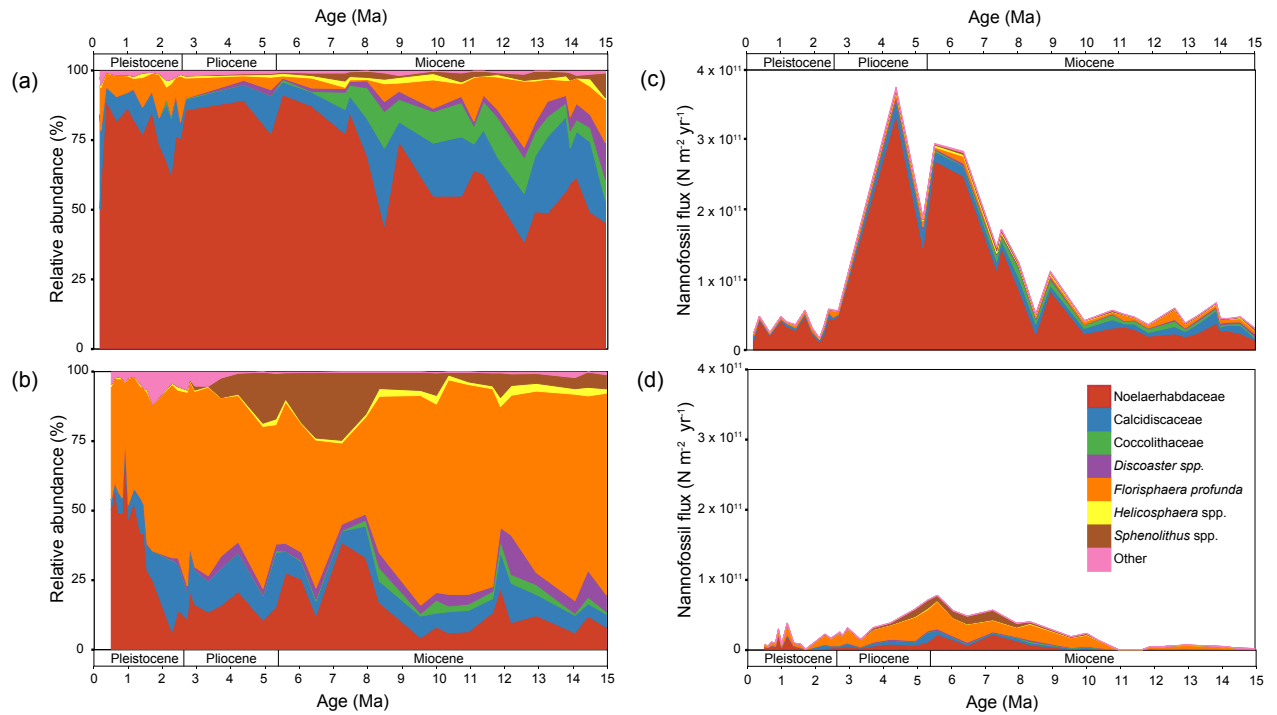


Figure S3. Coccolithophore community composition and total coccolith fluxes at the two investigated deep-sea sites. Relative abundance (%) of main nannofossil taxa at (a) South Atlantic Site 525 and (b) Equatorial Indian Ocean Site 707. Total fluxes (N m⁻² yr⁻¹) of main nannofossil taxa at (c) Site 525 and (d) Site 707.

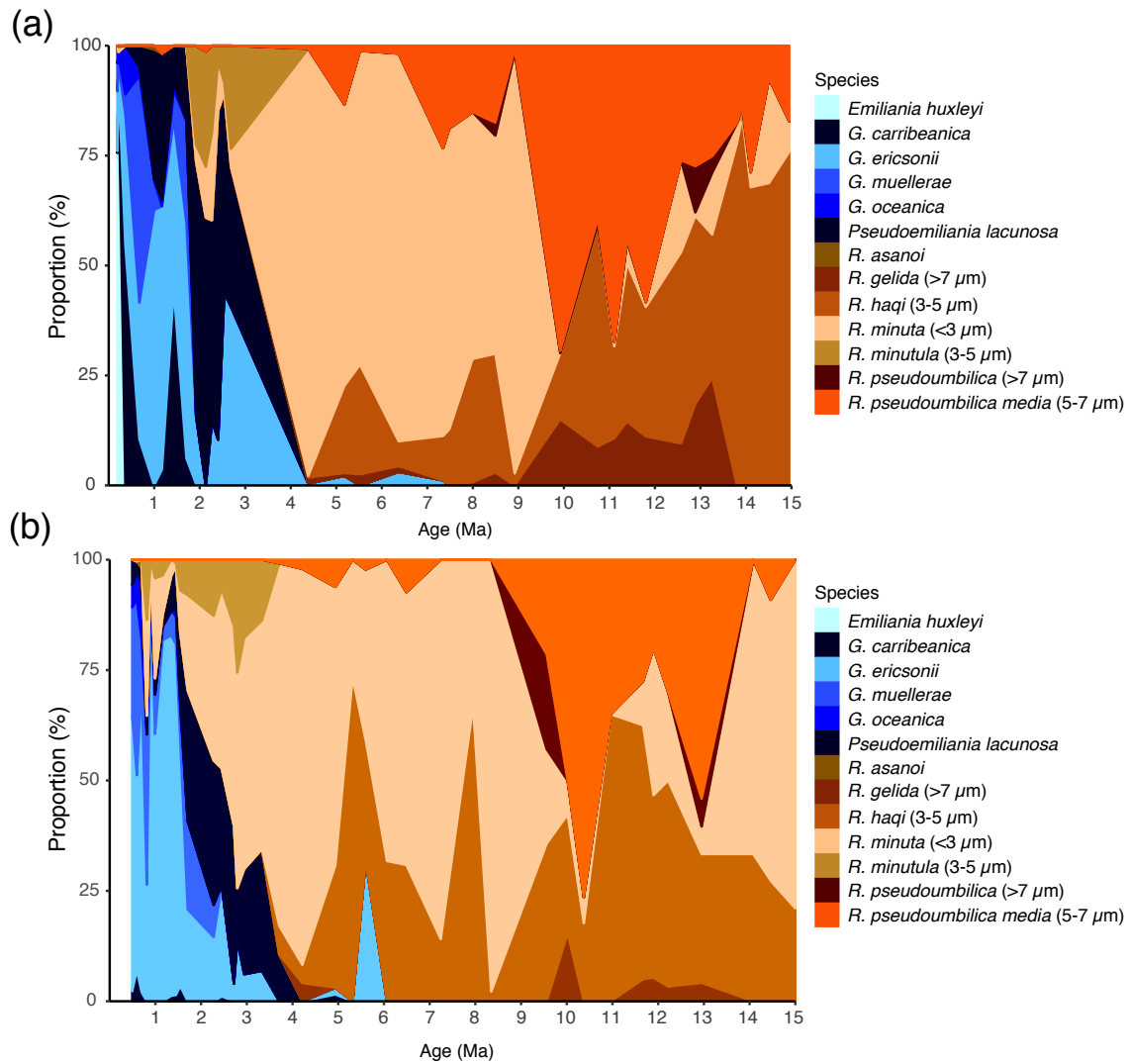


Figure S4. Proportions of morphospecies within the Noelaerhabdaceae family (genera *Emiliana*, *Gephyrocapsa*, *Pseudoemiliana* and *Reticulofenestra*). (a) South Atlantic Site 525. (b) Equatorial Indian Ocean Site 707.

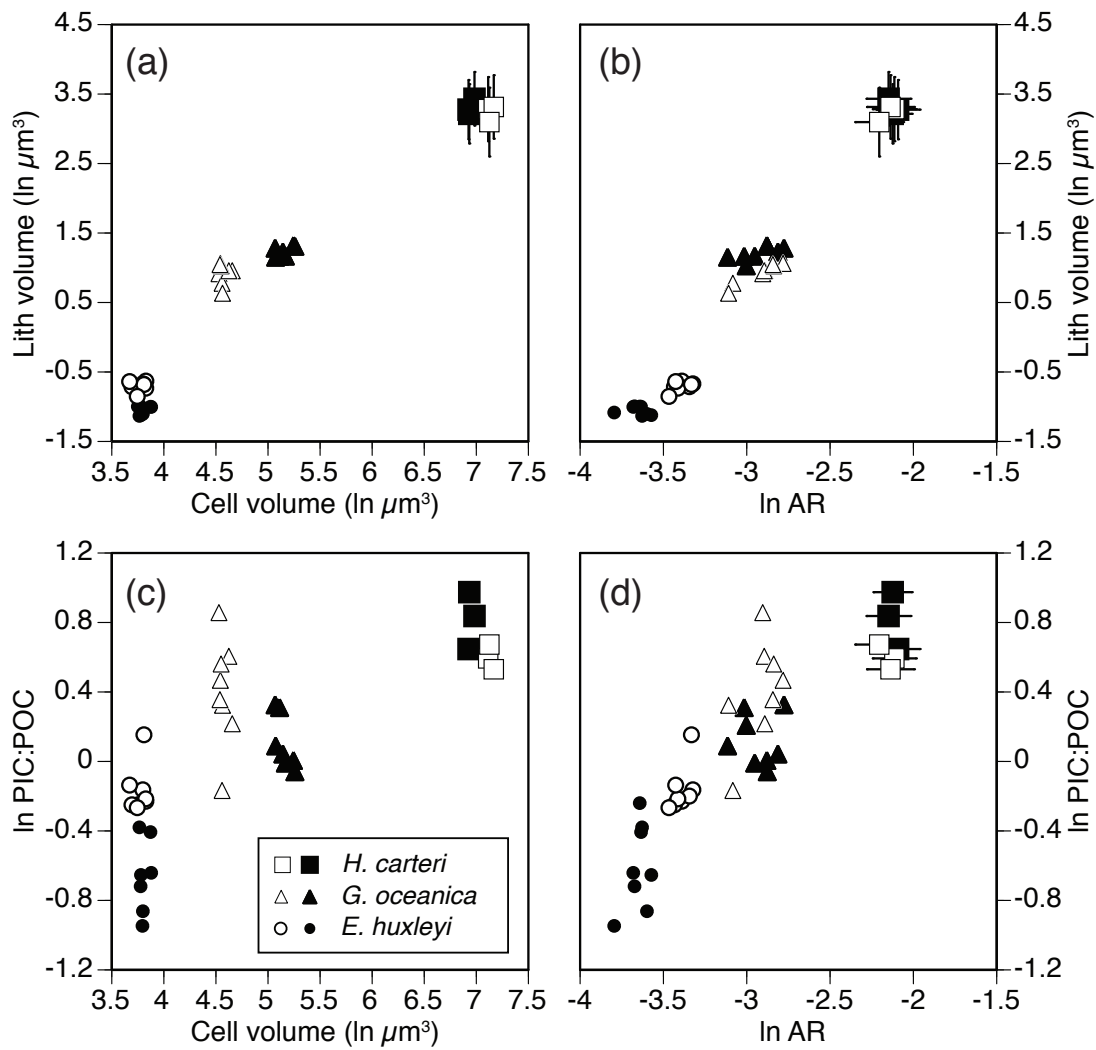


Figure S5. Cross-plots of physiological and coccolith morphology parameters as measured in different strains of three modern coccolithophore species. **(a)** Cell volume ($\ln \mu\text{m}^3$) vs. coccolith volume ($\ln \mu\text{m}^3$). **(b)** Coccolith volume vs. aspect ratio (\ln -scale). **(c)** Cell volume vs. PIC:POC ratio (\ln -scale). **(d)** Coccolith aspect ratio vs. PIC:POC ratio. Error bars in (a,b) and (d) represent ± 1 s.d. of coccolith measurements in replicate cultures. Data sources: Šupraha et al., 2015 (*H. carteri*) and McClelland et al., 2016 (*G. oceanica* and *E. huxleyi*).

Table S1. Age-depth models of DSDP Site 525 and ODP Site 707. Nannofossil biostratigraphic datums are calibrated to the geological timescale of Gradstein et al., (2012). Depths marked in bold were reported in Suchéras-Marx and Henderiks, (2014). FO=first occurrence; LO=last occurrence.

| Nannofossil datum | Age (Ma) | 525 Depth (mbsf) | 707 Depth (mbsf) |
|--|----------|------------------|------------------|
| FO <i>Emiliana huxleyi</i> | 0.29 | 1.21 | |
| LO <i>Pseudoemiliana lacunosa</i> | 0.44 | 2.42 | 0.43 |
| LO <i>Reticulofenestra asanoi</i> common | 0.91 | 3.64 | |
| LO <i>Helicosphaera sellii</i> | 1.26 | 6.07 | 7.23 |
| LO <i>Calcidiscus macintyreii</i> | 1.60 | 7.34 | |
| FO <i>Gephyrocapsa</i> spp. (<5.5µm) | 1.62 | | 10.76 |
| LO <i>Discoaster brouweri</i> | 1.93 | 9.30 | 13.46 |
| LO <i>Discoaster pentaradiatus</i> | 2.39 | | 16.06 |
| LO <i>Sphenolithus</i> spp. subtop | 3.54 | | 28.46 |
| LO <i>Reticulofenestra pseudoumbilicus</i> | 3.7 | 28.52 | |
| FO <i>Amaurolithus</i> spp. | 7.42 | 88.80 | |
| FO <i>Discoaster quinqueramus</i> | 8.12 | | 89.26 |
| FO <i>Discoaster berggrenii</i> | 8.29 | 103.78 | |
| FO <i>Discoaster pentaradiatus</i> | 9.37 | 121.46 | |
| FO <i>Discoaster hamatus</i> | 10.55 | | 113.12 |
| LO <i>Sphenolithus heteromorphus</i> | 13.53 | 170.81 | 123.82 |
| LO <i>Helicosphaera ampliapertura</i> | 14.91 | 193.15 | |
| FO <i>Sphenolithus belemnos</i> | 19.03 | | 136.70 |

Table S2. T-test comparison of coccolith volume index (CVI), area, major diameter and AR between the two strains of *H. carteri* studied in Šupraha et al., (2015).

| Strain | Area (μm^2) | | Thickness (μm) | | CVI (μm^3) | | Major (μm) | | AR | |
|---------|--------------------------|-------|-----------------------------|------|-------------------------|-------|-------------------------|------|--------|------|
| | 1323 | 1334 | 1323 | 1334 | 1323 | 1334 | 1323 | 1334 | 1323 | 1334 |
| Mean | 38.93 | 37.64 | 0.74 | 0.71 | 29.62 | 27.82 | 8.42 | 8.10 | 0.12 | 0.11 |
| d | 1.10 | | 2.08 | | 1.46 | | 2.08 | | 2.20 | |
| df | 325.92 | | 317.22 | | 328.39 | | 324.87 | | 327.32 | |
| p-value | 0.273 | | 0.037 | | 0.144 | | 0.039 | | 0.029 | |

Table S3. Site-to-site t-test comparisons of coccolith volume index (CVI, μm^3) on the genus (*Helicosphaera* spp.) and the morphospecies-level.

| Site | <i>Helicosphaera</i> spp. | | <i>H. carteri</i> | | <i>H. sellii</i> | | <i>H. granulata</i> | |
|---------|---------------------------|-------|-------------------|-------|------------------|-------|---------------------|-------|
| | 525 | 707 | 525 | 707 | 525 | 707 | 525 | 707 |
| Mean | 46.22 | 34.92 | 41.96 | 35.22 | 36.69 | 26.06 | 61.18 | 43.01 |
| t | 20.36 | | 10.83 | | 7.96 | | 15.15 | |
| df | 3863.8 | | 2427.2 | | 324.91 | | 890.57 | |
| p-value | 2.2e-16 | | 2.2e-16 | | 2.9e-16 | | 2.2e-16 | |