Comments of the reviewer are in black.
Answers of the authors to the reviewer are in green.

Reviewer #2 – Lennart de Nooijer

Dear editor,

I carefully read the manuscript you provided me with from Richirt and co-workers on layers of opal found coating the inside of foraminiferal shells. In short, I think this is a fine piece of work, of broad interest and can be published with minor corrections. Below, I outlined a few issues that can be clarified to turn this manuscript into a very nice publication!

Sincerely,
Lennart de Nooijer

Minor issues:

Line 36: 'sponges or protists' can be 'sponges and protists'
done

Line 56: 'from the North Pacific'
We changed for "*from the abyssal North Pacific*" on line 57

Line 57: 'investment' is a bit odd here: consider replacing by 'coating' or something similar.
While Brady used the term "investment" in his report of 1884, we changed for "external coating".

Line 78: 'has'
done

Line 99: 'resumes' can be 'summarizes'
done

Line 149: 'aspect'? Replace by 'appearance'
Done, we changed all occurrences of "aspect" in the manuscript.

Line 160: 'prior to the measurements'
According to reviewer #1 suggestion, we changed for "prior to analysis".

Line 180: 'connections'
Done

Line 247: I don't understand the 'aspect' here.
We meant "appearance" using the term "aspect" throughout the manuscript. To avoid confusion, all occurrences of "aspect" were changed for "appearance" in the manuscript.

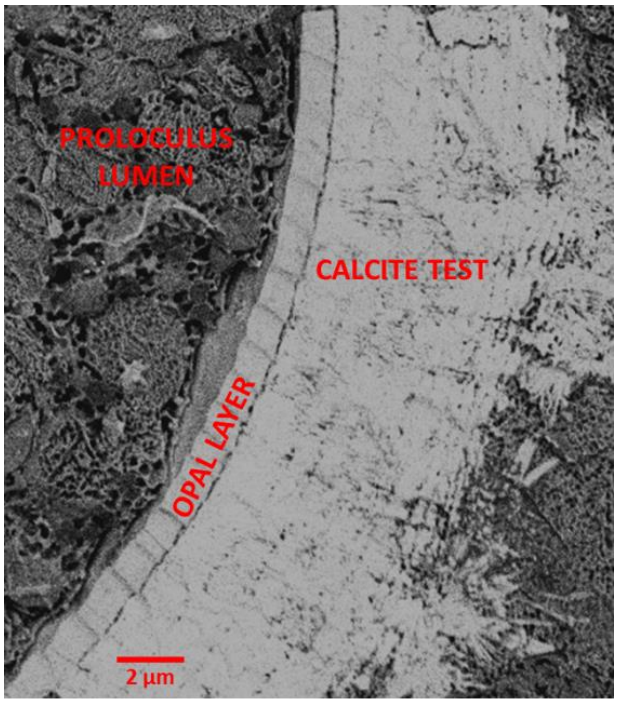
Line 289: This is not necessarily the case. It could easily well be that the formation of this inner opal layer is continuous and that the decrease in thickness with size (figure 6) reflects simply the changes in surface-to-volume ratio. Now I think about it: if they would have added a little layer with a constant rate, the decrease in thickness with size would probably describe a different curve (e.g. smaller chambers would have a much more similar thickness than larger chambers). It may be, as the authors suggest, that a little layer is added with each chamber addition event, but I don't see how this would lead to the power function of figure 6. Anyway, the 'ontogenetic effect' (line 289) is a bit out of place here. There is a trend with size, and according to the fitted curve, some explanations are more likely than others. I think

this paragraph could be extended: what is the size/weight or surface/volume curve for Bulimina's of different ages look like and could they indicate what controls the opal coating and the change in its thickness?

CT-scan 3D model reconstructions from other *Bolivina spissa* specimens from the same sampling site are available, these data and will be submitted in another manuscript elsewhere.
In brief, while S/V ratios of successive chambers follow an inverse power law, similarly to the opal layer thickness:
1 – The power values are not fitting so well between S/V of chambers and opal thickness (about -0.3 for chamber S/V decrease and -1 for the opal layer thickness). This may indicate a differential intensity of opal formation in different chambers.
2 - This similar trend is not verified when considering the proloculus, where the opal layer thickness is maximum, and the S/V ratio shows much lower values compared to subsequent chambers (almost minimum).
These observations indicate that the opal deposition process is not continuous/constant thought time and are rather resulting from an ontogenetic effect. However, because these ideas are still speculative and need further validation, we would like to not extend further this part in this manuscript.

We also want to point out here that a possible ontogenetic effect is only suggested but not demonstrated, as already specified in the initial manuscript for both occurrences on lines 289:
"The opal layer thickness from the proloculus towards the apertural side of the test follows an inverse power law (Fig. 6), ***suggesting that it is resulting from an ontogenetic effect****."*
and on lines 313-314:
"The opal layer thickness follows an allometric relationship (i.e., inverse power law), from the proloculus (thick) to newer chambers (thin), commonly found in organisms' growth pattern and ***suggesting that the layer is resulting from an ontogenetic process****...."*

The inverse power law relationship comes from the idea that the thickness of each opal layer deposited would not be equivalent between successive depositions, such as it is the case for successive calcite layers deposition in bilamellar species. The calcitic outer lamella covering the entire test when adding a chamber is progressively decreasing in thickness, so that the more layers are added, the more increasingly difficult it becomes to trace (Bé & Lott, 1964 for a planktic bilamellar foraminifera species).
An example of this decrease in outer lamellae thickness of calcite on cryo-SEM image in the proloculus (not provided in the initial manuscript, will also be submitted elsewhere soon):



This was partly suggested and unclear in the initial manuscript lines 282-288. We added a sentence to precise what we meant on line 301-303 of the revised manuscript:
"This decreasing trend in thickness is analogue to the calcitic test in Foraminifera having a lamellar wall (such as Bolivina), which cover the entire test with new calcitic material (i.e., outer lamellae) when adding a new chamber, resulting in a decreasing thickness of the calcitic test from the proloculus toward the newest chamber (Hansen, 2003). ***The outer lamella covering the entire test when adding a chamber is progressively decreasing in thickness, so that the more layers are added, the more increasingly difficult it becomes to trace (Bé & Lott, 1964).*** *In some of our B. spissa specimens, we could observe such a decreased outer lamellae thickness toward external calcite layers on cryo-SEM images."*

Line 307-308: this is a very interesting observation! I think the Methods and Results sections should explicitly mention that also other species were analyzed, but did not show the opal coating. This is important for the discussion.
We added in Material & Method sections 2.2 and 2.3 the following respective sentences:
Lines 123-124:
"Additional individuals belonging to the genus Uvigerina, Chilostomella *and* Globobulimina *and isolated from the same sample site were also studied with a similar procedure."*
Lines 135-136:
"Additional individuals belonging to the genus Bulimina *and isolated from the same sample site were also imaged with the same procedure."*

We added in the results sections 3.1 on lines 197-198:
"None of the individuals belonging to the other investigated genus (i.e., Uvigerina, Chilostomella, Globobulimina *and* Bulimina*) showed a Si layer."*

We also added supplementary observations made in previous literature (TEM studies) and showing a similar structure than in our *Bolivina spissa* specimens in the discussion section on lines 482-484 and added the associated references in the reference list:

"Furthermore, prior TEM observations of* Bolivina pacifica *(Fig. 4 in Bernhard et al., 2010) and* Bolivina argentea *(Figure 1a in Bernhard et al., 2012) revealed similar structures to those observed in* B. spissa *in this study, suggesting the potential presence of an opal layer beneath the calcitic test of other* Bolivina *species."

Line 364-366: To me, the co-occurrence of opal and calcite in these specimens means that the formation of opal is not (necessarily) an 'alternative' to calcification.
We agree with this remark and lower further the statement of the sentence on lines 390-392:

"*This indicates that calcification is not limiting in these environments and suggests that the opaline and calcitic parts of the test* ***could*** *serve different and/or complementary function(s)*."

Lines 410-413: could it also be that the formation of this opal layer is an 'unwanted' by-product of another process? It has been suggested that calcification as such (in foraminifera) started as a way to get rid of intracellular, harmful [Ca2+].
It may indeed be the case for also Si. The Precambrian Ocean orthosilicic acid concentrations were suggested to be several orders of magnitude higher than today and at this time (coinciding with the estimated foraminiferal emergence), and cells had to develop mechanisms to cope with potential unwanted and harmful autopolymerisation of silica inside the cytoplasm. One of the proposed mechanisms is Si excretion using Si transporters, which are common in eukaryotic cells (and also present in Rotaliida species not known to secrete any opal, see Marron et al., 2016; Hendry et al., 2018, both references in the initial manuscript submitted). However, this mechanism is very speculative and relatively unlikely in the modern ocean, where autopolymerisation of orthosilicic acid inside the cell is not problematic anymore for modern Foraminifera. Finally, this is questioning the evolutionary history of this trait, which need further dedicated studies, such as mentioned in the initial manuscript on lines 351-354.

We modified the beginning of the discussion section 4.3 on lines 370-376 to mention the idea suggested by the reviewer, but couldn't find any reference specifically for foraminifera:

"***The calcitic shell of foraminifera may potentially originate from the detoxification of harmful*** Ca^{2+} ***ions within the cell (Simkiss, 1977; Kaźmierczak et al., 1985),*** *the resultant test serving various functions such as protection against predation, buoyancy control, or facilitation of reproduction.* ***Similarly, the opal layer may also be initially secreted as a detoxification byproduct (Marron et al., 2016), with additional function(s) beneficial for their success in deep-sea environments.*** *Undoubtedly, the test also acts as protective physical barrier against unfavourable physical or chemical conditions of the environment (Marszalek et al., 1969; Wetmore, 1987),* ***particularly considering the chemical and mechanical characteristics of opal".***

Lines 421-423: I don't understand this: if most of the opal is secreted after the calcite is formed, how can that process affect the del-18O of the calcite? Which is fixed at the time of calcification.

The reviewer is right, observations suggest that the formation of the layer below the calcitic part of the test occurs afterward calcite precipitation (lines 295-297 in the original manuscript):

"The absence of an opal layer in the newest calcified chambers indicates that its formation must occur after the precipitation of the calcitic shell."

However, the hypothetical SDVs detected in the cytoplasm, where deposition of silica should occur, may possibly co-occur with calcification. These hypotheses are speculative and need further validation, as stated at the end of the same paragraph in the original manuscript.

We modified the sentence on lines XXX-XXX to specify that the putative SDVs might be active during calcification, prior opal final deposition beneath the calcitic test:

"This might result from the more intense Si precipitation on the proloculus side (thicker opal layer) compared to the apertural side of the test in B. spissa*,* ***assuming simultaneous calcite precipitation and opal formation within putative SDVs****".*