

1 **Calcareous nannofossils and paleoclimatic evolution across the Eocene-Oligocene** 2 **transition at IODP Site U1509, Tasman Sea, Southwest Pacific Ocean**

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18 **Key Points**

- 19 • Significant changes in calcareous nannofossil assemblages occurred at the onset of the Eocene-
20 Oligocene transition (EOT; ~34 Ma)
- 21 • Calcareous nannofossil diversity decreased during the EOT and warm-oligotrophic taxa were
22 replaced by cold-eutrophic taxa
- 23 • Increased paleoproductivity and surface ocean cooling are inferred from the calcareous
24 nannofossil assemblage

25 **Abstract**

26
27 The Eocene-Oligocene transition (EOT; ~34 Ma) was one of the most prominent global cooling events of
28 the Cenozoic, coincident with the emergence of continental-scale ice-sheets on Antarctica. Calcareous
29 nannoplankton experienced significant assemblage turnover at a time of long-term surface ocean cooling and
30 trophic conditions, suggesting cause-effect relationships between Antarctic glaciation, broader climate
31 changes, and the response of phytoplankton communities. To better evaluate the timing and nature of these
32 relationships, we generated calcareous nannofossil and geochemical datasets ($\delta^{18}\text{O}$, $\delta^{13}\text{C}$ and %CaCO₃) over a
33 ~5 Myr stratigraphic interval recovered across the EOT from IODP Site U1509 in the Tasman Sea, South
34 Pacific Ocean. Based on trends observed in the calcareous nannofossil assemblages, there was an overall
35 decline of warm-oligotrophic communities, with a shift toward taxa better adapted to cooler more eutrophic
36 conditions. Assemblage changes indicate four distinct phases caused by temperature decrease and variations
37 in paleocurrents: late Eocene warm-oligotrophic phase, precursor diversity-decrease phase, early Oligocene
38 cold-eutrophic phase, and a steady-state cosmopolitan phase. The most prominent shift in the assemblages
39 occurred during the ~550 kyr-long *precursor diversity-decrease phase*, which has relatively high bulk $\delta^{18}\text{O}$
40 and %CaCO₃ values, and predates the phase of maximum glacial expansion (Earliest Oligocene Glacial
41 Maximum – EOGM).

Plain Language Summary

Around 34 Ma, Earth experienced a major glaciation in the southern hemisphere, leading to the formation of permanent continental-scale ice-sheets on Antarctica. The Antarctic glaciation is supposed to have been triggered by a gradual decline of carbon dioxide with tectonic changes playing a secondary role. The separation of Antarctica from Australia and South America, and thus the development of the cold Antarctic Circumpolar Current, may have further intensified the conditions necessary for glacial expansion. This new scenario had profound consequences on climate, with direct effects on the global oceans and marine biota. In the past decades, geoscientists have tried to understand the glacial, tectonic and climatic history of Antarctica, but many questions remain unresolved. In particular, what were the impacts on the oceans in key areas between the Southern Ocean (high-latitudes) and the Equator (low-latitudes), such as the Tasman Sea? We use changes observed in the fossil record of calcifying unicellular algae (calcareous nannofossils) and stable oxygen and carbon isotopes to reconstruct the past surface-ocean evolution of this area. Our results suggest that biological productivity increased as cooler surface-water conditions developed in the Tasman Sea. This was likely driven by invigorated northward-moving nutrient-rich bottom-waters formed in the colder circum-Antarctic area.

1 Introduction

The Eocene–Oligocene transition (EOT, ~34 Ma) was a major change in Earth surface systems characterized by a decrease in global temperatures and large-scale glaciation of Antarctica (Lear et al., 2000; Pearson et al., 2009; Zachos et al., 1996; 2001). The EOT is associated with significant changes in fundamental components of the Earth’s system, including sea level, atmospheric CO₂, orbital configuration, land to sea ratio, continental weathering rates, ocean chemistry, circulation and productivity, as well as changes in the role of many positive climate feedbacks, which triggered transient and permanent modifications in marine and terrestrial fauna and flora (e.g. Coxall & Pearson, 2007; Coxall & Wilson, 2011; Hutchinson et al., 2021). During the EOT, a significant paleoceanographic change occurred, both in the Equatorial Pacific Ocean and Southern Atlantic Ocean, leading to a deepening of the carbonate compensation depth (CCD) by more than 1 km (Coxall et al., 2005; Coxall & Pearson, 2007; Pälike et al., 2012; Taylor et al., 2023), which resulted in increased exchange of carbonate from shallow waters to the deep-sea domain.

As evident from benthic foraminiferal stable oxygen isotope ($\delta^{18}\text{O}$) records, the transition from late Eocene greenhouse to early Oligocene coolhouse conditions occurred in two prominent orbitally-paced increases in $\delta^{18}\text{O}$ that reflect a combination of deep ocean cooling and increased continental ice volume (Coxall & Pearson, 2007). Records such as Mg/Ca of benthic foraminifera and the distribution patterns of temperature sensitive dinoflagellates indicate that most of the cooling (~2°C; Lear et al., 2008) occurred during the first step (Step 1), whereas the second step, named the “earliest Oligocene oxygen isotope step” (EOIS, Hutchinson et al., 2021), recorded a major increase in Antarctic ice volume (Houben et al., 2012; Lear et al., 2008; Haiblen et al., 2019). By definition, the start of the EOT (34.50 Ma; Viganò et al., 2024a), which comprises Step 1 and the EOIS, is marked by the extinction of calcareous nannofossil *Discoaster saipanensis* (Hutchinson et al., 2021). Close to the start of the EOT, a transient interval of more positive $\delta^{18}\text{O}$ values, named “Late Eocene

79 Event” (LEE), is observed in some records and interpreted as a transient cooling and/or glaciation (Hutchinson
80 et al., 2021). The end of the EOT is marked by the top of EOIS (33.66 Ma; Viganò et al., 2024a) and the onset
81 of the earliest Oligocene Glacial Maximum (EOGM), an interval of positive $\delta^{18}\text{O}$ values that corresponds with
82 Chron C13n and is interpreted to reflect the onset of continental-scale ice sheets on Antarctica (Coxall et al.,
83 2005; Coxall & Pearson, 2007; Hutchinson et al., 2021; Liu et al., 2004; Miller et al., 1991; Zachos et al.,
84 1996).

85 Two groups of hypotheses exist to explain the onset and development of Antarctic ice sheets: (1) “the
86 ocean-gateway hypothesis” and (2) “the CO_2 hypothesis”. The “ocean-gateway hypothesis”, first proposed in
87 the late 1970’s, suggests that the opening of Southern Ocean gateways (Exon, Kennet, Malone et al. 2001;
88 Hodel et al., 2022, Livermore et al., 2007; Scher et al., 2015) allowed for a rapid intensification of the Antarctic
89 Circumpolar Current (ACC), which reduced poleward heat flux and thermally isolated Antarctica (Kennett,
90 1977; Sijp et al., 2004). Subsequent modelling and proxy studies suggest that a global decline in atmospheric
91 CO_2 drove global cooling and ice sheet growth at the EOT (Anagnostou et al., 2016; DeConto & Pollard, 2003;
92 Pagani et al., 2011; Pearson et al., 2009; Zachos & Kump, 2005), with ice-sheet feedbacks and changes in
93 paleoceanography playing a secondary role (Hutchinson et al., 2021; Sauermilch et al., 2021).

94 In addition to the information derived from geochemical proxies, fundamental insights on the paleoclimatic
95 and paleoceanographic evolution during the late Eocene – early Oligocene come from the spatiotemporal
96 distribution and abundances of calcareous nannofossils, mainly consisting of the fossil tests of
97 coccolithophores (Agnini et al., 2017). In modern oceans, haptophyte algae are important primary producers
98 of organic and inorganic carbon (Rost & Riebesell, 2004). These extremely abundant single-celled planktonic
99 algae form a calcareous test (coccosphere) composed of small (1-30 μm) calcite plates (Perch-Nielsen, 1985).
100 In virtue of their peculiar biological/physiological traits, calcareous nannofossils preserved in ocean sediments
101 are sensitive indicators of past oceanographic and climatic changes to the marine photic zone. Relatively few
102 studies have documented the calcareous nannofossil turnover during the late Eocene to early Oligocene interval
103 (Bordiga et al., 2015; Dunkley Jones et al., 2008; Fioroni et al., 2015; Jones et al., 2019; Persico & Villa, 2004;
104 Villa et al., 2008, 2014, 2021). This knowledge gap partly arises because deep sea sedimentary sequences
105 across this time interval often contain condensed sections that result from significant changes in calcium
106 carbonate preservation.

107 The shift from the late Eocene warmhouse to the coolhouse climate state of the EOT was accompanied by
108 a decrease in nannofossil diversity that reached a minimum in the early Oligocene (Bown et al., 2004),
109 coinciding with the lowest rates of nannoplankton evolution of the entire Paleogene (Haq, 1973). This diversity
110 decline has traditionally been interpreted to reflect global cooling (Aubry, 1992). The mechanism by which
111 global cooling induces calcareous nannofossil global diversity decline can be explained considering 1)
112 latitudinal (thermal) differentiation between sub-polar and equatorial regions, 2) inter-specific displacement
113 and/or 3) a combination of these factors. In the first scenario, strong latitudinal thermal gradients may have
114 hindered the formation of ecological refugia, thereby contributing to a decrease in diversity. In the second case,
115 the competition between diatoms and coccolithophores likely prevented coccolithophore diversification at
116 higher latitudes, where diatoms emerged as the main phytoplankton group (Bown et al., 2004; Falkowski et

117 al., 2004; Spencer-Cervato, 1999). The competitive advantage of diatoms leading to diversification over
118 coccolithophores may lie in their ability to cope with increased turbulence and nutrient supply in the upper
119 ocean, conditions that usually characterize glacial periods (Iglesias-Rodríguez et al., 2002; Tozzi et al., 2004).
120 These conditions, together with the absence of ecological refugia, may have contributed to the observed global
121 decrease in calcareous nannofossils during this time.

122 A complementary explanation for the nannoplankton diversity decline is the nutrient-controlled contraction
123 of the oceanic trophic resource continuum (TRC) resulting from enhanced eutrophic conditions (Hallock,
124 1987). The sustained eutrophication, although temporary (Coxall et al., 2011), and decreased temperatures
125 during this interval are considered the possible forcing factors for evolutionary turnover and sequential
126 extinctions observed among calcareous nannoplankton (e.g. Aubry, 1992). The long-term decrease in diversity
127 of calcareous nannofossils is mainly related to an increase in the extinction rates during the late Eocene, which
128 became more intense towards the Eocene-Oligocene boundary (EOB) indicating an increase in an
129 environmental pressure, such as a change in ocean thermal structure and/or increased nutrient availability (e.g.
130 Aubry, 1992; Bown et al. 2004).

131 At present, our understanding of the paleoceanographic evolution of the Tasman Sea and the calcareous
132 nannofossil response during the EOT is limited. The study of this part of the Pacific Ocean is particularly
133 relevant for at least three reasons: 1) it stands as one of the rare sites in the Southwest Pacific characterized by
134 near continuous deposition that remains unaffected by the Marshall unconformity (e.g. Carter, 1985; Carter et
135 al., 2004; Carter & Landis, 1972); 2) it lies in a critical position between equatorial and subantarctic ocean
136 currents (Sutherland et al., 2022); 3) it represents an ideal case study, embodying open ocean sediment
137 deposition with a well-established chronology, that is advantageous to facilitate correlation across extensive
138 geographic regions (Viganò et al., 2024a) and to potentially infer the impact of the opening of the Tasman
139 Gateway (TG) and the early establishment of the Antarctic Circumpolar Current (ACC) on marine biota. Here
140 we present highly resolved calcareous nannofossil and geochemical (bulk $\delta^{13}\text{C}$, bulk $\delta^{18}\text{O}$, % CaCO_3) records
141 from International Ocean Discovery Program (IODP) Site U1509 (Sutherland et al., 2019), spanning from the
142 late Eocene to the early Oligocene. We analyze changes in the diversity and abundance of the calcareous
143 nannofossil assemblages to reconstruct the local paleoclimatic evolution and connections with Antarctic
144 glaciation and regional paleoceanographic changes. Records generated at Site U1509 provide new insights into
145 how calcareous phytoplankton reacted to the onset of global coolhouse conditions, and contribute to a better
146 understanding of this transition in a key area.

147

148 **2 Material and methods**

149 **2.1 Paleogene marine sedimentary records in the Tasman Sea and IODP Site U1509**

150 The Tasman Sea lies between Australia, New Caledonia and New Zealand (Figure 1), and formed when
151 Zealandia rifted from Australia and Antarctica between the late Cretaceous and early Eocene, about 83 to 53
152 Ma (Gaina et al., 1998; Hu et al., 2022; Sutherland et al., 2017). Since its inception, sediments, mostly marine,
153 have accumulated on the underlying seafloor. The seafloor of the Tasman Sea area has a complex bathymetry

154 that includes prominent rises and basins, largely shaped during the initiation of the Tonga-Kermadec
155 subduction zone (Dallanave et al., 2020; Sutherland et al., 2020).

156 Initial drilling in the region encountered major hiatuses of variable durations from the late Eocene to early
157 Oligocene (Moore et al., 1978) that are associated with the widespread regional Marshall unconformity (e.g.
158 Carter, 1985; Fulthorpe et al., 1996). These hiatuses traditionally have been interpreted to reflect enhanced
159 seafloor erosion caused by intensification of bottom currents during the progressive opening of gateways for
160 the Southern Ocean and the Antarctic glaciation (Close et al., 2009; Sauermilch et al., 2019; Houben et al.,
161 2019; Kennett et al., 1972, 1975a). Extensive seismic studies across the Tasman Sea (Dallanave et al., 2020,
162 2022; Etienne et al., 2018; Sutherland et al., 2017) now suggest that these unconformities relate to major
163 vertical motions of the seafloor caused by tectonism.

164 Site U1509 (34°39.13'S, 165°49.66'E) (Figure 1) was drilled at 2911 m water depth in the Tasman Sea
165 during IODP Expedition 371, where the northeast base of Lord Howe Rise converges with the west margin of
166 New Caledonia Trough (NCT) (Sutherland et al., 2019). The estimated paleodepth for this site is lower bathyal
167 (2000 m) during the upper Eocene and slightly deeper (abyssal) during the Oligocene (Sutherland et al., 2019,
168 2022).

169 The 690 m thick sedimentary sequence recovered at Site U1509 via rotary drilling (Hole U1509A) can be
170 divided into two lithostratigraphic units (Sutherland, Dickens, Blum et al., 2019). Lithostratigraphic Unit I (0
171 - 414.57 m CSF-A) consists of ~415 m of Pleistocene to upper Paleocene calcareous ooze, chalk, and
172 limestone, and Lithostratigraphic Unit II (414.57 - 689.68 m CSF-A) consists of ~275 m of Paleocene to Upper
173 Cretaceous claystone. Three subunits are recognized in Lithostratigraphic Unit I. Subunit Ia (0 - 99.60 m CSF-
174 A) is ~100 m-thick and mainly consists of Pliocene to late Oligocene calcareous ooze and chalk with varying
175 foraminifera abundances. Subunit Ib (99.60 - 139.28 m CSF-A) is an upper to lower Oligocene greenish grey
176 calcareous chalk, with abundant nannofossils and common to abundant foraminifera (~40 m). Subunit Ic
177 (139.28 - 414.57 m CSF-A) consists of ~275 m of lower Oligocene to upper Paleocene calcareous chalk and
178 limestone with varying amounts of siliceous microfossils and chert nodules (Sutherland et al., 2019), and it
179 frames the current study. Here we investigated the 183.30 m to 267.30 m interval of Subunit Ic, where depths
180 are expressed in meters CSF-A (see <https://www.iodp.org/policies-and-guidelines> for depth conventions). This
181 depth interval has a revised age model (Viganò et al., 2024a) that is calibrated to the Geological Time Scale
182 2020 (GTS20; Gradstein et al., 2020), and generally agrees with shipboard interpretations (Sutherland et al.,
183 2019), but with higher biostratigraphic and magnetostratigraphic resolution. Deposition of this depth interval
184 occurred between the late Eocene and early Oligocene (35.29 to 30.57 Ma). Stratigraphically, the EOT lies
185 between 256.96 m (extinction of *Discoaster saipanensis*) and 246.75 m (top of EOIS) (Viganò et al., 2024a).

186 Contrary to other locations in the Southwest Pacific, Site U1509 documents a relatively expanded record
187 of the EOT with an average linear sedimentation rate of ~2 cm/kyr; although some recovery gaps are present,
188 they are particularly notable during the early Oligocene (Sutherland et al., 2019; Viganò et al., 2024a). Study
189 of the EOT at this site is thus a unique opportunity for two important reasons: (1) the stratigraphic continuity,
190 and (2) the subtropical location of Site U1509 (~45° S) close to the sub-Antarctic/Antarctic area where the
191 glaciation took place.

192 **2.1.1 Present-day oceanography**

193 At present the oceanography of the study area is influenced by the Antarctic bottom water and the shallow
194 wind-driven East Australian Current (EAC) (Ridgway & Dunn, 2003; Sutherland et al., 2018), which flows
195 along eastern Australia between ca. 18°S and 32°S (Oke, Roughan, et al., 2019), with seasonal variations in
196 strength in response to changes in basin-scale winds (Chiswell et al., 1997). At about 32°S, part of these
197 vortexes propagates into the central Tasman Sea, giving rise to the Tasman Front (TF) (Chiswell et al., 2015;
198 Sutherland et al., 2022) and the Subtropical Front (STF), which pass south of New Zealand (Bostock et al.,
199 2015). The TF represents a surface thermal boundary that periodically moves from north to south, located
200 between eastern Australia and northern New Zealand at 31°–36°S latitude (Oke, Pilo, et al., 2019; Oke,
201 Roughan, et al., 2019).

202 The STF is instead a distinctive surface-water mass present in the Indian, Pacific, and Atlantic oceans
203 generally below 40°S (Hamilton, 2006) and characterized by high primary productivity (Bostock et al., 2015
204 and references herein). The STF represents a dynamic boundary where warm subtropical waters mix with
205 cooler and macronutrient-rich subantarctic waters (Hamilton, 2006; Bostock et al., 2013). Further south, two
206 main circumpolar fronts constitute the ACC: the Subantarctic and Polar Fronts (Rintoul, Hughes, & Olbers,
207 2001).

208

209 **2.1.2 EOT paleogeography and paleocirculation**

210 The late Eocene – early Oligocene paleolatitude for the northern Zealandia Site U1509 is estimated to be
211 ~44–45°S (Dallanave et al., 2022), which is considerably further south than its present-day latitude,
212 approximately at 34.65°S. This is a result of extensive tectonic activities that led to the northward movement
213 of Australia and its separation from Antarctica since the late Eocene (e.g. Cande & Stock, 2004).
214 Approximately at the same time a major reconfiguration of basin and ridge topography occurred within the
215 Tasman Sea area, with the permanent subsidence (>2000 m) of the NCT caused by subduction initiation of the
216 Australia-Pacific convergent plate boundary (Sutherland et al., 2010).

217 At higher latitudes, as the buildup of ice sheets on east Antarctica began (e.g. Zachos et al., 1994) and the
218 opening of the TG occurred, cool surface waters developed south of the proto-STF (pSTF) as part of a
219 developing early-ACC (Hodel et al., 2022; Nelson & Cooke, 2001; Sauermilch et al., 2021), possibly reaching
220 both southern Australia and New Zealand (Kamp et al., 1990). In the latest Eocene, there is evidence of a gyre
221 known as the proto-Ross Gyre (pRG) in the Pacific sector of the Southern Ocean (Exon, Kennett, Malone et
222 al., 2000; Huber et al., 2004; Stickley et al., 2004), while in the Atlantic sector of the Southern Ocean, a proto-
223 Weddell Gyre (pWG) existed even earlier (Vernet et al., 2019) (Figure 1). The EAC, originated from tropical-
224 equatorial currents in the Indian Ocean and from the South Pacific Gyre (SPG), was likely the source of warm
225 waters bathing the Tasman Sea and the Campbell Plateau (Hodel et al., 2022). The opening of the TG in the
226 late Eocene allowed cool high-latitude Indian Ocean waters to mix with warmer southward-flowing subtropical
227 waters of the SPG (Murphy et al., 1986) (Figure 1).

228

2.2 Calcareous nannofossils data

229
230 A total of 124 tooth-pick samples were prepared for calcareous nannofossil assemblage analysis. The
231 average sampling resolution is every 66 cm, increasing to ca. 33 cm across the EOT. Samples were prepared
232 following the standard procedure for smear slide preparation (Bown & Young, 1998) and then analyzed using
233 a Zeiss transmitted light microscope (LM) with a 1250× magnification.

234 Relative abundance patterns (%) of the total assemblage were obtained counting at least 300 specimens per
235 sample. The taxonomy follows that presented by Aubry (1984, 1988, 1989), Perch-Nielsen (1985), Bown
236 (2005), Bown and Dunkley Jones (2012), and Newsam et al. (2017), except for *Dictyococcites* and
237 *Reticulofenestra* for which we use the taxonomy expressed by Agnini et al. (2014). Key calcareous nannofossil
238 datums can be placed into the biostratigraphic frame using three different Cenozoic zonation schemes, NP
239 (Martini, 1971), CP (Okada & Bukry, 1980), and CN of Agnini et al. (2014). The taxonomy of
240 reticulofenestrids is not well constrained due to the heterogeneity of this group (Young, 1990). We assign
241 broadly elliptical/subcircular coccoliths characterized by a faintly visible or imperceptible net in the central
242 area to *Reticulofenestra*, and elliptical forms with a relatively solid central plug closing the central area to
243 *Dictyococcites* (e.g. Backman, 1980; Perch-Nielsen, 1985; Haq, 1971; Young, 1990). The comprehensive
244 discussion of the calcareous nannofossil biohorizons used in these schemes and their ages at Site U1509 are
245 discussed by Viganò et al. (2024a).

246 Principal component analysis (PCA) was performed on the most abundant calcareous nannofossil taxa (%)
247 of the assemblage using the statistical software PAST (PAleontological STatistic; Hammer et al., 2001). PCA
248 is a multivariate statistical technique that reduces the dimensionality of large data sets so that the variance is
249 explained by a small number of principal components (PCs; Harper, 1999). The fundamental concept behind
250 PCA is to enhance the interpretability of large calcareous nannofossil datasets by reducing their variability
251 along PCs. This helps identify the major loading taxa and evaluate environmental factors affecting changes in
252 the calcareous nannofossil assemblage. For this analysis, we considered the following 15 taxa: *Chiasmolithus*,
253 *Clausicoccus*, *Coccolithus*, *Cyclicargolithus*, *Dictyococcites*, *Discoaster*, *Ericsonia*, *Isthmolithus*,
254 *Lanternithus*, *Reticulofenestra*, *Sphenolithus*, *Zygrabolithus*, *Helicosphaera*, *Cribozentrum*, *Blackites*. These
255 taxa comprise 98% to 100% of the assemblage, excluding “Reworking” (reworked specimens) and “Others”
256 (genera with extremely low abundances).

257 We also conducted an additional PCA focused solely on placoliths to assess their specific contribution
258 during the EOT. This supplementary analysis is particularly significant since placoliths dominate the
259 nannofossil assemblage at this site (86-98% of the total assemblage), thus potentially providing additional
260 insights beyond those obtained from the PCA on the entire assemblage. This analysis involved the following
261 18 taxa: *Cribozentrum*, *Chiasmolithus*, *Clausicoccus*, *Coccolithus pelagicus* (6-13 μm), *Cyclicargolithus*
262 *floridanus*, *Dictyococcites* (<4 μm), *Dictyococcites* (4-10 μm), *D. bisectus* (>10 μm), *D. cf. D. bisectus* (<10
263 μm), *D. cf. D. filewiczii*, *D. filewiczii*, *Dictyococcites hesslandii*, *Ericsonia formosa*, *Reticulofenestra* (<4 μm),
264 *Reticulofenestra* (4-10 μm), *Reticulofenestra* (10-14 μm), *Reticulofenestra daviesii*, *Reticulofenestra umbilicus*
265 (>14 μm).

266

2.3 Paleocology and diversity indices

267
268 Calcareous nannofossils are considered excellent paleoenvironmental proxies and paleoceanographic
269 indicators, due to their broad geographic distribution and sensitivity to paleoenvironmental parameters (e.g.,
270 Aubry, 1992; Bordiga et al., 2015; Bown, 1998; Dunkley Jones et al., 2008; Persico & Villa, 2004; Villa et al.,
271 2008; 2021; Wei & Wise, 1990). Key EOT calcareous nannofossil taxa were grouped according to similar
272 paleoecological traits, such as preference for warm-water oligotrophic and cold-water eutrophic conditions.
273 We considered taxa with firmly established ecological affinities. These studies relied on both relative changes
274 in the calcareous nannofossil assemblage and comparisons with various geochemical and paleoenvironmental
275 proxies. In particular, discoasters (e.g., *D. saipanensis* and *D. barbadiensis*) are considered warm-water
276 oligotrophs that are common in abundance at low-middle latitudes (Villa et al., 2008; Wei & Wise, 1990) and
277 *Ericsonia formosa* displays a clear preference for low-middle latitudes and it usually thrives in warm waters
278 (Monechi et al., 2000; Wei & Wise, 1990) and oligotrophic environments (Bralower, 2002; Tremolada &
279 Bralower, 2004; Villa et al., 2008). On this basis, we grouped these taxa as warm-oligotrophic water indicators.
280 Among the cold-eutrophic group, we included *Reticulofenestra daviesii*, *Chiasmolithus* and *Clausicoccus*
281 *subdistichus* group. The species *R. daviesii* is particularly abundant at high latitudes and considered as a cold-
282 eutrophic taxon (Fioroni et al., 2015; Monechi et al., 2000; Persico & Villa, 2004; Villa et al., 2008; 2014),
283 although Bordiga et al. (2017) proposed that this species may exhibit a more adaptable and tolerant behavior.
284 The genus *Chiasmolithus* is well-established as a cold-water taxon (Bukry, 1973; Persico & Villa, 2008; Villa
285 et al., 2014; Wei & Wise, 1990), possibly adapted to moderate to high nutrients environments (Aubry, 1992;
286 1998; Bralower, 2002). Recent observations from ODP Site 756 (Viganò et al., 2023a) indicate a cold-
287 eutrophic affinity of *Clausicoccus subdistichus* gr. (i.e., *C. subdistichus* and *C. fenestratus*).

288 The calcareous nannofossil diversity was estimated across the study interval using multiple diversity
289 indices, since the application of one single index likely oversimplifies the real biodiversity signal. The indices
290 adopted include the number of taxa (S), Dominance (D), Shannon-Weaver index (H) (Shannon & Weaver,
291 1949) and Evenness (E) (Buzas & Gibson, 1969). They are described in detail in the Supporting Information
292 (Text S2). A significant relationship exists between diversity (indicated by the H index) and environmental
293 stability. Low diversity and higher dominance (D) are often associated with more unstable, stressed, and
294 eutrophic environments or during periods of disturbance (Wade & Bown, 2006; Wade & Pearson, 2008). In
295 contrast, an assemblage with high evenness (E) has a relatively similar number of individuals in each species,
296 suggesting ecological stability or constant/stable environmental conditions. For this purpose, multiple diversity
297 indices were used as proxies to assess environmental stability and/or disturbance. Additionally, recognizing
298 the possible connection between taxonomic diversity and preservation, we include calcium carbonate content
299 (%) and additional information related to the nannofossil assemblages, such as the resistance or fragility of
300 particular species and their relative abundances, to ensure the overall preservation quality throughout the entire
301 interval.

302

2.4 Geochemical data

A total of 183 samples were powdered using an agate mortar and inserted into polyethylene capsules. For each sample, a mass of ~0.300 mg was weighed using a Mettler Toledo AT21 precision balance and placed into a borosilicate vial for analysis of bulk sediment carbon and oxygen stable isotope composition and calcium carbonate content (% CaCO₃). CaCO₃ content functions as a preservation indicator, helping to verify potential dissolution events along the studied interval. Simultaneously, $\delta^{13}\text{C}_{\text{bulk}}$ serves as a proxy for monitoring oceanic trophic changes and associated primary productivity as well as possible reservoir exchanges (e.g., Coxall & Wilson, 2011; Tagliabue & Bopp, 2008).

$\delta^{18}\text{O}_{\text{bulk}}$ primarily evaluates mixed-layer ocean conditions in term of temperature and glacial extension (Reghellin et al., 2019), and secondarily establishes correlations between these inferred conditions and observed changes in the calcareous nannofossil assemblages of the studied site. The oxygen isotopes measured in the bulk are also utilized to compare conditions in the mixed layer with those at the bottom, represented by the $\delta^{18}\text{O}$ benthic signature. Ultimately, $\delta^{18}\text{O}$ bulk data provides a valuable tool for correlation and evaluation of the completeness of the studied section based on the globally recognized stepwise pattern of oxygen isotopes, proving essential for site-to-site correlations (Viganò et al., 2023a).

The geochemical analysis was carried out at the University of Padova (Department of Geosciences) using a Delta V Advance Isotopic Ratio Mass Spectrometer equipped with a Gas Bench II device. Each sample was flushed with He5.5 and then treated with 10 drops of orthophosphoric acid (EMSURE ® ≥ 99%) for ca. 3 hours at 70°C. Isotopic values are reported in standard delta notation relative to the Vienna Pee Dee Belemnite (VPDB). For the calibration and the quality check, we used, respectively, two internal standards: Maq1 (white Carrara marble; $\delta^{13}\text{C}= 2.58\text{‰}$; $\delta^{18}\text{O}= -1.15\text{‰}$ VPDB) and Gr1 (marble; $\delta^{13}\text{C}= 0.68\text{‰}$; $\delta^{18}\text{O}= -10.44\text{‰}$ VPDB). On average, each run sequence comprised 45 samples, in addition to 12 Maq1 samples and 3 Gr1 samples, each one with a weight range between 0.200-0.250 mg. The precision of standards was better than 0.06‰ for $\delta^{13}\text{C}$ and better than 0.09‰ for $\delta^{18}\text{O}$, across all sample runs. Every run also contained a ramp of 8 samples of Maq1 with weights ranges from 0.050 to 0.500 mg equally distributed along the run. The Maq1 ramp samples allow the construction of a linear regression equation (based on the of CO₂ beam intensity - mV- and the standard weight - mg) to extrapolate CaCO₃ content of the investigated samples (Spofforth et al., 2010).

3 Results

3.1 Calcareous nannofossil assemblage records

Late Eocene to early Oligocene calcareous nannofossil assemblages at Site U1509 contain four main taxa, *Reticulofenestra*, *Coccolithus*, *Dictyococcites* and *Cyclicargolithus*. *Reticulofenestra* is the dominant component, with a mean abundance of ~67% across the ~5 Myr interval (Figure 2). *Reticulofenestra* specimens were subdivided into size groups (<4 μm, between 4-10 μm and 10-14 μm) to capture possible differences in the abundance behavior of these subgroups (Figure 3), as changes in coccolith size, observed both in recent and fossil taxa, can be linked to environmental variations (e.g. sea-surface temperatures, pCO₂, light availability or trophic load) (Finkel et al., 2010; Henderiks & Pagani, 2008; Ma et al., 2023). Additionally, coccolith-size shifts have been actively driven by selective pressures associated with Cenozoic climate

341 variations (Herrmann & Thierstein, 2012). The taxonomy of *Reticulofenestra* is typically intricate and
342 ambiguous, leading to the frequent use of size groups (e.g., Toffanin et al., 2011). The use of these groups in
343 paleoecology is also motivated by the fact that small reticulofenestrids are considered to be better adapted to
344 eutrophic conditions. In modern oceans, small-size reticulofenestrids tend to thrive in regions characterized by
345 high productivity (e.g. Hagino & Okada, 2001; Winter et al., 1994).

346 At Site U1509, the small, medium and large forms of *Reticulofenestra* have mean abundance values of
347 ~26%, ~35% and 0.65% within the total assemblage. The larger forms (10-14 μm) record a slight decrease in
348 abundance towards the top of the interval, medium-size forms (4-10 μm) have constant values throughout the
349 interval, whereas smaller forms (<4 μm) increase in abundance towards the top (Figure 3).

350 Other relatively abundant calcareous nannofossils show trends over the EOT interval. *Coccolithus*
351 represents the second most abundant taxon with a mean value across the study interval of 11.4% (Figure 2).
352 Again, its abundance pattern varies, with a maximum peak of 20.7% in the upper part, although average values
353 of ~13% are recorded between 267.30 and 240.90 m. *Dictyococcites* is a common component of the
354 assemblages (on average ~8%; Figure 2). As for the reticulofenestrids, three groups have been differentiated
355 based on their size (<4 μm , between 4-10 μm and 10-14 μm), and some specific taxa are also separated out
356 (*D. bisectus* >10 μm , *D. cf. bisectus* <10 μm , *D. filewiczii* and *D. hesslandii*). These taxa are characterized by
357 highly variable abundance through the study section (Figure 3), although the higher abundances are those of
358 *D. cf. D. bisectus* (<10 μm) and *D. bisectus* (>10 μm), with mean values of 4.2% and 1.8%, respectively. All
359 these taxonomic entities have been differentiated due to the distinct behavior documented in the literature (e.g.
360 Bown & Dunkley Jones, 2012; Wei & Wise, 1990). *Cyclicargolithus* shows a distinct up-core abundance
361 increase from ~2% in the lower part of the sections (below 217.36 m) to 8.4% in the overlying part of the
362 section (Figure 2). Other key components in the assemblage, although with lower overall relative abundances,
363 are detailed below. *Clausicoccus* (i.e., *C. subdistichus* and *C. fenestratus*) rapidly increases in abundance (from
364 7.1% to 11.5%) at 250.35 m with high values (~6.3% on average) that persisted up to 221.41 m and then
365 rapidly dropped to a mean value of 0.1%. *Ericsonia* is rare at the base of the section, from 267.30 to 247.49
366 m, with a mean value of 1.4%. Above 247.49 m, *Ericsonia* displays a scattered decrease in abundance.
367 Similarly, to *Ericsonia*, *Isthmolithus* is continuously present at the base of the interval with percentages <1.7%.
368 *Discoaster* is rare to frequent in the lower part of the study interval with a peak in abundance (5.5%) between
369 267.30 and 256.96 m. *Discoaster* progressively decreases stratigraphically upward to values <1.2%.
370 *Cribocentrum* displays a similar pattern with a positive peak of 2.3% at 265.485 m and then gradually
371 disappears. *Lanternithus* is a minor component of the lower Oligocene assemblage but displays a significant
372 positive peak in abundance (up to 5.3%) at 242.70 m near the base of Chron C13n. Other components of the
373 assemblage include *Blackites* (mean value 1.6%), *Sphenolithus* (mean value 1.5%), *Zygrhablithus* (mean value
374 1.1%), *Chiasmolithus* (mean value 0.4%), and *Helicosphaera* (mean value 0.1%). The category 'Others'
375 includes very rare and discontinuous taxa, e.g., *Bramletteius*, *Coronocyclus*, *Markalius*, *Pontosphaera*,
376 *Thoracosphaera*, *Umbilicosphaera* and indeterminate placoliths.

377 For the studied interval, at Site U1509, a low percentage (<1%) of the assemblage consists of middle-late
378 Eocene reworked calcareous nannofossils (Figure 2). Examples of these are *Chiasmolithus consuetus*, *C.*

379 *eoaltus*, *C. expansus*, *C. grandis*, *C. solitus*, *Cribozentrum isabellae*, *Discoaster lodoensis*, *D. multiradiatus*,
 380 *D. praebifax*, *D. kuepperi*, *Neococcolithus dubius*. Reworked specimens are mainly recorded in the upper part
 381 of the section, from 223.20 m to 183.30 m. The Supporting Information (Plates S1-S6) contains a compilation
 382 of LM microphotos displaying selected calcareous nannofossil taxa including reworked specimens. These
 383 images enable the identification of specific morphological features and the assessment of the preservation state
 384 of calcareous nannofossils.

385

386 **3.2 Principal component analysis and diversity index**

387 The cumulative variation captured by the principal components likely represents the majority of the
 388 variation of the original variables, assumed here to represent various environmental parameters. Therefore,
 389 PCA serves as a valuable tool for simplifying complex data, offering an approach not directly obtainable
 390 through time-series data or assemblage plots.

391 The PCA analysis (Q-mode) performed on the entire assemblage record provided two principal
 392 components, PC1 and PC2. PC1 is characterized by a high negative loading on *Reticulofenestra* (-0.36) and
 393 *Cyclicargolithus* (-0.30), whereas it is positively loaded on *Coccolithus* (0.37) and *Ericsonia* (0.35). PC2 is
 394 loaded by *Clausicoccus* (0.51), *Lanternithus* (0.42) and *Zygrhablithus* (0.41). *Discoaster* and *Ericsonia* are
 395 also significant (-0.22). PC1 scores gradually decrease up section, marked by a major shift at 257.10 m (Figure
 396 6). PC2 includes two gradual shifts toward more positive values at 257.10 m and at 250.50 m respectively. At
 397 240.90 m, PC2 starts to decrease.

398 The two main variance factors in the Q-mode PCA analysis conducted on placoliths, PC1 and PC2, account
 399 for 24.3% and 11.6% respectively. PC1 is characterized by high negative loading on *Reticulofenestra* <4 μm
 400 (-0.36), *Dictyococcites* 4-10 μm (-0.31) and *Cyclicargolithus floridanus* (-0.28), whereas high positive
 401 loadings are found for *E. formosa* (0.36) and *C. pelagicus* (0.28). PC2 is high positively loaded by
 402 *Clausicoccus* (0.51) and *Chiasmolithus* (0.43) and high negatively loaded by *Dictyococcites hesslandii* (-0.43)
 403 (Figure 7).

404 The Shannon H index and the number of taxa (S) show an overall decrease across the investigated section,
 405 from 2.32 to 1.20 and from 30 to 12, respectively. Dominance (D) and Evenness (e^H/S) have a mean value of
 406 0.24 and 0.33 and display a marked shift at 257.10 m and at 250.50 m, respectively (Figure 6).

407

408 **3.3 Relative changes in the calcareous nannofossil assemblage**

409 Changes and trends in the calcareous nannofossil data allowed us to identify four intervals and 2
 410 subintervals (1-4, Figure 4) across the ~5 Myr study record:

- 411 1) **Interval 1** (267.30 – 257.10 m)
- 412 2) **Interval 2** (256.81 – 250.50 m)
- 413 3) **Interval 3** (250.19 – 221.41 m), further subdivided into two sub-intervals:
 - 414 • **Interval 3A** (250.19 – 240.90 m)
 - 415 • **Interval 3B** (240.59 – 221.41 m)
- 416 4) **Interval 4** (213.60 – 183.30 m).

417 Distinguishing features of the four intervals are as follows.

418 **Interval 1.** Interval 1 is defined by relatively abundant rosette-shaped *Discoaster* and low abundances of
 419 *Clausicoccus* (0-3.5%). From the base to close to the top of this interval, *Discoaster* varies in abundance from
 420 1.4 to 5.5%. At 257.10 m, discoasters abruptly decrease, with values never exceeding 1.2% for the rest of the
 421 succession. Common components of Interval 1 also include *D. filewiczii* and *D. hesslandii*. Secondary
 422 components of this interval are *Cribrocentrum* (0-2.3%) and *Ericsonia* (0-4.8%).

423 **Interval 2.** Interval 2 displays a sharp decline of discoasters (at 257.10 m) that abruptly decrease with values
 424 never exceeding 1.2% for the rest of the succession, and a first slight increase of *Clausicoccus subdistichus* gr.
 425 During this interval, the assemblage of Interval 1 is gradually replaced by cold water meso/eutrophic taxa, as
 426 documented by the remarkable increase of *R. daviesii* from ~3 to 12%.

427 **Interval 3A.** Subinterval 3A is characterized by the sudden increase of *Clausicoccus subdistichus* gr. starting
 428 from 33.95 Ma (Bc; Viganò et al., 2024a). The average abundance of this taxon is 2.8% but positive peaks in
 429 abundance (up to 13%) are observed in this subinterval. *Lanternithus minutus* shows positive peaks (up to
 430 4.3%) during this subinterval.

431 **Interval 3B.** Subinterval 3B is characterized by a gradual decline of *Clausicoccus subdistichus* gr.,
 432 *Lanternithus* and *R. daviesii*. *Coccolithus* also displays a remarkable decrease, returning to pre-interval values
 433 at the onset of Interval 4.

434 **Interval 4.** Interval 4 is denoted by the virtual absence of *Clausicoccus subdistichus* gr. and a significant
 435 increase in the abundance of *Cyclicargolithus*. The Oligocene assemblages consist of few dominant taxa with
 436 high numbers of individuals (i.e., *Reticulofenestra* and *Cyclicargolithus*).

437

438 **3.4 Bulk sediment geochemical data**

439 **3.4.1 Carbonate content**

440 Interval 1 (267.30 – 257.10 m) is characterized by the lowest CaCO₃ content of the entire succession,
 441 varying between 53% and 84% and averaging 71%. Interval 2 (256.81 – 250.50 m) is characterized by the
 442 highest values, ranging between ca. 70% and 100%. Above this interval, in the uppermost ~3.4 Myr (Intervals
 443 3 and 4; i.e. from 250.19 to 183.30 m), the carbonate content has a mean value of 78.3% (\pm 6.8%). Therefore,
 444 the carbonate content increased during Interval 2 but not during the EOGM, in contrast to the findings of other
 445 sites, specifically in the Indian (ODP Site 756; Viganò et al., 2023a) and in the Pacific Ocean (Coxall et al.,
 446 2005; Coxall & Wilson, 2011).

447

448 **3.4.2 Carbon stable isotopes**

449 Bulk carbon stable isotope values range between 1.45‰ and 2.64‰ with an average value of 2.04‰ (\pm
 450 0.24‰ σ) (Figure 5). In the lower part of the section (Interval 1; 267.30 – 257.10 m), $\delta^{13}\text{C}$ values have an
 451 average value of 2.21‰ (\pm 0.15‰). The following Interval 2 (256.81 – 250.50 m) is characterized by slightly
 452 lower mean values (2.15‰ \pm 0.11‰). From 250.19 – 221.41 m (Interval 3), the $\delta^{13}\text{C}$ curve displays a trend to
 453 lower values with an average of 2.10‰ (\pm 0.23‰). In the lower part of Interval 3 (Subinterval 3A; 250.19 –
 454 240.90 m) several positive peaks reaching values up to 2.64‰ occur just above the base of Chron C13n. From

455 213.60 to 183.30 m (Interval 4), $\delta^{13}\text{C}$ values are relatively low and stable with a range of 1.45 to 2.46 and an
456 average of 1.82‰ ($\pm 0.18\text{‰}$).

457

458 **3.4.3 Oxygen stable isotopes**

459 Bulk oxygen stable isotope values vary between -0.11‰ and 0.78‰ across the studied interval, with a mean
460 value of $0.41\text{‰} \pm 0.21\text{‰}$ (σ). Interval 1 (267.30 – 257.10 m) is characterized by the lowest $\delta^{18}\text{O}$ mean values
461 ($0.10\text{‰} \pm 0.10$). Oxygen isotope values sharply increase by $\sim 0.4\text{‰}$ between ~ 256.5 and ~ 257.4 m, marking
462 the base of Interval 2 (256.81 – 250.50 m) (Figure 5). This shift is interpreted to correlate with the so-called
463 “Late Eocene Event” (LEE) recorded at Sites 522 and 1218 (Katz et al., 2008), which may represent a failed
464 glaciation or a transient cooling event (Hutchinson et al., 2021). The onset of this event is coincident with the
465 extinction of *D. saipanensis* as recorded at Site U1509 (34.50 Ma; Viganò et al., 2024a) and is in perfect
466 agreement with the data from Site 1218 (Coxall et al., 2005). The first step of increase in $\delta^{18}\text{O}$ values (Step 1)
467 was not clearly identified at Site U1509. Presumably, it lies in a recovery gap between cores 29R and 28R
468 (Figure 5). At the base of Interval 3 (250.19 – 221.41 m), a major positive $\delta^{18}\text{O}$ shift begins (EOIS) at 248.40
469 m (~ 33.8 Ma), roughly coincident with the increase in abundance of *C. subdistichus* gr., for which the Bc is
470 33.95 Ma (Viganò et al., 2024a). The base of EOIS has a $\delta^{18}\text{O}$ value of 0.28‰ while the top of this shift (33.66
471 ± 0.04 Ma; Viganò et al., 2024a) has a maximum $\delta^{18}\text{O}$ value of 0.72‰ . EOIS thus represents a rapid $\delta^{18}\text{O}$
472 increase (0.44‰) within the lowermost part of Chron C13n. The highest values of $\delta^{18}\text{O}$ are recorded within
473 Chron C13n, which roughly correlates with the global phase of maximum positive $\delta^{18}\text{O}$ values (EOGM)
474 (Hutchinson et al., 2021). Throughout Interval 4 (213.60 – 183.30 m), $\delta^{18}\text{O}$ values display an average value of
475 0.45‰ ($\pm 0.10\text{‰}$).

476

477 **4 Discussion**

478 **4.1 Paleoclimatic evolution**

479 At Site U1509, calcareous nannofossil assemblages reveal several shifts in the relative abundance and
480 diversity indices across the Eocene-Oligocene, suggesting a link between paleoclimate changes and the
481 response of the local phytoplanktonic community. The paleoclimatic interpretation inferred from diversity
482 indices and the PCA analysis received additional support by the relatively high CaCO_3 wt% (Figure 5), which
483 indicates that no significant dissolution occurred throughout the study interval. Dissolution can indeed impact
484 the composition of coccolith assemblages (Roth, 1994), resulting in diminished diversity values and potentially
485 misleading environmental interpretations (Viganò et al., 2023b). Thus, principal components (PC1 and PC2
486 scores) are interpreted to reflect changes in sea surface temperature and paleoproductivity, respectively, based
487 on the ecological preferences recorded for the primary loading taxa.

488 Notably, a significant change in the assemblage correlates with the base of Chron C13n, which in turn
489 roughly corresponds to the onset of the EOGM (max $\delta^{18}\text{O}_{\text{bulk}}$ values). Specifically, the decrease in warm-water
490 oligotrophic taxa (i.e., *D. barbadiensis*, *D. saipanensis* and *E. formosa*) is consistent with both enhanced
491 eutrophic conditions and decreased temperatures, with the assemblage shifts primarily controlled by
492 temperature and nutrients (Figure 6). The shift towards cooler and eutrophic assemblages appears to be a

493 consistent feature of the EOT (Viganò et al., 2023a), since published records from both low-middle and high
494 latitudes suggest increased nutrient availability and cooler conditions (Bordiga et al., 2015; Diester-Haass,
495 1995; Diester-Haass, Robert, & Chamley, 1998; Diester-Haass & Zahn, 1996, 2001; Dunkley Jones, 2008;
496 Fioroni et al., 2015; Jones et al., 2019; Persico & Villa, 2004; Villa et al., 2008, 2014, 2021). Based on trends
497 and shifts in the diversity indices, PCA scores, and paleoecological groups, we subdivided the study interval
498 into four phases: (1) late Eocene warm-oligotrophic phase, (2) precursor diversity-decrease phase, (3) early
499 Oligocene cold-eutrophic phase and (4) steady-state cosmopolitan phase.

500

501 **Late Eocene Warm-Oligotrophic Phase (35.29 to 34.50 Ma)**

502 This phase is constrained to the lower part of the section (267.30 – 257.10 m) and is dominated by warm-
503 water and oligotrophic taxa and by highly even and diverse assemblages. Positive peaks in warm-water taxa
504 suggest sustained late Eocene warmer and oligotrophic conditions and/or a pre-glaciated world. Species
505 richness recorded at Site U1509 is relatively high during this phase reaching 30 taxa, and changes significantly
506 up-section. The high values observed in the Shannon index values during this phase indicate a highly diverse
507 community structure. At the same time PC2 displays relatively low values, ranging from a minimum of -2.61
508 to a maximum of 0.46, likely reflecting a stable-warm and oligotrophic environment (Bown et al., 2004). The
509 presence of a highly diversified community, mainly characterized by warm-oligotrophic taxa, is consistent
510 with relatively low $\delta^{18}\text{O}$ values documented in the interval. The end of this phase is marked by the onset of the
511 Late Eocene Event (LEE), which is coincident with the extinction of the warm-water *D. saipanensis* (34.50
512 Ma; Viganò et al., 2024a) and denotes the base of the EOT. The extinction of the last representative of the
513 rosette-shaped discoasters can be interpreted as the first biotic response to cooling during the LEE (Dunkley
514 Jones et al., 2008; Hutchinson et al., 2021; Pearson et al., 2008) (Figure 6).

515

516 **Precursor Diversity-Decrease Phase (34.50 to 33.95 Ma)**

517 During this interval (256.81 – 250.50 m), the marked decrease in warm-oligotrophic taxa suggests either
518 extreme sensitivity of calcareous nannoplankton to minor perturbations in the thermo/nutricline, or a climate
519 threshold that was able to trigger dramatic upheavals in the nannoplankton community. The marked decline of
520 warm-oligotrophic taxa is likely related to a global transition towards cooler and higher nutrient conditions.
521 During the precursor diversity-decrease phase, nannoplankton rapidly reacted to the cooler and eutrophic
522 conditions displaying a contraction of the k-selected community and the expansion of select r-mode
523 opportunist taxa (MacArthur & Wilson, 1967; Pianka, 1970). Major changes in calcareous nannofossil
524 assemblages are also recorded by diversity indices, especially emphasized by the dramatic decrease in species
525 richness (number of taxa) and Shannon indices. The shift from the Eocene warm-oligotrophic community to
526 the Oligocene cold-eutrophic community occurred in a time window of ~550 kyr, suggesting a strong and
527 prompt response of the nannofossil assemblage to oceanographic changes.

528

529 **Early Oligocene Cold-Eutrophic Phase (33.95 to 32.24 Ma)**

530 In the lower part of this interval (250.19 – 240.90 m), PC1 and warm-oligotrophs significantly decrease
531 and PC2 progressively increases. This phase indicates a period of intense perturbation within the marine
532 ecosystem. At Site U1509, the acme intervals of *Clausicoccus subdistichus* gr. and *Lanternithus* represent a
533 distinct feature of the EOT that is considered to be related to a better adaptation of these taxa to the enhanced
534 eutrophication hypothesized for the EOGM. These acme intervals are temporary events lasting for ~1.78 and
535 1.17 Myr, respectively, and the specific ecological affinities of *Clausicoccus subdistichus* gr. and *Lanternithus*
536 allow these taxa to rapidly adapt to the new environmental conditions, becoming a fundamental component of
537 earliest Oligocene assemblages. During this phase, Shannon index values gradually return to pre-transitional
538 values, reaching new relatively stable values.

539 The early Oligocene cold-eutrophic phase, initiated in the uppermost part of Chron C13r (~33.95 Ma), is
540 interpreted to reflect deep changes in ocean circulation which led to an intensified nutrient availability in
541 surface waters, providing competitive advantage for cold eutrophic taxa. Positive peaks in cold eutrophic taxa,
542 such as *Chiasmolithus* and *R. daviesii*, are recorded within Chron C13n, which corresponds to extreme glacial
543 conditions (EOGM), suggesting a strong connection between climate and nanoplankton changes.

544

545 **Steady-State Cosmopolitan Phase (32.24 to 30.57 Ma)**

546 The steady-state cosmopolitan phase (213.60 – 183.30 m) is characterized by the persistent dominance of
547 taxa belonging to the cosmopolitan/survivor-opportunist group, such as *Reticulofenestra* and *Cyclicargolithus*.
548 Decreasing Shannon index values confirm discrete changes in the assemblage structure, as reported also by
549 the gradual decrease in the number of taxa. The virtually constant diversity values (H) documented during this
550 phase possibly reflect a more stable ocean configuration, suggesting a gradual shift to a ‘new’ adjusted state
551 with different stable assemblages.

552

553 **4.2 Comparative nannofossil analysis across high and mid-low latitudes**

554 Within any given region, the nature of the water masses and their relationship with calcareous
555 nanoplankton distribution and abundance, depends mainly on three closely interconnected factors: 1) the
556 paleolatitude of the region 2) the degree of isolation, and 3) the types of currents. The relationship between
557 paleolatitude and calcareous nannofossil distribution was meticulously documented by Wei and Wise (1990),
558 who demonstrated the establishment of pronounced latitudinal gradients by the middle Eocene in the South
559 Atlantic. A latitudinal thermal gradient was also previously identified by Kennett (1978) in the Southern
560 Ocean, where he recognized distinct plankton biogeographic provinces during the Cenozoic.

561 In the following sections, we aim to interpret our findings in light of the key outcomes derived from studies
562 conducted at high and mid-low latitudes on calcareous nannofossil assemblages.

563

564 **High-latitude Insights:** At Site 748 (Kerguelen Plateau), Villa et al. (2008) highlighted a notable shift in
565 the Temperate-warm-water taxa (Twwt) index indicating a sudden and significant cooling event concurrent
566 with the rapid $\delta^{18}\text{O}$ positive shift (here termed EOIS). This cooling event is followed by a general indication
567 of cool surface-waters throughout the Oligocene based on nannofossil assemblages.

568 In a more recent investigation covering the Atlantic (Maud Rise) and Indian (Kerguelen Plateau) sectors of
569 the Southern Ocean (paleolatitudes of ~65°-60°S), Villa et al. (2014) presented additional evidence supporting
570 a gradual cooling trend. A more intense cooling was deduced through the identification of the establishment
571 of a 'cool-water assemblage' at the base of Chron C13n. Interestingly, the authors noted the onset of a
572 significant Southern Ocean eutrophication as early as 36.23 Ma (GTS20) (lower Chron C16n.2n), persisting
573 throughout the Oligocene.

574 A noteworthy observation is that the eutrophication of the Southern Ocean began 1.7 million years earlier
575 than at Site U1509. This temporal discrepancy suggests a nuanced and gradual pattern of eutrophication
576 unfolding across different latitudes. The observed delay implies that the Southern Ocean experienced an earlier
577 phase of enhanced nutrient availability and increased biological productivity compared to Site U1509. Such
578 temporal variations in eutrophication initiation may be linked to diverse environmental dynamics and
579 oceanographic conditions prevailing at high and middle latitudes. Factors such as local climate, ocean
580 circulation patterns, and proximity to nutrient sources could contribute to the observed temporal lag of
581 eutrophication. Further investigations into the specific mechanisms driving these variations will provide
582 valuable insights into the complex interplay between environmental factors and biological responses across
583 different latitudinal zones.

584

585 **Regional Insights:** In the late Eocene, the central Tasman Sea, as evidenced by DSDP Site 283, displayed
586 indications of warm surface waters characterized by the presence of rare discoasters and high diversity of
587 calcareous nannofossils (Edwards & Perch-Nielsen, 1975). Conversely, ODP Site 1172, situated in the eastern
588 Tasmanian margin, exhibited a scarcity of late Eocene discoasters and a low diversity of calcareous
589 nannofossils, implying cooler water conditions (Exon, Kennett, Malone et al., 2001). These contrasting
590 findings suggest a surface-temperature gradient between the two sites during the late Eocene. It is likely that
591 warm-subtropical currents flowing southward were dominant at Site 283, while they failed to reach Site 1172,
592 leading to cooler water conditions (Kennett & Exon, 2004).

593 It is crucial to recognize that most previous nannofossil records from the Southwestern Pacific, including
594 data from Site 592 (southern Lowe Howe Rise; Kennett et al., 1982), are primarily qualitative and often
595 focused on shipboard biostratigraphic data. Consequently, past paleoenvironmental inferences at these sites
596 rely on the presence or absence of taxa, particularly warmth-indicating taxa (e.g., discoasters) rather than
597 quantitative distribution (%). At the Campbell Plateau (DSDP Site 277), paleotemperature reconstructions
598 based on the *Discoaster/Chiasmolithus* ratio indicate a warming trend from the late middle Eocene onwards,
599 followed by cooling in the latest Eocene (Waghorn, 1981; Kennett & Exon, 2004). The similarity of our data
600 with previous qualitative records from the Southwestern Pacific suggests the presence, during the late Eocene,
601 of a widespread warm subtropical proto-East Australian Current (pEAC) extending from the NCT to the central
602 Tasman Sea with few exceptions (e.g. Site 1172). This trend aligns with the proposed tropical temperatures at
603 middle-high latitudes during the middle and late Eocene (Liu et al., 2009; Bijl et al., 2010). Notably,
604 reconstructions based on radiolarian assemblages at the same sites indicate the existence of cold-water taxa
605 and endemic Antarctic taxa since the middle Eocene, but they distinguish the conditions observed at Site 283

606 (warmer) from those at Site 1172 (cooler) (Pascher, 2017; Pascher et al., 2015). The heightened sensitivity of
607 radiolarians to temperature variations allows for the presence of cold taxa even with extremely limited
608 temperature changes since the middle Eocene.

609 In summary, the paleotemperature reconstructions in the region align seamlessly with the nannofossil
610 dataset, suggesting consistently mild temperatures in the middle to late Eocene, possibly due to the southward
611 extension of the East Australian Current (EAC).

612

613 **Mid-low latitude Insights:** Bordiga et al. (2015) associated the progressive decline of the absolute
614 abundances of nannofossils during the EOT at Site 1263 (Walvis Ridge, Atlantic Ocean), to the Southern
615 Ocean cooling. Nannofossil data from Java (NKK1 borehole, equatorial Indo-Pacific) indicated a significant
616 drop in diversity coinciding with the extinction of rosette-shaped discoasters and a shift from strongly
617 oligotrophic to more mesotrophic conditions (Jones et al., 2019). A recent study from the Equatorial Indian
618 Ocean (Site 709) suggested increased sea-surface nutrient availability (starting from the base of Chron C15n)
619 possibly linked to the formation at high latitudes of nutrient-rich waters (Villa et al., 2021).

620 The major changes observed in the calcareous nannofossil assemblage at Site U1509, in terms of both
621 timing and nature, closely resemble those recorded in the Indo-Pacific (Java; Jones et al., 2019), in the
622 equatorial (ODP Site 711; Fioroni et al., 2015) and in the mid-latitude Indian Ocean (Site 756, Viganò et al.,
623 2023a), indicating a basin-wide Indo-Pacific response. This connection between low and middle latitudes
624 could be attributed to the establishment of a shallow marine connection between the southern Indian Ocean
625 and the Pacific across the South Tasman Rise (e.g., the Tasmanian Gateway), that could have triggered the
626 production of cold-eutrophic bottom waters near the beginning of the Oligocene (Stickley et al., 2004).

627

628 **4.3 Major changes in calcareous nannofossil placoliths**

629 The EOT represents a tipping point in the Cenozoic climate evolution, documenting the transition from
630 warm-oligotrophic to cold-eutrophic communities, dominated by a few opportunistic taxa. Data collected at
631 Site U1509 allow us to analyze the tempo and mode of this transition, as well as the biodiversity community
632 structure. However, the role of placoliths, the dominant component of calcareous nannofossil assemblages,
633 still remains undetermined. To resolve this, an additional PCA was performed on this component, which is
634 distributed in different quadrants (i.e. time) in the PCA biplot (Figure 7).

635 In particular, *C. pelagicus*, *Cribo centrum*, *D. hesslandii*, *D. filewiczii* and medium size (4-10 μm)
636 reticulofenestrads, as well as the above-mentioned *E. formosa* are distributed in the right lower quadrant in the
637 PCA plot suggesting an affinity for warm and oligotrophic conditions. Samples documenting the transitional
638 (i.e. the *precursor diversity-decrease phase*) and EOGM conditions are instead distributed in the right upper
639 quadrants and are characterized by the higher abundances of the following placoliths: *D. bisectus* gr., large-
640 sized reticulofenestrads (10-14 μm and >14 μm), *Clausicoccus*, *Chiasmolithus* and *R. daviesii*.

641 Finally, the earliest Oligocene assemblages, distributed in the left upper quadrant are dominated by small
642 reticulofenestrads (<4 μm), small to medium size *Dictyococcites*, and *C. floridanus*. This latter assemblage
643 supports the hypothesis that, once the steady-state cosmopolitan phase was reached, the new environmental

644 conditions, characterized by cold and nutrient enriched waters, persisted for at least 1.8 Myrs. Further support
645 for an early Oligocene eutrophication at Site U1509 is provided by the presence of small opportunist
646 reticulofenestrads (<4 μm), as observed at ODP Site 756 (Viganò et al., 2023a).

647

648 **4.4 Bulk and Benthic Stable Isotopes**

649 To improve the comprehension and applicability of bulk carbonate stable isotopes in paleoenvironmental
650 analysis, we have compared our bulk isotopic record from Site U1509 in the Southwestern Pacific with the
651 benthic reference record from the eastern equatorial Pacific Site 1218 (Coxall & Pearson, 2007; Coxall &
652 Wilson, 2011). Despite lower resolution through the sequence and the discontinuous recovery in the early
653 Oligocene, the $\delta^{13}\text{C}_{\text{bulk}}$ and $\delta^{18}\text{O}_{\text{bulk}}$ records at Site U1509 mirror the more complete and highly resolved
654 $\delta^{13}\text{C}_{\text{benth}}$ and $\delta^{18}\text{O}_{\text{benth}}$ profiles at Site 1218. The comparison of these two records highlights the well-known
655 vertical gradient existing between surface and bottom waters (e.g. Boyle, 1988). As expected, the absolute
656 values are heavier in the benthic records (e.g. $\delta^{18}\text{O}_{\text{benth}}$ 0–1.5‰) representing bottom water conditions, while
657 the lighter bulk sediment isotopic signal ($\delta^{18}\text{O}_{\text{bulk}}$ 0–0.8‰) primarily reflects sea-surface conditions. These
658 results likely provide further support to the idea that bulk stable carbon and oxygen isotopes derived from
659 sediments containing abundant and well-preserved nanofossils may be used as a proxy for mixed layer
660 conditions (Reghellin et al., 2015; 2019; Viganò et al., 2023b). Nevertheless, the importance of biological
661 fractionation (“vital effects”) of a single taxon still remains, and a global signature could be enhanced,
662 attenuated, or completely blurred by the ecological and metabolic behavior of different taxa belonging to the
663 same microfossil group. In addition, minor differences/issues can come from instrumental offsets when
664 comparing absolute stable isotopic values, even with the use of standards intended to mitigate this effect. To
665 fully grasp and address this offset, a comprehensive analysis necessitates the comparison of multiple bulk and
666 benthic records, which unfortunately are seldom available in the existing literature. A preliminary observation
667 indicates that the global benthic signal is discernible in the bulk record, suggesting a connection between the
668 observed patterns in the two profiles and global processes. A significant aspect supporting this interpretation
669 is that both the general trend and specific details such as minor cooling phases (e.g., LEE; Figure 5) can be
670 recognized in the bulk isotopic signal, mirroring those present in the benthic isotope curve.

671 In the sub-Antarctic Deep Sea Drilling Project (DSDP) Site 277, with a paleodepth between 2200–2800 m,
672 the C and O stable isotopes display the typical surface-to-deep gradient (Pascher et al., 2015) with benthic
673 (*Cibicidoides* spp.) records that significantly differ from the bulk and deep-dweller planktonic foraminifera
674 (*Subbotina* spp.) records, which instead perfectly overlap. In addition, $\delta^{18}\text{O}_{\text{bulk}}$ values across the EOT at Site
675 277 range from 0.44‰ to ~1‰ (Pascher et al., 2015), and are comparable, within instrumental error, to those
676 of Site U1509 (~0.2‰–0.7‰).

677 It is noteworthy that calcareous nannoplankton taxa inhabiting high latitudes (e.g., Site 277; Pascher et al.,
678 2015) demonstrate significant differences from those thriving at low-middle latitudes (e.g., Site U1509, this
679 study; Site 756, Viganò et al., 2023a). However, discernible differences in the isotopic profiles are not
680 apparent, implying that the vital effect is likely a minor contributor to the bulk isotope signature.

681

682 **4.5 Paleooceanographic evolution in the Southwestern Pacific**

683 The observed variations in biotic and geochemical proxies at Site U1509 indicate an initial change of the
684 Southern Ocean circulation during the latest Eocene, coinciding with the onset of the EOT. In the following,
685 we integrate our results into a broader context of regional to global climate specifically considering results
686 from radiolarian studies in the region (Pascher, 2017), the recent discoveries related to surface water currents
687 in the late Eocene (Sauermilch et al., 2021; Nooteboom et al., 2022) and the timing and duration of the
688 eutrophication in the equatorial Pacific (Coxall et al., 2011).

689 The late Eocene decline in warm-oligotrophic water taxa at Site U1509 aligns with available data from the
690 Tasman Sea, which generally indicates a late Eocene cooling. This cooling led to the northward expansion of
691 the proto-Ross Gyre (pRG) to the Campbell Plateau (Site 277) (Pascher, 2017), and the presence of distinct
692 latitudinal thermal gradients based on dinocyst associations (Bijl et al., 2018). Additionally, extensive non-
693 deposition occurred in the central (Site 283), southwest (Sites 281 and 1172), and northern Tasman Sea (Sites
694 206 and 207), possibly indicating strengthened northward flowing bottom currents and/or tectonic processes
695 related to subduction (Site 206 and 207, Sutherland et al., 2010). An exception was Site 592, approximately
696 200 km south of Site U1509, which, along with U1509, preserved a complete Eocene-Oligocene transition
697 (EOT) sequence.

698 The observed early Oligocene enhanced eutrophication at Site U1509 may be linked to a crucial aspect of
699 the late Eocene-early Oligocene paleoceanographic reorganization: the development of a proto-Antarctic
700 Circumpolar Current (pACC) (e.g. Kennett et al., 1975a; Sarkar et al., 2019; Sauermilch et al., 2021; Scher et
701 al., 2015). This evolution likely intensified nutrient transport by southern water masses, leading to heightened
702 upwelling systems at middle-high latitudes (Kennett et al., 1975a). However, recent models highlight that even
703 slight depth adjustments in ocean gateways significantly impact ocean circulation patterns and Antarctic
704 surface waters (Sauermilch et al., 2021). According to these models, the openings primarily result in the
705 weakening of oceanic gyres rather than the establishment of a robust pACC, which was notably weaker than
706 it is today.

707 In conclusion, the eutrophication lasting ~700 kyrs observed at Site U1509 and corresponding to the earliest
708 Oligocene Glacial Maximum (EOGM), strongly correlates with the enhanced productivity reported in the
709 Eastern Equatorial Pacific at ODP Site 1218 (Coxall and Wilson, 2011), lasting approximately ~500 kyrs and
710 associated with Chron C13n. This shared feature suggests a connection between low and middle latitudes,
711 likely attributable to the expansion of deep mixing at high latitudes and the presence of common nutrient-rich
712 waters.

713 Although not explicitly recognized, the decrease of warm and oligotrophic conditions, which are followed
714 by a drop of calcareous nannofossil diversity and by the progressive increase of cold and eutrophic conditions,
715 are indeed evident and documented in numerous available datasets, indicating a significant and coherent global
716 evolution during the late Eocene to early Oligocene, as well as a strong teleconnection between low, middle,
717 and high latitudes (Coxall & Wilson, 2011; Cramer et al., 2009; Dunkley Jones et al., 2008; Houben et al.,
718 2013; Salamy & Zachos, 1999; Tibbett et al., 2021; Villa et al., 2021).

719

5 Conclusions

Nannofossil assemblage data from Site U1509 reveal a major shift from a late Eocene community characterized by warm and oligotrophic taxa, to an early Oligocene opportunistic assemblage, better adapted to colder and more eutrophic conditions. Our results suggest that this shift in the assemblage composition was driven by sea-surface water cooling and eutrophication caused by the inception of a proto-ACC, which resulted in the northward transport of cooler and nutrient-rich Antarctic waters. These mid-latitude changes highlight the impact of high latitude cryosphere changes on lower latitude ecosystems. The four paleoclimate intervals identified at Site U1509 reveal synchronous shifts in mid-latitude physical oceanography and marine phytoplankton communities. The most prominent turnover in the calcareous nannofossil assemblages took place during the *precursor diversity-decrease phase*, a transitional time interval of ~550 kyrs within the EOT.

In the Tasman Sea, the increase in marine productivity reached maximum values at the EOGM, as inferred from the high abundance of cold-eutrophic taxa and PC2 score, implying larger-scale effects on the nannoplankton community associated with sea ice formation and ocean overturning. However, nannofossil assemblage data indicate that increased nutrient availability and productivity may have persisted until at least ~32 Ma, although with reduced intensity. The Oligocene steady-state cosmopolitan phase is characterized by three dominant genera and by cold temperature and eutrophic conditions.

The role of the tectonic opening of the Southern Ocean gateways in the Antarctic glaciation is often considered neglected (DeConto & Pollard, 2003; Huber et al., 2004; Zhonghui et al., 2009); however our data highlights that evolving oceanographic conditions and invigorated oceanic circulation around Antarctica (Houben et al., 2019; Sauermilch et al., 2021), including deep and surface ocean cooling (Bohaty et al, 2012; Cramwinckel et al., 2018; Sauermilch et al., 2021; Sijp et al., 2011), undoubtedly influenced and shaped calcareous nannofossil assemblages during the EOT (e.g. Viganò et al., 2023a; Villa et al., 2021).

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755 **Open Research**

756 Data presented in this study are archived at PANGAEA (<https://doi.pangaea.de/>) under the CC-BYCC0:
757 Creative Commons Zero 1.0 Universal License. Calcareous nannofossil assemblage data (%) can be found in
758 Viganò et al. (2024b). Bulk stable isotopes and carbonate content are available in Viganò et al. (2024c).

759

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1225

1226 **Figure captions**

1227 **Figure 1** (a) Study interval at Hole U1509A: depth (m, CSF-A), epoch, core, recovery, image, and lithology
 1228 (Subunit Ic - calcareous chalk and limestone with biosilica and chert) are reported; (b) Present day location of
 1229 Site U1509 (black star) and other Exp. 371 Sites (U1508-1511) are reported (white stars) in the Southwest
 1230 Pacific. White circles = relevant DSDP and ODP sites (modified from Sutherland et al., 2018); (c) Polar-
 1231 centered paleogeographic reconstruction at 34 Ma (modified from Viganò et al., 2024a). Arrows represents
 1232 cooler (blue) and warmer (red) oceanic currents, as synthesized by Hodel et al. (2022) and compared with
 1233 recent findings from Sauermilch et al. (2021) and Nooteboom et al. (2022). The approximate position of the
 1234 pSTF is inferred from Nelson and Cooke (2001). Site U1509 (this study) and Site 756 (Viganò et al., 2023a)
 1235 (used for comparison) are reported. pACC: proto-Antarctic Circumpolar Current; pTF: proto-Tasman Front;
 1236 pSTF: proto-Subtropical Front; EAC: East Australian Current; pRG: proto-Ross Gyre; TG: Tasman Gateway;
 1237 DP: Drake Passage; pWG: proto-Weddell Gyre; pIOG: proto-Indian Ocean Gyre.

1238 **Figure 2** Relative abundances (%) of the main components of the calcareous nannofossil assemblages at
 1239 Site U1509. To the right, percentage of reworking and number of reworked specimens (n) in 1 mm^2 . To the
 1240 left, depth (CSF-A, m), recovery (Sutherland et al., 2019), magnetostratigraphy, calcareous nannofossil
 1241 biozonations (CP Zone: Okada & Bukry, 1980; NP Zone: Martini, 1971; CN Zone: Agnini et al., 2014),
 1242 planktonic foraminifera biostratigraphy (E Zone: Wade et al., 2011) and chronostratigraphy. Other nannofossil
 1243 genera (%) discussed in the main text are provided in the Supporting Information (Figure S1).

1244 **Figure 3** Additional relative abundances (%) of formal and informal (size) groups belonging to calcareous
 1245 nannofossil genera *Reticulofenestra* and *Dictyococcites* at Site U1509.

1246 **Figure 4** Synthesis of the featured calcareous nannofossil genera (%) along with bulk carbon and oxygen
 1247 stable isotope and carbonate (%) records at Site U1509. Different colored bands highlight the four intervals in
 1248 which calcareous nannofossils show significant abundance variations. To the left: depth (m, CSF-A), core and

1249 recovery (Sutherland et al., 2019), magnetostratigraphy, calcareous nannofossil (CN) Zone (Agnini et al.,
1250 2014) and chronostratigraphy. Bulk stable O and C isotopes and carbonate content (%) are shown with a 5-
1251 point running average.

1252 **Figure 5** Bulk stable isotopes (oxygen and carbon) and carbonate content (%) from IODP U1509 versus
1253 magnetostratigraphy, chronostratigraphy, calcareous nannofossil (CN) Zone (Agnini et al., 2014) and related
1254 EOT terminology. Tie-points are available Chron boundaries (Viganò et al., 2024a) calibrated to the GTS20
1255 timescale (Gradstein et al., 2020).

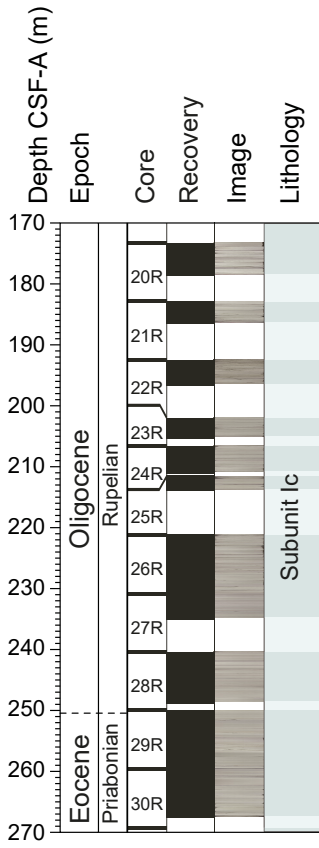
1256 **Figure 6** Warm oligotrophic taxa (%), cold eutrophic taxa (%), quantitative diversity indices (number of
1257 taxa, dominance, Shannon index and evenness), PC1 and PC2 scores, and geochemical data are plotted against
1258 core and recovery (Sutherland et al., 2019), magnetostratigraphy, calcareous nannofossil (CN) Zone (Agnini
1259 et al., 2014) and chronostratigraphy. PC1 and PC2 scores here refers to CN genera data (%). All plots are
1260 shown with a 5-point running average, except for PC1 and PC2 scores. The main paleoenvironmental phases
1261 are also reported.

1262 **Figure 7** Principal component analysis (PCA) plot of placoliths (%) from IODP Site U1509 in terms of the
1263 first and second component (PC1 and PC2). Placoliths are subdivided into 18 taxa.

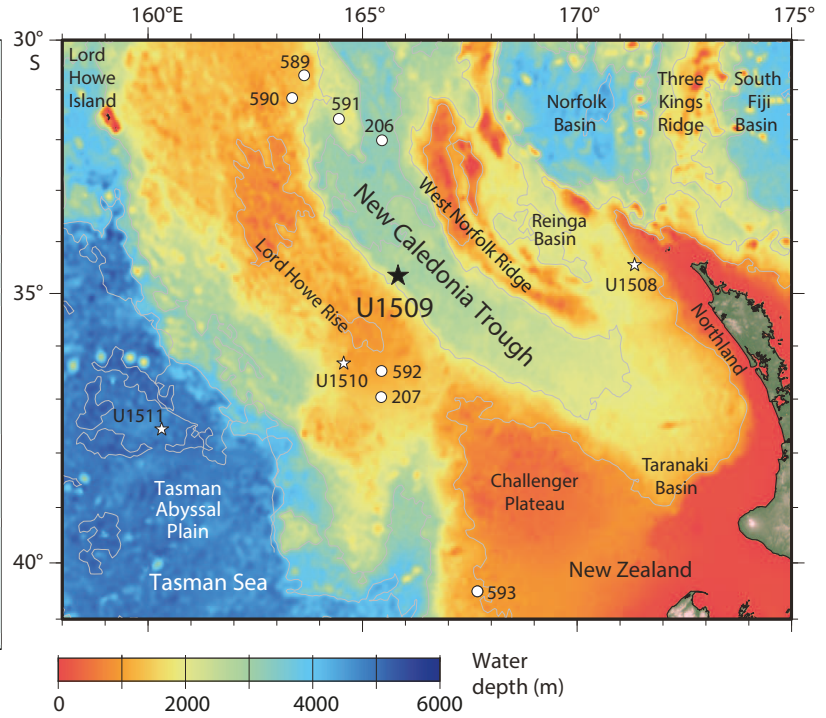
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(a) Hole U1509A



(b) Present day



(c) 34 Ma Reconstruction

