Comments of the reviewer are in black.
Answers of the authors to the reviewer are in green.
Line numbers referring to modifications are indicated for the revised manuscript **with track changes**.

Reviewer #1

The present paper by Richirt et al. reports on the discovery of an opal layer along the inside of the calcitic tests of cosmopolitan benthic species *Bolivina spissa*. The authors used a variety of electron microscopy techniques to document the opal layer as well as the putative organelles involved in its precipitation. The authors conclude that the opal layer was precipitated by the foraminifera itself, in a manner analogous to the precipitation of the calcitic test, and that this layer is most likely a defence mechanism against predation. Additionally, the authors suggest that the presence of an opal layer might 1) offer a novel paleoclimate proxy based on its stable isotope composition and 2) negatively affect paleoclimatic reconstructions based on the bulk composition of the test.

Overall, the manuscript provides a robust investigation into the morphology, composition and function of a bi-mineralic foraminifera test. The introduction reads very well and serves as an introduction to biosilification generally, the importance of foraminifera in paleoclimatic reconstructions, and as a good historical overview of silification in foraminifera. The figures (and supplementary figures) presented in the manuscript are generally very well organised and present a very convincing case for the presence of the opal layer. Section 4.2 is particularly well-written, with the numbered list concisely laying out all the arguments for why the authors think the opal layer is secreted by the foraminifera itself and not due to environmental or passive processes. The TEM images and documentation of the putative SDVs found in *B. spissa* are an exciting finding and it was very smart on the part of the authors to compare images of the putative SDVs in *B. spissa* with SDVs in another silicifying organism. The argumentation in the discussion follows a logical structure, and the conclusions reached on the presence of the opal layer, its presumed precipitation mechanism and its function are very compelling. There are some minor problems with the use of English, but these did not distract from the pleasure of reading a well-structured and clearly written manuscript. Suggestions for improving the language in the manuscript are provided in the line-by-line comments attached.

We thank the reviewer for conducting this thorough review and for providing a multitude of valuable suggestions.

The only major issue with the paper concerns the isotopic data and section 4.4: Implication(s) for palaeoproxies and biogeochemical cycles. Firstly, how were the samples cleaned to remove potential contamination from sedimentary particles and organic contaminants particularly, which can interfere with the CO_2 produced during analysis of stable isotopes during bulk analysis? Standard cleaning typically involves ultrasonication in methanol, deionized water and boiling with hydrogen peroxide to remove organic contaminants. See Roberts et al. (2018) for an overview on cleaning methods.

We followed the same protocol as in Ishimura et al. (2012), which reported undetected CO_2 gas generation from soft tissue over several days when reacting with phosphoric acid at 25°C. The absence of sedimentary particles was confirmed by the transparency of the test and the absence of authigenic material under a stereomicroscope before analyses.

We now indicate the procedure in the Material and Method section 2.6 on lines 164-166 as:
"In total, 17 specimens of B. spissa ***with transparent shells*** *were isolated from NSB site sediment (Supplementary Fig. 1),* ***cleaned with Milli-Q water, and carefully examined under a stereomicroscope to confirm the absence of authigenic particles (Ishimura et al., 2012)."***
And on lines 171-173 in the same section:
"*Samples were reacted with phosphoric acid (H_3PO_4) to decompose $CaCO_3$ and produce CO_2.* ***Note that with the same method, Ishimura et al. (2012) reported that no CO_2 was evolved through the reaction between H_3PO_4 and organic materials at 25°C over several days****."*

Secondly, the authors report lower $d^{18}O$ and $d^{13}C$ values on the proloculus side compared to the aperture side and relate this to the thickness of the opal layer. However, looking closely at the $d^{18}O$ data for example, only seven samples have data for both sides. 3 tests show the proloculus side lower than the aperture side by a difference significantly greater than the analytical precision of the mass spec (±0.1 ‰), 1 test shows a small difference, 2 tests show no significant difference and 1 test shows the reverse trend. If the authors wanted to seriously look at differences in the stable isotopic composition from the proloculus to the aperture side and use it as evidence for the effect of the opal layer on paleoclimate reconstructions based on stable isotopes, SIMS or LA-ICP-MS measurements of each chamber would be more appropriate. Additionally, as stated in the manuscript, these differences could be related to ontogenetic differences or calcification at different seawater temperatures, so a study looking into this would need to be carefully designed to account for these factors.

The reviewer is right to remark that the decreased $d^{18}O$ values in the proloculus part of the test is not observed for all specimens (half of the specimens for which both sides were measured showed this trend, as pointed out by the reviewer). For this reason, we specified already in the submitted manuscript that the isotopic shift was not observed systematically in section 4.3 on lines 382-385 (line numbers from the original manuscript).

In the revised version of the manuscript, when discussing about isotopic shifts in the section 4.4 we edited the sentences to make it clearer that we are discussing about possibilities. In addition, we now specify also in section 4.4 on lines 459-463 that this decreased $d^{18}O$ values is not observed for all specimens, meaning that these observations must be validated by increasing sample numbers and using other more dedicated methods, as suggested by the reviewer.
"However, the decreased $\delta^{18}O$ values in the proloculus part of B. spissa was not observed in all specimens, suggesting there could be other mechanism(s) responsible for such light isotopic compositions. Increasing the number of specimens analysed and conducting high spatial resolution analyses of isotopic compositions, such as Secondary Ionisation Mass Spectrometry (SIMS) or laser ablation ICP-MS, will provide further insight into a potential isotopic composition shift regarding chamber position."

Third, while discussing how the presence of the opal layer might potentially influence proxy calibrations and their interpretation by mixing of the calcite and opal signals, the authors cite Borelli et al. (2018) who suggested that the presence of incorporated silicate grains within the tests of another benthic foraminifera species might affect paleoclimate reconstructions. However, both Borelli et al. and the present manuscript fail to account for the fact that the internal Si-O bond requires significantly more energy to break, and therefore extremely powerful oxidising reagents (fluorine-based compound such as ClF_3 or BrF_5) are required for the analysis of oxygen isotopes in silica-based skeletons such as diatom frustules (Leng and Barker, 2006). Therefore, it appears unlikely that the presence of an opal layer would influence paleoclimate reconstructions based on the bulk oxygen isotope compositions of foraminifera tests that are typically extracted using phosphoric acid. Removal of this section or alternatively, a more robust evaluation of this claim would improve the manuscript.

Following the reviewer suggestion, we removed this statement from the manuscript (lines 464-466).

Finally, considering how thin the opal layer in *B. spissa* is shown to be in this study, and particularly coupled with the fact that, as the authors themselves state, this opaline layer has not been found in other very well-studied foraminifera, the evidence is thin for suggesting that Foraminifera could play a 'substantial' role in silicon cycling. The language in this section could therefore be softened somewhat. Suggesting the use of the opal layer as a new paleoproxy is already a significant and novel contribution that potentially opens up a new field of study and is a good place where to end off the manuscript.

Following the reviewer's remark, we softened the language as follow in the penultimate sentence of the discussion in section 4.4:

"If silicification in foraminifers was finally found to be more widespread than previously known, either among the genus Bolivina *or possibly among other Foraminifera genera, this group could also participate in Si cycling, adding up to the already significant role of other Rhizaria in this cycle (Llopis Monferrer et al., 2020)."*

We also removed the last sentence of the conclusion section, as proposed by the reviewer.

Line-by-line comments:

Words in **bold** show the suggested insertions/deletions/corrections.

21- … the Foraminifera **themselves secrete** the opal layer. The **opal** layer…
done
22- …growth pattern **to** the…
done
33- Please rephrase this sentence as quartz is the most common silicate mineral and therefore it reads a bit funny.
done
34- Biologically assimilable sounds clunky, consider using **biologically available** or **bioavailable**.
done
36- replace both instances of or with **and**
done
37- remove **their**
done
38- remove **The** and **were proposed to**
done
43- …of a shell**, also called a "test"**, which…
done
43- I recommend combining these two sentences, for example like this …or **consist** of precipitated minerals**, most commonly calcium carbonate** (CaCO3) (ref).
done
48- test**s**
done
49-50- I suggest turning this phrase around to make it easier to read… carbon cycle through carbonate production (ref) and remineralisation, especially in poorly oxygenated environments (ref).
done
51- Although **the** exact calcification process is still **up for** debate…
done
55- …in **the** late…, test**s**
done
56- …of **the** North… replace was with **were**
done
59- replace having with **with**
done
60- particle**s**
done
62- depth**s**
done
63- replace because with **as**
done
64- …being the only **representative of the** newly…
done
66- …the test together**,** such as…

done
77- …but were **inferred to be of** sedimentary…
done
78- replace have with **has**
done
79- replace or with **and**, ---having a **siliceous test have been** reported….
done
83- there are various instances of the use of "**the**" before Sagami Bay. Please remove all of these. It should read… 1410m depth in Sagami Bay (Japan).
All occurrences were corrected
84- replace from with **for**
done
88- remove **further**
done
89- …using **Foraminifera test** composition…
done
98- Figure 1: **M**ap of…
done
99- Table 1 **summarises** the sample**'**s origin…
done
100- interval**s**, **techniques**
done
102- …type of **analysis**…
done
105- live checks sounds clunky, please rephrase.
We rephrased:
"After confirming that individuals were alive (based on the presence of sediment aggregation at the aperture and cytoplasm coloration), isolated specimens were processed following the protocol described in Okada et al. (2024)."
109- …observation**s were**…
done
112- replace water ice with **ice crystals**
done
113- to avoid confusion, … a high-resolution SEM image for **each individual**…
done
114- …possible **overlap** of EDS peaks **from** the coating metals.
done
117- replace were with **was**
done
118- …the main **component** of **calcitic tests**… Suggest changing sentence to:
Scaling and/or rotation of EDS maps were performed…
done
119- replace mapping with **map**, and from with **of**
done
12o- … origin, **the** aluminium…, replace to with **from**, …(typically clay minerals, the major constituent of sediment at the sampling site)
done
128- **Subsequently**, …
done
130- In addition, **optical images**…
done
131- …after **the** decalcification…
done
133- …the **contents of the cytoplasm** using…
done

135- please rephrase this sentence as live checked is clunky. A suggestion could be, Directly on board, living specimens(n = 8) were isolated from different…
done
145- SEM or STEM?, …mode **operating** at an acceleration…
done
148- remove **size**
done
152- …prior **to** measurement
done
153- …microscope **Fourier transform** infrared spectrometer…
done
155- Remove Because
done
155- CaF2 absorbs below 1000 cm-1, **therefore** no band assignments **were** done…
done
159- …apertural side, **a** few chambers…
done
160- …prior **to analysis**.
done
172-…imaged **with** low-vacuum…
done
175- … the **final (newer)** chambers…
done
170-175- I suggest re-labelling Figure 2 such that the text then references the figures in order. For example this would then read as: Macrospheric (haploidic) and microspheric (diploidic) specimens were observed with a stereomicroscope (Fig. 2a & 2**b**) before being imaged in low-vacuum SEM settings (Fig. 2c & 2**d**). The figure caption for Figure 2 could potentially be re-written as: Macrospheric (**top row**) and microspheric (**bottom row**) Bolivina spissa specimens, imaged with **a** stereomicroscope (**a and b**) and low-vacuum SEM before (**c and d**) and after (**e and f**) decalcification to expose the Si layer below the calcitic shell.
done
180- connexions replaced with **connections**.
done
180- The initial reference to Figure 3a can be expanded to make it clear to the reader that the smooth walls visible in the image are in fact the Si layer, before discussion of the funnel-like structures most likely composed of organic material. A label should also be added to Figure 3a to make this immediately visually clear to the reader that these structures are the Si layer.
We modified the sentence as follow:
"Figure 3a shows the Si layer connections between consecutive chambers ***after the removal of the calcitic test by decalcification.****"*
We added a label on Figure 3a.
180- **Protruding** funnel-line structures were **visible** at the pores' locations…
done
181- Suggest rewriting this sentence as: These funnel-like structures were not made of Si but were probable remains of organic material, as cryo-SEM (ref) and TEM observations (ref) show the Si internal coating terminates at the pore plate.
188- connexions replaced with **connections**.
done
191- Figure 4 illustrates the **workflow used** to obtain…
done
192- …maps **of** non-sedimentary origin which **were**…
done
193- The calcium distribution **(Fig. 4a, b)** was correlated to areas of lower electrondensity

on the SEM images **(Fig. 4c, d**), representing the calcitic tests of individuals.
done
194- Sedimentary aluminosilicate**s were** removed by subtracting **the** aluminium signal…
done
195- decide whether to use silicon or Si and use throughout.
We replaced silicon by Si
196- replace from with **of**
done
197-… the **Si** signal…
done
200- **The** proloculus…
done
204- ….the Al signal **from the** Si signal (i.e., removing aluminosilicate**s**)
done
204- Replace from with **of**
done
206- chamber**s**
done
207- **the** proloculus **to** apertural side
done
207- suggest changing this sentence to… was correlated to cryo-SEM observations showing decreasing Si layer thickness towards younger chambers.
We changed for "*was confirmed by cryo-SEM observations showing decreasing Si layer thickness toward younger chambers*".
209- fracture**s,** remove **nature**
done
210- …structure**s were** observed between the Si layer **and** the calcitic shell…
done
211- In very rare cases, we observed a **gap** between the two layers**,** such as **in** Fig. 5e, which we ascribe to preparation artifact**s** (i.e. **the** cutting step).
done
213- …of **a** representative…
done
215- **M**agnified…
done
216- the a with **the**
done
216- …typical **of** glassy material**s**.
done
217- …the **gap** between…**results** from **a preparation artifact.**
done
218- **The** thickness…
done
221- replacer newer with **younger**
done
222- replace while with **when,** replace over with **of the**
done
223- replace definition with **resolution**
done
228- replace minute with **small**
done
232- replace are with **is,** remove **the**
done
233- The broad band**s** at ~3400 cm-1 and ~1635 cm-1… please add a reference here

if possible
Same references as in the previous sentence were added.
234- …a broader band **at 1070 cm-1** with a shoulder…
done
236- **Representative** FTIR spectra…
done
243- suggest rewriting as: ultra-thin sections of B. spissa individuals were imaged with TEM.
done
244- Suggest rewriting this sentence as: From a total of eight individuals imaged, two showed structures filled with matter showing the characteristic conchoidal fracture patter of silicon-based materials.
done
245- Suggest rewriting as: This conchoidal fracture pattern, visible after sectioning with a diamond knife, was similar to the conchoidal fractures visible within the Si layer coating the inside of the calcitic shell of all eight specimens.
We modified as following:
"From a total of eight individuals imaged, two showed structures filled with material showing the characteristic conchoidal fracture pattern of Si-based materials (Fig. 8b–g)".
253- replace resembling with **similar to**
done
255-…detritus, **occur abundantly** in…
done
258-…for **the** proloculus side…for **the** apertural…
done
259- values **ranging from** +0.13…
done
260- …which **ranged** from…
done
260-…of **the** aperture side **were** comparable **to the** isotopic equilibrium value of calcite at **a** depth of…
done
262- replace represent with **showed**
done
268- Decide whether to use Si layer or silicon-rich layer and use consistently Throughout
We chose Si layer and modified the manuscript accordingly.
269- on cryo-SEM and TEM images, no brackets. Replace from with **of**
done
270 replace from with for, silicon-using with silicon-**based**
done
271-…which **were in good agreement with spectra of** reference opal
done
275- B. spissa **appears homogenous**, without…
done
276- Please provide a figure reference here. The sentence on M. legis a bit too long and therefore reads poorly, consider breaking up into two sentences.
We split the sentence in 2, and added the plates reference.
280-…genera, **as is the case for the carbonate** tests of Miliolida and Rotaliida…
done
284- … **analogous** to the calcitic test**s** in…
done
287- replace could observe with observed a
done
289- replace is resulting with **results**

done
289- …trend**, similar** in …
done
290- of **an** opal coating
done
291- …steps, **comparable to**…
done
292-… thickness is **at a** maximum… Please add a figure reference.
done
293- replace few last with **youngest**
done
295- Figure reference
We added references to Fig. 5a and 8a. We also added a new supplementary figure 7 for other examples showing the absence of Si layer in youngest chambers and modified other supplementary figures numbers accordingly.
296- **an** opal layer, replace afterward with **after**
done
298- I suggest changing the title of section 4.2 to: Is **the opal** layer precipitated by the foraminifera itself?
done
302-…and hence exposed…
done
306- replace showing with **demonstrating** and when with **while**
done
307- …site**,** such as…
done
310- replace from with **of**
done
310- suggesting change to: (e.g., of sedimentary or biogenic origin, i.e., secreted by another organism and subsequently incorporated by the foraminifer)
done
313- pattern**s**
done
315- I suggest that the sentence is reorganised to remove the brackets and that the figure references are placed after the corresponding text.
We reorganised and split the sentence as follow:
"Supplementary TEM observations reveal peculiar organelles occurring in the cytoplasm, containing material exhibiting the typical conchoidal fracture pattern on TEM images and opal composition in EDS spectra (Fig. 8 & Supplementary Fig. 4). These findings further corroborate the assertion that the foraminifer secrete the opal layer itself."
318- replace resembling with **similar to**
done
318-…opal in **the frustules of diatoms** (ref)
done
319- What do you mean with 'shell building'? Please clarify.
We mean the shell of other organisms and modified accordingly
324- have never before been reported
done
325- …**SDV**-like organelles in two **out of** eight…
done
326- **SDV**-like (no s in this case)
done
327….both in younger and older chambers of the same specimen (Fig. 8), suggesting that opal layer…
done

we added 2 references in the next sentence to state that similarly, calcification occurs in anoxic settings.

"These organelles were observed in individuals from 1-2 cm depth interval in the sediment, where oxygen is absent (Glud et al., 2009), suggesting that opal precipitation may occur in anoxic settings, ***as it was shown for calcite precipitation (Nardelli et al., 2014; Orsi et al., 2020).*** *"*

334- …**reported on in the literature**.

done

342- Please clarify what you mean with 'nesting Rotaliida and Miliolida in naked foraminifers'

done, we rephrased as:

"These phylogenetic relationships, in which Rotaliida and Miliolida are nested within naked foraminifers, suggests that biosilicification was acquired independently throughout their evolution history."

343- Similarly, **it has been previously** suggested that

done

349- Please clarify what you mean with 'in most of other Foraminifera represents'.

We mean other Foraminifera that do not show the trait. We removed "represents".

349- It is the first time SIT as an acronym is used and should therefore be written out… ancestor **have previously been** identified…

now specified in the text

356- If you use 'was proposed' then please add references at the end. I'd recommend changing this to: Foraminifera tests serve various functions...

done

361- replace in with **to**

done

364- sign**s**

done

367- Please remove the word **reportedly**, it makes it sound like there is an element of doubt about these claims of mechanical strength etc.

done

367- I recommend rewriting this sentence as a light weight and strength do not lead to structural integrity, which is a different property altogether. I suggest: ...are known to possess remarkable mechanical properties such as light weight, strength and structural integrity, among other functions

done

369- **Despite not observing** any… Remove capitalization of Diatoms and use **diatom frustules** instead.

done

371- I think this sentence should be turned around or else ended differently as currently it sounds as if the authors are putting forward a theory that they already have identified some potential arguments against but are nevertheless advocating for until proven otherwise.

Agree, we simplified the sentence to be more straightforward:

"However, the occurrence of other species having a more fragile test compared to B. spissa at the same location, such as Chilostomella, does not support this hypothesis."

383-…calcify **at higher temperatures** compared…

done

384- remove **it**

done

388- … 5 times **more resistant** to abrasion...

done

389- Foraminiferivory is quite a niche term not found in many recent publications. I suggest replacing it with predation.

done

390- …reported to drill holes into the shell**s** of bivalve**s** by…

done
391- test**s**
done
393- remove **a**
done
396- replace protection with **protective**
done
397….preventing predators **from accessing** cell contents… in Sagami Bay are potential prey
done
400- remove **it was suggested that**
done
405- replace sometimes with **occasionally** and remove **such**
done
410-… be **for protection against** predators…
done
413- ...other, potentially non-exclusive, functions.
done
415- B. spissa**,** where C and O isotopic compositions were close…
done
417- **overwritten**
done
421- …temperature**s** during **the**…
done
423-… suggested that variations **in** intracellular…
done
428- purpose**s**
done
429-…B. spissa **has been** used…
done
430- replace done with **performed**
done
431- …shells as a geochemical proxy.
done
444- It is not immediately clear what is meant with 'for global nitrogen cycle'. Please clarify and rephrase.
We removed this statement and reorganized slightly the end of this section to clarify the idea that Foraminifera, which are already known to be involved in C and N cycling, may also play a role in Si cycling.
449- …cycles, **the** biological carbon pump, **and** marine food…
This sentence was removed from the manuscript.
456- replace afterward with **after,** remove phases, and replace presume with **Propose**
done
458- test**s**
done
458- However, other (non-exclusive) functions could exist and need to be investigated further.
done
459- Suggest rewriting this sentence as The presence of this until now overlooked opal layer below the calcitic test of the cosmopolitan B. spissa raises questions on the extent of silification in Foraminifera.
done
460- This sentence is a bit long and could be split into two.
done

463- …below **the calcitic test**…
Done

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

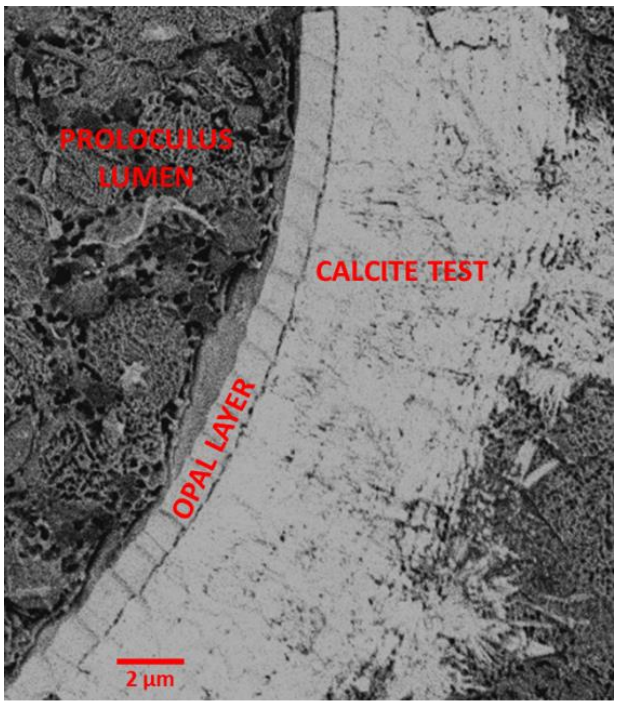
Line 247: I don't understand the 'aspect' here.
We meant "appearance" using the term "aspect" throughout the manuscript. To avoid confusion, all occurrences of "aspect" were changed for "appearance" in the manuscript.

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 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 line 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 (and the reference associated in the reference list) to precise what we meant on line 304-306 of the revised manuscript:
"This decreasing trend in thickness is analogous to the calcitic tests 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 observed 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 genera 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 genera (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 395-397:

"*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 383-389 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 line 459 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****".*