

## ***Interactive comment on “Weaving of biomineralization framework in rotaliid foraminifera: Implications for paleoenvironmental reconstructions” by Y. Nagai et al.***

### **Anonymous Referee #2**

Received and published: 16 August 2018

A paper by Nagai et al. presents novel and detailed observations on pseudopodial activities and structures during chamber formation in calcareous forams. They also verified that organic layers are a part of pseudopodia/cytoplasm. In addition, the authors also present the important finding for pore formations on calcareous walls, which have not yet been understood in detail. I think highly of their works which made progress to understand the biomineralization processes in foraminifers. I am sure that their findings would provide many ideas and hints with paleoceanographers and biogeochemists using foraminifers as tools.

I think the manuscript is generally well-structured and well-written. However, I feel the

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Results section and Figures are still not easy to understand for most readers. The authors should take more careful about descriptions of observations, figures and their explanations. I also prefer more explanations in the figure caption to understand without reading the relevant text. I also suggest adding more close-up photographs and movie in particular for Figs. 1&2 as supplementary materials.

I assess some unsolved questions on foram biomineralization can be solved by this paper. However, some new questions are arising after reading this paper. For examples,

1) Are three organic layers produced by separate/independent pseudopodia? I think the initial stage is very important to understand this process. I suggest that the authors should present SEM photographs prior to starting the initial stage as well.

2) Thickness between OOL and POS remain the same or increasing throughout this process? In L256-257, the authors noted chamber thickening.

3) Why is the space between IOL and POS narrower than that between OOL and POS?

4) How can pore plates on POS and pore funnels on OOL align at the same locations if these are separately formed?

5) Are pore plates on POS biconvex on both side?

6) Vesicles are usually included in foraminiferal cytoplasm/pseudopodia. Why are these vesicles independently found on the surface of the OOL and POS? Were vesicles originally contained inside the OOL/POS?

7) Concerning semi-closeness/closeness at site of calcification during early/late stages, maybe your finding is related to passive/active ion transport to change Mg/Ca, but it is still speculative unless the authors verify changes in passive and active ion transport at different stages. I wonder the authors are missing the importance of the space between IOL and POS, which maybe more closed and has not yet fully understood even in this paper. Is there any possibility that differences in Mg/Ca corresponding to an IOL-POS space and a POS-OOL space? To solve this question, I suggest

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showing Mg/Ca distribution map with OOL/POS lines in Fig. 7. I also wonder if elemental compositions in vesicle have any clues to solve this question.

I anticipate that some questions can be answered in the revised manuscript, but for others I look forward to future works by the authors.

Specific and technical comments

L1: Weaving? implications for paleoenvironmental reconstructions? I suggest the title rewrite as "Functional role of pseudopodia on biomineralization in rotaliid foraminifers: Implications for paleoceanographic proxies."

L22-24: I suggest rewriting as "Elemental and/or isotopic signatures of calcareous tests of Foraminifera are commonly used to reconstruct paleoenvironmental conditions."

L25: differ greatly between taxa/species/individuals/inter-chambers/intra-chambers/layers?

L26: proportional contributions from ... > relative contributions between ...

L27: still investigated > still under investigation/unknown/not clear

L30: Better to specify what you found for the first time

L33: POS should be explained when first mentioned in the abstract

L40: I do not think so unless the authors verify changes in passive and active ion transport at early and later stages, respectively.

L41: The "vital effect" has broad meanings. Better to specify. You may mean differences in elemental and/or isotopic ratios along chamber walls.

L42-44: Better to conclude how your findings are helpful to interpret and calibrate paleoceanographic proxies and biogeochemical cycles.

L47, Keywords: should have more important words.

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L52-66, the first paragraph: this paragraph is jumbling about rotallids and forams in general, most of which are not directly related to the topic of this manuscript. I guess most BG readers know about forams. So better to start from the introduction of biomineralization of forams.

L71-73: Better to set this sentence as a topic sentence

L75: by experiment > by culturing experiments

L84-87: I think in situ observations and culturing experiments of foraminifera have a long history and many researches, as described in the next paragraph. I suggest deleting these sentences.

L91-95: too long noun, better to rewrite as "Superfine structure observation by ... have been reported in order to ..."

L100-108: The authors should more justify to use the general term "pseudopodium/a" because foraminiferal pseudopodia are usually named as granuloreticulopodia. I would only agree with the authors if foraminifers do not produce any dynamic net-like structures with no any granules visible during chamber formation.

L116-117: The "POS" used to be called as POM (Primary Organic Membrane) in Hemleben et al. (1986).

L116-118: OOL and IOL are not first mentioned

L124: The new term "organic scaffolding" are not easy to imagine and not mentioned as an important term throughout the manuscript. I suggest the author redefine the term "anlage" to confine organic layers.

L126-127: Use POS, OOL and IOL consistently throughout the text except for first mentioned.

L130: natural state?

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L130-131: electron microscopy>SEM/TEM?  
L132, (SOC): Move to L129 that is first mentioned  
L150-151: paleoenvironments>palaeoceanographic proxies; predicting responses to ongoing climate change > how?  
L153: Better to rewrite as “within a hyaline calcareous wall using the benthic foraminifera”  
L157, SEM: Define when first mentioned.  
L167: De Nooijer et al., 2009? Check all years of references in the text. I found some typos in other refs as well.  
L208: The first paragraph of the Results section is just an outline and unnecessary. Delete or partly move to the method section.  
L223: the last existing calcified chamber > the last chamber  
L224: characteristic > morphology  
L226: delete “from then”  
L231, an aggregation of cytoplasm: Indicate where and which part in the figure,  
L233: retracts until where?  
L238-239: fine and short pseudopodia? I cannot see it. Need more close-up photos.  
L240-241: A brighter band of particles? I cannot see it.  
L242: beyond? inside?  
L243: smooth? Fig. 1C looks smoother than 1D  
L251, Calcium carbonate: How do you know it?  
L252: I think the overall outline and size are fixed at earlier stages (the middle stage).

C5

L253: Hard to see pseudopodial movements. Do you have a movie?  
L253: Open triangles in Fig. 2A?  
L256-257: How do you know the chamber wall getting thicker?  
L262: The usual type of pseudopodia movement means reticulopodia?  
L266-267: Move to the method section  
L266-277: This paragraph with Fig. 3 should move to the Discussion section because Fig. 3 are mostly schematic models and your interpretations based on observations.  
L274: gray in Figures 2-4?  
L274: um?  
L292, vesicle: How do you identify it? Vesicles are usually included in foraminiferal cytoplasm/pseudopodia. Why are these vesicles independently found on the surface of OOL and POS?  
L295, pseudopodia: how do you identify it?  
L311, needle-like structure: Show in the figure.  
L316-317: Show in the figure  
L328-329: Which are algal cysts in Fig. 5A?  
L335: period>stage  
L353: OOL had toward the inner side?  
L376-377: Indicate which photos clearly show this.  
L387: Cader>Cadre, Ni>Ní based on references  
Figs. 1&2: Add color legend in the figure; indicate initial, middle, late stages in the figure; hard to see any bubbles and pseudopodia inside chambers; in Fig. 1C, the

C6

frame of new chamber are magenta?; I prefer more explanations in the caption to understand without reading the relevant text; open triangle?; Did you identify calcareous wall by polarized microscope?: any more magnified images? You should have used a fluorescent dye to observe cytoplasm more clearly.

Fig. 3: move after Figs. 4-7; indicate initial, middle, late stages in the figure; for A, indicate which part of close-up in B; colors of outer (blue) and inner sides (purple) are confusing with vesicle and pseudopodia.; the shape of carbonate crystals looks like needles. is it OK?; What are the purple colored polygonal shape on the POS?

Figs. 4-6: Indicate differences between dotted lines and thick lines.

Fig. 7: Indicate OOL and IOL lines; Add Mg signal and Mg/Ca data

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