

## Late Neogene evolution of modern deep-dwelling plankton

Flavia Boscolo-Galazzo<sup>1‡\*</sup>, Amy Jones<sup>2</sup>, Tom Dunkley Jones<sup>2</sup>, Katherine A. Crichton<sup>1‡</sup>, Bridget S. Wade<sup>3</sup>, Paul N. Pearson<sup>1</sup>

<sup>1</sup>Cardiff University, School of Earth and Environmental Sciences, Cardiff (UK).

<sup>2</sup>Birmingham University, School of Geography, Earth and Environmental Sciences, Birmingham (UK).

<sup>3</sup>University College London, Department of Earth Sciences, London (UK).

‡Now at Bergen University, Department of Earth Science and Bjerknes Center for Climate Research, Bergen (Norway).

‡Now at Exeter University, Department of Geography, Exeter (UK)

\*corresponding author: [flavia.boscologalazzo@uib.no](mailto:flavia.boscologalazzo@uib.no)

The fossil record of marine microplankton provides insights into the evolutionary drivers which led to the origin of modern deep-water plankton, one of the largest component of ocean biomass. We use global abundance and biogeographic data combined with depth habitat reconstructions to determine the environmental mechanisms behind speciation in two groups of pelagic microfossils over the past 15 million years. We compare our microfossil datasets with water column profiles simulated in an Earth System model. We show that deep-living planktonic foraminiferal (zooplankton) and calcareous nannofossil (mixotroph phytoplankton) species were virtually absent globally during the peak of the middle Miocene warmth. Evolution of deep-dwelling planktonic foraminifera started from subpolar-midlatitude species during late Miocene cooling, via allopatry. Deep-dwelling species subsequently spread towards lower latitudes and further diversified via depth sympatry, establishing modern communities stratified hundreds of meters down the water column. Similarly, sub-euphotic zone specialist calcareous nannofossils become a major component of tropical and sub-tropical assemblages during the latest Miocene to early Pliocene. Our model simulations suggest that increased organic matter and oxygen availability for

27 planktonic foraminifera, and increased nutrients and light penetration for nanoplankton, favored  
28 the evolution of new deep water niches. These conditions resulted from global cooling and the  
29 associated increase in the efficiency of the biological pump over the last 15 million years.

## 30 **1. Introduction**

31         The biodiversity of planktonic and nektonic organisms is difficult to explain given the  
32 uniform character and vastness of pelagic environments, where genetic isolation seems difficult to  
33 maintain (Norris, 2000). Planktonic microorganisms with mineralized shells have often been used  
34 as a model to study the mode and tempo of species origination in the open ocean, due to the  
35 abundance, widespread distribution, and temporal continuity of their fossil record (e.g., Pearson et  
36 al., 1997; Norris, 2000; Bown et al., 2004; Ezard et al., 2011; Norris et al., 2013). Because of the  
37 great fossilization potential of their calcium carbonate tests across much of the global ocean, their  
38 relatively simple and well-established taxonomy, and highly resolved biostratigraphy, planktonic  
39 foraminifera and calcareous nanofossils are amongst the most thoroughly studied . Planktonic  
40 foraminifera are heterotrophic zooplankton, with different species specialized to feed on different  
41 types of food, from other plankton to sinking detritus. In the modern ocean, planktonic  
42 foraminifera live stratified across a range of depths spanning from the surface to hundreds of  
43 meters down the water column (Rebotim et al., 2017; Meilland et al., 2019). Properties such as  
44 food quantity and quality, oxygen, light and pressure all change markedly across the first few  
45 hundreds of meters of the ocean. Depending on such down-column variability in environmental  
46 conditions, planktonic foraminifera can actively control their living depth of preference, which  
47 remains relatively stable during their adult life-stage (Hull et al., 2011; Weiner et al., 2012;  
48 Rebotim et al., 2017; Meilland et al., 2019; Duan et al., 2021). A key advantage of using planktonic  
49 foraminifera for evolutionary studies is the ability to extract ecological information from their shell

50 chemistry. This provides invaluable information about species-specific functional ecology (e.g.,  
51 feeding strategy) and habitat preferences (e.g., surface versus deep waters), which in combination  
52 with biogeographic, taxonomic, biometric, and stratigraphic data have often been used to infer  
53 speciation and extinction mechanisms (Norris et al., 1993; Norris et al., 1994; Pearson et al., 1997;  
54 Hull and Norris, 2009; Pearson and Coxall, 2012; Woodhouse et al., 2021 ) and reconstruct  
55 phylogenetic relationships (Aze et al., 2011).

56 Calcareous nannoplankton also have a highly resolved and continuous fossil record; they  
57 are the most abundant microfossils in oceanic pelagic sediments, and similar to planktonic  
58 foraminifera, their spatial distribution ranges from tropical to subpolar latitudes (Poulton et al.  
59 2017). In the modern ocean they also live stratified in the water column, with species adapted to  
60 euphotic waters, and species adapted to live deeper (Poulton et al., 2017). In contrast to planktonic  
61 foraminifera, nannoplankton are predominantly autotrophic, performing photosynthesis in water  
62 where light penetration is sufficient, although there is evidence for heterotrophy (mixotrophic  
63 behavior) in some extant (Godrjian et al., 2020) and fossil (Gibbs et al., 2020) species. In euphotic  
64 waters, organic matter production from nannoplankton is at the base of pelagic food chains and of  
65 the functioning of the ocean biological carbon pump. Taxonomic, biometric and stratigraphic data  
66 have been used to establish phylogenetic relationships between fossil nannoplankton species  
67 (Young and Bown, 1997).

68 Little emphasis has been given to the long-term drivers of evolutionary patterns observed  
69 in fossil plankton from species to phylum level, although more recently, a broad connection with  
70 changing climate and ocean properties has been suggested (e.g., Ezard et al., 2011; Norris et al.,  
71 2013; Frass et al., 2015; Henderiks et al., 2020; Lowery et al., 2020). Boscolo-Galazzo, Crichton  
72 et al. (2021) showed that over the last 15 million years the remineralization of particulate organic

73 carbon (POC) in surface waters declined markedly driven by climate and ocean cooling (Kennett  
74 and Von der Borch, 1985; Kennett and Exon, 2004; Cramer et al., 2011; Zhang et al., 2014; Herbert  
75 et al., 2016; Sosdian et al., 2018; Super et al., 2020), increasing the efficiency of the ocean  
76 biological pump in delivering organic matter at depth. Such a mechanism was key to promote the  
77 evolution of life in deep waters, allowing the development of the modern twilight zone ecosystem  
78 (Boscolo-Galazzo, Crichton et al., 2021). The goal of this study is to combine the fossil record of  
79 two ecologically complementary calcareous microplankton groups seldom analyzed together,  
80 planktonic foraminifera and nannoplankton, and together with model simulations, help disentangle  
81 the evolutionary drivers of modern deep-dwelling plankton. We use the planktonic foraminiferal  
82 dataset from Boscolo-Galazzo, Crichton et al. (2021) and extend our analysis to calcareous  
83 nannofossils in coeval sediment samples to assess their abundance and distribution pattern. We  
84 compare the results from these two groups and contrast them against time and site-specific model  
85 water column profiles for POC and oxygen (O<sub>2</sub>) obtained from the cGENIE Earth System model.  
86 Further, using stable isotopes, depth habitat reconstructions, abundance and biogeography data we  
87 reconstruct the speciation mechanisms which led to the evolution of modern deep-dwelling  
88 planktonic foraminiferal species.

89

## 90 **2. Methods**

### 91 **2.1 Planktonic foraminifera**

92 In this study we focus on the deep-dwelling groups of macroperforate planktonic  
93 foraminifera of the hirsutellids, globorotaliids, truncorotaliids and globoconellids, which in the  
94 modern ocean calcify and live mostly in the twilight zone of the ocean, i.e. between 200-1000 m  
95 (Birch et al.,

96 2012; Rebotim et al., 2017), and have a more complete fossil record than deep-dwelling  
97 microperforate planktonic foraminifera (Kennett and Srinivasan, 1983).

98 Planktonic foraminiferal data and depth habitat reconstructions (Fig. 1) are from Boscolo-  
99 Galazzo, Crichton et al. (2021). They were obtained from globally and latitudinally distributed  
100 DSDP (Deep Sea Drilling Project)/ODP (Ocean Drilling Program)/IODP (Integrated Ocean  
101 Drilling Program & International Ocean Discovery Program) sites and from cores drilled onshore  
102 and offshore Tanzania, all characterized by abundant calcareous microfossils (Boscolo-Galazzo,  
103 Crichton et al., 2021). The work was focused on seven target ages (15 Ma, 12.5 Ma, 10 Ma, 7.5  
104 Ma, 4.5 Ma, 2.5 Ma, 0 Ma/Holocene). To avoid sample-aliasing, bulk sediment stable isotopes  
105 were measured on an average of ten samples per target age at each site. The sample displaying  
106 mean oxygen stable isotope values was chosen for subsequent analyses (Boscolo-Galazzo,  
107 Crichton et al., 2021). Taxonomy follows: Berggren (1977), Kennett and Srinivasan (1983), Bolli  
108 et al. (1989), Scott et al. (1990), Berggren (1992), Pearson (1995), Majewski (2010), Fox and  
109 Wade (2013), Spezzaferri et al. (2015), Wade et al. (2018), Lam and Leckie, (2020a), with  
110 phylogenetic genus names from Aze et al. (2011).

111 Ages were determined based on biostratigraphic analysis mostly following the biozonation  
112 scheme by Wade et al. (2011).

113 Foraminiferal picking for stable isotope measurements were conducted from the size  
114 fractions: 180-250  $\mu\text{m}$ , 250-300  $\mu\text{m}$ , 300-355  $\mu\text{m}$  (Boscolo-Galazzo, Crichton et al., 2021). Stable  
115 isotopes were measured on an average of 15 different species per sample, using ~25 specimens for  
116 common species, and as many specimens as possible for rare species. Stable isotopes were  
117 measured at Cardiff University. Stable isotope results are shown in Fig. S1 to S9 in the  
118 Supplement. Only data from the largest of the three measured size fractions are shown when data

119 from more than one size fraction are available. Data from size fractions other than those above are  
120 shown only when a species did not occur within the preferred size interval. Foraminiferal  
121 abundance counts were carried out in two size fractions, 180-250  $\mu\text{m}$  and  $>250 \mu\text{m}$ , counting up  
122 to 300 specimens in each. Total abundances were derived by summing up abundances from these  
123 two size fractions.

124 Boscolo-Galazzo, Crichton et al. (2021) reconstructed planktonic foraminiferal depth  
125 habitat (Fig. 1) using a combined model-data approach, solving the paleotemperature equation of  
126 Kim and O'Neill (1997) for each data point using measured foraminiferal  $\delta^{18}\text{O}$ , global ice volume  
127 estimates, and the cGENIE modeled salinity field to determine local water  $\delta^{18}\text{O}$ , and then use the  
128 model temperature-depth curve to determine depth. The full method is described in Boscolo-  
129 Galazzo, Crichton et al. (2021).

130

## 131 **2.2 Calcareous Nannofossils**

132 Quantitative calcareous nannofossil data were collected from the same samples as used for  
133 planktonic foraminiferal analysis or, when this was not possible, stratigraphically adjacent samples  
134 (Table S1 in the Supplement). A cascading count technique was used to maximise nannofossil  
135 diversity recovery and quantification of low abundance species (Styzen, 1997). Nannofossils were  
136 counted per field of view (FOV) until a minimum of 400 specimens were achieved for each sample.  
137 However, if a high abundance species exceeded an average of 25 specimens per FOV, it was  
138 excluded from subsequent counts in that sample and its abundance scaled-up based on its average  
139 abundance and the total numbers of FOV counted. Only specimens directly counted contributed  
140 to the minimum count threshold of 400 specimens. An additional scan of two slide transects were  
141 undertaken to record rare species not observed during the extended count and are included in the

142 total species richness and diversity analyses. Samples for nannofossil analysis were prepared using  
143 the smear slide technique (Bown & Young, 1998). Calcareous nannofossils were observed using  
144 both plane-polarised (PPL) and cross-polarised light (XPL) on a Zeiss Axioscope light-microscope  
145 at x1000 magnification. Identification and taxonomy used herein follows Young et al. (1997) and  
146 is coherent with the recent Neogene calcareous nannofossil taxonomy (Ciummelli et al., 2016;  
147 Bergen et al., 2017; Blair et al., 2017; Boesiger et al., 2017; Browning et al., 2017; de Kaenel et  
148 al., 2017).

### 149 **2.3 Plankton Ecogroups**

150 In order to compare the datasets obtained from the planktonic foraminiferal and nannofossil  
151 analysis, we grouped species into ecogroups based on depth-habitat preferences. Planktonic  
152 foraminiferal ecogroups are defined based on paleodepth habitat reconstructions from Boscolo-  
153 Galazzo, Crichton et al. (2021): the euphotic zone ecogroups includes species with an average  
154 depth habitat shallower than 200 m (the bottom of the euphotic zone), the twilight zone ecogroup  
155 includes species with an average depth habitat coinciding with the twilight zone (200-1000 m).  
156 The twilight zone ecogroup is largely composed of species within the globoconellids, the  
157 *Globorotalia merotumida-tumida* lineage, the hirsutellids and the truncorotaliids, but also includes  
158 species from other genera, such as *Globigerinella calida*, *Globorotaloides hexagonus*, *G.*  
159 *variabilis*, and *Pulleniatina obliquiloculata*. *Dentoglobigerina venezuelana* has a changeable  
160 depth habitat through time (Matsui et al., 2016; Wade et al., 2018); following the depth habitat  
161 reconstructions from Boscolo-Galazzo, Crichton et al., (2021) it was grouped as euphotic zone  
162 species for target ages 15, 12.5, 7.5, 4.5 Ma and as twilight zone species for target age 10 Ma.  
163 Species were excluded from the grouping when they are known to have a marked seasonality in  
164 abundance and depth habitat (*Globigerina bulloides*, *Globigerinella praesiphonifera* and the

165 neogloboquadrinids) (e.g., Jonkers and Kucera, 2015; Greco et al., 2019), and if they were too rare  
166 and depth habitat reconstruction was not possible (*Candeina nitida*).

167 Three ecogroups for calcareous nannofossils are used: upper-euphotic, lower-euphotic and  
168 sub-euphotic. The upper-euphotic group is represented by: *Discoaster* spp., *Rhabdosphaera*  
169 *xiphos*, *Reticulofenestra* spp. and *Gephyrocapsa* spp. (excluding *G. ericsonii*); the lower-euphotic  
170 ecogroup contains: *Rhabdosphaera clavigera*, *Gephyrocapsa ericsonii* and *Ceratolithus* spp.,  
171 finally the subeuphotic ecogroup includes: *Florisphaera profunda* and *Calciosolena murrayi*.  
172 Because species specific stable isotope measurements and depth habitat reconstructions are  
173 difficult for calcareous nannofossils, species depth-habitat preference was assigned based on the  
174 literature (Poulton et al., 2017; Tangunan et al., 2018). In particular, Poulton et al. (2017) described  
175 vertically separated coccolithophores communities sampled during a meridional cruise in the  
176 Atlantic Ocean. Here we use their criteria for assigning nannofossil species into ecogroups,  
177 whereby in the upper-euphotic zone ecogroup we include species found to live in waters with  
178 >10% surface irradiance, in the lower-euphotic zone ecogroup we group species found to live in  
179 waters with 10-1% irradiance, and in the sub-euphotic zone ecogroup we group species found to  
180 live in waters with <1%, i.e. too low to support photosynthesis (Poulton et al., 2017). *Discoaster*  
181 become extinct in the early Pleistocene, therefore, its depth habitat remains under debate as the  
182 group has no extant relative (Schueth and Bralower, 2015; Tangunan et al., 2018). However,  
183 geochemical evidence from oxygen isotope values of *Discoaster* and planktonic foraminifera  
184 (*Globorotalia menardii*, *Dentoglobigerina altispira* and *Globigerinoides obliquus*), reveal  
185 comparable values and signifies that *Discoaster* likely inhabited the upper euphotic zone  
186 (Minoletti et al., 2001).



187 For each target age, the relative abundance of ecogroups was calculated summing up the  
188 abundance counts of all the individual species pertaining to an ecogroup at each site, hence,  
189 ecogroup abundance data represent global mean values. For both nannofossils and planktonic  
190 foraminifera, the percentage of each ecogroup per time bin was converted into pie-charts (Fig. 2-  
191 5). Diversity indexes for both foraminiferal and nannofossil ecogroup were calculated using the  
192 statistical software Past (Hammer et al., 2001) (Fig. 6).

## 193 **2.4 cGENIE model**

194 We extracted model output for Particulate Organic Carbon (POC) and oxygen  
195 concentration from the cGENIE simulations for each of the seven target ages as described fully in  
196 Boscolo-Galazzo, Crichton et al. (2021). To facilitate a general discussion of near-surface changes,  
197 we divided the data latitudinally by calculating the arithmetic mean for low latitudes ( $<16^\circ$   
198 latitude), two mid-latitude bands (mid 1:  $16^\circ$  to  $40^\circ$ , mid 2:  $40^\circ$  to  $56^\circ$ ) and high latitudes ( $>56^\circ$ ).  
199 The cGENIE simulations take account of changing boundary conditions including  $\text{CO}_2$  forcing,  
200 bathymetry and ocean circulation (Crichton et al., 2020). The model's ocean biological carbon  
201 pump is temperature dependent, where temperature affects both nutrient uptake rates at the surface  
202 and remineralization rates of sinking particulate organic matter down the water column (Crichton  
203 et al., 2021).

## 204 **3. Results**

### 205 **3.1 Plankton Ecogroups**

206 For both calcareous nannoplankton and planktonic foraminifera, the variation in abundance  
207 of euphotic zone and deeper-dwelling ecogroups show global patterns recognised across sites.  
208 Additionally both group indicate a long-term directionality towards increased abundance of deep-  
209 dwelling ecotypes. Among planktonic foraminifera, the twilight zone ecogroup increases in

210 abundance through time starting at 7.5 Ma (Fig. 6). The relative abundance of the twilight zone  
211 ecogroup in the middle Miocene is 15% and it increases to ~30% in the Holocene time slice (Fig.  
212 6). The average abundance of the euphotic zone species ecogroup in the middle Miocene is 85%  
213 and it decreases through time until reaching 60% in the Holocene (Fig. 6). In the twilight zone  
214 ecogroup we observe an increase in the total number of species from about 1-2 species at 15 Ma,  
215 to 14 species in the Holocene (Fig. 6). In the middle Miocene this group comprised 1/6 of the total  
216 number of species in our samples, while in the Holocene it represents almost the half. All the  
217 diversity indexes show a late Miocene to Holocene increasing trend for the twilight zone ecogroup  
218 (Fig. 6).

219         Calcareous nannofossil assemblages are dominated by the upper-euphotic ecogroup from  
220 15 to 10 Ma at all sites (Fig. 2-5). At 7.5 Ma the sub-euphotic ecogroup first becomes a significant  
221 component of assemblages at Indian Ocean sub-tropical Sites U1482 and to some extent Site 242,  
222 but it is not until the 4.5 Ma time slice that the sub-euphotic ecogroup becomes a significant  
223 component of assemblages at the majority of locations (Sites 516, 871/872, 242, U1338, U1482,  
224 U1489; Fig. 2-5). By the Holocene time slice, coccoliths of sub-euphotic species are dominant at  
225 most locations, except at Eastern Equatorial Pacific Site U1338 (Fig. 6 and 4). At the southern  
226 high latitude Site 1138 there is no significant contribution from coccoliths of either lower-euphotic  
227 or sub-euphotic species at any point, although there is no data from the Pliocene to Holocene time  
228 slices at this location (Fig. 5). Global average compositions of calcareous nannofossil assemblages  
229 reflect the changes noted above, with a marked and rapid decline in the relative contribution of the  
230 upper-euphotic ecogroup, and a corresponding increase in the sub-euphotic zone ecogroup through  
231 the Pliocene and to Holocene (Fig. 6).

232 **3.2 Planktonic foraminiferal deep-dwelling species: depth habitat, abundance and**  
233 **biogeography**

234 **3.2.1 Hirsutellids**

235 The only hirsutellid species occurring in our Miocene samples is *Hirsutella scitula*. At 15  
236 Ma this morphospecies is common only at Site 1138 (~8%), sporadically occurs at Site 516 (<1%)  
237 and is absent at the other investigated sites (Fig. 7). Oxygen isotopes range from 0.5 to 1.3‰ (Fig.  
238 S1-S2). Depth-habitat reconstructions for Site 1138 are unattainable from  $\delta^{18}\text{O}$  data due to the  
239 overprinting effect of subpolar front shifts at this location, but habitat reconstruction at Site 516  
240 suggests a paleodepth habitat shallower than 200 m. By 12.5 Ma, *H. scitula* appears at Site U1338  
241 and U1489 in very low abundance (<0.5%) (Fig. 7). At Site U1489 the species was so rare that it  
242 was encountered when picking for stable isotopes and no more when counting for species  
243 abundances, despite the use of different splits of the residue. No differences were observed  
244 between 12.5 and 10 Ma in the biogeography of *H. scitula* (Fig. 7). However, by 7.5 Ma, *H. scitula*  
245 occurs at all our low latitude sites (Fig. 8) with oxygen isotopes ranging from -0.5 to 2.0‰ (Fig.  
246 S3-S9), which according to depth habitat reconstructions translates to 250 and 500 m water depth  
247 (Boscolo-Galazzo, Crichton et al., 2021). This is similar to that of *Globorotaloides hexagonus*  
248 (Fig. 1), the only twilight zone dweller we observed at tropical sites at 15 Ma, displaying stable  
249 isotopes ranging from 0 to 1‰, which translates to depths around 300-500 m. In the late middle  
250 Miocene the stable isotope values of *G. hexagonus* increase to 2-2.5‰ (Fig. S3-S9). Similarly, the  
251 oxygen isotope values of tropical *H. scitula* increased through time, reaching 2-3‰ in the youngest  
252 target ages. In line with this, the reconstructed depth habitat of *H. scitula* and *G. hexagonus*  
253 increases through time in a stepwise fashion, and in the Holocene it reaches down to 800-1500 m  
254 (Fig. 1) (Boscolo-Galazzo, Crichton et al., 2021). *Hirsutella scitula* becomes gradually more

255 common at low latitude sites through the Miocene-Pliocene, although it never becomes abundant.  
256 In our record, *Hirsutella margaritae* and *H. theyeri* first appear in the early Pliocene at a depth  
257 between 200-300 m (Fig. 1) (oxygen isotopes range -1 to -0.5‰), similar to that of *H. hirsuta*  
258 (oxygen isotopes range 0 to 1‰) when it first appears in the Holocene (Fig. S3-S9).

### 259 **3.2.2 Truncorotaliids**

260 The earliest appearances of *Truncorotalia crassaformis* in our record corresponds to our  
261 4.5 Ma time slice at Site 1138 in the Indian Ocean sector of the Southern Ocean where it represents  
262 >20% of the assemblage, and in coeval sediments at mid-latitude Site 516 in the southwest  
263 Atlantic, where it represents ~9% of the assemblage (Fig. 9), with oxygen isotopes ranging from  
264 1.5 to 3.0‰ (Fig. S1-S2). At Site 516 we observe the co-occurrence of *T. oceanica* (~14%) and *T.*  
265 *crassaformis* in the early Pliocene (4.5 Ma), and of *T. viola* (~5%) and *T. crassaformis* (~13%) in  
266 the late Pliocene (2.5 Ma) (Fig. 10). We did not observe *T. oceanica* and *T. viola* anywhere else.  
267 *T. oceanica* and *T. crassaformis* display almost overlapping  $\delta^{18}\text{O}$  values and depth habitat (Fig.  
268 S2) but *T. crassaformis* has 0.5‰ lower  $\delta^{13}\text{C}$  values. Oxygen stable isotope data (1‰; Fig. S2)  
269 and habitat reconstructions for Site 516 indicate that a subsurface habitat (>200 m) was already  
270 occupied by *T. crassaformis* at the beginning of its evolutionary history (Fig. 1). The late Pliocene  
271 appearance of *T. viola* at Site 516, which differs from *T. crassaformis* in having a more convex  
272 umbilical side, a triangular outline and a subacute profile, is associated with a shift to more positive  
273 oxygen isotope values of *T. crassaformis* (1.5‰) and to slightly greater depths (Fig. 1).

274 We find *T. crassaformis* by the late Pliocene at our investigated tropical and subtropical  
275 sites (2.5 Ma, Site U1338, U1489, 872, U1490, 242, U1482) (Fig. 10-11), with oxygen isotope  
276 values ranging from 1.0 to 2.0‰ which translate to depth habitats of 400-600 m (Boscolo-Galazzo,  
277 Crichton et al., 2021; Fig.1). The appearance of *T. crassaformis* at our low latitude sites is coeval

278 with the appearance in our record of *Truncorotalia tosaensis*, morphologically transitional  
279 between *T. crassaformis* and *T. truncatulinoides* (Lazarus et al., 1995) (Fig. 10). *Truncorotalia*  
280 *tosaensis* displays oxygen isotopes values ranging from 0 to 0.5‰ (Fig. S1-S9), which translate to  
281 300-350 m depth (Fig. 1). Consistent with earlier findings (Jenkins and Srinivasan 1986; Lam and  
282 Leckie 2020b; Lazarus et al., 1995), we record the first occurrence of *T. truncatulinoides* in the  
283 late Pliocene in the south-west Pacific (2.5 Ma, Site U1482), and only later in the North Pacific (0  
284 Ma, Site 872), Indian Ocean (0 Ma, Site 242) and South Atlantic (0 Ma, Site 516) (Fig. 11).  
285 *Truncorotalia truncatulinoides* records oxygen isotope values ranging from -1 to 2‰, more  
286 negative than coeval *T. crassaformis* (Fig. S3-S9). *Truncorotalia truncatulinoides*, although  
287 reported in the modern tropical ocean as one of the species living at the greatest depths, occupies  
288 a shallower depth habitat than *T. crassaformis* when it first appears in our tropical to subtropical  
289 records (2.5 Ma).

### 290 **3.2.3 Globorotaliids**

291 With globorotaliids here we refer to the *Globorotalia merotumida-tumida* lineage  
292 composed by *G. merotumida*, *G. plesiotumida*, *G. tumida* and *G. ungulata* (Kennett and  
293 Srinivasan, 1983). This group first appears with *Globorotalia plesiotumida* in our 10 Ma time slice  
294 at Site 871 (Fig. 7). At all the investigated low latitude sites, we find *G. tumida* by 4.5 Ma with  
295 abundances between 2-24% (Fig. 9). *Globorotalia plesiotumida* co-occurs with *G. tumida* only at  
296 Sites 872 and 242 corresponding to our 4.5 Ma time slice (Fig. 9; Fig. S9). In our records, *G.*  
297 *tumida* consistently displays oxygen isotope values between -1 to 0‰ (Fig. S3-S9), and an average  
298 depth habitat around 250 m, with a shallowest occurrence at 50 m and a deepest occurrence around  
299 600 m (Fig. 1). Similar oxygen isotope values and depth habitat preference are recorded for *G.*  
300 *plesiotumida* and *G. ungulata* (Fig. 1 and Fig. S3-S9).

### 301 **3.2.4 Globoconellids**

302 In our records, *Globoconella miozea* is a dominant component of planktonic foraminiferal  
303 assemblage at Site 1138 at 15 Ma (65%) and occurs in moderate abundance at Site 516 (5%) (Fig.  
304 7). The distribution of globoconellids appears restricted to southern high to mid-latitudes during  
305 the middle Miocene and the late Miocene to Pliocene. At Site 1138 globoconellids decrease in  
306 abundance through time, with *Globoconella panda* the only late Miocene (7.5 Ma) species (<1%;  
307 with  $\delta^{18}\text{O}$  of 3‰), followed only by *Globoconella inflata* in the Holocene (4.2%; with  $\delta^{18}\text{O}$  of  
308 3.5‰) (Fig. 11). On the contrary, at Site 516 globoconellids increase in abundance through time  
309 becoming a characteristic feature of the planktonic foraminifera assemblage as noted in previous  
310 studies of this area (Berggren, 1977; Norris et al., 1994) (Fig. 7-11). In the Holocene *G. inflata* is  
311 most abundant at mid-latitude Site 516 (22.7%; with  $\delta^{18}\text{O}$  of 1‰), but also occurs in the subtropical  
312 (<0.5%; with  $\delta^{18}\text{O}$  of 0.9‰) and subpolar Indian ocean (4.2%; with  $\delta^{18}\text{O}$  of 3.5‰) (Fig. 11; Fig.  
313 S1-S3). Depth habitat reconstructions for the globoconellids show a deepening trend through time  
314 although less marked compared to those of other deep-dwelling groups considered in this study  
315 (Boscolo-Galazzo, Crichton et al., 2021; Fig. 1). At Site 516, the depth habitat is just above 200 m  
316 for middle Miocene *Globoconella miozea* ( $\delta^{18}\text{O}$  0.9‰), just below 200 m for late Miocene *G.*  
317 *miotumida* and *G. conomiozea* ( $\delta^{18}\text{O}$  1‰), and around 350 m for late Pliocene *Globoconella*  
318 *puncticulata* ( $\delta^{18}\text{O}$  1.6‰) the precursor of *G. inflata*, which shows a similar depth habitat at this  
319 site ( $\delta^{18}\text{O}$  1-1.2‰; Fig. S2), (Fig. 1). At Site 242 the average depth reconstruction for the Holocene  
320 *G. inflata* is of ~450 m ( $\delta^{18}\text{O}$  0.9‰; Fig. S3), similar to that of *T. crassaformis* and *T.*  
321 *truncatulinoides* (Boscolo-Galazzo, Crichton et al., 2021; Fig. 1).

## 322 **4. Discussion**

### 323 **4.1 Evolution of a deep-plankton ecological niche linked to late Neogene cooling**

324 We observe a trend of increasing ecological importance of deep-dwelling species in both  
325 calcareous nannofossil and foraminiferal communities from the late Miocene to Recent. The  
326 foraminiferal twilight zone ecogroup shows a marked increase in abundance and diversity at 7.5  
327 Ma, at the same time as the first significant appearance of the sub-euphotic ecogroup within  
328 coccolithophore assemblages, and coinciding with a possible acceleration of global cooling  
329 (Cramer et al., 2011; Crichton et al., 2020). Fossil deep-dwelling coccoliths are dominated by one  
330 species, *Florisphaera profunda*, which is often very abundant in Plio-Pleistocene sediments. This  
331 makes our sub-euphotic ecogroup a low diversity – high abundance assemblage, whose origin  
332 significantly impacts the relative abundance balance between ecogroups, but with little change in  
333 diversity metrics towards the modern. Although there are morphological variants of *F. profunda*  
334 in the modern oceans, potentially representing sub-species or pseudo-cryptic species (Quinn et al.,  
335 2005), these are not distinguished in fossil assemblages and documenting their divergence times  
336 requires either further molecular genetic or detailed morphological analyses. The one clear signal  
337 in our diversity analyses is a late Miocene – early Pliocene peak in upper-euphotic species richness,  
338 followed by a marked decline through the late Pliocene to the Holocene. The late Miocene-  
339 Pliocene peak diversity is present in previous global compilations of total nannofossil diversity  
340 (Bown et al. 2004; Lowery et al. 2020), but here we show that this signal is driven by first a  
341 diversification and then progressive extinction almost entirely within upper-euphotic taxa.

342 Modern planktonic foraminifera evolved in two main diversification pulses in the middle  
343 Miocene (16-14 Ma) and during the late Miocene-Pliocene transition (6-4.5 Ma) (Kennett and  
344 Srinivasan, 1983; Kucera and Schönfeld, 2007). Our species abundance and diversity data show  
345 that this diversification was mostly driven by the origin of lineages of deep/subsurface dwelling  
346 species (Fig. 7-11 and Fig. 6). Diversity among euphotic zone species remained constant from the

347 middle Miocene to the early Pliocene, then declined (Fig. 6). This pattern, similar to calcareous  
348 nannofossils, may explain early records pointing to a decrease in Pliocene to Recent planktonic  
349 foraminiferal diversity (Wei and Kennett, 1986).

350         The observed evolutionary patterns in planktonic foraminifera and coccolithophores can  
351 be explained by the development of environmental conditions favourable to deep living plankton  
352 with cooling. For the deep-dwelling planktonic foraminifera, survival requires the availability of  
353 food at depth, and with the exception of few species adapted to oxygen minimum zones (Davis et  
354 al., 2021), the absence of severe oxygen depletion. In the published literature there is a general  
355 tendency for planktonic foraminiferal  $\delta^{18}\text{O}$  values to be tightly grouped during times of warm  
356 climate, becoming more spread-out during cooling episodes, for instance the transition from mid-  
357 to late Cretaceous (Ando et al., 2010) and early to middle Eocene (John et al., 2013). In light of  
358 our findings such tight  $\delta^{18}\text{O}$  values, may indicate times when food and oxygen availability at depth  
359 were limited, allowing planktonic foraminifera to live at shallower depths only. Because our data  
360 are restricted to certain time slices and locations, we only capture a part of the overall pattern for  
361 the Neogene, but other examples of depth-related evolution in the Neogene include the *Fohsella*  
362 *peripheroronda* – *F. fohsi* lineage in the middle Miocene (Hodell and Vayavananda, 1993; Norris  
363 et al., 1993) and the appearance of various deep-dwelling digitate species in the Plio-Pleistocene  
364 (Coxall et al., 2007).

365         Deep-dwelling coccolithophores, most notably *Florisphaera profunda*, require dissolved  
366 macronutrients (N, P) and at least some degree of light penetration (Quinn et al., 2005; Poulton et  
367 al., 2017). The requirement for light penetration to depth is most clearly shown by the absence  
368 of *F. profunda* beneath high surface productivity regions where nutrients are not limited at depth  
369 but light is rapidly attenuated by more abundant mixed layer microplankton (Beaufort et al., 1999).



370 For coccolithophores, the cooling-driven shift from nutrient recycling within to below the mixed  
371 layer, may have provided the ecological driver for species to live in deeper, more nutrient-rich  
372 waters, but, as mixed layer waters cleared, also allowed the irradiance necessary for photosynthesis  
373 to penetrate to these new deeper habitats. Additionally, the capability of coccolithophores to absorb  
374 carbon and nutrients from seawater under low light conditions (Godrjian et al., 2020; 2021) may  
375 have also aided in the occupation of new deep water niches.

376 Our model outputs from cGENIE is consistent with this interpretation, organic matter  
377 export (POC at 40 m in Fig. 12) reduced with cooling, suggesting an overall decrease in primary  
378 productivity at all 4 latitudinal bands considered in this study. Fewer particles in surface waters  
379 would have allowed greater light penetration (Fig. 12), at the same time, the model indicates  
380 enhanced organic matter delivery at >200 m with cooling. Greater organic matter delivery below  
381 the euphotic zone, suggests a deeper remineralization depth and increased dissolved nutrients  
382 availability at depth. This is most clearly shown in the modelled low and mid latitudes near-surface  
383 waters. Oxygen availability also increased, particularly below 100 m depth in low latitudes and  
384 further down the water column, below 200 m depth (Fig. 12).

385 Nonetheless, planktonic foraminifera and nannoplankton have distinct trophic statuses  
386 (zoo- versus phytoplankton), further coccolithophore species require light for their dominantly  
387 photosynthetic mode of life. In our data, such differences between the requirements of zoo- and  
388 phytoplankton deep-dwellers is clearly observed in the biogeographic patterns. Sub-euphotic  
389 coccolithophores are consistently more abundant in low nutrient sub-tropical locations (e.g. DSDP  
390 Site 242 and IODP Site U1482; Fig. 2). The end-member of this biogeographic difference is ODP  
391 Site 1138, in the Southern Ocean. Here, twilight foraminifera dominate most time intervals,  
392 presumably due to high export production. However, lower-euphotic and sub-euphotic

393 coccolithophores are effectively excluded, due to turbid, high-productivity surface waters (Fig. 5).  
394 This pattern is also supported through a comparison of high to low productivity tropical sites in  
395 the Holocene – the Eastern Equatorial Pacific Site U1338 has abundant twilight foraminifera, but  
396 relatively low abundances of sub-euphotic coccolithophores (Fig. 4), whereas the more  
397 oligotrophic Site U1482 (Fig. 2) has lower abundances of twilight foraminifera and higher  
398 abundances of sub-euphotic coccolithophores.

399         Despite these ecological differences between zoo- and phytoplankton, we suggest there is  
400 a shared environmental driver for the evolution of deep-dwelling coccolithophores and planktonic  
401 foraminifera linking the evolution of deep-dwelling specialists in each group. Efficient near-  
402 surface recycling of organic carbon in past warm climates, such as the middle Miocene (Fig. 12),  
403 precluded the occupation of the deep habitat for both groups by reducing both organic carbon  
404 transfer (food limitation for foraminifera and foraminiferal preys such copepods) and light  
405 penetration (photosynthesis for coccolithophores) to depth (Fig. 2-6). Global cooling since the  
406 middle Miocene (Kennett and Von der Borch, 1985; Kennett and Exon, 2004; Cramer et al., 2011;  
407 Zhang et al., 2014; Herbert et al., 2016; Sosdian et al., 2018; Super et al., 2020), however, led to a  
408 decreased export of organic matter and a deepening of the mean organic matter remineralization  
409 depth, which in turn favoured the evolution of deep water niches in planktonic foraminifera (Fig.  
410 1) and nannoplankton via increased availability of organic matter, oxygen, and likely nutrients at  
411 depth (Fig. 12), and clearing of surface waters.

#### 412 **4.2 Mechanisms of speciation of deep-dwelling planktonic foraminifera**

413         The hirsutellids gave rise to a large late Neogene radiation among planktonic foraminifera,  
414 leading to the origin of modern phyletic groups such as the menardellids, globoconellids,  
415 truncorotaliids, and the globorotaliids of the *Globorotalia merotumida* - *tumida* lineage (Kennett

416 and Srinivasan, 1983; Scott et al., 1986; Aze et al., 2011). The majority of the modern  
417 representatives of these groups are lower euphotic zone to twilight zone species. The hirsutellids  
418 originated about 18 Ma (Wade et al., 2011) from *Globorotalia zealandica* (Kennett and Srinivasan,  
419 1983), the first representative of the group being *Hirsutella praescitula* (Kennett and Srinivasan,  
420 1983; Aze et al., 2011). Extant hirsutellids include *H. scitula*, *H. hirsuta* and *H. theyeri*; genetic  
421 data available for *H. hirsuta* indicate a single genotype (Schiebel and Hemleben, 2017). Modern  
422 *H. scitula* and *H. hirsuta* are deep water forms. Depth habitat reconstructions show *H. scitula* at  
423 the greatest water depth (Boscolo-Galazzo, Crichton et al., 2021; Fig. 1), consistent with it being  
424 reported to have a deeper average depth habitat than *H. hirsuta* in the modern ocean (Birch et al.,  
425 2013; Stainbank et al., 2019), where it feeds on suspended organic matter (Itou et al., 2001).  
426 *Hirsutella hirsuta* has been reported to feed on dead diatoms and to predominantly live at depths  
427 around 250 m (Schiebel and Hemleben, 2017), consistent with our habitat reconstructions placing  
428 *H. hirsuta* and other hirsutellids shallower than *H. scitula*, between the bottom of the euphotic  
429 zone and the upper twilight zone. Our depth habitat reconstruction for *H. scitula* at Site 516 for  
430 the 15 Ma time slice, indicates an initial euphotic zone depth habitat preference for this species.  
431 By the 7.5 Ma time slice, we find *H. scitula* at most of our low latitude sites, at depth comprised  
432 between 300-500 m (Fig. 1). We suggest that the spread of *H. scitula* from high-mid latitudes  
433 towards the tropics after the middle Miocene warmth (Fig. 7-11) tracks increasing availability of  
434 POC and oxygen at depth (Boscolo-Galazzo, Crichton et al., 2021; Fig. 12), allowing this species  
435 to find food in deep tropical twilight zone waters, profiting from a new ecological niche. We  
436 suggest that moving from a surface to a deep-water habitat was for *H. scitula* an ecological  
437 innovation which allowed the species to move outside its high-mid latitude areal, consistent with  
438 observations from the modern ocean documenting *H. scitula* dwelling at progressively deeper

439 depth from high to tropical latitudes (Schiebel and Hemleben, 2017). The proceeding ocean  
440 cooling (and increasing efficiency of the biological pump) explains the stepwise depth habitat  
441 increase of *H. scitula* through time at tropical and subtropical sites (Fig.1): more food became  
442 available at increasingly greater depth in association with improved oxygen availability (Fig. 12),  
443 allowing the species to expand its habitat vertically other than geographically. At low latitude sites  
444 this was accompanied by speciation, with the appearance of *Hirsutella margaritae* and *H. theyeri*  
445 in the early Pliocene, and of *H. hirsuta* in the Holocene. These new species display depth habitats  
446 shallower than *H. scitula* at their appearance. Hence, we suggest that it was the occupation of a  
447 new deep water habitat in tropical waters that triggered speciation from *H. scitula* through depth  
448 sympatry, i.e. genetic isolation attained in the same area but at different depths (Weiner et al.,  
449 2012). Earlier studies on speciation among planktonic foraminifera based on the fossil record,  
450 highlighted a predominance of sympatric speciation, possibly linked to changes in ecology (Norris  
451 et al., 1993; Lazarus et al., 1995; Pearson et al., 1997). This has more recently been supported by  
452 genetic analysis, which reveals a consistent depth separation between intra-specific genotypes at a  
453 global scale, suggesting that depth sympatry could be a universal mechanism generating diversity  
454 among microplankton (Weiner et al., 2012).

455 Truncorotaliids start their evolutionary history at ~4.31 Ma (Raffi et al., 2020), when  
456 *Truncorotalia crassaformis* splits from *Hirsutella cibaoensis* (Kennett and Srinivasan, 1983; Aze  
457 et al., 2011), one of the first new species originating from *H. scitula* after its spread to low latitudes  
458 (Kennett and Srinivasan, 1983). Our reconstructed geographic and temporal distribution for *T.*  
459 *crassaformis* suggests that the early Pliocene split from the hirsutellids happened in cold subpolar  
460 water. By the early Pliocene *Truncorotalia crassaformis* was an abundant component of the  
461 subpolar assemblage at Site 1138 (>17%) and had already successfully spread to middle latitudes

462 (>8%) but does not occur in our low latitude samples (Fig.9). Although modern planktonic  
463 foraminifera have a high dispersal potential (Darling et al., 2000; Norris and De Vergas, 2000),  
464 our data suggest that the evolution of *Truncorotalia crassaformis* from the hirsutellids might have  
465 happened through allopatry, with low latitude population of *H. cibaoensis* becoming isolated from  
466 subpolar populations and eventually evolving into *T. crassaformis* (e.g., following the  
467 development of diachrony reproductive seasons in high and low latitude populations). Similar to  
468 *H. scitula*, from subpolar latitudes, *Truncorotalia crassaformis* appears to have subsequently  
469 spread to lower latitudes occupying progressively deeper habitats (Fig. 1; Fig. 7-11), and  
470 originating numerous daughter species, some of which are intermediate morphospecies with  
471 limited geographic distributions no longer extant today (Lazarus et al., 1995). At Site 516 we find  
472 *Truncorotalia viola*, with lighter  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$  values than *T. crassaformis* (Fig. S2), pointing to  
473 a clear ecological differentiation. Together with the marked morphological differentiation between  
474 the two, this suggests *T. viola* may have been a different biological species. *Truncorotalia*  
475 *truncatulinoides*, the most representative species of this group, appears to have originated from *T.*  
476 *crassaformis* at about 2.7 Ma in the tropical southwest Pacific, and subsequently spread in the  
477 global ocean (Dowsett, 1988; Lazarus et al., 1995). According to our reconstruction, depth  
478 sympatry associated with gradual morphological changes characterizes speciation among the  
479 truncorotaliids, as depth habitat deepening of the ancestor/precursor is clear in the transition *T.*  
480 *crassaformis*-*T. viola* and *T. crassaformis*-*T. tosaensis*-*T. truncatulinoides* in our record (Fig. 1).  
481 The possibility to colonize deeper water habitats may have led to progressive reproductive  
482 isolation between “deeper” and “shallower” populations of *T. crassaformis*, resulting in biological  
483 speciation. Depth sympatry as speciation mechanism for the truncorotaliids was already proposed  
484 by Lazarus et al. (1995) based on biogeography, but without a definitive test.

485 In the modern ocean the *Globorotalia merotumida-tumida* lineage is represented by *G.*  
486 *tumida* and *G. ungulata*, distributed in tropical to temperate regions. Genetic analysis has revealed  
487 that they are two distinct biological species with a single genotype each (Schiebel and Hemleben,  
488 2017). Little is known about the ecology of these two species, although *G. tumida* is known to  
489 dwell in subsurface waters at the deep chlorophyll maximum (Schiebel and Hemleben, 2017).  
490 According to the phylogeny of Aze et al. (2011), *Menardella menardii* gave rise to the  
491 *Globorotalia merotumida-tumida* lineage around 9 Ma. However, *Menardella menardii* is absent  
492 in our late Miocene samples while other menardellids such *Menardella limbata* and *M. pertenuis*  
493 are common at several of our investigated middle latitudes to tropical sites. By 4.5 Ma,  
494 *Globorotalia tumida* had evolved and is common at all of our low latitude sites (Fig. 9), while *M.*  
495 *menardii* is extremely rare, occurring only at Sites U1338 and U1490 (0.1%), becoming more  
496 common only by the Holocene. Based on this biogeographic pattern, we propose that the *G.*  
497 *merotumida-tumida* lineage originated from a late Neogene menardellid, such for instance *M.*  
498 *limbata* which is morphologically very similar to *G. merotumida*. Given that *G. plesiotumida* and  
499 *G. tumida* display a deeper habitat (> 200 m) than *M. limbata* and other Miocene menardellids  
500 (100-200 m), we suggest that such transition may have happened through depth sympatry in  
501 tropical waters, with forms which remained reproductively isolated in the twilight zone generating  
502 the *G. merotumida-tumida* lineage. Morphometric measurements on *M. limbata* and *G.*  
503 *merotumida* shells are required to test for an evolutionary relationship between these two species.  
504 According to our depth habitat reconstruction, *G. plesiotumida* and *G. tumida* occupied a similar  
505 depth habitat at 4.5 Ma, so it is not clear from our dataset which evolutionary mechanism may  
506 have led to the origination of the latter from the first. Hull and Norris (2009) analyzed speciation  
507 within this lineage and suggested that the evolution from *G. plesiotumida* to *G. tumida* happened

508 abruptly within 44 kyr. *Globorotalia ungulata*, appears in our record by the late Pliocene and often  
509 display a habitat shallower than *G. tumida*, suggesting depth sympatry as the evolutionary  
510 mechanism leading from *G. tumida* to *G. ungulata*. However, because depth habitat  
511 reconstructions for these two species are more variable and shallower than that of other twilight  
512 zone species, more data are required to more conclusively infer speciation mechanisms.

513         The globoconellids originated in the late early Miocene (~17 Ma) with *Globoconella*  
514 *miozea*, which is considered to descend directly from *Hirsutella praescitula* (Kennett and  
515 Srinivasan, 1983; Scott et al., 1990; Norris et al., 1994; Aze et al., 2011). *Globoconella miotumida*  
516 originated in the middle Miocene and, after the extinction of *G. miozea* at about 10 Ma, remained  
517 the only representative of the globoconellids until the latest Miocene. At this time, the evolutionary  
518 turnover within the group accelerated and a number of different morphospecies originate from *G.*  
519 *miotumida* and go extinct very rapidly, until the appearance in the late Pliocene of *G. inflata* which  
520 persists until today (Wei and Kennett, 1988; Wei, 1994; Aze et al., 2011). Compared to other  
521 Neogene to Recent taxa, the globoconellids display a more restricted geographic distribution  
522 throughout their evolutionary history. They tend to be common at mid latitude hydrographic fronts  
523 (Schneider and Kennett, 1999; Schiebel and Hemleben, 2017; Lam and Leckie, 2020; Brombacher  
524 et al., 2021), except *G. puncticulata* and *G. inflata*, which extend into low latitude regions (Norris  
525 et al., 1994). The geographic distribution of globoconellids as shown here suggests that this group  
526 was already specialized to live at hydrographic fronts in the middle Miocene, possibly feeding on  
527 phytoplankton. Starting at ~5.5 Ma, cooling and the possibility to feed on sinking detritus in deeper  
528 waters (Boscolo-Galazzo, Crichton et al., 2021) may have stimulated evolutionary turnover within  
529 this otherwise rather static group. The closely spaced temporal succession of morphospecies at this  
530 time may reflect ongoing evolutionary experiments in an attempt to profit from new ecological

531 possibilities opening up at depth and outside the area of the group. The depth habitat  
532 reconstructions for *G. puncticulata* and *G. inflata* suggests that from the Pliocene this group started  
533 to progressively adapt to greater depths, consistent with the distinctive change in morphology  
534 between *G. sphericomiozea* (and other Miocene globoconellids) and its Pliocene descendants *G.*  
535 *puncticulata* and *G. inflata* (Kennett and Srinivasan, 1983), as well as with previous stable isotope  
536 reconstructions for these species (Schneider and Kennett, 1996). We suggest that an evolutionary  
537 transition began with the morphospecies *G. puncticulata*, transitional between *G. sphericomiozea*  
538 and *G. inflata* and led to the late Pliocene speciation of *G. inflata*. It is not clear from our data  
539 whether depth sympatry or allopatry allowed the speciation of *G. inflata*, as *G. puncticulata* and  
540 *G. inflata* show similar depth habitat in our record. It may have been a combination of the two,  
541 given *G. inflata* genotypes display a characteristic allopatric distribution in the ocean (Morard et  
542 al., 2011).

543 Our data indicate that combining stable isotopes and model-derived water column  
544 temperature is a promising approach to quantify the depth habitat of extinct planktonic  
545 foraminiferal species. When combined with abundance and biogeographic data, depth habitat  
546 reconstructions offer insights into speciation mechanisms not resolvable with the use of one  
547 technique alone (e.g., stable isotopes). Our reconstructions indicate that both allopatry and depth  
548 sympatry played a role in the origin of modern deep-dwelling planktonic foraminiferal species.  
549 Both allopatry and depth sympatry appear to have been involved with the cladogenesis of the  
550 truncorotaliid and the globorotaliid lineages, while depth sympatry seems to be mostly involved  
551 for intra-lineage speciation.

552

## 553 **5. Conclusions**



554 Our global abundance and biogeographic data combined with our depth habitat  
555 reconstructions allow us to piece together the environmental drivers behind speciation in two of  
556 the most extensively studied group of pelagic microfossils, planktonic foraminifera and calcareous  
557 nannofossils over the last 15 million years. The evolution of the new Neogene deep-water lineages  
558 of the hirsutellids, globorotaliids, globococcolithids and truncorotaliids, and of nanoplankton lower-  
559 euphotic zone and sub-euphotic zone species, resulted in the vertical stratification of species seen  
560 in the modern ocean, in particular at low latitudes. For planktonic foraminifera such vertical  
561 stratification of species, hundreds of meters below the surface, originated through depth sympatry  
562 as well as cladogenesis of new lineages via both sympatry and allopatry.

563 Our study places the evolution of modern plankton groups in the context of large scale  
564 changes in ocean macroecology driven by the global climate dynamics of the past fifteen million  
565 years. The late Miocene to present evolutionary history of planktonic foraminifera and  
566 nanoplankton was linked, wherein increased efficiency of the biological pump with cooling since  
567 the middle Miocene was a shared evolutionary driver. Lower rates of organic matter  
568 remineralization in the upper part of the water column allowed the creation of new ecological  
569 niches in deep waters, by increasing food delivery and oxygen at depth for heterotrophic planktonic  
570 foraminifera, and by clearing surface waters and augmenting the concentration of macronutrients  
571 at depth for nanoplankton.

572

573 **Data availability:** All data associated with this article are available in the Supplement or have  
574 been previously published. The code is tagged as v0.9.18 and is available at DOI:  
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576

577 **Author contributions:**

578 Conceptualization, F.B.G. and P.N.P.; investigation, F.B.G. (planktonic foraminifera) and A.J.  
579 (nannofossils); software, K.A.C.; writing–original draft, F.B.G.; writing–review and editing,  
580 F.B.G., A.J., T.D.J, K.A.C., P.N.P., B.S.W.; visualization, F.B.G., A.J., K.A.C.; project  
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582

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584 The authors declare that they have no conflict of interest.

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592

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841 **Figure 1.** Depth-habitat reconstructions for middle Miocene to present planktonic foraminiferal  
842 species at the investigated sites. Surface dwellers (species living at depths shallower than 200 m)  
843 are indicated with a grey dot, deeper species are indicated with colored symbols. Relative size of  
844 symbols represents the size fractions of the sample. Reproduced from Boscolo-Galazzo, Crichton  
845 et al. (2021).

846 **Figure 2.** Foraminiferal and nannofossil ecogroup abundance at Site 242 and U1482.

847 **Figure 3.** Foraminiferal and nannofossil ecogroup abundance at Site 871/872 and U1490.

848 **Figure 4.** Foraminiferal and nannofossil ecogroup abundance at Site U1338 and U1489.

849 **Figure 5.** Foraminiferal and nannofossil ecogroup abundance at Site 516 and 1138.

850 **Figure 6.** Planktonic foraminiferal and nannofossil ecogroups relative abundance and diversity  
851 indexes.

852 **Figure 7.** Abundance and biogeography of planktonic foraminiferal species at 15, 12.5, and 10  
853 Ma. In the pie-charts twilight zone planktonic foraminiferal species are in bold. Sites where  
854 twilight zone planktonic foraminifera were found are highlighted in green in the maps. The crossed

855 square symbol in the maps indicate that the time interval of interest was not recovered for a given  
856 site. Continental configuration follows: Ocean Drilling Stratigraphic Network (GEOMAR, Kiel,  
857 Germany).

858 **Figure 8.** Abundance and biogeography of planktonic foraminiferal species at 7.5 Ma. In the pie-  
859 charts twilight zone planktonic foraminiferal species are in bold. Sites where twilight zone  
860 planktonic foraminifera were found are highlighted in green in the maps. The crossed square  
861 symbol in the maps indicate that the time interval of interest was not recovered for a given site.  
862 Continental configuration follows: Ocean Drilling Stratigraphic Network (GEOMAR, Kiel,  
863 Germany).

864 **Figure 9.** Abundance and biogeography of planktonic foraminiferal species at 4.5 Ma. In the pie-  
865 charts twilight zone planktonic foraminiferal species are in bold. Sites where twilight zone  
866 planktonic foraminifera were found are highlighted in green in the maps. The crossed square  
867 symbol in the maps indicate that the time interval of interest was not recovered for a given site.  
868 Continental configuration follows: Ocean Drilling Stratigraphic Network (GEOMAR, Kiel,  
869 Germany).

870 **Figure 10.** Abundance and biogeography of planktonic foraminiferal species at 2.5 Ma. In the pie-  
871 charts twilight zone planktonic foraminiferal species are in bold. Sites where twilight zone  
872 planktonic foraminifera were found are highlighted in green in the maps. The crossed square  
873 symbol in the maps indicate that the time interval of interest was not recovered for a given site.  
874 Continental configuration follows: Ocean Drilling Stratigraphic Network (GEOMAR, Kiel,  
875 Germany).

876 **Figure 11.** Abundance and biogeography of twilight zone planktonic foraminiferal species at 0  
877 Ma. In the pie-charts twilight zone planktonic foraminiferal species are in bold. Sites where



878 twilight zone planktonic foraminifera were found are highlighted in green in the maps. The crossed  
879 square symbol in the maps indicate that the time interval of interest was not recovered for a given  
880 site. Continental configuration follows: Ocean Drilling Stratigraphic Network (GEOMAR, Kiel,  
881 Germany).

882 **Figure 12.** cGENIE model output for changes in Particulate Organic Carbon (POC) flux, and  
883 dissolved Oxygen in near-surface ocean waters from the middle Miocene to Present, with a  
884 temperature-dependent biological carbon pump. Inset map shows the modelled Present surface  
885 ocean temperatures. Depths are the middle of cGENIE's top three ocean layers.

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