

Author's response (on behalf of all the co-authors) to the review of:

The 3D submicron-scale skeletal reconstruction of *Nannoconus* (Cretaceous calcareous nannofossil) - Insights into biomineralization

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Our manuscript investigates Ptychographic X-ray Computed Tomography (PXCT) as applied to *Nannoconus*, a calcareous nannofossil that was the primary bicarbonate producer in Early Cretaceous oceans. This pre-assessed manuscript went through a second round of review where - one of the reviewers, already assessed the manuscript and thought that the authors addressed
20 all the comments received in a satisfactory manner.

A second, new, reviewer, Isaline Demangel, also assessing the work, noted the achievement of this study, but at the same time, suggested to clearly reframe the objectives of this study and reorganise the discussions in a “structured, cohesive” manner. The reviewer mentions that the manuscript appears to lie between a methodological and an interpretative paper, without fully achieving either objective. However, the PXCT results are distinctly structured to establish a clear methodological framework
25 prior to interpretation. The results follow a step-wise and reproducible workflow for 3D skeletal reconstruction of *Nannoconus*: segmentation of a lamella and definition of geometric parameters, followed by (1) generation of inclination and tilt, (2) creation of point series, (3) formation of layers/segments, and (4) construction of the final 3D skeleton. This progression, thus, clearly defines the methodological framework before any interpretative discussion. Additionally, this framework is implemented directly in the Python-based code provided in the supplementary information, reinforcing the methodological rigor and
30 reproducibility of this reconstructive approach.

The reviewer also raised three major and some minor comments. We have incorporated the comments in the revised manuscript. Here we present the responses to reviewer comments; in red the reviewer comments, in black, our responses. Any related revisions to the manuscript (i.e., in the revised manuscript) are clearly indicated by figure, page, and line numbers in **bold black**, wherever necessary. On the behalf of all the co-authors, I thank the reviewer, for the encouraging remarks and concerns over the manuscript.

Sincerely,

Rajkumar Chowdhury

Major comments

Comment: The manuscript aims to address the gap in knowledge regarding the producing organism and its biomineralisation process. However, the data presented primarily document the skeletal microstructure rather than the biological identity or process itself. The introduction should therefore be reframed to clarify that the study provides structural constraints on possible biomineralisation mechanisms rather than direct biological evidence.

Response: We understand the ambiguity in the introduction and reframed it presenting the emphasis on the investigation of the microstructural constraints to provide perspectives on the biomineralization of the *Nannoconus*'s skeleton. The revised part is incorporated in lines **52-54** as follows:

The knowledge of the structural constraint of the 3D skeletal arrangement obtained from the aforementioned reconstruction has been used to provide perspectives on *Nannoconus* biomineralization process.

Comment: The study is based on a single *Nannoconus* specimen. While the results are valuable as a demonstration of the method's potential, broader generalisations about *Nannoconus* lamellar architecture or biomineralisation should be made cautiously. Future PXCT analyses on multiple specimens, including lamellae from different positions within the skeleton and from different geological intervals, will be necessary to confirm whether the observed features are consistent across individuals.

Response: The 2D SEM images of various *Nannoconus* species from multiple geological settings and age broadly indicate that: 1. The skeleton consists of an interlocking arrangement of lamellae, and, 2. different species are characterized by distinct skeletal morphologies. The 3D reconstruction of the *N. globulus* skeleton obtained through PXCT clearly explains the skeletal microstructure through several geometric parameters. Specifically: 1. The interlocking lamellar pattern results from the stacking of lamellae at two distinct inclinations, and 2. The general skeletal morphology is controlled by changes in radius. On the other hand, two distinct lamellae, segmented from different parts of the skeleton, present similar morphology, indicating

that the skeleton is composed of morphologically homogenous lamellae. Consequently, the analysis of a single *Nannoconus* specimen provides sufficient geometric parameters to interpret the 3D microstructure across various species. The resulting probable inferences on biomineralization, based on these geometric parameters, are therefore valid across *Nannoconus* species belonging to a range of geological intervals.

This part is added in the introduction of the revised manuscript in the lines **50-53**:

Subsequently, we analyzed these volumes using image visualization software and successfully reconstructed the complete 3D skeleton of a single *Nannoconus*, specimen specifically, *N. globulus* for the first time. This strategy serves as a methodological framework for reconstructing the *Nannoconus*'s skeletal structure in general.

In the section of discussion in the lines **485-491**:

It is noteworthy that the 2D SEM images of various *Nannoconus* species from different geological settings and ages show that their skeletons consist of interlocking lamellae, and species differ in overall skeletal morphology. The PXCT-based 3D reconstruction of *N. globulus* clarifies this microstructure: the interlocking pattern results from lamellae stacked at two distinct inclinations, and changes in radius control the general morphology. Thus, a single specimen provides sufficient geometric parameters to interpret 3D microstructure across species, and the resulting biomineralization inferences are broadly applicable to *Nannoconus* species through diverse geological intervals.

Comment: The discussion is currently vague and unstructured, moving between topics without developing clear arguments. It would benefit from being reorganised into distinct, coherent paragraphs. Additionally, the manuscript does not address the potential effects of diagenesis on the skeletal microstructure. Although the author states that well-preserved specimens were selected, overgrowths are visible and reported. It would therefore be useful to discuss the preservation state and whether the observed features are primary or influenced by diagenesis to support the PXCT interpretation.

Response: The discussion of the 3D skeletal reconstruction of *Nannoconus* is organized around the following themes: 1. evaluation of skeletal models, i.e., determining which of the two proposed models: the layer model or the segment model, provides the most valid description of the *Nannoconus* skeleton, and explains the reasoning behind this choice. 2. controls on skeletal morphology, i.e., using the segment model as the valid framework, identifying the geometric parameters that control the skeletal morphology across different *Nannoconus* species. 3. probable biomineralization process, i.e., inferring the most plausible biomineralization mechanism responsible for assembling the lamellae into segments, and ultimately constructing the full skeleton. Each of these themes in the discussion are now restructured with new headings to clarify the contents. They are added in lines: **347, 376, and 415** respectively as:

5.1 Which of the two models best explains the *Nannoconus* skeleton?

5.2 Which parameters control *Nannoconus*'s skeletal morphology?

5.3 How were the segments of the *Nannoconus* skeleton biomineralized?

90 The *Nannoconus* specimen selected for the PXCT experiment is well-preserved under SEM. The external 2D views show clearly distinguishable lamellae with no visible diagenetic alteration. However, the tomographic PXCT images reveal overlapping lamellae within the interior of the skeleton, which obscures their boundaries. This indicates that, despite the specimen's apparent "good state of preservation" in SEM, the high resolution and tomographic imaging obtained through PXCT can reveal diagenetic effects that are not visible in 2D. Hence, to ensure an accurate reconstruction, the geometric parameters, such as radius, inclination, and tilt, were measured repeatedly, and the 3D skeletal model was generated through multiple trials to achieve the closest possible resemblance to the real skeleton. As a result, diagenetic effects detected internally do not affect the validity of the 3D reconstruction or the subsequent biomineralization interpretations. In the revised manuscript these comments are included:

In the section of Materials and Methods in lines 128-129 as:

100 **Here, "good state of preservation" refers to skeletons which are structurally fully intact without any breakage and devoid of post-depositional growth of calcite, that often obscures the microstructure of nannofossils.**

In the section of segmentation of a lamella in lines 196-202 as:

105 **These images reveal overlapping lamellae within the interior of the skeleton, likely resulting from diagenetic alteration (a process involving the dissolution of the biocalcite, produced during the biomineralization and subsequent reprecipitation). Although the specimen was considered "well-preserved" based on SEM images, the high spatial resolution of the PXCT reveals clear diagenetic effects within the *Nannoconus*'s skeleton. As a consequence, individual lamellae are sometimes difficult to segment (i.e., virtually separate) in the tomographic images. Despite having a spatial resolution of ~30–40 nm, this resolution is at the same scale of the thickness of the lamellae themselves and is therefore insufficient for distinctly segmenting a single lamella.**

110 At the end of the section of discussion in the lines 477-484 as:

The *Nannoconus* skeleton is reconstructed from a specimen of *N. globulus*. Although the specimen is generally well-preserved as seen in SEM images, PXCT observations at higher resolution reveal finer diagenetic alterations within the skeletal interior. Nevertheless, our reconstruction process enables the segmentation of individual lamellae and the generation of a 3D skeleton using geometric parameters, including tilt and inclination angles as well as radial length.

115 **To ensure accuracy, these geometric parameters i.e., radius, inclination, and tilt, were measured repeatedly, and the**

3D skeleton was iterated through multiple trials to obtain the closest possible resemblance to the real skeleton. As a result, the internally detected diagenetic effects do not affect the validity of the 3D reconstruction allowing subsequent interpretations of biomineralization.

Minor Comments

120 The manuscript contains several syntactical and grammatical issues.

Several statements are unsupported by references.

Some paragraphs are repetitive, and certain sections could be shortened for conciseness.

The manuscript would benefit from some restructuring to enhance readability. For example, points 2 and 3 on stratigraphy could be incorporated into the brief introductory paragraph of Section 2.

125 **Response:** We have carefully checked and corrected many syntactical and grammatical issues. No additional references were added in the text; however, some existing references were repositioned to more clearly support the corresponding statements. These adjustments are visible in the marked-up version of the manuscript.

To make the text more concise, the following revisions were implemented:

130 **1. The texts in Subsection 2.2, including Points 2.2.1 and 2.2.2, have been shortened so that each part is now presented as a single paragraph.**

2. As suggested, the subsection 2.3 (i.e., Stratigraphy) has been removed, and its content has been incorporated into the introductory paragraph of Section 2.

3. Figure 12 has been moved to the end of Subsection 5.3 to present the discussion in a clearer manner.