

Biogeosciences Discuss., referee comment RC2  
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## Comment on bg-2021-74

Inge van Dijk (Referee)

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Referee comment on "Host-influenced geochemical signature in the parasitic foraminifera *Hyrrokkin sarcophaga*" by Nicolai Schleinkofer et al., Biogeosciences Discuss., <https://doi.org/10.5194/bg-2021-74-RC2>, 2021

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With great interest I read the manuscript 'Host influenced geochemical signature in the parasitic foraminifer *Hyrrokkin sarcophagi*' by Sleinkofer et al. The authors investigated the chemistry of both host and parasitic foraminifer in great detail, and I think the manuscript is in general well-written and structured. I have some specific comments on the model used to explain the geochemical signature of the foraminifer, and the chosen model parameters. Furthermore, I would also like to see some changes in the method description. I would be happy to review the revised version of the manuscript after moderate revisions.

### Major comments:

In general, the manuscript is missing quite some details on the sample preparation / cleaning or number of measurements per specimen or per species (see comments below). I have no doubt the analysis were done correctly, but I would like to see these details added to the revised manuscript for reproducibility.

For the EPMA analysis, I believe some more investigation is necessary to support the claims of the authors on the co-variation of certain elements (e.g. high Mg corresponds with high Na and S.) This can be done several ways, e.g. plotting the map counts of Mg versus other elements, or calculating line transects of the different elements (see for example my own protocol for foraminifera in van Dijk et al., 2019, <https://doi.org/10.3389/feart.2019.00281>).

For the mixing model, I would like to see some changes in the model parameters, mainly for Mn and Sr. The chosen DMn from Mucci, 1988 is based on inorganic precipitation, and

DSr from Raitzsch et al., 2010 is from a high-Mg species, which in general have higher D values. I would propose to use DSr derived from a mid-Mg species like Amphistegina, which is in the same range of Mg/Ca. This will likely greatly change the results of Fig. 6 and the current interpretation and discussion. The model is a bit too simplified in my opinion, and it ignores a lot of observations on foraminiferal calcification of direct uptake of the elements during calcification through trans membrane transport (Nehrke et al., 2013; Toyofuku et al., 2017). Even when following seawater endocytosis model of Erez, 2003, the seawater is not unmodified when it is available for the foraminifer, as the model assumes. For example, Mg ions are likely pumped out of the seawater, before it is available at the site of calcification as calcifying fluid. I understand that there are still a lot of unknowns in foraminiferal calcification processes and the authors cannot include all in their mixing model, but maybe they can add a step/fractionation from seawater to calcifying fluid (which can be based on e.g. the offset between distribution coefficients observed for inorganic calcite and foraminifera).

I would like to see some discussion on the mechanisms of boring (see also comments below). Did the authors find evidence for mechanistic boring, or does the foraminifera bore by acidifying the microenvironment, as observed for calcifying foraminifera due to proton pumping (de Nooijer et al., 2009; Glas et al., 2012; Toyofuku et al., 2017). And if so, while the foraminifera is dissolving the shell, it automatically changes the chemistry of the seawater around the foraminifer, providing Ca and other elements for calcification, thus recycling the hosts carbonate.

### **Specific comments:**

Figure 1: Was this specimen embedded in resin and cut/polished? Please give some details about the preparation/treatment of the sample.

L89: This is a bit unclear for me. What does HAO stand for, and why is HAW (with callus according to L88) included in HA, which are *H. sarcophaga* that infested *A. excavata* without callus formation.

L91-93: For this study, you picked/selected only specimen that were firmly attached to the host?

L94-113: I prefer to put sample preparation with the paragraph of the method, e.g. L94-98 in 2.4. It makes the methods section much easier to read.

L96: Which type of resin was used and was the embedding done in vacuum to avoid air bubbles etc.? Where the shells cleaned in anyway?

L97: I am normally polishing until 0.2 um using a diamond emulsion. With 3 um, is the surface smooth enough? Was a previous method followed for the preparation?

L103 and again at 108: No kind of e.g. reductive and/or oxidative cleaning?

L105: Were the Eppendorfs acid cleaned?

L125: type of microscope?

L135-142: Samples were carbon coated? How many maps of how many specimens were taken? What kind of areas were selected

L141: You performed maps, lines or point measurements, and how many? With maps, how did you exclude resin / pores?

L149-157: Sample were diluted to solutions of e.g. 10 ppm Ca?

L152 and 159/160: What was the accuracy of the standards?

L159: What was the measurement precision?

L187: What test was used to compare the point measurements by EPMA (Fig. 2)

L190: Was a Bonferroni correction applied to the obtained p-values?

L197: Supplement S1, not S2.

L203: You mean especially in the foraminifer?

L205: Is there also a high magnification image available of the SRZ? Maybe this can be added as an insert in Figure 1, to show the thickness of the organic layers.

L206-211: The authors describe in detail the shape and size of e.g. the bore canal and layering in SRZ. Did they also obtain images from other SRZ and canals on other specimens to check the variability?

L223: How many measurements per sample? – could be added to the method section

L225, L228, 235 and in general throughout 3.3. as well as 3.4.: Are these similarities or differences significant or not? These statements need to be supported by statistics (e.g. Kruskal-Wallis test with obtained (Bonferroni corrected) p values).

L231: Na/Ca in both region E1&2 is exactly 14.8 mmol/mol? Or on average for both area? Please clarify, or better, give values for both regions and show they are not significantly different.

3.3.: Could you add the standard deviation to the mean ratio in the text? For comparison with other studies this comes in handy.

3.4.: There is a lot of information in the EPMA maps, but the authors use merely 2 sentences to describe the results. Please add more information, like what is the width of the high intensity layers, and do they correspond exactly to the fluorescence image (I presume Fig 1 and Fig3A are the same specimen?). What are min and max values of the different elements and values of the high and low intensity bands. Also: how were porous areas removed from the calculations, e.g. observed with Ca counts in Fig.3 panel A.

L247: The covariation or collocation of elemental bands is difficult to distinguish by eye. Authors claim Na and S are enriched at the same location, but looking at Na and S of Fig. 3, I disagree. For instance, Na and S of Panel A: the green band of S in the middle of the map seems to correspond to the blue band (low intensity) in the Na map. How did the authors check the covariation of elements? Merely by eye is not sufficient in my opinion.

L253: Again, how many measurements are included in fig 4?

L261: Maybe add d18O and d13C values as a line to Fig. 4

L324: how many specimens and host?

L329: Any idea if this is a chemical or a mechanical penetration of the host shell?

L369/Table 3. The authors use partitioning coefficient of inorganic precipitated calcite. Please use published values of foraminifera i.e. 1) for small benthic foraminifera see Barras et al., 2018, or 2) for *Amphistegina lessonii* (which has similar Mg/Ca as the studied species), which can be calculated from van Dijk et al., 2020 *Frontiers in Marine Science* 8 (DMn ~1). Also, it should be noted that the partitioning of Mn is not stable and increases with decreasing seawater Mn/Ca (e.g. Barras et al., 2018).

L549-551: Is this confirmed by the EPMA measurements of this study?

L560: As mentioned before, in e.g. Fig3A I could not clearly see this simultaneous increase in Mg with other elements. Consider making line transect over this area to see and compare the location of the high intensity bands of different elements. This is also necessary to understand if S is increased in the carbonate, or present in organics between carbonate layers (The authors did not perform EPMA on samples which were oxidative cleaned for comparison?)

L551: Again, did the authors made this comparison by eye, or by e.g. plotting them overlapping?

L617: How will the data be made available? Through doi / supplement?

### **Figures/Tables:**

S1\_Meigen Test. Please add a proper caption to this image, in the same style as the other figures.

Fig. 2 and 4: Maybe add the n above the boxplot. Explain that we are looking at boxplots, e.g. Boxplot distributions (line = median, boxes = interquartile range, whisker = min/max values). Are the measurements on different samples significantly different or not?

Fig. 3: Include more details in the caption. These are SEM images? What does A and B stand for (I presume different specimens)? Elements are in counts? What were the min and max counts per element (maybe give each of the their own quantified scale bar.

Fig. 4&7: Please consider choosing another color for either HAW or L.pertusa.

Table 2/3. Tables and Figure should be readable without the main text. So please add the full names of HAW, HAO, D etc in these captions.