

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

Y. Nagai et al.

nagai.y@jamstec.go.jp

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Dear Reviewer,

We are pleased to submit the revised version of our Research Article manuscript “Weaving of biomineralization framework in rotaliid foraminifera: Implications for paleoenvironmental reconstructions” (bg-2018-295). We appreciated your constructive criticisms and comments, and we thank you for providing this opportunity for us to improve this manuscript and submit a revised version.

A point-by-point response to comments is included below. All files for the revised

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manuscript (tracked changes and clean versions, figures, table, supplementary files) are contained in the .ZIP file uploaded with this revision.

We hope the present version is acceptable for publication in Biogeosciences.

Best regards,

Yukiko Nagai JAMSTEC

RESPONSE TO REVIEWER 2

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.

Many thanks for your positive comments and we appreciated your very thorough review of our manuscript.

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

We can agree with these comments in general, and we have increased textual explanations and details on figure captions according to the comments (which are detailed

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below). We also added higher magnification photos and a video as supplemental materials. As reviewer 2 pointed out, the distribution of pseudopodia during chamber formation is much better visible with a video.

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.

We consider that all three organic sheets are formed with branched pseudopodia extending from the aperture. We expect these pseudopodia themselves are ultimately expanded from single root, but separate branches are forming each organic sheet. According to our recent study (Nagai et al., 2018), these three sheets (OOL, POS and IOL) appears initially to be independent but all sheets actually converge at the pore plate. We must agree that the very initial stage of these sheet construction must be seen at higher resolution as the next step. Though we have already tried to prepare to fix the specimens for that, trials so far have failed because the samples are extremely fragile. But we will keep trying! Added the following to Discussion to make this clear:

'According to a recent study (Nagai et al., 2018), the three sheets (OOL, POS and IOL) appears initially to be independent but all sheets actually converge at the pore plate, indicating they probably ultimately expand from a single root. This, however, warrants confirmation by higher resolution investigation of the very start of the initial stage in future studies.'

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

The distance between OOL and POS increased along the growth of the calcareous wall. Added: 'the chamber wall of which thickens (overall distance between OOL and POS increased over time).'

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3) Why is the space between IOL and POS narrower than that between OOL and POS? This is caused by the difference of growth rate of calcareous material between the inner side and the outer side. Assuming that the materials (Ca²⁺, Carbon, among others) are transported from the seawater, it can be presumed that the inner side will become thinner because the chamber wall is formed and material transportation is more restricted in the inner side. Added the following to Discussion:

'The reason why the space between IOL and POS is narrower than between OOL and POS (meaning the inner calcareous layer is thinner than the outer) is presumably caused by the difference of growth rate of calcareous material between the inner side and the outer side. Assuming that the materials for chamber formation are transported from the seawater, it can be presumed that the inner side will become thinner because the chamber wall is formed and material transportation is more restricted in the inner side.'

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

To really clarify this issue, we need to look at the initial stage of formation to see if these are truly separately formed. If from the beginning of construction, all three sheets (OOL, POS and IOL) are converged at the pore site, then such alignments are not necessary. This is a subject of future detailed study on the early stages.

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

Pore plates seems to be dented at the IOL side (see Fig. 3C in Nagai et al. 2018). Added the following in Results: 'Pore plates seems to be dented at the IOL side, from a previous study (see Fig. 3C in Nagai et al. 2018).'

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?

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Indeed, we agree that vesicles are usually found inside the cell, as the reviewer mentioned. There is no sufficient evidence in the present study to prove that these are vesicles, and thus we changed 'vesicles' to 'spherical structures' across the entire manuscript.

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 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.

We approve this point by the reviewer, and agree that the actual amount of seawater exchanged is a key subject to explore in the future. Regarding the differences in Mg/Ca between IOL-POS vs POS-OOL space, measuring the elemental distribution in such a narrow gap is very difficult with existing techniques. This is because chemical compositions are not well preserved during the conventional process of sample prep for electron microscopy. We are currently carrying out EDS analyses with the same interest in mind, but have not achieved sufficient results for a publication. We hope to investigate the elemental composition of liquid components and evaluate the importance of the closedness of the inter-layer spaces in future studies.

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

Many thanks. We have attempted to do so, as detailed above.

Specific and technical comments

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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."

We can agree in part, in that saying 'reconstructions' is going too far. So we changed the second part of the title as suggested. It now reads: 'Weaving of biomineralization framework in rotaliid foraminifera: Implications for paleoceanographic proxies". We hope this is ok.

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

Changed as suggested.

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

Added '... , as well as between taxa, species, individuals, etc.'

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

Corrected.

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

Changed to '... still under investigation'.

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

To make it clear we moved 'for the first time' to the end, it now reads: 'We document triple organic layers sandwiching carbonate precipitation sites for the first time'.

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

Agreed and changed to 'primary organic sheet'.

L40: I do not think so unless the authors verify changes in passive and active ion

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transport at early and later stages, respectively.

We agree with you and therefore changed the expression to make it more mild: 'provides insight towards resolving' instead of 'resolved'.

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

Specified as follows: 'The 'vital effect', specifically elemental and isotopic ratios along chamber walls, . . .'

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

To address this we reorganised the last few sentences of the introduction, as follows, which we hope helps: 'Our study provides insight towards resolving a key 'missing piece' in understanding foraminiferal calcification through culture experiments and in-depth observations of living animals. Our findings contribute to interpreting and understanding biogeochemical proxies by showing that the 'vital effect', specifically elemental and isotopic ratios along chamber walls, is directly linked to spatio-temporal organization of the 'biomineralization sandwich' controlled by the three major organic layers.

L47, Keywords: should have more important words.

Biogeosciences does not actually require keywords, so now they have been removed.

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.

We can agree with this and have deleted the first paragraph.

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

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Done.

L75: by experiment > by culturing experiments

Corrected as suggested.

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.

Deleted as suggested.

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

Modified as suggested.

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 foraminifera do not produce any dynamic net-like structures with no any granules visible during chamber formation.

Your suggestion is true, but we prefer to use pseudopodia as it is a more general term. We modified the sentence as follows to make this clear: 'Foraminiferal pseudopodia are usually named granuloreticulopodia (see Travis and Bowser, 1991) to define a granular reticulated pseudopodium responsible for feeding, digestion and locomotion; in the present paper we will simply use pseudopodia as it is a more general term.'

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

Changed to '...the one in the middle was initially named the 'Primary organic membrane' (POM) (Hemleben et al., 1986) but later changed to 'Primary organic sheet' (POS) (Erez, 2003)'

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L116-118: OOL and IOL are not first mentioned

True. Changed to simply OOL and IOL instead of spelling out the whole name.

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.

Since Anlage has been defined differently by different people, we disagree and would like to refresh with a new term to avoid confusion in the future.

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

Changed to simply OOL and IOL instead of spelling out the whole name.

L130: natural state?

Changed to ‘well-preserved morphology’.

L130-131: electron microscopy>SEM/TEM?

Changed as suggested.

L132, (SOC): Move to L129 that is first mentioned

Changed as suggested.

L150-151: paleoenvironments>palaeoceanographic proxies

Changed as suggested

; predicting responses to ongoing climate change > how?

Deleted this part of the sentence.

L153: Better to rewrite as “within a hyaline calcareous wall using the benthic foraminifera”

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Changed as suggested.

L157, SEM: Define when first mentioned.

This was defined already in the introduction.

L167: De Nooijer et al., 2009? Check all years of references in the text. I found some typos in other refs as well.

Corrected and checked all years of references.

L208: The first paragraph of the Results section is just an outline and unnecessary. Delete or partly move to the method section.

Deleted.

L223: the last existing calcified chamber > the last chamber

Modified as suggested.

L224: characteristic > morphology

Modified as suggested.

L226: delete “from then”

Deleted.

L231, an aggregation of cytoplasm: Indicate where and which part in the figure,

Added reference to Figure 1B.

L233: retracts until where?

Added ‘until the surface of the newly forming chamber’.

L238-239: fine and short pseudopodia? I cannot see it. Need more close-up photos.

We added an enlarged part to Figure 1C, we hope this helps.

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L240-241: A brighter band of particles? I cannot see it.

Changed to simply saying 'bright band'.

L242: beyond? inside?

Changed to 'inside' as suggested.

L243: smooth? Fig. 1C looks smoother than 1D

Deleted this part of the sentence.

L251, Calcium carbonate: How do you know it?

Added to 'material, inferred to be calcium carbonate,' at beginning of the paragraph.

L252: I think the overall outline and size are fixed at earlier stages (the middle stage).

We removed 'size' but the outline morphology is actually changing since the middle stage so we left it.

L253: Hard to see pseudopodial movements. Do you have a movie?

We have added a movie as Supplementary Video.

L253: Open triangles in Fig. 2A?

Deleted 'open triangles' (this referred to an earlier version of the figure, apologies).

L256-257: How do you know the chamber wall getting thicker?

This is clearly visible in the supplementary video, so we added reference to that video: '(from Figure 2B–C; also see Supplementary Video)'

L262: The usual type of pseudopodia movement means reticulopodia?

Added 'usual type of pseudopodia movement (typical of reticulopodia)'.

L266-267: Move to the method section

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We do not think this can be moved to Methods because without results from the observation of the stages, we would not have been able to divide the stages as such. So we have left this section here.

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.

Moved.

L274: gray in Figures 2-4?

Corrected.

L274: um?

Corrected.

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?

This is a good point in that we cannot clearly identify these spherical structures as vesicles with certainty, at this point. Therefore we have changed the name to 'spherical structures' throughout the manuscript and deleted the assumption that these represent vesicles.

L295, pseudopodia: how do you identify it?

We can only identify pseudopodia by morphology, that the elongated structures are inferred to be pseudopodia. Added the following to make this clear: 'elongated structures, inferred to be pseudopodia,'

L311, needle-like structure: Show in the figure.

Added panels to Figure 5E to show this.

L316-317: Show in the figure

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Added arrowheads in Figure 5E to show the gaps.

L328-329: Which are algal cysts in Fig. 5A?

These overlay the OOL, so we added 'Algal cysts including *Dunaliella* individuals can be seen overlaying the OOL'.

L335: period>stage

Corrected.

L353: OOL had toward the inner side?

This is a part of a line we thought we have removed, apologies. Deleted now.

L376-377: Indicate which photos clearly show this.

Added '(Figure 4C-E, Supplementary Video)'

L387: Cader>Cadre, Ni>Ní based on references

Corrected.

Figs. 1&2: Add color legend in the figure;

Added.

indicate initial, middle, late stages in the figure;

Indicated

hard to see any bubbles and pseudopodia inside chambers;

This is more visible in the Supplementary Video which we have added now, these represent structures within the chambers.

in Fig. 1C, the frame of new chamber are magenta?;

No, in 1C there is no calcified parts yet, and magenta indicates calcified wall. So this

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is only present in 1D. We made this more easily visible in 1D now.

I prefer more explanations in the caption to understand without reading the relevant text;

We added more comprehensive explanations to Figs 1 & 2 in the revised captions, as follows. It should be understandable without having to read the relevant text now.

'Figure 1: Time series observation of chamber formation by optical microscopy, as seen in the individual observed on December 7th, 2017 (see Table 1). The initial stage of chamber formation, where the organic framework is built, depicted by A-B. A: Beginning of chamber formation, defined as 0 minute from the start, indicated by a dense radiating spray of pseudopodial network. B: 9 minutes, when an aggregation of cytoplasm becomes visible around the aperture of the last existing chamber. As this cytoplasm expands, the pseudopodial network starts to retract to the surface of the new chamber to complete the framework. The middle stage, where the organic framework is prepared for calcium carbonate precipitation which begins at near the end of this stage, takes place between 15 minutes to 60 minutes, as depicted by C-D. C: 27 minutes, cytoplasm concentrates and outline of newly forming chamber wall now clearly visible, pseudopodia still just visible on the surface. D: 41 minutes, pseudopodial retracts inside the forming chamber wall. Left: optical microscopy image. Right: the same image with schematic overlay; colour legend: deep purple = pseudopodia; light purple = cytoplasm; magenta = calcium carbonate in the newly forming chamber; yellow = previously formed chambers.

Figure 2: Time series observation of chamber formation by optical microscopy (continued), as seen in the individual observed on December 7th, 2017 (see Table 1). The late stage of chamber formation, where calcium carbonate is extensively precipitated and chamber wall is thickened, taking place from around 60 minutes after the start of chamber formation (total time varies considerably among individuals). A: 65 minutes, pseudopodia expands again to form a dense network but in thicker strands than

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seen in previous stages. B: 100 minutes, a network of pseudopodia is seen in the new chamber, the chamber wall of which thickens. C: 124 minutes, chamber wall thickening continues. D: 180 minutes, chamber wall thickening is nearly and the pseudopodial network begin to disappear, indicating that the end of the chamber formation process is near (actual completion was at 248 minutes for this individual). Left: optical microscopy image. Right: the same image with schematic overlay; colour legend: deep purple = pseudopodia; light purple = cytoplasm; magenta = calcium carbonate in the newly forming chamber; yellow = previously formed chambers.'

open triangle?;

Deleted, this was from an earlier version which should have been removed before submission.

Did you identify calcareous wall by polarized microscope?:

We inferred the brighter parts to be calcium carbonate based on images from differential interference contrast (DIC) microscopy.

any more magnified images?

We added images in original resolution as supplementary figure.

You should have used a fluorescent dye to observe cytoplasm more clearly.

That is a possibility for future research, but we consider that the activity of pseudopodia is already clearly seen in the supplementary video taken with DIC.

Fig. 3: move after Figs. 4-7;

Moved.

indicate initial, middle, late stages in the figure;

Indicated.

for A, indicate which part of close-up in B;

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B is not a close-up of A but instead is a cross-section.

colors of outer (blue) and inner sides (purple) are confusing with vesicle and pseudopodia.;

Background colours are deleted.

the shape of carbonate crystals looks like needles. is it OK?;

Yes. We have added a reference to Figure 4E, newly added to show needle-like crystals.

What are the purple colored polygonal shape on the POS?

These are holes, we removed the colours.

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

Added 'Thick lines indicate membranous pseudopodia and dotted lines indicate framework pseudopodia.'

Fig. 7: Indicate OOL and IOL lines;

Added this on Panel C.

Add Mg signal and Mg/Ca data

The Mg signals were too weak, therefore we left it out.

Please also note the supplement to this comment:

<https://www.biogeosciences-discuss.net/bg-2018-295/bg-2018-295-AC2-supplement.zip>

Interactive comment on Biogeosciences Discuss., <https://doi.org/10.5194/bg-2018-295>, 2018.