

Review of the preprint entitled “*Morphometric changes in two Late Cretaceous calcareous nannofossil lineages support diversification fueled by long-term cooling*” by Mohammad Javad Razmjooei, Nicolas Thibault, Anoshiravan Kani (DOI 10.31233/osf.io/nfyc9).

The manuscript by Razmjooei and co-authors is interesting, adding new insights in the morphometric changes of two Late Cretaceous calcareous nannofossil lineages (*Cribrosphaerella* and *Microrhabdulus*) from the Shahneshtin section (Zagros Basin, Iran). Based on their relative abundances and size patterns, the authors highlight the possible first occurrence of *C. hilli* (lines 252-255) in the late Campanian of the studied site and define *Microrhabdulus* sp. nov. 1 and *Microrhabdulus* sp. nov. 2 as new species of the *Microrhabdulus* group. The comparison of the micropalaeontological data obtained here to global calcareous nannoplankton diversity and temperature reconstructions of the Albian–Maastrichtian time interval is interesting, giving a wider perspective of the interpretations following the Cope’s and Bergmann’s rules.

Besides, the manuscript is well written, and I think this manuscript has the potential to interest a broad scientific community, particularly when dealing with the relationships between calcareous nannofossil morphometrics and climate (temperature) changes in the past. However, the robustness of the dataset needs to be better set up, so some interpretations will be less questionable (see my comments in the section (A) below). Besides, I agree that global temperature changes might be an important forcing factor behind the morphometric changes highlighted for *Cribrosphaerella* and *Microrhabdulus* groups. However, I am wondering if other (global vs local) climate parameters couldn’t have played significant roles as well (see my comments in the section (B) below). At last, I propose some minor corrections in (C).

(A) *Specimen preservation*

The fact that the preservation of the calcareous nannofossil assemblage identified as moderate is only discussed lines 101-104 (chapter 3 “Material and methods”), is clearly unhelpful and I believe that a chapter devoted to the preservation of the assemblage and more particularly of the studied specimens (i.e. *Cribrosphaerella* and *Microrhabdulus*) is warranted. It is crucial when focusing on morphometrics, and particularly when interpretations are based on specimen length variations of about 1-1.5 μm . Besides, the authors refer to Razmjooei et al., (2020b) when dealing with the preservation of the calcareous nannofossil assemblages. In this previous paper, the authors highlight “moderate to poor preservation” according to criteria of Roth (1978), as well as “very low species richness”, with the “absence of small coccoliths such as small *Biscutum*, *Z. erectus* and *P. stoveri*”, “all indicative of a significant impact of diagenesis on the nannofossil assemblage”.

Therefore, I suggest the authors to better summarize the founding of Razmjooei et al., (2020b). For example, what does “quantitative and palaeoecological studies” (line 101) mean when dealing with calcareous nannofossil preservation? I suggest also the authors to develop a paragraph dealing with the preservation of *Cribrosphaerella* and *Microrhabdulus* in particular (Actually, I totally disagree with the fact that since the studied lineage are $>3 \mu\text{m}$, they are prevented from any major influence of diagenesis on size” (line 104). Diagenesis and dissolution (or overgrowth) impact calcareous nannofossil whatever the size, and $3 \mu\text{m}$ is actually quite small. Also, *M. undosus* are frequently fragmented in the samples (line 200-201). Does that mean that its abundances might be biased as well? Does that reflect significant impact of dissolution? As an example, the number of foraminifera fragment is an index to highlight dissolution imprint on quaternary assemblages (higher fragments reflecting higher dissolution impact). Could it be the case here? Lines 204-206: “the maximum length observed in each sample still represents a valuable index as this parameter is more likely to represent the length of complete, non-fragmented specimens”. Can you develop? For example, what is the actual length of specimens in literature? Is it

comparable to the length obtained here? In all cases, the definition of new *Microrhabdulus* species is based on specimen widths and lengths (lines 279-281) and these parameters must clearly not be ascribed to dissolution or overgrowth overprints. SEM observations and pictures should be added to the paper to be more convincing, as well.

Besides, while the differences between potential morphotypes are highlighted via density plots (Matlab® script of Thibault et al., 2018) and histograms of PAST® (Hammer et al., 2001) i.e. proven tools in the field, I am still puzzled by the meaning of statistics performed on less than 50 specimens, (up to 19 for *C. ehrenbergii*; and up to 6-9 for *M. undosus* group). Can you explain what “the statistic difference between potential morphotypes is tested [...] for three distinct stratigraphic intervals that bear enough specimens for reliable statistics” mean?

(B) Nannoplankton diversity and climate changes

In the introduction (lines 57-60), the comparison of *Cribrosphaerella* and *Microrhabdulus* relative abundances and sizes with global nannofossil diversity (Bown et al., 2004) and a long-term signal of TEX86 (O'Brien et al., 2017), is presented as an illustration of the relationship that might exist between global (decreasing) temperature and global (increasing) calcareous nannofossil diversity during the Cretaceous. However, it is rather presented as the main (and only?) forcing factor behind *Cribrosphaerella* and *Microrhabdulus* distributions during Campanian – Maastrichtian. Other forcing factors might exert control on them at global and local scales. Therefore, I propose the authors to adopt a clearer position regarding the relationship between *Cribrosphaerella* and *Microrhabdulus* groups and climate changes. Indeed, there are two options:

i) the aim of the paper is to better constrain *Cribrosphaerella* and *Microrhabdulus* taxonomies and define and present new calcareous nannofossil species, testing the role of global cooling as an opening for future interpretations. In that case, I would clearly define it as the aim of the study (lines 56-60). Also, I would most clearly present chapter 5.4 as the hypothesis the authors want to test here (probably at the beginning of the discussion (5.2), not at the end (5.4)), bearing in mind that other parameters (not tested here) might play a significant role as well. I would be extremely caution when dealing with the relationship between *Cribrosphaerella* and *Microrhabdulus* behaviors and temperature (rephrase for example: “We infer here that our observations illustrate an intimate link between climatic cooling and speciation, and strongly support that the Late Cretaceous nannoplankton peak in diversity was essentially fueled by cooling », lines 378-381, and elsewhere in the manuscript.)

ii) the aim of the paper is to understand the relationships between *Cribrosphaerella* and *Microrhabdulus* patterns and climate changes based on their abundances and morphometrics (as exposed lines 56-60). In such a case I would suggest integrating in the discussion, local vs global climate parameters that might exert control on the studied nannofossils (local temperature and nutrient conditions, atmospheric pCO₂, sea-level), probably better integrating results and interpretations from Razmjooei et al., 2020b).

(C) Some minor corrections:

Line 44: Do you mean morphospecies?

Line 46: I suggest you to remove “subtle”

Line 57: the ecological preferences of *Cribrosphaerella ehrenbergii* and *Microrhabdulus undosus* (particularly with temperatures) presented lines 384-396, should be presented here. Since their abundances and morphometrics are interpreted in terms of temperatures changes, it would worth mentioning it in a few words within the introduction.

Line 65: what is the environment of the studied section (neritic, hemipelagic, pelagic)?

Line 82: Please refer to Fig. 2.

Line 115: "fewer specimens were measured". Please, give the exact number and discuss the limit of such number.

Line 118: "the biometric measurements have been performed manually (?), under a light microscope..."

Figure caption 2: the oxygen isotope signal documented here is not reported in the figure. Is there any uncertainties associated to the absolute ages?

Tables 1 and 2: please, add the units (μm) of the morphometrics in Figure captions. What is the significance of two digits after the decimal point (for mean length and mean width of both groups) vs one digits after the decimal point (fmax length of *M. undosus*).

Line 157: relative abundance of *C. ehrenbergii* averages 5%

Line 158: then reach minima of less than 1%

Lines 166-167: reaching values as high as 17%

Line 168: from CC26a and UC20cTP to Cretaceous-Paleogene boundary

Line 178: Figure 4 should be cited before Figure 5, or Figure 4 and Figure 5 should be permuted.

Line 186: *Microrhabdulus spp.* represents only a minor component of the calcareous assemblage. Why is that? What are his ecological preference?

Lines 191-192: ...upper Campanian. Above the Campanian/Maastrichtian...

Lines 193: two double peaks of abundance

Lines 220-221: The appearance of *Microrhabdulus* sp. nov. 2 (maybe linked to a temperature change?) is not associated with changes in *C. ehrenbergii*. Is there any explanation?

Line 225: Do you mean longer, or wider morphotype instead of thicker? Thicker refer to the morphotype thickness, which is another parameter (not measured here).

Lines 249-250: what is usually, the maximum size of *C. ehrenbergii* et *C. hilli*? Is it comparable to the sizes obtained here?

Lines 255-258 and lines 350-353: SEM observations would clearly help improving this part and therefore, the manuscript.

Figure 8 caption: "thick forms". Do you mean "wide/large forms"?

Line 354: ... and the length of *C. ehrenbergi* remains very stable

Lines 354-355: I don't totally agree. Generally, *C. ehrenbergi* appears longer after the shift compared to before. What does that imply regarding Gould and Eldredge (1977) assumption cited here?

Lines 365-267: please, be caution when referring to a rapid shift in *C. ehrenbergi* and *M. undosus* groups in the other parts of the manuscript.

Figure 10: It would worth adding the average length of *Microrhabdulus* in the figure, as well. Could it be possible to also add the rapid temperature changes documented lines 432-435?

Figure 10 caption: can you mentioned the red, green, pink/ scare, circle, diamonds?

Lines 442-445: If that is so, then I suggest you to change the title of the article and document climate instability instead of long-term cooling. In all cases, what do you mean by climate instability? Do you only refer to temperature instability or it could be associated to other parameters? And which ones?

Line 485: ...oligotrophic areas, the global peaks in their diversity...