

Response to Ralf Schiebel (Reviewer#2)

We would like to thank Dr. Ralf Schiebel for reviewing our manuscript and for providing his constructive comments and corrections. A point-by-point response to the comments is included below.

Haruka Takagi
(on behalf of all co-authors)

Line 21: The author have possibly not observed "symbiont growth", and rewording to "symbiont abundance" may be more correct.

Reply 2-1: We will correct it as suggested.

Line 29: Following the paper of Jakob et al. (2017), planktic foraminifer shells may be composed of vaterite and other calcium carbonate species, and "calcareous" may be the correct term.

Reply 2-2: We will correct it as suggested.

Line 46: *Globigerina bulloides* has certainly not been reported photosymbiotic. Please delete from the list.

Reply 2-3: In a recent study by Bird et al. (2017), *Globigerina bulloides* type IId has been reported to possess cyanobacterial symbionts. Although their finding contradicts to the old observational results of this species showing that *G. bulloides* is symbiont-barren (Hemleben and Spindler, 1983; Gastrich, 1987), we respected their results and listed *G. bulloides*. However, the cyanobacterial symbiosis is an exceptional case among planktonic foraminifera, so we change the sentence only for eukaryotic algal symbiosis. The change of the text will be as "....., twelve have so far been reported to be photosymbiotic with eukaryotic algae (.....,,,,)". and delete *G. bulloides* from the list in Line 46. For Table 2 including the information of inferred cyanobacterial symbiosis of *G. bulloides*, we will leave it as is.

Line 48: Change *hirsta* to *hirsuta*

Reply 2-4: Thank you for pointing it out. We will correct it to "*hirsuta*".

Line 50: Change “in all previous studies“ to “in some previous studies”.

Reply 2-5: We will change it as suggested.

Line 150: Change “in his study” to “in her study”.

Reply 2-6: We appreciate the correction. We will correct it.

Lines 182-183: Please delete the sentence "Therefore, although genetic information and detailed microscopic evidence are needed in the future, we categorize them here as dinoflagellate-bearing species." Second guess does not improve the quality of a scientific, and there is no need to do so in this place.

Reply 2-7: Thank you for the advice. We will delete the sentence.

Line 201: Please replace by “ σ PSII was relatively clearly low in dinoflagellate-bearing species. . .”

Reply 2-8: We will replace “clearly” to “relatively”.

Line 203: Chapter “3.4 Principal component analysis and clustering” would need a brief introduction. Please first write what you tested, i.e. objects and variables, and then present data. In general, this paragraph needs to be better explained and better organized for easy understanding.

Reply 2-9: In the method section (2.4 Statistical analysis), we explained the purpose of PCA and its variables. However, as you suggested, it is better to briefly mention it again at the beginning of Chapter 3.4 for easier understanding. We will revise the chapter accordingly. In addition, since the variables we used for the analysis was not listed altogether (ratio of symbiotic individuals, F_v/F_m value, and Chl *a*/biomass are in Table 1, whereas correlation coefficient of test size-Chl *a* relationship is in Figure 6), we will include the last one in Table 1 as well.

In the Discussion and Conclusions chapters, the writing style deteriorates, and some rewording would be necessary. I would recommend using the present tense throughout, since it makes a nicer reading.

Reply 2-10: Following your advice, we will use the present tense for Discussion and Conclusions chapters. The text will be carefully checked again and will be reworded/rewritten for nicer reading. We would like to express our appreciation for your careful reading and detailed corrections in these chapters.

Lines 230-231: “Based on the result of the PCA and cluster analyses, 30 foraminiferal species were characterized and categorized into four groups (Fig. 9).” This not correct; Statistics cannot create new results, but confirm results. Please rewrite the sentence accordingly.

Reply 2-11: Thank you for your advice. We will rewrite the sentence as “The cluster analysis using photosymbiotic variables showed that 30 species fall into four groups, and PCA extracted features relevant to the cluster structure.”.

Lines 241-242: Please rephrase to “Though our study did not identify their genotype, we revealed that this species never possessed symbionts even when collected from shallower water depth (< 100m).”

Reply 2-12: We will rephrase the sentence as suggested.

Line 242: “A recent study. . .”

Reply 2-13: We will correct it as suggested.

Line 248: “Five species were newly confirmed as symbiotic in this study;...”

Reply 2-14: We will correct it as suggested.

Line 249: “All species in the Cluster 1 and 2 including...”. Since we are not primarily interested in Clusters by different groups of foraminifera, you may name these groups for a better understanding. “All species in the macroperforate spinose group with dinoflagellate symbionts, and the macroperforate spinose foraminifers with non-dinoflagellate symbionts...” reads much better, because it contains important information. Please change all of the following text accordingly.

Reply 2-15: We agree that using cluster names describing their features would make it much easier to understand. However, such morphological groups span multiple clusters; e.g., macroperforate spinose species belong to either Cluster 1, 2, 3 or 4. Conversely, Cluster 2 includes all major morphological/ecological groups (macroperforate spinose group with dinoflagellates, macroperforate spinose group with non-dinoflagellates, macroperforate non-spinose group, and microperforate group). Therefore, it is not easy to name the clusters, and we would like to leave

the cluster names as they are, except for some parts that can be rephrased as suggested (e.g., Line 257, "...species in the Cluster 1..." to "*G. conglobatus*, *G. sacculifer* and *O. universa* (Cluster 1)...").

Line 253: delete "itself"

Reply 2-16: We will delete it as suggested.

Line 254: replace "directly clarified" by "determined"

Reply 2-17: We will correct it as suggested.

Line 255: replace "growth" by "size"

Reply 2-18: In this sentence, we would like to explain that the positive correlation in test size-Chl *a* content relationship is an indication of the increase of symbiont number. Therefore, we think 'growth' cannot be replaced by 'size'. We would like to leave it unchanged.

Line 256: replace "should be a specific diagnostic of" by "may indicate"

Reply 2-19: We will correct it as suggested.

Line 257: replace "perform" by "support"

Reply 2-20: We will correct it as suggested.

Line 258-259: delete "It may imply more phototrophic nature of these species.", since this is second guess

Reply 2-21: We will delete the sentence as suggested.

Line 264: please say which species sometimes found without symbionts

Reply 2-22: We will add the species name in the parenthesis; "... (all species except for *S. dehiscens* and *G. conglobatus* includes specimens whose chlorophyll is non-functional, Fig. 5)".

Line 267: "We speculate that these small specimens were. . ."

Reply 2-23: We will correct it as suggested.

Line 268: "...symbiont-barren individuals in this group was small."

Reply 2-24: We will correct it as suggested.

Line 273: "...on phototrophy that can quantitatively represent photosymbiosis."

Reply 2-25: We will correct it as suggested.

Line 277-278: ". . .the examined species were not able to increase their biomass as the host grew." How do you know? This is possibly second guess, and should be deleted from the manuscript. Please delete also the following argumentation "If these are the case, possession of symbionts...".

Reply 2-26: We will delete these sentences as suggested.

Line 293-294: "However, caution should be paid for the narrow size range of *T. humilis* (97–168 μm) (Fig. 6)." This is possibly also the case for *T. humilis* smaller than 97 microns.

Reply 2-27: In this sentence, we wanted to make a notice that the specimens used for the regression analysis were all very small compared to the other species, which may cause the low correlation between test size and Chl *a* content in *T. humilis*. To make this point clear, we will rephrase the sentence as follows, "However, caution should be paid for the narrow size range of *T. humilis* we analyzed, which may cause the low correlation."

Line 304: delete "utter"

Reply 2-28: We will correct it as suggested.

Line 304: "Each foraminiferal species..." I doubt that this is the case for each species; please see your Fig. 11.

Reply 2-29: Thank you for pointing it out. For non-symbiotic species, it is true that the species does not fall into "in-between" heterotroph and phototroph, but 100% heterotroph. We will correct the sentence as "Each foraminiferal species that possesses symbionts can be located.....".

Line 314-316: The significant positive correlation between test size and Chl a content (Figs. 6 and 10) shows the increasing number of symbionts with host size, and a quantitative relationship in the host and symbionts based on their scaling exponent (Table 2).

Reply 2-31: We will correct the sentence as suggested.

Line 317: "If the test shape is less spherical, . . ."

Reply 2-32: We will correct it as suggested.

Line 318: . . . (the increase in cytoplasm. . .

Reply 2-33: We will correct it as suggested.

Line 321-323: ". . .increased in nearly proportional to the host's test volume. This kind of size scaling across different species of planktonic foraminifera suggests a robust relationship between the host and symbionts."

Reply 2-34: We will correct the sentences as suggested.

Line 326: ". . .almost five times more Chl a than the microperforate non-spinose group, and 10 times more than the..."

Reply 2-35: We will correct the sentence as suggested.

Line 329: ". . .spines may facilitate..."

Reply 2-35: We will correct it as suggested.

Line 330: "efficient illumination..."

Reply 2-36: We will correct it as suggested.

Lines 333-334: "Moreover, clear clusters correspond to each morphogroup macroperforate spinose, macroperforate non-spinose, and microperforate non-spinose."

Reply 2-37: We will correct it as suggested.

Lines 334-335: delete: "It is also an interesting feature firstly revealed in this study."

Reply 2-38: We will delete it as suggested.

Lines 339-340: "If such microenvironmental conditions surrounding the intracellular symbionts are measurable or numerically modeled, our understanding of the differences and the controlling factor of symbiont density would be improved."

Reply 2-39: We will correct it as suggested.

Line 342: "When species are grouped according to symbiont type, dinoflagellate..."

Reply 2-40: We will correct it as suggested.

Line 344: "parameters are significantly"

Reply 2-41: We will correct it as suggested.

Line 366: ". . . nutrients in ambient seawater. . ."

Reply 2-42: We will correct it as suggested.

Line 370: “. . . established in *G. ruber* (pink). In fact, the. . .”

Reply 2-43: We will correct it as suggested.

Line 379: “The present study extends our understanding. . .”

Reply 2-44: We will correct it as suggested.

Line 381: “Nineteen species, showed...”

Reply 2-45: We will correct it as suggested.

Lines 383-384: “Finally, we propose a new framework of photosymbiosis in planktonic foraminifera as a continuous spectrum of photosymbiosis.”

Reply 2-46: We will correct it as suggested.

Lines 390-393: “Interestingly, photophysiology may be basically determined by the type of symbiont, regardless of the phylogenetic position of the host and its test morphology. Physiological parameters, in particular σ PSII, seem to correspond to the overall depth habitat of the host foraminifera.”

Reply 2-47: We will correct it as suggested.

Table 1: pachyderma”, barren?

Reply 2-48: In the description of *N. pachyderma* in Hemleben et al. (1989), it is written that “Symbionts have not been observed.”. However, we do not know the reference for this information. We will leave the cell of “Microscopy-based algal type” as it is (i.e., “Not reported”), and will make a remark that “Absence of symbionts inferred” with a reference of Hemleben et al. (1989).

Fig. 1, line 574: “tropical eastern Atlantic”

Reply 2-49: We will change it as suggested.

Fig. 2: very nice!

Reply 2-50: Thank you!

Figures 9, 10, and 11: for didactical reasons, always give the same color for the same group

Reply 2-51: As suggested, we will change the symbol color in Figure 11 to the same color as in Figure 9. However, in terms of Figure 10, the groups are defined based on the morphological and known symbiont group, not the clusters in Figure 9. Therefore, to avoid confusion, we used a new set of colors each of which does not correspond to that in Figure 9. In addition, we mention in the caption in Figure 10 that “the groups do not correspond to the clusters in Figure 9”.

Fig. 11 in the figure: “Acquired”

Reply 2-52: We will correct it.