1	Orbital tuning for the middle Eocene to early Oligocene Monte Cagnero Section (central Italy):
2	paleoenvironmental and paleoclimatic implications
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23 Abstract

24 During the middle Eocene to early Oligocene Earth transitioned from a greenhouse to the-icehouse 25 climate state. The interval comprises the Middle Eocene Climatic Optimum (MECO; ~40 Ma) and a 26 subsequent long-term cooling trend that culminated in the Eocene-Oligocene transition (EOT; ~34 27 Ma), and with the Oi-1 glaciation. Here, we present a refined calcareous nannofossil biostratigraphy 28 and an orbitally tuned age model for the Monte Cagnero (MCA) section spanning from the middle 29 Eocene to the early Oligocene (~41 to ~33 Ma). Spectral analysis of magnetic susceptibility (MS) 30 data displays strong cyclicities in the orbital frequency band frequency allowing us to tune the 31 identified 405 kyr long eccentricity minima in the MS record to long eccentricity minimatheir 32 equivalents in the astronomical solution. Our orbitally tuned age model allows us to estimate the 33 position and duration of polarity chrons (C18 to C13) and compare them with other standard and 34 orbitally tuned ages. We were also able to constrain the timing and duration of the MECO event, 35 which coincides with a minimum in the 2.4 Myr and 405 kyr eccentricity cycles. Our study 36 corroborates the previous estimated age for the base of the Rupelian stage (33.9 Ma) and estimates 37 the base of the Priabonian stage in the MCA section to be at 37.4 Ma. Finally, calcareous 38 nannofossils with known paleoenvironmental preferences show a general gradual shift from 39 oligotrophic to eutrophic conditions with an abrupt change -dominance reversal at ~37 Ma. Besides, 40 nannofossil assemblages suggest that enhanced nutrient availability preceded water cooling at the 41 late Eocene. Altogether, this evidence points to a poorly developed water column stratification prior 42 to the cooling trend.

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Keywords: Neo-Tethys Ocean; Astrochronology; Trophic conditions; Eocene-Oligocene boundary;
MECO event

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47 Highlights:

• Magnetic susceptibility displays imprinted orbital signals used for orbital tuning

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- Orbitally tuned age model developed for the Middle Eocene to early Oligocene interval
- Refined calcareous nannofossil biostratigraphy has been established
 - Dominance reversal<u>Abrupt change</u> from oligotrophic to eutrophic conditions at ~37 Ma
 - Poorly developed water column stratification prior to the late Eocene cooling trend
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54 **1. Introduction**

55 The middle Eocene to early Oligocene was a key transition in Earth's climatic history 56 represented by the shift from a greenhouse to an icehouse climate, when global climate changed from 57 warm, comparatively ice-free conditions, to the colder, more glaciated state of the Neogene (Zachos 58 et al., 2008). This interval is characterized by a gradual long-term cooling trend in which there is a 59 superimposed important climatic event, known as the Middle Eocene Climatic Optimum (MECO; 60 Bohaty and Zachos, 2003; Jovane et al., 2007a; Bohaty et al., 2009; Westerhold et al., 2020). Unlike 61 of the hyperthermal events such as the Paleocene-Eocene Thermal Maximum (PETM), and the 62 Eocene Thermal maximum 2 and 3 (ETM2 and ETM3), the MECO is by far the more controversial 63 event to interpret (Bohaty and Zachos, 2003; Sluijs, 2013; Giorgioni et al., 2019). This ~500 kyr-64 long event, with the peak warming lasting <100 kyr and centered at ~40.0 Ma, is characterized by a global decline in oxygen isotopic records-values (δ^{18} O), being interpreted as a 4 to 6 °C warming of 65 66 bottom and surface waters, and an evident shallowing of the carbonate compensation depth (CCD; 67 Bohaty and Zachos, 2003; Bohaty et al., 2009). Despite this warming event, the global cooling trend 68 continues continued until it reached its maximum during the Eocene-Oligocene transition (EOT) at ~34.0 Ma (Jovane et al., 2007b; Westerhold et al., 2014). δ^{18} O values increase over 1.0% during the 69 70 transition (e.g., Kennett, 1977; Zachos et al., 1996; Coxall et al., 2005) and are interpreted as an 71 Antarctic ice-sheet growth of 60 to 110% of the present volume and a 3 to 4 °C bottom waters cooling (Zachos et al., 1996, Lear et al., 2008). Concomitant to the onset of Antarctic glaciation, a >1 72 73 km drop in the CCD occurred (Coxall et al., 2005). Both the ice-sheet growth and the CCD 74 deepening occurred in two pulses lasting ~40 kyr each and positioned 300 to 400 kyr apart, pointing

75 to a probable orbital pacing (Coxall et al., 2005). The cause of this major global climate 76 reorganization is classically interpreted to be the thermal isolation of Antarctica due to the onset of 77 Antarctic Circumpolar current, as a consequence of the Southern Ocean gateways opening (e.g., 78 Kennett, 1977; Kennett and Exon, 2004; Barker et al., 2007). A more recent hypothesis for a main 79 driving factor suggests that a pCO₂ threshold was crossed (e.g., DeConto and Pollard, 2003; Ladant 80 et al., 2014), but the reason why pCO_2 declined is vet unresolved. A possible explanation is that the 81 Arabia-Eurasia collision and closure of the Tethys Ocean gateway triggered different mechanisms 82 that reduced atmospheric pCO_2 (Allen and Armstrong, 2008; Jovane et al., 2009).

83 Astronomically driven climatice forcing fluctuations can be preserved as changes in the 84 sediment properties within the stratigraphic record and the correlation of this cyclic variations 85 recorded in climate proxies to astronomical models (extensively known as astronomical tuning) has 86 become a standard tool for calibrating different epochs of the Geologic Time Scale (GTS) (e.g., 87 Gradstein et al., 2012). The astronomical time scale (ATS) covered almost the entirely Cenozoic 88 except for the middle-late Eocene, which is known as the middle-late Eocene gap (Hilgen et al., 89 2012). This is probably due to the CCD shallowing, which made carbonate-rich successions scarce 90 for this time interval (Pälike et al., 2012). Although there are numerous studies attempting to close 91 this gap (Jovane et al., 2010; Westerhold and Röhl, 2013; Westerhold et al., 2014, 2015; Boulila et 92 al., 2018) there is not yet a consensus on the solution. Despite these discrepancies, recognizing 93 Earth's astronomical cycles in sedimentary records and placing them in time by the astronomical 94 tuning approach is yet one of the most powerful techniques due to its high temporal resolution 95 (Hinnov, 2013). These orbital cycles can lead to changes in chemical, physical, paleontological, and 96 sedimentological properties, and consequently, be detected in the sedimentary record by different 97 proxies. Among all, one of the widest currently used proxy is magnetic susceptibility (MS) due to its 98 rapid, low-cost, and non-destructive data acquisition (e.g., Kodama and Hinnov, 2014; Li et al., 99 2019a). Since its precursor studies, MS proved to be an effective and reliable technique for cyclostratigraphy, especially when apply to marl-limestone successions (Boulila et al., 2008a, 2008b,
2008c).

102 The Monte Cagnero (MCA) Section is the Global Boundary Stratotype Section and Point 103 (GSSP) for the base of the Chattian stage (28.1 Ma; Coccioni et al., 2018). Besides, ilt comprises a 104 pelagic carbonate succession made of marl-limestone alternations, which characterizes a similar is 105 within the same geological stratigraphic setting as with the those of important near sections, such as 106 the Massignano Section, GSSP for the base of the Rupelian stage (33.9 Ma; Premoli Silva and 107 Jenkyns, 1993) and Alano Section, recently ratified GSSP for the base of the Priabonian stage (37.7 108 Ma; Agnini et al., 2020). The orbital control on the MCA Section has already been demonstrated by 109 the cyclostratigraphic analysis on the EOT interval presented by from Hyland et al. (2009), which 110 showed the presence of Milankovitch related cycles during the EOT using in the CaCO₃ content 111 record. Our main goal is to extend this cyclostratigraphic approach towards the base of the MCA 112 Section covering the interval studied by Jovane et al. (2013), which spans the middle Eocene to early 113 Oligocene, and test whether or notwhether it is a suitable candidate section for studying the time 114 interval comprising the MECO to the EOT. Besides, another goal is to investigate paleoclimatic and 115 paleoenvironmental changes based on calcareous nannofossils assemblages.

116 Here, we developed a cyclostratigraphic analysis for the MCA Section using a high-117 resolution magnetic susceptibility record, which spans approximately 8 Myr from Chron C18n.2r 118 (middle Eocene) into the to Chron C12r (early Oligocene). Constrained by a radiometric date, The the 119 resultant cyclostratigraphic framework analysis allowed us to reliably was subsequently orbitally 120 tuned the MS record to the ZB18a astronomical solution (Zeebe and Lourens, 2019) at the long 405 121 kyr eccentricity level. The new orbitally tuned age model together with the calcareous nannofossils 122 data allowed us to refine constrain the age constraints for of biozones, polarity chrons, and climatic 123 events, and to discuss major paleoenvironmental changes within the studied interval.

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- 125 **2. Geological and stratigraphic setting**

126	The MCA Section (43°38'50"N, 12°28'05"E; 727 meters above sea-level) is a continuous
127	Paleogene sedimentary record preserved in the Scaglia limestone, which consists of pelagic
128	carbonate successions of the Umbria-Marche Basin, northeastern Apennines, Italy (e.g., Coccioni et
129	al., 2008; Hyland et al., 2009; Coccioni et al., 2013; Jovane et al., 2013; Savian et al., 2014; Coccioni
130	et al., 2018; Fig. 1a). The studied section ranges from 58 to 128 meters stratigraphic level from the
131	base (msl, 70 m thick) and comprises calcareous marl and marly limestone lithologies from the
132	Scaglia Variegata and Scaglia Cinerea formations. Based on previous magnetostratigraphy (Jovane et
133	al., 2013), the studied interval is correspondents to the middle Eocene - early lower Oligocene,
134	therefore spanning Earth's greenhouse-to-icehouse transition and and including important climatic
135	events such as the MECO and the Oi-1 (Fig. 1b). The stratigraphic interval corresponding to the
136	MECO event has been already well constrained at the MCA Section by Savian et al. (2014). The
137	EOT on the other could not yet been reliably characterized due to the lack of δ^{18} O data. Following
138	Hutchinson et al. (2021), we assumed here the EOT as a ~790 kyr event with its onset
139	stratigraphically correlated to the extinction of the calcareous nannofossil Discoaster saipanensis.
140	The Eocene-Oligocene boundary (EOB) is well established within the section at 114.1 msl defined
141	by the extinction of the planktonic foraminiferal Family Hantkeninidae (Premoli Silva and Jenkyns,
142	1993; Coccioni et al., 2008; Hyland et al., 2009; Jovane et al., 2013).



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Figure 1. Paleogeographic and paleoclimate settings. a) Paleogeographic reconstruction for 35 Ma (from http://www.odsn.de) with the approximate location of the MCA Section. b) Global compilation of Cenozoic $\delta^{18}O_{\text{benthic}}$ records (Westerhold et al., 2020) with a locally weighted smooth over 1 Myr (red curve) indicating the long-term cooling, which culminated in the Oi-1 glaciation event, and the rapid warming event of the MECO superimposed on this cooling trend.

150 **3. Material and methods**

151 3.1. Magnetic susceptibility

Low-field mass normalized magnetic susceptibility (χ, hereafter MS) is extensively used as a paleoclimate proxy for cyclostratigraphic analyses since it is a rapid, non-destructive, and low-cost method (Kodama and Hinnov, 2014). Besides, it is considered <u>as</u> a reliable method for resolving astronomical <u>astroclimatic</u> cycles that may be related to fine-grained terrestrial influx in mixed carbonate-clay successions, once increased susceptibility implies a relative increase of ferrimagnetic and/or paramagnetic minerals associated with terrigenous detrital input (e.g., Boulila et al., 2008a, 2008b, 2008c; Kodama and Hinnov, 2014; Li et al., 2019a). The MCA interval that extends from 58 to 72 msl has MS data available with ~5 cm resolution (Savian et al., 2014). Therefore, we opted for
measuring the interval between 72 to 128 msl at the same spatial resolution. MS measurements were
carried out at the National Oceanography Centre Southampton (NOCS). Measurements were
eolleeted-realized with a Kappabridge KLY-3 (AGICO) magnetic susceptibility metersusceptometer.
Combining both datasets, we generate a MS series with 1230 data points. Based on the average
sediment accumulation rate (SAR) derived from magnetostratigraphy (Jovane et al., 2013), the MS
series has an average temporal resolution of ~5.8 kyr.

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167 *3.2. Calcareous nannofossils*

168 Calcareous nannofossil samples have been collected every 25 cm for the lower part of the 169 section (58-78 msl) and every 50 cm up to 128 msl. Quantitative analyses have been performed on 170 180 samples. Smear slides were prepared from unprocessed samples according to standard 171 techniques (Bown and Young, 1998) and analyzed under a polarized light microscope at a 172 magnification of 1250X. Abundances were determined by counting 300 specimens of nannofossils in a variable number of visual fields. A supplementary counting has been performed on two traverses in 173 174 order to recognize rare important biostratigraphic markers. For the biostratigraphic events recognized 175 and the taxonomic criteria adopted we refer to Agnini et al. (2014 and references therein).

176 To statistically explore calcareous nannofossil data, we used a multivariate analysis approach. 177 Principal component analysis (PCA) was performed using the PAST (PAleontological STatistics) software (Hammer et al., 2001) in order to understand major changes in calcareous nannofossil 178 179 assemblages. Prior to the analysis, we grouped rare or discontinuous taxa at the genus level and 180 excluded the remaining taxa that presented less than 0.5% of standard deviation of their relative 181 abundances to eliminate rarely occurring species (Dunkley Jones et al., 2008). Relative abundances 182 were then rescaled to sum to 100% and zero values were replaced by non-negative values (0.01%). 183 Finally, we performed an additive log-ratio (ALR) transformation of relative abundances data using 184 Cyclicargolithus floridanus as the denominator, as it is one of the dominant species with continuous occurrences throughout the section. To test the reliability of the PCA outcome, we also performed aclassical Q-mode cluster analysis on the same dataset.

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188 3.3. Time-series analysis

189 Prior to time-series analyses analysis, MS series was log-transformed, detrended, linearly 190 interpolated, and resampled at 5 cm (depth-domain) and 6 kyr (age-domain) sampling resolutions. 191 Since tChe MCA Section has available calcium carbonate content (CaCO₃) measurements, data with 192 sampling resolution varying between 5 to 11 cm are available, from 58 to 72 msl (Savian et al., 193 2014) and from 108 to 128 msl; (Hyland et al., 2009; Savian et al., 2014).; coherence Coherence and 194 phase relationships were calculated between MS and CaCO3 records with the R-package "biwavelet" 195 (Gouhier et al., 2019) based on the MATLAB coherence and phase analysis tool (Grinsted et al., 196 2004). Spectral analyses, as well as data filtering, were processed in the Acycle software (Li et al., 197 2019b). Spectral and evolutionary spectral analyses of depth- and age-domain MS series were 198 conducted by applying the multitaper method (MTM) with five 2π prolate tapers (Thomson, 1982) 199 and with conventional AR1 red noise modeling, and with the R-package "astrochron" (Meyers, 200 2014). Evolutionary wavelet analysis was performed with the R-package "biwavelet" (Gouhier et al., 2019), based on the wavelet program written by Torrence and Compo (1998), and the evolutive fast 201 202 Fourier transform (FFT), respectively analysis was carried out with the Acycle software (Li et al., 203 2019b). To isolate orbital-linked periodicities, filtered signals were extracted "Astrochron" was also 204 used to extractusing Gaussian bandpass-filters of the MS series, windowsin order to isolate orbital-205 linked periodicities. The MS filtered signal was manually tuned to the ZB18a astronomical solution 206 (Zeebe and Lourens, 2019) in the QAnalySeries software (Kotov and Pälike, 2018).

207

4. Results

209 4.1. Magnetic susceptibility

210 Magnetic susceptibility measurements indirectly show the concentration and composition of 211 ferromagnetic, diamagnetic, and paramagnetic minerals (Kodama and Hinnov, 2014). These measurements vary between ~0.6 and 22.6 x 10^{-8} m³/kg, following mainly changes in lithology and 212 increasing towards the top of the section (Fig. 2). Since the section exhibits alternations between 213 214 marls and limestones, it is hypothesized that MS is inversely proportional to the CaCO₃. Therefore, we statistically compare the MS record to published CaCO3 records (Hyland et al., 2009; Savian et 215 216 al., 2014). Although there is not CaCO₃ data throughout the entire section, comparing the MS record 217 with the available CaCO₃ records allow us to obtain a reliable response, which covers about half the 218 length of the studied interval, with data at the base and the top of the section. Besides the visual 219 analysis, cross wavelet coherence and phase analysis from the two intervals also demonstrates that 220 MS varies in anti-phase with CaCO₃ content (Figs. S1 and S2). Furthermore, we extrapolate this 221 phase relation for the interval without CaCO₃ measurements in order to determine the phase relation 222 between MS and eccentricity at the tuning step.





Figure 2. MCA stratigraphic records from 58 to 128 msl spanning the middle Eocene through early Oligocene. From left to right: planktonic foraminifera (PF; Jovane et al., 2013) and calcareous nannofossil (CN; this study) biozonation following the schemes proposed by Wade et al. (2011) and Agnini et al. (2014), lithostratigraphic sequence, characteristic remanent magnetization inclination and magnetostratigraphic interpretation (Jovane et al., 2013), CaCO₃ content from Hyland et al. (2009; red) and Savian et al. (2014; green), magnetic susceptibility, and calcareous nannofossil events (this study). The most reliable magnetic polarity reversals are indicated by red stars.

Biostratigraphic uncertainty is represented by unfilled rectangles. B = base; T = top; Bc = basecommon; Tc = top common; X = crossover.

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234 4.2. Calcareous nannofossil biostratigraphy

Calcareous nannofossils are abundant throughout the section and moderately to poorly 235 236 preserved. The studied interval spans the Bartonian - early Rupelian, and most of the expected 237 calcareous nannofossil events have been identified. Table 1 summarizes the identified biohorizons 238 and their reliability throughout the studied interval, following the calcareous nannofossil biozonation scheme proposed by Agnini et al. (2014), which provides new events as substitutes for previous 239 240 standard schemes considered partly unreliable. A detailed description of each datum and the identification of classical biohorizons following the biozonation schemes proposed by Martini (1971) 241 242 and Okada and Bukry (1980) can be found in the supplementary material.

Depth (m; msl)			Nama	Diastrationarkia datawa	Datum confidence	
Тор	Bottom	Mid-point	Nanno zone boundary	Biostratigraphic datum	Datum confidence	
128.00	127.50	127.75	Base CNO2	T E. formosa	high	
125.50	125.00	125.25		B C. altus	high	
115.50	115.00	115.25		X C. subdistichus >/<5.5 μm	high	
114.50	114.00	114.25	Base CNO1	Bc C. subdistichus >5.5 μm	high	
109.00	108.50	108.75	Base CNE21	T D. saipanensis	high	
108.00	107.50	107.75		T D. barbadiensis	high	
105.00	104.50	104.75	Base CNE20	T C. reticulatum	high	
100.00	99.50	99.75		Bc I. recurvus	low	
91.00	90.50	90.75	Base CNE18	Tc C. erbae	high	
85.50	85.00	85.25	Base CNE17	Bc C. erbae	high	
84.00	83.50	83.75		B C. oamaruensis	low	
74.25	74.00	74.12	Base CNE16	T S. obtusus	high	
64.50	64.25	64.37		B S. obtusus	high	
58.75	58.50	58.62	Base CNE15	B D. bisectus	high	

243 **Table 1.** Calcareous nannofossil biostratigraphic datums for the MCA Section between 58 and 128

244 msl. B = base; T = top; Bc = base common; Tc = top common; X = crossover.

245

246 4.3. Calcareous nannofossil abundance changes and paleoclimatic events

247 Calcareous nannofossil assemblages show several fluctuations in abundance that were related

to environmental variability that characterized the middle Eocene to early Oligocene transition (Fig.

249 3). Different groups have been recognized in the MCA assemblages. The Coccolithaceae are

250 represented by the genera Clausicoccus, Coccolithus, Chiasmolithus and Ericsonia. Clausicoccus 251 (mainly small sized) is recorded as scarce (<1%) and discontinuous up to the middle part of the 252 section. From 100 msl up to the section Clausicoccus increases in size and abundance. The small 253 sized specimens (<5.5 µm) rapidly increase and show peaks of 5-7%, then they decrease in 254 abundance in proximity of the EOT and are replaced by larger forms of *Clausicoccus* >5.5 to 8 µm. 255 The genus *Coccolithus* consists of *C. pelagicus* and *C. eopelagicus*. The abundance record of the 256 group shows a decreasing trend above the MECO and then a slight increasing trend up to 100 msl. 257 Above this level, C. pelagicus becomes one of the major components of the assemblage (about 20%) 258 up to the end of EOT, afterwards a slightly decrease was is observed. The genus Chiasmolithus is 259 rare and discontinuously present throughout the section. Few species belonging to this genus are 260 identified, namely C. grandis, C. oamaruensis, C. solitus and C. altus. The low percentages (<1%) 261 and the sporadic occurrence prevent here their use as reliable biostratigraphic markers. Ericsonia 262 formosa is continuously present with higher abundances (around 10%) in the lower part of the 263 section within the MECO. The overall trend shows a marked decrease from 90 msl up to the top of 264 the section, where the Top of *E. formosa* (127.50 msl) has been observed.

265 The Noelaerabdaceae are represented by the genera Cribrocentrum, Cyclicargolithus, 266 Dictyococcites and Reticulofenestra. Cribrocentrum is present from the lower to the middle part of 267 the section, it reaches an acme and then drops in abundance at ~ 87 msl. This genus, characterized by 268 three marker species C. reticulatum, C. erbae and C. isabellae, shows very pronounced variations in 269 abundance from 1% up to 50% during the acme of C. erbae. Cribrocentrum reticulatum occurs from 270 the base and gets extinct at 104.75 msl, where it defines the base of CNE20 Zone. Cribrocentrum 271 erbae is continuously present from 76 msl, showing a rapid increase in abundance between 85.5 and 272 91 msl (marker of CNE17 Acme Zone). Cribrocentrum isabellae occurs rarely, with very low 273 abundances and seems to be an unreliable bioevent. Cyclicargolithus floridanus is among the main 274 calcareous nannofossil constituents of the MCA Section, and shows peaks of abundance (up to 48%) 275 during the MECO. The genus Dictyococcites is represented by the two species D. bisectus and D.

276 scrippsae. Dictyococcites bisectus occurs from the base with percentages around 10%, but very low 277 percentages (<1%) during the MECO have been observed. The abundance of Dictvococcites scrippsae vary abruptly (from <1% to 30%) during the MECO interval, but has also wide 278 279 fluctuations thereafter up to the top of the section. The genus Reticulofenestra comprises R. daviesii, 280 *R. dictyoda* group (specimens with length >5 and <14 μ m and specimens of *R. minutula* <5 μ m) and 281 R. umbilicus (>14 µm). Reticulofenestra daviesii is rare and shows sporadic occurrence in the lower 282 part of the section; it becomes continuous from 92 msl with an increase in abundance around the 283 EOT. Overall, R. dictyoda together with R. umbilicus show spikes of abundance during the MECO, 284 R. umbilicus shows a decreasing trend upwards, while R. dictyoda shows an increase in abundance during the EOT. 285

Discoaster, mainly represented by D. barbadiensis, D. saipanensis and D. deflandrei, 286 287 exhibits a clear decreasing trend from the lower part of the section upwards that culminates with the 288 extinction (Top) of the D. barbadiensis and D. saipanensis (rosette-shaped Discoaster). A sharp 289 decline of Discoaster is noticeable from 84.5 msl. Discoaster barbadiensis is commonly more 290 abundant than D. saipanensis and shows two peaks in abundance during the MECO. Following the 291 extinctions of the rosette-shaped Discoaster at 108 msl, the abrupt decrease in abundance of the 292 whole genus gives rise to a very evident and widespread paracme event, useful for biostratigraphic 293 purpose (Wei and Wise, 1990). Discoaster remain below their usual Cenozoic percentages for about 294 4 Myr, with an abundance of less than 1% in the upper part of the MCA Section. This paracme spans 295 from the top of the NP19-20 to the base of the NP24 (i.e., from C13r to C11n; Maiorano and 296 Monechi, 2006; Tori, 2008).

The genus *Sphenolithus* shows high percentages (roughly 30-40%) during the MECO and post MECO, while upwards a significant decreasing trend has been observed. Several species have been identified: *S. obtusus, S. moriformis, S. predistentus, S. radians, S. spiniger* and specimens that can be related to the *S. predistentus-distentus* lineage. The occurrence of *S. furcatolithoides* above its 301 range (CN14 Zone) can be interpreted as reworking or a final tail of distribution as evidenced in
302 several sections (Fornaciari et al., 2010).

303 Holococcoliths and, in particular, Lanternithus minutus and Zyghrablithus bijugatus are 304 present throughout the section showing several peculiar changes in abundance. Lanternithus minutus 305 occurs throughout the section, is rare during the MECO, shows a slight increase starting from 75.5 to 306 90 msl, then decreases rapidly above it and significantly rebounds at the top of rosette-shaped 307 Discoaster, below the EOT, and through the upper part of the section. Zygrhablithus bijugatus is 308 continuously and more or less commonly present, with peaks of abundance in the MECO interval 309 and up to 80 msl; above this level the abundances drop and then increases again below the EOT, with 310 a trend similar to L. minutus.

The genus *Helicosphaera* occurs discontinuously in very low percentages (<1%) gathering specimens referable to *H. compacta*, *H. euphratis* and *H. salebrosa*. The same occurs to the calcareous dinoflagellate *Thoracosphaera*, occurring discontinuously throughout the section with percentages <1%.





Figure 3. Relative abundance (%) of significant calcareous nannofossil groups and species against orbitally tuned ages. Planktonic foraminiferal (PF; Jovane et al., 2013) and calcareous nannofossil (CN; this study) biozonation following the schemes proposed by Wade et al. (2011) and Agnini et al. (2014), respectively. Black and red horizontal dashed lines indicate the Eocene-Oligocene boundary (EOB) and the *Discoaster* extinction event (DEE), respectively. <u>Blue interval indicates the EOT as a</u> ~790 kyr event started at the extinction of *D. saipanensis* (Hutchinson et al., 2021). <u>The oO</u>range interval indicates the MECO based on its stratigraphic constraint as proposed by Savian et al. (2014).

325 *4.4. Principal component analysis of abundance data*

826 The PCA allows for identifying the hypothetical variables that can explain most of the 327 variance enclosed in the analyzed dataset (Davis, 1986), highlighting groups of samples or taxa 328 which are associated to particular environmental changes. Working in the sample space (with taxa as 329 variables), we have gathered two new matrices, which respectively represent the spatial dispersion of 330 the samples (scores plot and scatter diagram) and the loadings of the taxa on the principal 331 components, respectively (PCs; see Fig. 4). PCs 1 and 2 of the transformed abundance data explain 332 48.8% and 12.1% of the variance, respectively. A cross-plot and ranked bar charts of PC1 and PC2 333 loadings display how the calcareous nannofossil taxa define groups of samples (Fig. 4). The PCA 334 scatter diagram displays three clusters that comprise four groups of samples corresponding to 335 different time-intervals (Fig. 4a). The groups are determined by assemblages' variations over time as 336 indicated by the taxa correlations (Fig. 4b) and probably associated to taxa evolutionary turnover and 337 paleoclimatic changes. The recognized clusters are the following: (i) a cluster with negative PC1 is 338 composed of both Bartonian (first group, blue dots) and Priabonian samples (second group, red dots) 339 from the Priabonian/Bartonian boundary up to Bc I. recurvus; (ii) a second cluster (third group, pink 340 dots) is composed of Priabonian samples from Bc I. recurvus to the Discoaster Extinction Event 341 (DEE); (iii) a third cluster (fourth group, green dots) comprises the samples from the DEE to the top 342 of the section. (Fig 4a).

343 Based on the PCA analysis, we can observe a clear change among the assemblages. Samples 344 from the interval comprising the Bartonian up to the Bc I. recurvus show a similar assemblage and 345 are characterized by strong negative PC1 values (mainly characterized by Cribrocentrum spp., 346 Discoaster spp. and R. umbilicus) and variable PC2 values. Thereafter, a "transitional" interval from 347 the Bc *I. recurvus* up to the DEE is associated with changes in the assemblage, with the occurrence 348 of *Clausicoccus, Blackites, R. dictyoda* <14 µm group, *Helicosphaera* + *Pontosphaera* group, and *R.* 349 daviesii, suggesting a cooler and mesotrophic environment. This interval is characterized by 350 increasing PC1 values, ranging from strong negative toward positive values, and also variable PC2 351 values. Finally, a completely different assemblage has been observed above the DEE characterized 352 by the strongest positive PC1 values and slightly variable PC2 values, suggesting a cooler and 353 eutrophic Oligocene interval (Fig. 4a). Comparable assemblage behavior in PCA analysis has been 354 observed at the DEE event by Jones et al. (2019).



Figure 4. Principal component analysis of the calcareous nannofossil abundance data. a) Cross-plot of PC1 and PC2 showing the four groups of samples. b) Bar charts of PC1 and PC2 loadings showing positive (blue) and negative (red) correlations.

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360 We also performed an alternative PCA analysis on the transposed data matrix (R mode) with 361 the samples as variables, in order to reduce the complexity of the relations among taxa. In this case, 362 PC1 and PC2 explain 60.4% and 17% of the variance, respectively. The PCA scatter diagram shows the dispersion of taxa (scores from PC1 and PC2) and explains the relation between most significant 363 taxa and their paleoecological preferences (Fig. 5). Thus, we can interpret PC1 as a proxy for sea 364 365 surface temperature, while PC2 probably represents nutrient availability. On this assumption and, in agreement with the literature, Cribrocentrum spp., R. umbilicus >14 μ m, Discoaster spp. and E. 366 367 formosa had a preference for a warm and oligotrophic environment (Monechi et al., 2000; Bralower, 2002; Gibbs et al., 2006; Angori et al., 2007; Villa et al., 2008; Schneider et al., 2011; Nyerges et al., 368 369 2020), while C. eopelagicus and C. protoannulus seemed to prefer a cooler and oligotrophic 370 environment (Dunkley Jones et al., 2008). Reticulofenestra daviesii had a preference for cold waters 371 regardless of nutrients (Wei and Wise, 1990; Monechi et al., 2000; Villa et al., 2008; 2014; Fioroni et 372 al., 2015) as well as the *Helicosphaera* + *Pontosphaera* group and *Blackites* spp. that were weakly 373 related to nutrients (slightly mesotrophic) and had a preference for cooler environments (Nyerges et 374 al., 2020). Sphenolithus had a preference for warm waters (Haq and Lohman, 1976; Wei and Wise, 375 1990; Aubry, 1992; Wei et al., 1992; Monechi et al., 2000; Bralower, 2002; Tremolada and 376 Bralower, 2004; Villa and Persico, 2006; Schneider et al., 2011) and was, according to our results, 377 weakly related to nutrients, while Z. bijugatus, L. minutus and D. bisectus were better adapted to warm/temperate waters and also weakly related to nutrient. L. minutus and D. bisectus seemed 378 379 slightly mesotrophic adapted. Dictyococcites scrippsae and C. pelagicus had a preference for warm 380 and eu-mesotrophic waters (Haq and Lohman, 1976; Bukry, 1981; Wei and Wise, 1990); while 381 *Clausicoccus* spp. had a preference for cool and eutrophic waters (Tori, 2008; Nyerges et al., 2020). 382 Reticulofenestra dictyoda group <14 µm had a preference for waters rich in nutrients regardless of 383 the temperature (Dunkley Jones et al., 2008; Jones et al., 2019). Therefore, the results of PCA 384 analysis allow us to define paleoenvironmentally significant groups based on the paleoecological 385 preferences of taxa (Table 2). These results are also confirmed by cluster analysis performed on the 386 same dataset (supplementary material; Figs. S3 and S4), where Z. bijugatus and L. minutus show an 387 additional strict relation, that according to the literature can be related to their preference for near-388 shore/shelf region (Monechi et al., 2000; Nyerges et al., 2020).



Figure 5. Cross-plot of PC1 and PC2 scores of calcareous nannofossil abundance data and itspaleoecological interpretation.

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	Sea surface water	Trophic conditions		
Warm-water taxa	Warm/Temperate- water taxa	Cool-water taxa	Oligotrophic taxa	Meso-eutrophic taxa
Sphenolithus spp.	E. formosa	Blackites spp.	Cribrocentrum spp.	Clausicoccus spp.
D. scrippsae D. bisectus		C. protoannulus	C. eopelagicus	D. scrippsae
C. pelagicus	Z. bijugatus	R. daviesii	<i>R. umbilicus</i> >14 µm	<i>R. dictyoda group</i> <14 µm
Discoaster spp.	Cribrocentrum spp.	Clausicoccus spp.	Discoaster spp.	C. pelagicus
	L. minutus	Helicosphaera + Pontosphaera	C. protoannulus	Blackites spp.
			E. formosa	L. minutus
				D. bisectus

Table 2. Inferred paleoenvironmentally significant groups based on the paleoecological preferences

394 of calcareous nannofossil taxa.

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396 *4.5. Orbital tuning*

The MCA entire succession consists of rhythmic alternation of calcareous marl and marly limestones with siliciclastic component made up by terrigenous clay and silt (Jovane et al., 2013; Coccioni et al., 2018). Because of the proximity, we adopted here the same interpretation of Jovane et al. (2006, 2010) for the Contessa and Massignano sections that limestone layers (high CaCO₃ and low MS) were deposited during cool and dry periods, while marl layers (low CaCO₃ and high MS) were deposited during warm and wet periods. Additionally, there is no evidence of large dissolution 403 except during the peak warming within the MECO event in the MCA Section (Savian et al., 2014). 404 Thus, it is reasonable to assume a negative relation between CaCO₃ and eccentricity based on the 405 interpretation that high eccentricity enhanced chemical weathering and runoff, which leads to an 406 increase in terrigenous input and, consequently, a relative decrease in the CaCO₃ content. Once we 407 have-demonstrated that MS reflects mainly CaCO₃ variability in an anti-phase relation (Figs. S1 and 408 S2), our adopted strategy was to tune the minima in the long eccentricity filtered MS to the long 409 eccentricity minima from the astronomical solution. The tuning process was constrained by an 410 available 206 Pb/ 238 U date of 33.291 ± 0.057 Ma for the MCA Section at 123.1 msl (Sahy et al., 2017). 411 Concerning the target curve, despite different astronomical solutions disagree before ~50 Ma due to 412 chaotic behavior of the solar system (e.g., Laskar et al., 2004, 2011a, 2011b; Zeebe, 2017; Zeebe and Lourens, 2019), they all closely agree during the studied interval. We thus opted to use the ZB18a 413 414 astronomical solution (Zeebe and Lourens, 2019) as the target curve, since it represents the most 415 precise and up to date solution.

416 To establish a reliable astrochronology, we tuned the MS series to the stable long eccentricity 417 geochronometer, which is related to the Venus-Jupiter resonance interaction (g2-g5) with a period of 418 405 kyr (Hinnov, 2018). Magnetostratigraphic interpretation from Jovane et al. (2013) gives a mean 419 SAR of 0.86 cm kyr⁻¹ for the studied interval. Based on this SAR, we can assume that the stable long eccentricity cycle (405 kyr) has a mean thickness of ~3.5 m. MTM spectrum and wavelet 420 421 analysisevolutive FFT of the MS series in the stratigraphic domain corroborates this assumption and 422 displays a stable and strong signal around this periodicity (Fig. 6 and S5). The MTM analysis shows 423 two distinct peaks within the interpreted long eccentricity bandwidth, at 0.19 and 0.30 cycles m⁻¹, 424 corresponding to periodicities of ~5.2 and ~3.3 m (Fig. 6). Using a Gaussian bandpass filter centered 425 at the average frequency between those peaks With a Gaussian bandpass filter (0.25 cycles m^{-1}), we 426 extracted the long 405 kyr eccentricity signal from the MS series and tuned it to the long eccentricity 427 signal from the ZB18a astronomical solution (Fig. 7). It is noteworthy that the evolutive FFT shows a 428 drastic shift in the long eccentricity signal towards a lower frequency in the interval between ~75 to

- 429 <u>~80 msl (Fig. 6), implying an increased SAR within this interval. Although abrupt, this higher SAR</u>
- 430 interval was already identified in the bio- and magnetostratigraphic age model from Jovane et al.
- 431 (2013). Once interpreting this interval as two long eccentricities cycles would result in an interval
- 432 with lower SAR compared to the surrounding interval, it was interpreted as a single long eccentricity
- 433 <u>cycle (Fig. 7).</u>





Figure 6. Spectral analysis of the MS series in the stratigraphic domain. a) Log-transformed, detrended and 5-cm interpolated MS series with low-frequency noise removed after high-pass filtering (cut frequency of 0.1 cycles m⁻¹). b) MTM power spectrum and estimated noise spectrum and confidence levels. Interpreted long-, and short-eccentricity, obliquity, and precession bandwidths are labeled as E, e, O, and P, respectively. Orbital cycles were interpreted based on the bio- and magnetostratigraphic age model from Jovane et al. (2013). c) Evolutive FFT with a 10 m sliding window.Evolutionary wavelet analysis of the MS series with the 405 kyr long eccentricity

- 443 interpreted based on the magnetostratigraphic SAR of 0.86 cm kyr⁻¹ (Jovane et al., 2013). Warm
- 444 colors indicate high spectral power, and the white shaded areas represent the "cone of influence",
- 445 where edge effects become important (Grinsted et al., 2004).







460 Furthermore, we intended to perform a fine-tuning at the short eccentricity level, but due to a 461 weak short eccentricity signal in the MS series, even after the 405 kyr tuned (Fig. 8), we opted to use 462 only the long eccentricity tuning in order to maintain the robustness of the MCA age model. This 463 weak signal is probably due to the fact that MS reflects mainly CaCO₃, which in some Neo-Tethys 464 sections, such as the Alano Section, show a weak short eccentricity signal (Galeotti et al., 2019). 465 Galeotti et al. (2019) suggested the sum of changes in runoff and tectonic activity (Doglioni and 466 Bosellini, 1987; Carminati and Doglioni, 2012) as a possible cause. Another possible cause is related 467 to the low SAR leading to a smoothing in the precession signal and this which, in turn, would have 468 obliterated the eccentricity signal, as shown at Blake Nose (Röhl et al., 2001, 2003) and Walvis 469 Ridge sites (Lourens et al., 2005; Westerhold et al., 2007), in the Atlantic Ocean. We thus suggest a 470 future astrochronological refinement study for the MCA using a different proxy in order to resolve 471 the short eccentricity signal, such as carbon isotope records, which seems to better resolve this cycle 472 (e.g., Galeotti et al., 2019; Giorgioni et al., 2019). Despite of it, spectral analysis of the resultant 400 473 405 kyr orbitally tuned MS series displays strong signals in orbital frequencies of long eccentricity, 474 obliquity, and precession (Fig. 8), suggesting that our orbital tuning is reliable. Another important 475 feature to support its robustness is that our estimated age based solely on the tuning process for 123.1 476 msl is 33.304 Ma, a difference of only 13 kyr to the available high-resolution radiometric date for the 477 same stratigraphic level (Sahy et al., 2017). The presence of a precessional signal suggests that the 478 absence of a reliable signal within the short eccentricity band is most likely explained by the first of 479 the two mentioned hypotheses. It is also worth noting the presence of a prominent obliquity signal in 480 the power spectrum (Fig. 8a), despite the relatively low latitude of the MCA Section. The evolutive 481 FFT analysis shows that the obliquity seems to exert more influence on the MS record mainly near 482 the EOT interval (Fig. 8b), an orbital configuration similar to those identified by Galeotti et al. 483 (2016) in high latitudes, and recently by Messaoud et al. (2020) in another Neo-Tethys section. The 484 reliability of our age model is also corroborated by the matching between the 2.4 Myr amplitude 485 modulation component of the resultant tuned MS series with the one from the astronomical solution



486 (Fig. 7). Furthermore, the SAR derived from the independent orbital tuning (Fig. 7d) agrees with the



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Figure 8. Power spectra of MS record on astrochronology. a) MTM power spectrum and estimated noise spectrum and confidence levels. Long- and short-eccentricity, obliquity, and precession bandwidths are shown by gray areas labeled as E, e, O, and P, respectively. b) Evolutive FFT with an 800 kyr sliding window.

494 **5. Discussion**

The developed astrochronologic time scale allows us to revise the previous MCA chronologic framework based on magnetostratigraphy (Jovane et al., 2013) and better constrain important events recorded within the section, such as the MECO, the EOT, and the Oi-1 event. Our long eccentricity tuning, together with new high-resolution calcareous nannofossil biostratigraphy, suggest that the MCA Section is complete, at least at the scale of hundreds of thousands of years.

500

501 5.1. Comparison of MCA chron positions and durations

502 Based on the most up to date magnetostratigraphic interpretation for the MCA (Jovane et al., 503 2013), together with our developed astrochronologic age model, new magnetochrons polarity chrons 504 boundaries ages and durations between chrons C18n and C13n have been estimated and compared to 505 standard and tuning-derived magnetic polarity reversals ages for the same interval (Tables 3 and 4). 506 Firstly, we point outhighlight the similarity between our estimated ages with the ones from Hyland et 507 al. (2009) for the common time intervals. This fact corroborates both, the consistency of the MCA 508 astrochronology, at least in the upper part of the section, and once again that MS represents mainly 509 changes in CaCO₃ content. On the other hand, due to ambiguous data from magnetostratigraphy (Fig. 510 2), there are ages and durations discrepancies in the lowest part, and also unidentified reversals in the 511 middle part of the section, which require a refined magnetostratigraphic analysis in order to solve 512 this issue (Tables 3 and 4). Despite these discrepancies, the reliable magnetostratigraphic data in the 513 lowest part of the section allow us to estimate the age of the onset of magnetochron C18n 514 based on astrochronology. The onset age of magnetochron-Chron C18n is relatively younger when 515 compared to classical standards and tuning-derived ages, but it coincides surprisingly well with the 516 age proposed by the recent middle Eocene astronomical timescale from Boulila et al. (2018; Table 517 3), even though the geological record studied has a controversial stratigraphic interpretation 518 (Vahlenkamp et al., 2020). Despite that, once Since it this reversal is positioned within the MECO

519 interval, this reversal ageit is also important to support our further time constraint of the event in the

520 MCA Section.

Chron	Standard GPTS					Astronomically calibrated						
	CK95 (Cande and Kent, 1995)	GPTS 2004 (Ogg and Smith, 2004)	GPTS 2012 (Ogg, 2012)	MQSD20 (Malinverno et al., 2020)	ODP Site 1218 (Pälike et al., 2006)	Massignano (Jovane et al., 2006)	Monte Cagnero (Hyland et al., 2009)	Contessa Highway (Jovane et al., 2010)	PEAT Sites (Westerhold et al., 2014)	IODP Exp. 342 composite U1408-U1410 (Boulila et al., 2018)	Monte Cagnero (this study ^a)	
C13n (y)	33.058	33.266	33.157	33.076	33.232		33.230		33.214		33.231	
C13n (o)	33.545	33.738	33.705	33.675	33.705		33.750		33.726		33.712	
C15n (y)	34.655	34.782	34.999	34.875	35.126	34.640			35.102		34.836	
C15n (o)	34.940	35.043	35.294	35.199	35.254	34.960			35.336		35.202	
C16n.1n (y)	35.343	35.404	35.706	35.627	35.328	35.343			35.580		35.540	
C16n.1n (o)	35.526	35.567	35.892	35.863	35.554				35.718		-	
C16n.2n (y)	35.685	35.707	36.051	36.054	35.643				35.774		-	
C16n.2n (o)	36.341	36.276	36.700	36.728	36.355				36.351		-	
C17n.1n (y)	36.618	36.512	36.969	37.049	36.668				36.573		-	
C17n.1n (o)	37.473	37.235	37.753	37.741	37.520				37.385		36.982	
C17n.2n (y)	37.604	37.345	37.872	37.934	37.656				37.530		37.220	
C17n.2n (o)	37.848	37.549	38.093	38.150	37.907				37.781		37.372	
C17n.3n (y)	37.920	37.610	38.159	38.287	37.956				37.858		37.439	
C17n.3n (o)	38.113	37.771	38.333	38.477	38.159				38.081		37.973	
C18n.1n (y)	38.426	38.032	38.615	38.835	38.449				38.398	38.527	38.273	
C18n.1n (o)	39.552	38.975	39.627	39.734	39.554				39.582	39.458	38.861	
C18n.2n (y)	39.631	39.041	39.698	39.897	39.602				39.666	39.597	38.929	
C18n.2n (o)	40.130	39.464	40.145	40.366	40.084			40.120	40.073	39.989	39.959	

^a Tuned to the ZB18a astronomical solution (Zeebe and Lourens, 2019).

Table 3. Comparison of magnetochronpolarity chron-boundary ages in millions of years (Ma). In our estimated ages column, bold numbers represent the most reliable magnetostratigraphic interpretation, whereas regular font numbers represent ambiguous interpretation and hyphen represents the boundaries that were not identified. For the studied interval, the recently published GPTS 2020 (Ogg, 2020) used all ages from the Pacific Equatorial Age Transect (PEAT) sites (Westerhold et al., 2014).

Chron		Standard	GPTS		Astronomically calibrated						
	CK95 (Cande	GPTS 2004	GPTS 2012	MQSD20	ODP Site	Massignano	Monte	PEAT Sites	IODP Exp.	Monte	
	and Kent, 1995)	(Ogg and	(Ogg, 2012)	(Malinverno	1218	(Jovane et	Cagnero	(Westerhold	342 composite	Cagnero	
		2004)		et al., 2020)	(Palike et al., 2006)	al., 2006)	(Hyland et al., 2009)	et al., 2014)	(Boulila et al.,	(this study-)	
		,			, ,		, ,)		2018)		
C13n	0.487	0.472	0.548	0.599	0.473		0.520	0.512		0.481	
C13r	1.110	1.044	1.294	1.200	1.421			1.376		1.124	
C15n	0.285	0.261	0.295	0.324	0.128	0.320		0.234		0.366	
C15r	0.403	0.361	0.412	0.428	0.074	0.383		0.244		0.338	
C16n.1n	0.183	0.163	0.186	0.236	0.226			0.138		-	
C16n.1r	0.159	0.140	0.159	0.191	0.089			0.056		-	
C16n.2n	0.656	0.569	0.649	0.674	0.712			0.577		-	
C16n.2r	0.277	0.236	0.269	0.321	0.313			0.222		-	
C17n.1n	0.855	0.723	0.784	0.692	0.852			0.812		-	
C17n.1r	0.131	0.110	0.119	0.193	0.136			0.145		0.238	
C17n.2n	0.244	0.204	0.221	0.216	0.251			0.251		0.152	
C17n.2r	0.072	0.061	0.066	0.137	0.049			0.077		0.067	

C17n.3n	0.193	0.161	0.174	0.190	0.203	0.223	0.534
C17n.3r	0.313	0.261	0.282	0.358	0.290	0.317	0.300
C18n.1n	1.126	0.943	1.012	0.899	1.105	1.184 0.931	0.588
C18n.1r	0.079	0.066	0.071	0.163	0.048	0.084 0.139	0.068
C18n.2n	0.499	0.423	0.447	0.469	0.482	0.407 0.392	1.030

^a Tuned to the ZB18a astronomical solution (Zeebe and Lourens, 2019).

Table 4. Comparison of magnetochron-polarity chron boundary durations in millions of years (Myr). In our estimated durations column, bold numbers represent reliable magnetostratigraphic interpretation for base and top boundaries, whereas regular font numbers represent ambiguous interpretation for either base and/or top boundaries, and hyphen represents that base and/or top boundaries were not identified. For the studied interval, the recently published GPTS 2020 (Ogg, 2020) used all ages from the PEAT sites (Westerhold et al., 2014), resulting in the same durations.

534

535 5.2. MCA biostratigraphy

536 Integrating the planktonic foraminiferal (Jovane et al., 2013) and our revised calcareous 537 nannofossil biostratigraphies, together with the developed age model, we provide here an up to 538 dateup-to-date biostratigraphic framework for the MCA Section spanning the middle Eocene to the 539 early Oligocene interval. Jovane et al. (2013) identified all planktonic zones proposed by Wade et al. 540 (2011) for the same interval of our study, which spans zones E11 through O1. Revisiting the 541 calcareous nannofossil biostratigraphy and following the biozonation from Agnini et al. (2014), we 542 were able to define all the biozones that characterize the studied interval (CNE15 to CNO2), except for the base of CNE19, defined by the base of C. isabellae. Based on our developed orbitally tuned 543 544 age model, we have been able to place the important calcareous nannofossil and planktonic 545 foraminiferal biohorizons identified in the MCA Section on a timeframe and compare our estimated 546 ages with those of Agnini et al. (2014) and Wade et al. (2011) (Table 5).

547 Our estimated planktonic foraminiferal ages based on the biostratigraphy from Jovane et al. 548 (2013) are in agreement with those proposed by Wade et al. (2011). Differences are around 200 kyr, 549 except for the base of zone E15 with a relatively higher offset (Table 5). It is noteworthy the 550 similarity between the proposed and our estimated age for the extinction of *H. alabamensis*, marker for the EOB (Premoli Silva and Jenkyns, 1993). The calculated age of the EOB does not only match recent ones (e.g., Speijer et al., 2020) but is also virtually the same age – less than 20 kyr of difference – found by Hyland et al. (2009) in their MCA study, once again pointing outhighlighting the robustness of MCA astrochronology.

555 Estimated ages for calcareous nannofossil markers seem to agree even better than planktonic 556 foraminifera, which may result from the higher sampling resolution. Most of the estimated ages 557 closely agrees to those of Agnini et al. (2014) with less than ~150 kyr of difference (Table 5). This is 558 particularly true for zones CNO1 and CNO2 that display offsets of 40 and 60 kyr, respectively, which once again support our estimated age for the EOB. The exceptions are the bases of zones 559 560 CNE17, CNE18, and CNE20, which have differences of approximately 500, 900, and 300 kyr, respectively. Despite the relatively high age difference, we suggest the base of common C. erbae as 561 562 the most reliable datum in the MCA Section for the base of the Priabonian stage, since its GSSP was 563 recently ratified (Agnini et al., 2020) as the base of the Tiziano bed in the Alano Section with an 564 astrochronological age of 37.710±0.01 Ma (Galeotti et al., 2019).

			Age (Ma)	
Zone (base)	Biostratigraphic datum	CN zones (Agnini et al., 2014)	PF zones (Wade et al., 2011)	Monte Cagnero (this study)
CNO2	T E. formosa	32.92		32.98
CNO1	Bc C. subdistichus >5.5 μm	33.88		33.92
O1	T H. alabamensis		33.9	33.93
CNE21	T D. saipanensis	34.44		34.56
E16	T G. index		34.5	34.69
CNE20	T C. reticulatum	35.24		34.96
E15	T G. semiinvoluta		35.8	35.32
CNE18	Tc C. erbae	37.46		36.57
CNE17	Bc C. erbae	37.88		37.39
E14	T M. crassatus		37.7	37.43
CNE16	T S. obtusus	38.47		38.63
E13	T O. beckmanni		39.4	39.75
E12	B O. beckmanni		39.8	40.00
CNE15	B D. bisectus	40.34		40.50

Table 5. Estimated ages for calcareous nannofossil (CN) and planktonic foraminiferal (PF) zones compared to their respective standard ages. CN and PF biozonations are after Agnini et al. (2014)

567 and Wade et al. (2011), respectively.

569 5.3. Paleoenvironmental and paleoclimatic implications

570 Increasing sedimentation rates towards the top of the section (Fig. 7) (Jovane et al., 2013 and 571 this study) agrees with paleobathymetric estimates by benthic foraminiferal data (Guerrera et al., 572 1988; Parisi et al., 1988), which points out toindicate a gradual shallowing from lower bathyal (1000-573 2000 m) during the middle Eocene to upper bathyal depth (400-600 m) in the early Oligocene. This 574 could explain a relative increase in SAR simply by increasing the terrigenous input due to the 575 gradually more proximal sediment input setting. Following the interpretation that MS reflects mostly 576 terrigenous input in a pelagic carbonate succession, a sea level fall would lead to an increase in the 577 terrigenous/carbonate ratio, which can be observed in the raw MS data by its gradually increasing 578 values towards the top of the MCA Section (Fig. 2).

579 The MCA Section is one of the few middle latitude successions that records continuous, rich, and well preserved calcareous nannoflora, enabling the reconstruction of the biotic and 580 581 paleoceanographic changes from middle Eocene to lower Oligocene. We can observe significant 582 changes in the calcareous nannofossil assemblages within the studied interval (Fig. 3). Modifications 583 in the abundances of calcareous nannofossil taxa are mainly related to paleoenvironmental changes. 584 Paleotemperatures and paleofertility are the main factors that affect the presence and abundance of 585 taxa. Up to now, it does not exist a specific scheme that establishes the paleoecological preferences 586 of calcareous nannofossils, because species preferences could have changed through time and among 587 different biogeographical settings. However, a general agreement on the preferences of some taxa can be identified. To infer possible temperature and trophic variations of surface waters, most 588 589 calcareous nannofossils were allocated into groups of environmental affinities, largely following Haq 590 and Lohmann (1976), Aubry (1992), Gardin and Monechi (1998), Monechi et al. (2000), Bralower 591 (2002), Persico and Villa (2004), Tremolada and Bralower (2004), Gibbs et al. (2006), Villa et al. 592 (2008, 2014), Agnini et al. (2011), Jones et al. (2019) and Nyerges et al. (2020). Together with the 593 cited literature, the multivariate statistical analysis performed on the MCA calcareous nannofossil

dataset allowed the recognition of major community changes and the identification of five groups of
taxa with similar paleoecological preferences as shown in Figure 9 and Table 2.

596 Variations in relative abundances of the paleoenvironmentally significant groups revealed 597 important environmental changes and trends throughout the MCA Section (Fig. 9). Overall, we can 598 observe that the warm and warm/temperate-water taxa are quite abundant throughout the section 599 with a slightly decreasing trend in the upper part of the section. Cool-water taxa were are rare until 600 the upper Priabonian at ~35.5 Ma (100 msl) coinciding with the base of the range of *I. recurvus* and 601 an enrichment in meso-eutrophic taxa. These taxa increased in abundance during the EOT, 602 corroborating the long-term global cooling. A peak in abundance of oligotrophic taxa is observed in 603 the early Priabonian at ~37.3 Ma (86 msl) related to the acme of C. erbae (marker of the CNE7 604 zone). Despite that, oligotrophic taxa show a decreasing trend with an abrupt drop in abundance and 605 diversity related to the extinction of the rosette-shaped Discoasters, which has been recently defined 606 as the Discoaster Extinction Event (DEE; Jones et al., 2019). Jones et al. (2019) placed the event 607 between 34.44 and 34.77 Ma, while a recent study in the southwestern Neo-Tethys estimated an 608 orbitally tuned age of 34.35 Ma for the event (Messaoud et al., 2020). Based on our orbitally tuned 609 age model, the extinctions of D. saipanensis and D. barbadiensis occurred respectively at 34.58 and 610 34.69 Ma (Fig. 3), therefore agreeing better with the estimated ages from Jones et al. (2019). On the 611 contrary, meso-eutrophic taxa increased in abundance starting at ~37.0 Ma (89 msl), defining an 612 abundance reversal or a definitive crossover from oligotrophic to mesotrophic taxa dominance.

In the lower part of the section (up to the acme of the *Cribrocentrum*), several fluctuations in abundance between oligotrophic and mesotrophic taxa have been observed with a persistent dominance of warm-water taxa. <u>Despite Although</u> the MECO event could be defined as a warm oligotrophic interval, it still shows very dynamic cycles (Giorgioni et al., 2019). At the onset of the MECO event, a reduction of *D. bisectus* and *Sphenolithus* (oligotrophic taxa) was associated with a sharp increase in the abundance of *C. floridanus* (eutrophic taxon), *Z. bijugatus*, and small *Reticulofenestra* (Fig. 3). The MECO interval is then characterized by fluctuations in eutrophic and 620 oligotrophic taxa (Fig. 9), clearly linked to nutrient availability, in warmer SSTs, as suggested by the 621 marked increases in Discoaster and E. formosa. A similar shift from oligotrophic to eutrophic 622 conditions has also been identified at Alano Section (Luciani et al., 2010; Toffanin et al., 2011) and 623 in the Southern Ocean (Villa et al., 2014). After the MECO, the decreasing trend of warm-water taxa 624 reflects the cooling trend that characterizes the middle late Eocene (Zachos et al., 2008). Above the 625 acme of the C. erbae and the subsequent collapse of this genus, the progressive decline of warm-626 water and oligotrophic taxa continued. Assemblages remained mostly characterized by warm-water 627 taxa and were enriched with mesotrophic taxa linked to subsequent changes in the nutrient level up 628 to the base of the range of I. recurvus. Increased abundances of species adapted to colder and 629 medium-to-rich nutrient waters indicate a gradual cooling of SST that began at the base of the range 630 of *I. recurvus*. The DEE marked the definitive prevalence of cool eu-mesotrophic forms. During the 631 late Eocene the gradual reduction of the stratification of the water column (Tori et al., 2008) and the 632 cooling of SST seem to be the cause of the Discoaster crisis, which were deep dwellers taxa (Aubry, 633 1992). The interval spanning the EOT, and the Oi-1 event is characterized by a sudden turnover 634 toward dominantly meso-eutrophic taxa (Clausicoccus, D. scrippsae, R. dictyoda and C. pelagicus), 635 and an increase in abundance of R. daviesii, Blackites spp. and Clausicoccus spp. in response to 636 changing SST and nutrient supply (Coccioni et al., 2008; Villa et al., 2008, 2014). Calcareous 637 nannofossils show a significant reorganization of the assemblages with the replacement of the 638 dominant genera, a gradual decrease in species diversity, a decrease in the abundance of warm-water 639 taxa (Discoaster and Sphenolithus), and the collapse of oligotrophic taxa (Cribrocentrum, R. 640 umbilicus, Discoaster, and E. formosa). All these changes seem to be mainly related to a gradual 641 (step-by-step) eutrophication, environmental instability, and small changes of SST.

Thus, the high-resolution record of calcareous nannofossil reported here clearly suggests a loss of stratification, increased instability, and general eutrophication trend in the water column during this time interval. Nutrient enrichment shows a stable trend starting at \sim 37 Ma, therefore preceding the water cooling. This may mean that first there was a generalized loss of stratification in 646 the water column and, later, a decrease of SST. All these changes could be related to an increase in 647 seasonality, carbon cycle variability, and also to modification in oceanic circulation. Furthermore, 648 the increased abundance of *Z. bijugatus* and *L. minutus*, known as shallow-water taxa, corroborates 649 the sea level shallowing assumed by the increasing terrigenous/carbonate ratio interpreted from high 650 MS values in the upper part of the section.





653 Figure 9. Astrochronology time scale of the MCA Section established by 405 kyr tuning and groups 654 of paleoenvironmentally significant calcareous nannofossils. From left to right: Planktonic 655 foraminiferal (PF; Jovane et al., 2013) and calcareous nannofossil (CN; this study) biozonation 656 following the schemes proposed by Wade et al. (2011) and Agnini et al. (2014), 657 magnetostratigraphic interpretation after Jovane et al. (2013) with the most reliable magnetic polarity 658 reversals indicated by red stars, ZB18a astronomical solution (Zeebe and Lourens, 2019) with the 659 405 kyr (blue area) and the 2.4 Myr (green area) components, MS record, and the relative abundance 660 (%) of calcareous nannofossil groups (Table 2; Notice the scale difference for the cool-water taxa). 661 Black and red horizontal dashed lines indicate the Eocene-Oligocene boundary (EOB) and the 662 Discoaster extinction event (DEE), respectively. Dark blue interval indicates the EOT as a ~790 kyr

event started at the extinction of D. saipanensis (Hutchinson et al., 2021). Light blue interval 663 664 indicates the late Eocene cooling showed by calcareous nannofossil cool-water taxa. Purple interval 665 indicates the top acme of *C. erbae*, which marks the onset of the oligotrophic taxa decreasing trend. 666 The oOrange interval indicates the MECO based on its stratigraphic constraint as proposed by Savian 667 et al. (2014). Warm-water taxa comprehend Sphenolithus spp., D. scrippsae, C. pelagicus, and 668 Discoaster spp.; warm/temperate-water taxa comprehend E. formosa, D. bisectus, Z. bijugatus, 669 Cribrocentrum spp., and L. minutus; cool water taxa comprehend Blackites spp., C. protoannulus, R. 670 daviesii, Clausicoccus spp., and Helicosphaera+Pontosphaera; oligotrophic water taxa comprehend 671 Cribrocentrum spp., C. eopelagicus, R. umbilicus, Discoaster spp., C. protoannula, and E. formosa; 672 and meso-eutrophic water taxa comprehend Clausicoccus spp., D. scrippsae, R. dyctioda group, C. 673 pelagicus, Blackites spp., L. minutus, and D. bisectus.

674

675 5.4. Timing of the MECO event in the MCA Section

676 The MECO is recognized as one of the most rapid enigmatic warming events of the 677 Cenozoic, probably related to pCO₂ global changes (Bohaty and Zachos, 2003). Its duration was 678 firstly estimated to last over than 600 kyr (Bohaty and Zachos, 2003), but the recently estimated 679 duration decreased to about 500 kyr, with the peak warming lasting less than 100 kyr and placed 680 around 40.0 Ma (Bohaty et al., 2009). Savian et al. (2014) identified and placed recognized the 681 stratigraphic interval corresponding to the MECO event in the MCA Section between approximately 682 61.0 and 65.5 msl based on a multi-proxy study. Micropaleontology, geochemistry, rock magnetic, 683 and stable isotope data, constrained by bio- and magnetostratigraphic datums, allowed them to place 684 the entire event between ~ 61.0 and ~ 65.5 msl and the peak warming together with its aftermath 685 specifically between ~63.2 and ~65.5 msl. In addition to micropaleontological and geochemical 686 evidence, Savian et al. (2014) reported increased aeolian iron supply and enhanced putative 687 magnetofossil concentrations within this shorter interval, which altogether was interpreted as a 688 period of high primary productivity. Our age model allowed us to place this interval in time domain and compare it with other records in which MECO was identified. Based on the MECO constraint from Savian et al. (2014), together with our developed age model, we have been able to place the event approximately between 40.24 and 39.75 Ma (Fig. 10), which supports the duration of about 500 kyr with peak warming occurringed around 40.0 Ma. Besides, it consistently agrees with the recent placing of MECO event in the Neo-Tethys (Giorgioni et al., 2019).

Our study also <u>points outhighlights</u> the importance of the orbital forcing influencing the MECO climate warming due to its coincidence with a node of <u>lows in</u> the 405 kyr and 2.4 Myr components of eccentricity, as firstly suggested by Westerhold and Röhl (2013). Giorgioni et al. (2019) already showed the same behavior in the Neo-Tethys, and here we demonstrate it again for the MCA Section (Fig. 10). As discussed by Giorgioni et al. (2019), this orbital forcing could have enhanced the effects of the India-Asia collisional event in the Tethys (Jovane et al., 2009; Najaman et al., 2010), functioning as a trigger for climate warming and carbon cycle instability.



Figure 10. The MECO event (yellow) at the MCA Section with peak warming defined as the negative δ^{18} O anomaly (orange). a) ZB18a astronomical solution (Zeebe and Lourens, 2019) with the

2.4 Myr and the 405 kyr cycles extracted by a Gaussian bandpass filter centered at 0.00042 \pm 0.00004 kyr⁻¹ and 0.002469 \pm 0.0006 kyr⁻¹, respectively. b-e) CaCO₃, δ^{13} C, δ^{18} O and MS data from Savian et al. (2014). Magnetostratigraphic interpretation is from Jovane et al. (2013). Planktonic foraminiferal (PF; Jovane et al., 2013) and calcareous nannofossil (CN; this study) biozonation following the schemes proposed by Wade et al. (2011) and Agnini et al. (2014), respectively.

709

710 **6.** Conclusions

711 Based on previous studies of correlated sections, marls and limestones at the MCA Section 712 are inferred to have been deposited during warm/wet and cold/dry intervals, respectively. Together 713 with a radiometric date and magneto- and biostratigraphic tie points, we were able to construct a new 714 high-resolution age model for the MCA Section, by tuning the minima in the identified long 715 eccentricity cycle of the MS record to minima in the astronomical solution. Our orbitally tuned age 716 model allowed us to place the base of the Priabonian stage in the MCA Section at ~37.4 Ma, based 717 on the identification of the base of common C. erbae, and to independently confirm the base of the 718 Rupelian stage at ~33.9 Ma, as previously suggested. We constrained the MECO event duration to be 719 \sim 500 kyr with its peak warmth at \sim 40.0 Ma, coinciding with a minimum in the 2.4 Myr and 405 kyr 720 eccentricity components, also corroborating previous studies. The developed age model also points 721 out the necessity of revisiting the MCA magnetostratigraphy, particularly between chrons C18n to 722 C15r. Calcareous nannofossil paleoenvironmentally significant groups allowed the identification of a 723 gradual eutrophication with an abrupt change <u>dominance reversal</u> from oligotrophic to eutrophic 724 conditions at around 37 Ma. Assemblages show that nutrient enrichment preceded water cooling at 725 the late Eocene, suggesting that, prior to the cooling, a loss of water column stratification took place. 726 Furthermore, we strongly reinforce the need for a study within the EOT interval at the MCA Section 727 with high-resolution isotopic records in order to constrain important events such as the Oi-1 based on 728 isotopic signature. Despite these, the MCA is a continuous section in the hundreds of thousand years 729 scale, spanning the late Eocene through the early Oligocene. Therefore, it has the potential to become instrumental in understanding major paleoceanographic and paleoclimatic changes during thisinterval, over important events such as the MECO and the EOT.

732

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