7th, August, 2019

Dear Professor Dr. Hiroshi Kitazato
Associate Editor, *Biogeosciences*

We are pleased to submit a revised version of our manuscript "Characterizing photosymbiosis in modern planktonic foraminifera" (bg-2019-145).
We appreciated the constructive suggestions and comments from the two reviewers Dr. Howard J. Spero and Dr. Ralf Schiebel and open discussion by Dr. Martina Prazeres. We thank you for providing this opportunity for us to improve this manuscript and submit a revised version.

A point-by-point response to the comments, a list of changes, and a marked-up manuscript version showing the track changes are included below.

We hope that our corrections are sufficiently clear, and the present version is acceptable for publication in *Biogeosciences*. We would appreciate in advance for your further consideration.

Yours sincerely,
Haruka Takagi (on behalf of all co-authors)

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Response to Howard Spero (Reviewer#1)

We are very grateful to the reviewer Dr. Howard Spero for his positive and valuable comments on our manuscript. The issues raised by the reviewer are taken into consideration and in the following paragraphs, we present our reply to each of them.

Haruka Takagi
(on behalf of all co-authors)

Line 140 – please add to this sentence that 'non functional' chlorophyll could come from phytoplankton in the guts of zooplankton prey. This caveat eliminates the potential that a reader of your paper concludes that your data indicates that all foraminifera species ingest phytoplankton prey directly which is not the case for species such as *sacculifer*, *ruber* and *Orbulina*.

Reply 1-1: Thank you for the suggestion. We will add the sentence as suggested. We agree that it will avoid readers to misunderstand the trophic activity of foraminifera.

Line 145 – The chl content of a dinoflagellate symbiont cell is » than that in a pelagophyte or chrysophyte symbiont from thermocline dwellers. How do you determine symbiont 'density', which I interpret to mean number of symbionts, from Chl a content? Certainly a single dinoflagellate cell has » chl a than a very small chrysophyte cell. Hence there is little connection between chl and symbiont 'density'.

Reply 1-2: Thank you for the comment. In this part, we used the word 'density' for expressing 'per unit mass', which we admit that it is not a good wording. In order to state it precisely, we will change "As an indicator of symbiont density of an individual, …" to "To normalize by the size of an individual, …".

Line 200 – please provide a conversion for the fluorescence units you use - 10-20 m2 quanta-1 to the more generally used units - ïA¸ mol photons m2 s-1

Reply 1-3: The unit for σ_{PSII} is often '$Å^2$ $quanta^{-1}$' (Å = 10^{-10} m). Since Å is not an SI-unit, we used 'm' instead. We will add '$Å^2$ $quanta^{-1}$' next to '$\times 10^{-20}$ m^{-2} $quanta^{-1}$', in the definition table in Figure 3.

I am having trouble understanding the relationship between σPSII and photosynthetic saturation. For the readers, would it be possible to explain this light absorption efficiency term in a way that one can interpret it relative to the light field in the ocean. I observe that the results seem to be inverted relative to photosynthetic light saturation – a concept that many researchers understand. This should be explained better in the discussion (line 345). In this regard, on line 352 you note that this parameter indicates a higher acclimation potential to a low-light environment. How does this relate to Ik in a P/I curve for symbiont photosynthesis? Note that Jorgensen et al (1985), Spero and Parker (1985) and Rink et al. (2005; 1998) show P/I curves that could easily be related to the photosynthetic efficiency term here. Such a link would go a long way to relate previous research on symbiont photosynthesis with the new data you present here and in your other papers.

Reply 1-4: We appreciate your comment. We agree that this point is important when comparing our results to the previous studies.

Saturating irradiance, I_k, is defined as the point where the extrapolated initial slope (α) of the photosynthesis–irradiance curve (P-I curve) crosses the saturation level of photosynthetic rate (P_{max}), thus $I_k = P_{max}/\alpha$. α takes into account that the light absorbed by the algal cell is proportional to the functional absorption cross-section (σ_{PSII}) of the photosystem II (the effective area that a molecule presents to an incoming photon and that is proportional to the probability of absorption) and to the number of photosynthetic units (n), $\alpha = n * \sigma_{PSII}$ (Falkowski and Raven, 1997). Therefore, theoretically, I_k is inversely proportional to σ_{PSII}. In general, low-light acclimated algae shows low I_k, low P_{max}, and high α (thus high σ_{PSII}). Jorgensen et al. (1985), Spero and Parker (1985), and Rink et al. (1998) all showed that the I_k of dinoflagellate-bearing species was high, which is consistent to the low σ_{PSII} of dinoflagellate-bearing species in our results. Although I_k or α of pelagophyte-bearing species has not been reported so far, the high σ_{PSII} of pelagophyte-bearers indicates low-light acclimated photophysiology (Babin et al., 1996; Bouman et al., 2018). We will add this discussion in the revised version.

Line 190 – Does your *O. universa* data use pre-sphere *O. universa* or just spherical *O. universa*? Are the size measurements for *Orbulina* on the inner trochospiral test or the diameter of the sphere? If the latter, then the measurements are not that valuable as the sphere is seldom filled with cytoplasm in a plankton tow. Please indicate this in the text and tables.

Reply 1-5: The measured *Orbulina universa* specimens smaller than 400 μm were all trochospired (pre-sphere stage). The rest were spherical adult, and we measured their sphere diameter since the inner trochospired test was not always visible depending on the thickness/transparency of the sphere. We have confirmed that when the spherical adult specimens alone were used for the regression analysis, it also showed a significant positive correlation between Chl *a* content and the spherical diameter ($p << 0.01$, $R = 0.419$, $y = -5.63 + 2.51x$, $N = 69$). As you mentioned, and as is shown in Spero and Parker (1985), the symbiont content should be better correlated with

juvenile trochospired test size than with spherical diameter of *O. universa*. However, it may be the case for other species as well; e.g., the final sac chamber of *G. sacculifer* is seldom filled with cytoplasm, and the symbiont content may have a higher correlation with test size without a final chamber. In our study, we consistently used the maximum diameter of the test as the 'test size' whatever the growth stage is. We will explain it in the text, Figure 6, and Table S1. In addition, in Table S1, the juvenile specimens of *O. universa* will be marked with *.

Line 235. Please mention/discuss the results from Fehrenbacher et al (2018) that support a microhabitat for non-spinose species on organic aggregates. Marine snow is the primary organic particulate that transports phytoplankton cells from the surface to deep ocean. *G. scitula* and *crassiformis* may obtain chlorophyll from such material. Alternatively, many of the zooplankton prey of these foraminifera could participate in the nightly diurnal migration of the deep scattering layer where the zooplankton could feed on surface phytoplankton at night and migrate back to depth during the day where the forams could capture/ingest them.

Reply 1-6: Thank you for the insightful comment. We agree that their lifestyle (attaching to organic aggregates) is one of the factors they incorporate non-functional chlorophyll. We will include the possibility of marine snow grazing of non-spinose species citing Fehrenbacher et al. (2018). As we replied in Reply 1-1, phytoplankton in the gut of zooplankton prey is also an important path that indirectly incorporates non-functional chlorophyll. We will include this possibility as well.

Line 248 – contact Barbel Hoenisch at LDEO. She has unpublished observations on Sphaeroidinella dehiscens from Puerto Rico culture experiments that supports your observations on the 7 dehiscens you observed. She collected dozens of specimens using scuba and had them in culture until gametogenesis when they put on a cortex. All looked like sacculifer and contained dinoflagellate symbionts. You could ask for details and permission to provide Barbel's 'unpublished data' for the observations you describe.

Reply 1-7: We appreciate your suggestion and are happy to know that her observation supports ours. However, since informal references such as personal communication should be avoided in this journal, we would like to refrain from including such unpublished data.

Line 248 – are you 100% certain that the *G. tenella* and *G. rubescens* you claim to have collected have dinoflagellate symbionts and were not early/juvenile *ruber* or *sacculifer*? The latter look very different than the adult stages when the shells are only 100 um in size.

Reply 1-8: Since *G. ruber* pink is absent in the Pacific, foraminifera with pink pigmentation collected from the Pacific were 100% *G. rubescens*. They had dinoflagellate-like symbionts. For specimens collected from the Atlantic, we identified them based on the key taxonomic features such as four globular chambers in the last whorl, high arched umbilical aperture, and lack of supplementary aperture, in addition to the typical pink pigmentation and small test size. Likewise, *G. tenella* was identified based on its key features; four globular chambers in the last whorl, high arched umbilical aperture, single small supplementary aperture, and small test size. Most of the specimens we analyzed were larger than 100 μm, and can be distinguished from small *G. ruber* (s.s.) or *G. sacculifer* based on the above features. We recognize that confirmation of their molecular taxonomic position should be needed and this should be the next step.

Line 253 – add that the relationship observed by Spero and Parker was a logarithmic relationship. Again – is the relationship in Figure 6 for *Orbulina* comparing sphere diameter or trochospiral shell length? You may be able to compare your chl data with the regression in Spero and Parker to generate a true chl vs symbiont density relationship for the dinoflagellate symbionts in other species.

Reply 1-9: We will add the statement of the logarithmic relationship between test size and symbiont density. As we have explained in the above (Reply 1-5), we used the maximum test diameter regardless of the growth stage of foraminifera; i.e., trochospiral diameter for prespherical *O. universa* and sphere diameter for spherical *O. universa*. Using the relationship of Spero and Parker (1985), we can show the Chl *a* vs symbiont density. Since the linear regression of Spero and Parker (1985) was performed on half-log scaled cross-plot (test size is in linear scale and symbiont number is in log scale) whereas ours is double-log scaled (both test size and Chl *a* are in log scale), the relation between Chl *a* vs symbiont density is expressed as an exponential function in double-log scale (Fig. A). In this relationship, the Chl *a* content per symbiont cell varies significantly.

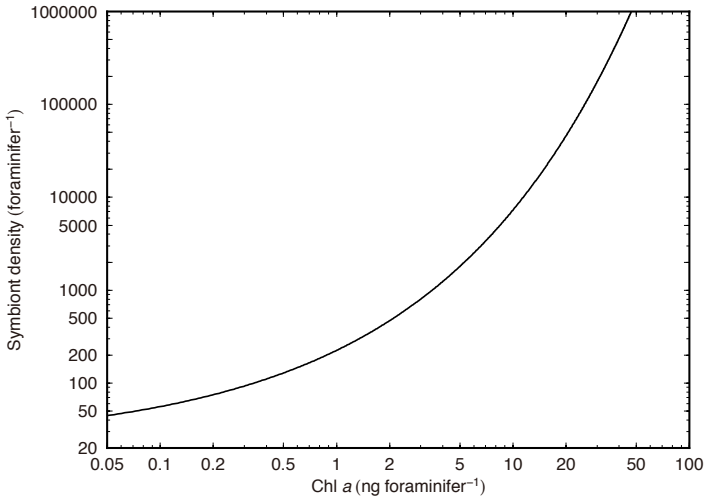

Figure A. Relationship between Chl *a* content and symbiont density derived from test size-Chl a relationship (this study) and test size- symbiont density relationship (Spero and Parker, 1985).

Alternatively, when we use a certain Chl *a* content of symbiont, e.g., 1-5 pg cell^{-1} (cf. Fitt et al., 2000, for *Symbiodinium* in corals), we can show a test size-Chl *a* content relationship derived from Spero and Parker (1985) and can compare it to ours (Fig. B). We will include the figure and a short discussion about it in the supplementary material.

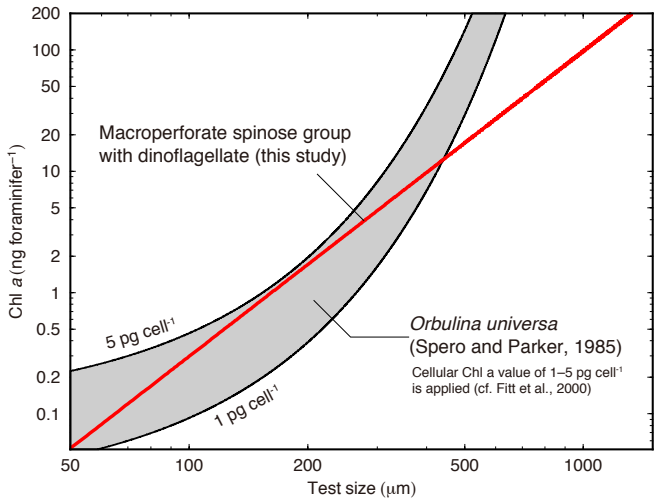

Figure B. Relationship between test size and Chl *a* content. Red line; relationship in dinoflagellate-bearing group (regression line for macroperforate spinose group with dinoflagellate in Figure 9). Gray area; relationship in *Orbulina universa* derived from Spero and Parker (1985) using a range of Chl *a* content per symbiont cell (cf. Fitt et al., 2000).

Section 4.2. This section discusses chlorophyll content in terms of host size, photosynthetic characteristics relative to chamber morphology or spinose vs non-spinose species. It is the opinion of this reviewer that this section fails to discuss the two most important parameters – differences in symbiont type (dinoflagellates have » more chl a per symbiont cell than does chrysophyte/pelagophyte symbionts) and depth habitat (the ambient light regime as a function of water depth controls light availability for the symbionts. Self-shading due to internal vs external symbiont distribution has little to no effect on available light as the shells are virtually transparent to light penetration given their thickness and the size of the foraminifera. Rather, the internal/external location difference will have an effect on nutrient availability or DIC supply for photosynthesis. Unlike the smaller symbionts in the deeper dwellers, the dinoflagellate symbionts in the mixed layer species would quickly exhaust their DIC supply if they were inside the foram test during the day rather than on the spines where DIC availability is only diffusion limited. This section should be modified accordingly.

Reply 1-10: Thank you for the insightful comment and discussion. In the second paragraph, we discussed the higher Chl *a* content in the spinose group than the non-spinose group from a morphological perspective (i.e., possession of spines). In fact, we think the difference cannot be simply related to the symbiont type because the

spinose group includes several types of symbionts (dinoflagellate for globigerinoidids, pelagophyte for *G. siphonifera* Type II, and prymnesiophyte for *G. siphonifera* Type I). Moreover, pelagophyte symbiont is possessed in the non-spinose group as well (*N. dutertrei*). As shown in Fig. 10, spinose group with dinoflagellate symbiont (red) and spinose group with non-dinoflagellate symbiont (orange) are similarly distributed, and both show higher Chl *a* content than the non-spinose groups. It indicates that the symbiont type is not the primary factor to make the difference. We believe that the symbiont type would affect the relationship to some extent, but considering our data, the effect is not apparent. In terms of the effect of depth (light environment relating to depth), it is hard to discuss here because specimens collected from various depth (< 100 m) are mingled in the test size-Chl *a* relationship. As we will comment in the following reply (Reply 1-11), statistical modeling such as GLMM or GAMM will be suitable approaches to reveal the effect of depth or symbiont taxonomy. In this section, we will not include a detail discussion on taxonomy or depth because of the above reason, but will mention the possibility of their effect.

We totally agree that the presence of spines and symbionts distribution on them have to do with nutrient availability and DIC supply (so we will include this point in the text). Likewise, it is our opinion that this does affect the illumination on each cell as well. We believe that the spherical distribution of symbionts on spines does affect the exposure to light, hence affect photosynthesis. As you pointed out, test wall characteristics such as macroperforate or microperforate may make little difference to the light penetration when they are sequestered inside the test. We will delete this point, but leave the effect of spines on illumination as it is.

Line 375 – do you see any differences in photophysiology when comparing specimens from oligotrophic environments with a deep mixed layer and clear water (deep light penetration) vs locations with a shallower chlorophyll maximum? This basic difference in light field in the water could explain some of the photophysiological differences between species and locations.

Reply 1-11: Overall, stations in the Atlantic (M140 stations) were more productive than those in the Pacific subtropical gyre (KH-17-4 stations). When we compare the data of these two cruises, the former tended to show higher F_v/F_m and lower σ_{PSII}. However, this tendency was not necessarily true for all species, thus we hesitate to discuss this possibility in the text. In our opinion, factors determining photophysiology is various, and we need further detailed analysis to relate the obtained data of photophysiology and controlling factors such as light penetration, nutrient, symbiont taxonomy, etc…. We believe that using statistical models such as GLMM or GAMM to see the relationship between photophysiology and environmental factors is the future step to better understanding on photosymbiosis.

Besides, in order to discuss more detail on interspecific photophysiological differences, comparison of the photophysiological parameters for specimens cultured under controlled condition, or the compilation of individual data collected from the similar environmental condition would be useful.

Figure 11 is very interesting, but is not discussed at all in the text of the manuscript. Nevertheless, I would like to point out that the spectrum of endosymbiosis concept drawn up in this figure does not take into consideration that the foraminifera lose their symbionts every generation and must reestablish the symbiosis every new generation. Also, I have been culturing planktic foraminifera for over 40 years and have never observed a *sacculifer*, *Orbulina* or *G. ruber* without symbionts. LeKieffre et al (2018) shows an amazingly tight inter relationship between symbionts and host foraminifera in Orbulina. The dinoflagellate bearing foraminifera species are incapable of surviving without their symbionts – The horizontal arrow that you have drawn in Fig. 11 does not reflect this 'all or nothing' symbiotic association which must be as necessary as zooxanthellate in reef building hermatypic corals.

Reply 1-12: Thank you for the comment and valuable information based on your years of observation. In the figure, we used the word "acquired phototrophy" for foraminifera, which we intended to show that the symbiotic relationship must be acquired at every new generation. To make it clearer, we will explain it both in the text and the caption of Figure 11 with saying 'sexually reproduced new generation must acquire symbionts from the environment'.

In fact, in the conceptual diagram of photosymbiosis, we wanted to draw a line dividing "all-or-nothing" (so-called obligate) relationship and flexible (so-called facultative) relationship. As shown in LeKieffre et al. (2018), the relationship between *Orbulina* and its symbionts must be strong and their trophic interaction should be called "obligate". However, we have no information on such interaction for the other species. In our method, we cannot go into such detailed interactional relationship. Since our knowledge of foraminiferal photosymbiosis is based on a set of snapshot information of algal possession at a certain time of their lifecycle, whether the observed phenomena is truly essential for survival cannot be concluded. Therefore, at this moment, we thought it is inappropriate to categorize the foraminiferal photosymbiosis into the criteria of obligate or facultative. That is why we simply mapped them based on the statistical result alone (i.e., the PCA results).

In the revised version, we will add a discussion about the perspectives of the necessity of symbiosis citing the result of LeKieffre et al. (2018) and other related studies. We believe future works will reveal the interrelationship between the host and symbionts for the other species, and will make the diagram more elaborate and sophisticated.

References:

Babin, M., Morel,A., Claustre, H., Bricaud, A., Kolber, Z. S., and Falkowski, P. G.: Nitrogen- and irradiance-dependent variations of the maximum quantum yield of carbon fixation in eutrophic, mesotrophic and oligotrophic marine systems, Deep-Sea Research I, 43, 1241–1272, 1996.

Bouman, H. A., Platt, T., Doblin, M., Figueiras, F. G., Gudmundsson, K., Gudfinnsson, H. G., Huang, B., Hickman, A., Hiscock, M., Jackson, T., Lutz, V. A., Mélin, F., Rey, F., Pepin, P., Segura, V., Tilstone, G. H., Van Dongen-Vogels, V., and Sathyendranath, S.: Photosynthesis-irradiance parameters of marine phytoplankton: Synthesis of a global data set, Earth System Science Data, 10, 251–266, 2018.

Falkowski, P. G., and Raven, J. A.: Aquatic photosynthesis, Blackwell Science, 1997.

Fitt, W. K., McFarland, F. K., Warner, M. E., and Chilcoat, G. C.: Seasonal patterns of tissue biomass and densities of symbiotic dinoflagellates in reef corals and relation to coral bleaching, Limnology and Oceanography, 45, 677–685, 2000.

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 “σPSII 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 σPSII, 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.

Response to Martina Prazeres

We would like to thank Dr. Martina Prazeres for her contribution on the discussion from the viewpoint of benthic foraminiferal symbiosis. A point-by-point response to the comments is included below.

Haruka Takagi
(on behalf of all co-authors)

Ln 56-57: Is kleptoplasty a possibility?

Reply 3-1: In planktonic foraminifera, kleptoplasty has never been reported despite its years of study. In this study, we investigated the functionality of chlorophyll but did not identify the algal symbionts. At this point, the possibility of functional kleptoplasty is left. However, we are suspicious about it because most of the species whose symbionts has not been identified to species level has once been observed under TEM and the plausible symbionts reported was at least algae (see Table 1), not chloroplasts.

Ln 249-250: Is this based on the chlorophyll functionality or have the symbionts been identified? Please, clarify.

Reply 3-2: It is solely based on the chlorophyll functionality. We change the sentence as follows; "… five species were newly confirmed as symbiotic based on the functionality of chlorophyll; *S. dehiscens*, …, …, ….".

Ln 257-258: Could it be the other way around? They require more pigment because photosynthesis is not that efficient?

Reply 3-3: Probably, we should delete the word "effective" which makes the confusion. In this sentence, we intended to mention that the more chlorophyll they have, the more photosynthesis they can perform. The effectiveness of photosynthesis is another story (in fact, the F_v/F_m value is the parameter of the effectiveness, and the value of these species are high).

Ln 270-272: Unless physiological studies have been conducted confirming the nature of the algal-host relationship, they might in fact all be 'facultative'.

Reply 3-4: We totally agree. That is why we do not want to use the word "facultative" or "obligate". Please also check the comment from Reviewer #1 and our reply to it (Reply 1-12).

Ln 276-278: If the host can acquire food, then increasing the algal biomass might not be necessary.

Reply 3-5: That is true. But it is also the case for species with positive correlation in test size-Chl *a* relationship. Planktonic foraminifera actively acquire food even if they have symbionts. They cannot rely solely on their symbionts. The extent of their dependence on symbionts is still unknown, which is an interesting subject in the future. In any case, this sentence will be deleted in the revised manuscript as suggested by Reviewer #2.

Ln 280-281: Yes, but it can also mean that what authors are calling 'obligated' symbiont-bearing species are actually mixotrophics (as in most cases), which are obligate symbionts but also acquire energy through feeding.

Reply 3-6: As explained in Reply 3-5, all planktonic foraminifera species are basically heterotrophic, and for symbiotic species, they can be called mixotrophic. We think this point should be clarified in the earlier part of the text. We will change the first sentence of Introduction to "Planktonic foraminifera are unicellular heterotrophic marine zooplankton with calcite tests.".

Ln 281-284: Please, clarify how this can be true. In benthic forams, there is no sure thing as 'certain algae', as host species are very conserved when it comes to choosing an algal partner (please see Prazeres & Renema 2019, Biological Reviews). Also, symbiosis is a very fine tuned relationship.

Reply 3-7: In this part, we mentioned the possibility of "retention of photosynthesis-capable algae". This is a hypothesis to explain the absence of test size-Chl *a* correlation for species in Cluster 3. We will state more clearly that it is a hypothesis. In addition, we will tone down this part as suggested by Reviewer #2.

Ln 288: I am not sure that's how symbiosis work, at least not in benthic forams. Please, clarify this assertion.

Reply 3-8: This sentence will be deleted as it is a second guess derived from the above hypothesis.

Ln 289-290: This is actually not a good reference, given that it not symbiosis at all, just kleptoplasty, which actually contradicts what authors are saying.

Reply 3-9: We are sorry for making confused. We cited them for the reference of a way of maintaining symbiosis (incorporation of algal cells, non-permanent retention, and replenishment). Since a similar thing is reported for

kleptoplasty, we here cited them. We will replace the reference right after the word "…kleptoplasts" in the sentence, which will make it more straightforward to read.

Ln 293: The types of symbioses mentioned here need to be defined early on. Whats the difference between: 'facultative' and 'transient'? Are they being used interchangeably?

Reply 3-10: The terms "obligate" and "facultative" are defined in Introduction (Lines 76-84) with a short historical review of these terms used in previous studies. In contrast, the terms we newly used from this section, "persistent" and "transient", are chosen to illustrate the mode of symbiosis indicated from our results. We think "obligate symbiosis" and "facultative symbiosis" are technical terms including the meaning of the dependency of the symbiotic relationship. Therefore, "facultative" and "transient" are not interchangeable in our text.

Ln 304-305: I would be very careful stating that planktonic forams are phototrophics, as they are more likely to be mixotrophic to some degree.

Reply 3-11: Thank you for the comment. It is true that they are mixotrophic. The word 'acquired phototrophy' here is interchangeable to 'mixotrophy'. Since the former was used in the paper we referred to show the concept of photosymbiosis (Stoecker et al., 2009), we preferred to use 'acquired phototrophy'. In addition, since the photosymbiosis in planktonic foraminifera should be established at every new generation, 'acquired' seems to be suitable to describe its nature (please see Reply 1-12 as well). In the sentence, we will change in the parenthesis as "… (higher extent of acquired phototrophy/mixotrophy) …".

Ln 338-340: This can also indicate mixotrophy, or a less dependency on the algal symbionts. It seems to me that the authors are assuming that all energy is coming from the symbiosis with algae, which might not be the case. Nowhere in the text that authors mention mixotrophy (except when talking about benthic forams). If this is not the case, the authors need to add citations with compeling evidence that planktonic forams that host dinoflagellates are only photoautotrophs.

Reply 3-12: Thank you for pointing it out. We never assume that planktonic foraminifera solely relies on phototrophy. We admit that the references for mixotrophy in Introduction (Line 76) were insufficient. We will add Stoecker (1998) and Caron (2000) since they discuss and review the mixotrophy in marine plankton including planktonic foraminifera.

Ln 351-352: Please, re-write. A sentence should never finish in a preposition. It is fine in spoken English but not in written English.

Reply 3-13: We will change the last part of this sentence as "…regardless of the phylogenetic position of the host".

Ln 360-362: In the case of planktonic forams, the symbiont selects the host? Please, clarify.

Reply 3-14: In contrast to benthic species, host-symbiont association so-far reported for planktonics is one-to-one relationship alone. It seems that they have strong symbiont specificity. However, the selection process of symbionts is still unknown. We do not know whether the host attracts symbionts or the symbionts attract host, or alternatively, the acquisition of symbionts is controlled by a chance; i.e., individuals that could not acquire its partner in early life stage will die.
In this part, we tried to interpret the host's habitat depth relating to the light preference of the symbionts. Since the host must acquire specific symbionts, we assume that the light requirement of the symbiont may regulate the host's depth. We think the term "control" may be confusing, hence we change this sentence as "… the symbiont acclimation potential may be one of the factors restricting the habitat depth of the host species".

Ln 381-382: Since the authors mentioned kleptoplasty in benthic foraminifera, and later on suggested for planktonic forams, just having active chlorophyll is not convincing, it is indicative.

Reply 3-15: Sorry for making confused. We did not suggest kleptoplasty in planktonic foraminifera, but suggest the algal retaining behavior we presume is similar to what is reported for kleptoplasty in benthic species. We will rewrite the earlier part (Line 289-290) as explained in Reply 3-9 to avoid a misinterpretation.

Figure 11: Typo. Please amend from 'Aquired' to "Acquired'.

Reply 3-16: We will correct it.

References:

Caron, D. A.: Symbiosis and mixotrophy among pelagic microorganisms, in: Microbial Ecology of the Oceans, edited by: Kirchman, D. L., Wiley-Liss, Inc., New York, 495–523, 2000.

Stoecker, D. K.: Conceptual models of mixotrophy in planktonic protists and some ecological and evolutionary implications, European Journal of Protistology, 34, 281–290, 1998.

A list of changes in the manuscript

(Line numbers are for the manuscript with track changes attached below)

1. L21: Replacement of "growth" to "abundance" (requested by RS)
2. L29: Addition of "heterotrophic" (according to the comment by MP)
3. L29: Replacement of "calcite" to "calcareous" (requested by RS)
4. L45–47: The words "with eukaryotic algae" are added to constrain the type of photosymbionts, and accordingly, "*Globigerina bulloides*" is deleted and the number of species changed from thirteen to twelve. (requested by RS)
5. L51: Replacement of "all" with "some" (requested by RS)
6. L55: Deletion of "foraminiferal" that is unnecessary in this context
7. L77: Addition of "planktonic" to make the meaning of sentence clearer
8. L79: Addition of references Stoecker (1998) and Caron (2000) (as a response to the comment by MP)
9. L110–113: Addition of the explanation of *O. universa* test measurement (requested by HJS)
10. L136: Replacement of "and" with "or" to make the meaning of sentence clearer
11. L145: Addition of the possibility of phytoplankton in the gut of zooplankton prey (requested by HJS)
12. L150–151: Rewording the purpose of the Chl *a*/biomass calculation (as a response to the comment by HJS)
13. L153: Replacement of "his" with "her" (requested by RS)
14. L164: Addition of "(Table 1)" to make the readers easier to find the data
15. L186–187: Deletion of the sentence "Therefore… dinoflagellate-bearing species" (requested by RS)
16. L205: Replacement of "clearly" with "relatively" (requested by RS)
17. L213–215: Addition of the sentence explaining the variables for PCA and clustering (requested by RS)
18. L221: Replacement of "revealed" with "confirmed" (requested by RS)
19. Discussion and Conclusion: Past tense basically changed to present tense (requested by RS)
20. L237–239: Rewording of the short explanation of the results of PCA and clustering (requested by RS)
21. L247–251: Addition of a supporting observation in the previous study to explain the possession of non-functional chlorophyll in deeper dwelling non-spinose species (requested by HJS)
22. L254: Replacement of "they were" with "when" (requested by RS)
23. L254: Replacement of "The" with "A" (requested by RS)
24. L260: Deletion of "On the other hand," (requested by RS)
25. L260: Replacement of "in this study" with "based on the functionality of chlorophyll" to make the meaning of the sentence clearer (as a response to the comment by MP)

26. L265–266: The words “in logarithmic scale” were added as suggested and Figure S4 (Figure B in the reply comment) was included in the supplementary materials to compare our results to Spero and Parker (1985). (requested by HJS).
27. L266: Replacement of “directly clarified” with “determined” (requested by RS)
28. L269: Replacement of “should be a specific diagnostic of” with “may indicate” (requested by RS)
29. L270: Addition of species name in the Cluster 1 (requested by RS)
30. L271: Replacement of “perform” with “support” (requested by RS) and deletion of “effective” (as a response to the comment by MP)
31. L272: Deletion of the sentence “It may indicate…species.” (requested by RS)
32. L277–278: Addition of the species name in the parenthesis (requested by RS)
33. L281: Replacement of “they” with “these small specimens” (requested by RS)
34. L288: Replacement of “represent a “strength” of” with “quantitatively represent” (requested by RS)
35. L289: Addition of the species name of the Cluster 3 (requested by RS)
36. L292–295: Deletion of the sentence “Contrary to…as the host grew” and rephrasing of the subsequent sentence to make the point clearer (requested by RS)
37. L296–297: Deletion of the sentence “Therefore, the lack of …algal prey” (requested by RS)
38. L298: Replacement of “speculate” with “hypothesize” (as a response to the comment by MP)
39. L298–300: Rephrasing the sentence to decrease speculations (as a response to the comment by RS and MP)
40. L302: Deletion of the sentence “If these are the case…content” (requested by RS)
41. L304–305: Deletion of the sentence “They could serve as…is long” (as a response to the comment by MP)
42. L305–306: Rephrasing the sentence to avoid confusion (as a response to the comment by MP)
43. L308: Addition of “to test the hypothesis” (as a response to the comment by MP)
44. L312–313: Addition of the explanation on the size of *T. humilis* and the possible effect on regression analysis (as a response to the comment by RS)
45. L321: Addition of “/mixotrophy” (as a response to the comment by MP)
46. L322: Deletion of “utter” (requested by RS) and addition of “that possesses symbionts” (as a response to the comment by RS)
47. L323: Addition of “(a certain extent of mixotrophy, Fig. 11)” to make the concept of acquired phototrophy clearer (as a response to the comment by RS and MP)
48. L325: Deletion of “in species which harbor active chlorophyll” which is unnecessary in this context
49. L326–334: Addition of the discussion on Figure 11 in terms of the necessity of photosymbiosis (requested by HJS)
50. L336: Addition of the family name (requested by RS)

51. L342–343: Rephrasing the sentence to make it more simple (requested by RS)
52. L345: Replacement of “flatter” with “less spherical” (requested by RS)
53. L347: Replacement of “growth rate of” with “increase in the” (requested by RS)
54. L350–351: Deletion of “increased” and “theory” from the sentence (requested by RS)
55. L353: The words “irrespective of their symbiont type” were added to indicate that the type of symbionts is not the major factor to explain the higher Chl *a* content in spinose species (as a response to the comment by HJS)
56. L354–356: Replacement of “higher” with “more” (requested by RS)
57. L359: Replacement of “could be seen as a character that” with “may” (requested by RS)
58. L360: Replacement of “lighting” with “illumination” (requested by RS)
59. L361–364: Addition of the discussion on the advantage of nutrient and DIC availability of spinose species (requested by HJS)
60. L366: Rephrasing the sentence to make it more simple (requested by RS)
61. L367: Deletion of the sentence “It is also… this study” (requested by RS)
62. L368–370: Addition of the explanation on possible factors causing the difference in test size-Chl *a* content relationship among different morphogroups, with a perspective on depth/light and nutrient (requested by HJS)
63. L371–374: Deletion of the discussion on test architecture and light penetration (requested by HJS)
64. L375–377: The original sentence was revised according to the newly added discussion.
65. L376: Replacement of “theoretically” with “numerically (requested by RS)
66. L379: Replacement of “based on the” with “according to” and deletion of “they possess” (requested by RS)
67. L381: Replacement of were all” with “are” (requested by RS)
68. L389: Deletion of “belongs to” (requested by MP)
69. L390–399: Photophysiological parameters previously reported (parameters based on P-I curve) and our FRRF-based parameters are compared. The relation of I_k and σ_{PSII} was explained. (requested by HJS)
70. L408: Replacement of “controlling” with “ constraining” (as a response to the comment by MP)
71. L417: Deletion of “however” (requested by RS)
72. L424–427: A sentence for future direction to better understand factors affecting photophysiology (use of statistical modeling) was added. (as a response to the comment by HJS)
73. L429: Replacement of “was aimed to extend” with “extends” (requested by RS)
74. L431: Deletion of “in contrast” (requested by RS)
75. L439: Deletion of “implying a strength” (requested by RS)
76. L440–442: Rephrased the sentence to make it more simple (requested by RS)
77. L443: Replacement of “especially” with “in particular” (requested by RS)

78. L447–452: “Author contribution” is included.

79. L461–463: Acknowledgement to HJS, RS, and MR is included for their contribution to the manuscript.

80. References: Addition of references resulting from the changes in the manuscript.

81. Table 1: Addition of correlation coefficient of test size-Chl *a* relationship that is one of the variables used for PCA and clustering (as a response to the comment by RS)

82. Figure 1: Replacement of “northeastern” with “tropical eastern” (requested by RS)

83. Figure 6: Note for test size measurement for *O. universa* is added. (requested by HJS)

84. Figure 10: New colors are applied to avoid confusion to the color code used in clustering (Figure 9) (requested by RS)

85. Figure 11: The same colors are used as in Figure 9 (requested by RS). In the caption, the necessity of acquiring symbionts from the environment for a new generation is explained (requested by HJS). Typo of “Acquired” is corrected (requested by RS and MP). The word “mixotrophy” is added next to the “acquired phototrophy” (as a response to the comment by MP).

86. Figure S4: This is a new figure comparing the test size-Chl *a* content relationship from this study and the one derived from Spero and Parker (1985).

87. Table S1: Juvenile pre-spherical *O. universa* specimens are marked with *.

Characterizing photosymbiosis in modern planktonic foraminifera

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Abstract. Photosymbiosis has played a key role in the diversification of foraminifera and their carbonate production through geologic history. However, identification of photosymbiosis in extinct taxa remains challenging and even among the extant species the occurrence and functional relevance of photosymbiosis remain~~s~~ poorly constrained. Here, we investigate photosymbiosis in living planktonic foraminifera by measuring active chlorophyll fluorescence with fast repetition rate fluorometry. This method provides unequivocal evidence for the presence of photosynthetic capacity in individual foraminifera and it allows us to characterize multiple features of symbiont photosynthesis including chlorophyll *a* (Chl *a*) content, potential photosynthetic activity (F_v/F_m), and light absorption efficiency (σ_{PSII}). To obtain robust evidence for the occurrence and importance of photosymbiosis in modern planktonic foraminifera, we conducted measurements on 1266 individuals from 30 species of the families Globigerinidae, Hastigerinidae, Globorotaliidae, and Candeinidae. Among the studied species, 19 were recognized as symbiotic and 11 as non-symbiotic. Of these, six species were newly confirmed as symbiotic and five as non-symbiotic. Photosymbiotic species have been identified in all families except the Hastigerinidae. A significant positive correlation between test size and Chl *a* content, found in 16 species, is interpreted as symbiont ~~growth~~ abundance scaled to the growth of the host, consistent with persistent possession of symbionts through the lifetime of the foraminifera. The remaining three symbiont-bearing species did not show such a relationship, and their F_v/F_m values were comparatively low, indicating that their symbionts do not grow once acquired from the environment. The objectively quantified photosymbiotic characteristics have been used to design a metric of photosymbiosis, which allows the studied species to be classified along a gradient of photosynthetic activity, providing a framework for future ecological and physiological investigations of planktonic foraminifera.

1 Introduction

Planktonic foraminifera are unicellular heterotrophic marine zooplankton with ~~calcite~~ calcareous tests. Since they are geographically widespread and abundant, and can be preserved in seafloor sediments as microfossils, foraminifera are one of the most important archives of surface ocean conditions in the past. They have been used to investigate pelagic marine

biodiversity dynamics from middle Mesozoic to the present (Bolli et al., 1985; Norris, 1991; Boudagher-Fadel et al., 1997; Hull, 2017; Yasuhara et al., 2017). Recent studies of macroevolutionary dynamics of planktonic foraminifera emphasized the importance of species ecology including photosymbiosis (endosymbiosis with autotrophic algae) as a key player determining temporal and spatial patterns of species diversity (Ezard et al., 2011; Fenton et al., 2016). However, identifying photosymbiosis in extinct species is difficult and requires indirect evidence such as size-dependent stable isotopic trends (e.g., Pearson et al., 1993; Norris, 1996). These indirect methods must be benchmarked by observations from living foraminifera, where the presence of symbionts can be determined directly. A knowledge on the prevalence, diversity, and phylogenetic position of photosymbiosis is also required to elucidate ecological and evolutionary strategies of the involved clades and to characterize key features of foraminiferal test geochemistry like $\delta^{13}C$ and $\delta^{11}B$ (e.g., Spero and DeNiro, 1987; Hönisch et al., 2003; Henehan et al., 2013; Ezard et al., 2015).

Photosymbiosis in modern planktonic foraminifera has been empirically identified based on microscopic observations of intracellular algae (Lee et al., 1965; Anderson and Bé, 1976; Gastrich, 1987), and molecular confirmation of algal DNA extracted from a single foraminifera cell (Gast and Caron, 1996; Gast et al., 2000; Shaked and de Vargas, 2006; Bird et al., 2017, 2018). As a result, among the ~~50 species of modern planktonic foraminifera, ~~thirteen~~ twelve have so far been reported to be photosymbiotic with eukaryotic algae (*Orbulina universa*, *Globigerinoides sacculifer*, *Globigerinoides conglobatus*, *Globigerinoides ruber*, *Globigerinella siphonifera*, *Turborotalita humilis*, ~~*Globigerina bulloides*,~~ *Neogloboquadrina dutertrei*, *Pulleniatina obliquiloculata*, *Globorotalia inflata*, *Globorotalia menardii*, *Candeina nitida*, and *Globigerinita glutinata*), and six to be symbiont-barren (*Hastigerina pelagica*, *Globigerina bulloides*, *Globorotalia truncatulinoides*, *Globorotalia hirsuta*, *Neogloboquadrina incompta*, and *Neogloboquadrina pachyderma*) (Table 1). The remaining ~30 species have not been systematically examined for the presence of symbionts. In a strict sense, in ~~all~~ some previous studies on a photosymbiotic association, the authors could not differentiate whether the intracellular algae they identified were symbionts or just captured preys to be digested. Although observations of features like mitosis (cell-division) of the intracellular algal cells are strong evidence that these were alive within the foraminifera, the presence of intracellular algae alone does not guarantee that they act as photosymbionts. Many ~~foraminiferal~~ species ingest phytoplankton prey (Anderson et al., 1979), which makes it difficult to differentiate symbionts or prey, especially by DNA analysis. Since many species of planktonic foraminifera do not survive well in culture, it is hard to conduct behavioral or physiological experiments to confirm their symbiosis. These limitations have hindered the progress of studies of photosymbiosis targeting various species of planktonic foraminifera.

One solution to identify functional photosymbiosis is to detect a physiological signature of photosynthesis within the cell. This has been done by measurements of oxygen production with microelectrodes (Jørgensen et al., 1985; Rink et al., 1998; Lombard et al., 2009) or a determination of photosynthetic carbon fixation by measurements of ^{14}C-tracer (Spero and Parker, 1985; Gastrich and Bartha, 1988). These studies were limited to established symbiotic species that are easy to culture (e.g., *O. universa*, *G. sacculifer*, and *G. siphonifera*). For the other species, especially non-spinose species (e.g., *N. dutertrei*, *P. obliquiloculata*, and *G. glutinata*), the physiological characteristics of their photosymbiosis have never been described. Therefore, our knowledge of modern photosymbiosis has been exclusively obtained from a small number of spinose symbiotic

species. A powerful alternative to directly and unambiguously determine the presence of active photosynthesis in the foraminifera is given by measurement of fluorescence induced by light capture in the photosystem II of the algal chlorophyll. These methods have been used in benthic symbiont-bearing foraminifera (e.g., Uthicke, 2006; Schmidt et al., 2011; Ziegler and Uthicke, 2011) and recently successfully adapted for application on single specimens of living planktonic foraminifera (Fujiki et al., 2014; Takagi et al., 2016, 2018). An active chlorophyll fluorometry performs non-destructive and non-invasive measurements of algal physiology based on real-time variable fluorescence profiles (Kolber and Falkowski, 1993), allowing us to quantify chlorophyll *a* content of a specimen, the health of its symbionts and their light-level adaptation (Fujiki et al., 2014; Takagi et al., 2018). The measurements can be performed almost immediately after collection, with minimal manipulations, thus minimizing damage to the foraminifera and circumventing culturing stress-induced artifacts. This approach can make a breakthrough in the study of photosymbiosis, not just because of its versatility but its potential to provide key quantitative attributes of the photosymbiosis.

Symbiotic relationships in planktonic foraminifera have been previously categorized as either obligate or facultative (Hemleben et al., 1989). Obligate photosymbiosis is essential for the host, making it functionally mixotrophic, an adaptive strategy to live in oligotrophic and well-lit parts of the ocean (Hallock, 1981; Stoecker, 1998; Caron, 2000; Lee, 2006). In facultative symbiosis, the foraminifera is not dependent upon it for survival and as a result, symbiotic algae in facultative symbiosis will be only found in some specimens of the host species. Facultative associations generally do not involve extensive metabolic adaptation of the host and can thus enhance the flexibility of nutritional sources with minimal energetic investment (Stoecker et al., 2009). In planktonic foraminifera, species always found with intact intracellular algae has been regarded as obligate symbiotic species, whereas species sometimes found with but sometimes without them has been termed as facultative symbiotic species (Hemleben et al., 1989). However, most of our knowledge of foraminiferal photosymbiosis is based on indirect evidence, insufficient to categorize planktonic foraminiferal photosymbiosis as either obligate or facultative. Rather, the persistence and functional relevance of the symbiotic relationship through a foraminiferal lifetime should be determined anew, using direct measurements, allowing us to correctly understand the function of each specific photosymbiotic relationship.

Here, we present the results of active chlorophyll fluorometry of 30 species of modern planktonic foraminifera obtained from 1266 individuals. The main purpose of this study is (1) to provide information on the biomass of symbionts (indicated by chlorophyll *a* content), (2) to qualify the functionality/fitness of symbionts (indicated by photophysiology), (3) to characterize the photosymbiotic features, and (4) to propose a new framework to characterize the photosynthetic activity of modern planktonic foraminifera.

2 Material and Methods

2.1 Sampling and identification of morphological species

Planktonic foraminifera were collected in central and western Pacific Ocean and north-eastern Atlantic Ocean (Fig. 1). We have sampled across much of the northern hemisphere tropical-subtropical gradient in both Pacific and Atlantic, to get the

endemic species and to replicate for the others. Samples from the Pacific Ocean were taken onboard during RV Mirai cruises MR13-04 and MR14-02, RV Kaiyo cruise KY14-09, RV Shinsei-maru cruise KS-16-9, and RV Hakuho-maru cruises KH-16-7 and KH-17-4 (Fig. 1a). The samples were collected either by vertical stratified towing (closing ring net or VMPS with 100-µm mesh) or by pumped seawater (sampling depth, ca. 5 m). The pumped seawater was continuously opened to a 100-µm-mesh net settled within a water tank to collect specimens as gently as possible. Some specimens were additionally collected from Tsugaru Strait, Sagami Bay, and off Sesoko Island by surface towing and vertical towing with a 100-µm-mesh net to increase the taxonomic range of our analysis. Samples from the Atlantic were taken onboard during RV Meteor cruise M140 (Fig. 1b). A multi-closing net system (Multi-Plankton-Sampler) with 100-µm mesh was used for stratified sampling of the water column. Samples from pumped seawater (sampling depth, ca. 8 m) were also collected in the same way as to the Pacific sampling.

Collected specimens were isolated immediately after collection with either brush or Pasteur pipets into Petri-dishes filled with 0.22-µm-filtered or 0.45-µm-filtered seawater, and rinsed several times. Specimens were identified to morphospecies level under a stereoscopic microscope, and the maximum test length (test size) were measured. We consistently measured the maximum test length whatever the growth stage is, hence for *O. universa*, we measured a trochospiral~~ed~~ diameter for pre-spherical juveniles and a sphere diameter for adult specimens. We identified 30 morphospecies from four families (Globigerinidae, Hastigerinidae, Globorotaliidae, and Candeinidae) (Fig. 2). *Sphaeroidinella dehiscens* was identified only after it thickened its test during the adult stage under culture, though the data we used here is from the very first measurement after collection before the identification. We differentiated *G. ruber* white variety and pink variety based on the pigmentation in earlier whorls of the tests. *Globigerinella siphonifera* was divided into two morphotypes (Type I and Type II) based on the criteria described in Faber et al. (1988) and Huber et al. (1997). From among the isolated individuals, viable specimens were selected for analysis with the following criteria; (1) penultimate chamber was filled with cytoplasm and (2) the specimen was sticky when touched with a brush or the rhizopods were observed under a microscope. Screening for the presence of photosymbiosis was conducted on as many species and specimens as possible, regardless of locality and sampling depth. Photophysiological measurements were carried out only on specimens collected from the upper 100 m of the water column (corresponding approximately to the photic zone). The specimens were kept individually in a well of a culture dish filled with filtered seawater until the measurement. The duration between the collection and the measurement was no longer than 12 hrs. During this time, most spinose species recovered their spines.

2.2 Fast repetition rate fluorometry measurements and photophysiological parameters

Fast repetition rate (FRR) fluorometry, a kind of active fluorometry, can obtain photophysiological information of host-algal symbiotic consortia using various parameters of photosystem II (PSII) (Fig. 3). FRR fluorescence transients were measured either using an FRR fluorometer DF-03 or DF-14 (Kimoto Electric Co., Ltd.) (Table S1). FRR fluorometers generate a series of blue flashlets of an excitation light intensity of 30 mmol quanta m^{-2} s^{-1} with a wavelength of 470 nm with a 25 nm-bandwidth (DF-03) or a wavelength of 450 nm with a 10 nm-bandwidth (DF-14). Saturation protocols were consisting of 50 flashlets of

2 μs duration at 4 μs intervals (DF-03) or 100 flashlets of 1 μs duration at 2 μs intervals (DF-14). A fluorescence induction curve based on the biophysical model of Kolber et al. (1998) was numerically fitted to transients of chlorophyll fluorescence to derive PSII parameters. The parameters include minimum fluorescence (F_0), maximum fluorescence (F_m), variable fluorescence [F_v (=F_m − F_0)], maximum photochemical efficiency indicating photosynthetic activity (F_v/F_m), and functional absorption cross-section of PSII indicating light absorption efficiency (σ_{PSII}) (Fig. 3). Before measurements, specimens were confirmed that no visible contamination of algae or particles were present at the test surface ~~and~~ or spines under a stereoscopic microscope. After 10-minutes dark adaptation, a specimen was transferred into a quartz glass cuvette with filtered seawater for the measurement.

2.3 Assessment of symbiont possession and parameters characterizing photosymbiosis

When chlorophyll fluorescence (F) was detected from an individual foraminifera, the status of chlorophyll was categorized based on the detection of variable fluorescence (F_v). F_v represents fluorescence transients during the saturation process of the reaction centers of PSII. It is detected only when the PSII captures photons and passes the product further through the chain of photosynthetic reactions; i.e., when actively photosynthesizing organisms are present in the specimen. When F_v was not detected but F value was significantly higher than the background level of the fluorometer, chlorophyll was regarded to be present but non-functional, signifying remnants of phytoplankton prey, or possibly phytoplankton in the gut of zooplankton prey. If no F was detected, the specimen had no chlorophyll (Figs. 3 and 4).

When functional chlorophyll was detected in a specimen, then the maximum fluorescence (F_m) value was used to estimate chlorophyll *a* (Chl *a*) content of the specimen based on a linear relationship between F_m and Chl *a* (cf. Fujiki et al., 2014; Takagi et al., 2016). Calibration line was established for each FRR fluorometer. A relationship between the Chl *a* content, an indicator of symbiont biomass, and the foraminiferal test size was then analyzed. To normalize with ~~by~~ the size of an individual, ~~As an indicator of symbiont density of an individual,~~ Chl *a* content per protein biomass (Chl *a*/biomass) was also calculated. The protein biomass was estimated based on species-specific relationships with test size (exponential equation) proposed by Movellan (2013). For species whose test size-biomass relationship was not presented in ~~his~~ her study, the protein biomass was estimated based on the relationship established by morphologically similar species (Table S2). As indicators of photosynthetic vitality and light absorption efficiency of symbionts, photophysiological parameters F_v/F_m and σ_{PSII} were used, respectively.

2.4 Statistical analysis

To compare the differences in the parameters (Chl *a*/biomass, F_v/F_m, and σ_{PSII}) among species, statistical tests for comparison of differences in medians (Kruskal-Wallis test and post-hoc Steel-Dwass test for multiple comparison) were conducted. Species with less than 20 specimens were not tested due to small sample size. Principal component analysis (PCA) was performed to characterize photosymbiotic features of the studied species, based on the four diagnostic variables of photosymbiosis obtained in this study; (1) ratio of symbiont-bearing individuals, (2) correlation coefficient between test size and Chl *a* content, (3) Chl

a content relative to the protein biomass (Chl *a*/biomass), and (4) F_v/F_m value. Species medians were used for the variables Chl *a*/biomass and F_v/F_m as representative values (Table 1). In terms of the correlation coefficient of test size-Chl *a* relationship, negative values were considered as zero. K-means clustering was also performed to categorize photosymbiosis and to visualize the results of the PCA. All the statistical analyses were performed using R (R version 3.3.1, R Core Team, 2016).

3 Results

3.1 Possession of symbionts

The results of the measurements on all 1266 specimens are shown in Table S1, including sampling locality, date, and the measured parameters. The incidence of each type of chlorophyll (functional, non-functional, and no chlorophyll) is summarized in Figure 5. Chlorophyll fluorescence, either functional or non-functional, was detected in 27 out of 30 species. The species *G. adamsi*, *N. incompta*, and *N. pachyderma* never showed any evidence for the presence of chlorophyll. Specimens of *G. scitula*, *G. crassaformis*, *G. truncatulinoides*, *H. pelagica*, *H. digitata*, *G. bulloides*, *T. quinqueloba*, and *T. fleisheri* never possessed functional chlorophyll, although many of them contained non-functional chlorophyll. Nineteen species contained functional chlorophyll, and can be considered symbiont-bearing: *O. universa*, *S. dehiscens*, *G. sacculifer*, *G. conglobatus*, *G. ruber* (white), *G. ruber* (pink), *G. rubescens*, *G. tenella*, *G. calida*, *G. siphonifera* Type I, *G. siphonifera* Type II, *T. humilis*, *P. obliquiloculata*, *N. dutertrei*, *G. inflata*, *G. menardii*, *C. nitida*, *G. glutinata* and *G. uvula*. Among these species, the percentage of symbiont-bearing individuals varied from 100 % (*S. dehiscens* and *G. conglobatus*) to 58 % (*G. calida*). Although the examined specimens included individuals collected at all depths, the percentages of non-functional or no-chlorophyll individuals were similar when removing the specimens collected below 100 m (Fig. S1). The incidence of symbiotic individuals was not significantly different between Pacific and Atlantic ($p >> 0.05$, Fisher's exact test for species with more than 15 individuals in each basin, see Fig. S2). Moreover, the ontogenetic (size) trend in possession of symbionts was not apparent (Fig. 6).

Globoturborotalita rubescens and *G. tenella* have never been reported to possess symbionts, but we observed ovoid reddish brown symbionts along with their spines just as they are usually seen in *O. universa*, *G. ruber*, *G. conglobatus*, and *G. sacculifer* (Fig. S3). ~~Therefore, although genetic information and detailed microscopic evidence are needed in the future, we categorize them here as dinoflagellate-bearing species.~~ The remaining symbiont-bearing species that have never been reported before were *G. calida* and *G. uvula*. Symbionts of these species are treated here as uncharacterized. As a precaution, the convincing symbiont-bearing species whose symbionts have not yet been identified by DNA analysis are treated as uncharacterized as well, *T. humilis*, *P. obliquiloculata*, *G. inflata*, *G. menardii*, *C. nitida*, and *G. glutinata* (Table 1).

3.2 Test size−Chl *a* content relationship, and Chl *a*/protein biomass

Out of the 19 species which had functional chlorophyll (symbiont-bearing species), 16 species showed a statistically significant positive correlation between test size and Chl *a* content ($p < 0.05$, Fig. 6), with Chl *a* content being a power function of test

size. The powers (scaling exponents) of the fitted functions varied from 1.33 (*G. tenella*) to 3.71 (*G. calida*) (Table 2). For the remaining three species, *T. humilis*, *P. obliquiloculata*, and *G. inflata*, their test size-Chl *a* relationships showed no significant correlation (Fig. 6).

The ratio of Chl *a* to protein biomass per individual showed clear differences among species (Fig. 7). *Globigerinoides conglobatus*, *G. sacculifer*, and *O. universa* showed significantly higher Chl *a*/biomass values (species median values were 4.8, 4.8, and 4.6 ng μg^{-1}, respectively), and *P. obliquiloculata* showed the lowest (median value 0.1 ng μg^{-1}). Spinose species tended to show higher Chl *a*/biomass values than non-spinose species.

3.3 Photophysiological state

Overall, F_v/F_m values tended to be high in dinoflagellate-bearing species (species median values 0.46–0.53) (Fig. 8a). Amongst all 19 symbiont-bearing species, F_v/F_m value was highest in *S. dehiscens* (0.53), and lowest in *G. inflata* (0.33). Species to species comparison showed that *P. obliquiloculata* alone showed significantly lower F_v/F_m values ($p << 0.01$).

On the other hand, σ_{PSII} was ~~clearly~~ relatively low in dinoflagellate-bearing species (median values 374–606 $\times 10^{-20}$ m^2 $quanta^{-1}$) and high in pelagophyte-bearing species (median values 618–749 $\times 10^{-20}$ m^2 $quanta^{-1}$) (Fig. 8b). The highest and lowest σ_{PSII} in median were recorded in *N. dutertrei* (749 $\times 10^{-20}$ m^2 $quanta^{-1}$) and *C. nitida* (347 $\times 10^{-20}$ m^2 $quanta^{-1}$), respectively. Based on the statistical testing of the species to species difference in medians, *N. dutertrei* and *G. siphonifera* Type II (pelagophyte-bearing) showed no difference ($p = 0.79$), and associated with the highest σ_{PSII}. *Globigerinoides ruber* (pink) alone showed significantly lower σ_{PSII} than the other dinoflagellate-bearing species ($p << 0.01$), and the value was comparable to that of *C. nitida* ($p = 1.0$).

3.4 Principal component analysis and clustering

To characterize photosymbiotic features, all studied species were tested for PCA with the four diagnostic variables of photosymbiosis; (1) ratio of symbiont-bearing individuals, (2) correlation coefficient between test size and Chl *a* content, (3) Chl *a* content relative to the protein biomass (Chl *a*/biomass), and (4) F_v/F_m value (Table 1). The first principal component (PC1) alone accounted for 84.2 % of the total variance, and the second principal component (PC2) for 10.2 % (Fig. 9). In the PC1 score, the loading coefficient was positive for all variables related to photosymbiosis used in the analysis (0.96 for the ratio of symbiont-bearing individuals, 0.91 for the positive correlation coefficient of test size-Chl *a* content relationship, 0.96 for the F_v/F_m median value, and 0.82 for the Chl *a* content relative to protein biomass). Considering the high contribution to explaining the total variance, and the positive loading for the four variables, the PC1 score well represented photosymbiotic characteristics among foraminiferal species. In fact, the cluster analysis ~~revealed~~ confirmed that four clusters of species were separated along the PC1 score. The lowest PC1 score (−2.2) was recorded by non-symbiotic species (Cluster 4). The distribution of species along the PC1 score was relatively wide for the Cluster 2 and 3 (0.7–2.2, −0.6–0.2, respectively), whereas almost the same for the Cluster 1 with the highest score (2.3–2.5). The Cluster 1 and 2 consisted of the species with significant positive correlations between test size and Chl *a* content. The Cluster 1 was separated from Cluster 2 primarily due

to their distinctly high PC2 score. The PC2 was characterized by Chl *a* content per protein biomass (Chl *a*/biomass) which exclusively had positive loading (0.57). Three species in the Cluster 1, *G. conglobatus*, *G. sacculifer*, and *O. universa*, were revealed to have significantly high Chl *a*/biomass as represented in Figure 7. The Cluster 2 consisted of 13 species which showed the widest distribution along with both PC1 and PC2 axes. Among the Cluster 2, the non-spinose species tended to show lower PC1 and PC2 scores compared to the spinose species. The Cluster 3 consisted of three species, *T. humilis*, *P. obliquiloculata*, and *G. inflata*. They were the species that possessed symbionts in most cases but without significant positive correlation in the test size-Chl *a* relationship. Overall, the clusters and the PC1 score depicted a clear tendency of photosymbiosis related features of the species.

4 Discussion

4.1 Characteristics and a new framework of planktonic foraminiferal photosymbiosis

The cluster analysis using photosymbiotic variables shows that 30 species fall into four groups, and features relevant to the cluster structure are extracted by PCA ~~Based on the result of the PCA and cluster analyses, 30 foraminiferal species were characterized and categorized into four groups~~ (Fig. 9). The Cluster 4 is a group of non-symbiotic species. Of the 11 species in this group, six species were tested on their photosymbiosis for the first time, and revealed to be non-symbiotic: *G. adamsi*, *T. quinqueloba*, *H. digitata*, *G. scitula*, *G. crassaformis*, and *T. fleisheri*. An interesting feature of this group ~~was~~ is that many species possess~~ed~~ non-functional chlorophyll (Fig. 5). For example, all ~~the~~ specimens in *G. scitula* and *G. crassaformis* ~~had~~ have a certain amount of chlorophyll inside, but it ~~was~~ is always non-functional and likely derived from prey. The occurrence of fresh (fluorescent) chlorophyll in these species ~~was~~ is surprising, considering that most of these specimens were collected from a water depth below 300 m (Table S1) where the chlorophyll concentration is low. They might incorporate sinking aggregates of phytoplankton remains as food (e.g., Anderson et al., 1979; Spindler et al., 1984), and chlorophyll or chloroplast itself might have remained undigested, resulting in non-functionality of chlorophyll. It is even reported that non-spinose deeper dwelling foraminifera are often found attached or embedded within marine snow and organic particulates (Fehrenbacher et al., 2018). We frequently observed a similar behavior/situation during the isolation of collected specimens. Such probable microhabitat mainly consisting of phytoplankton debris would facilitate to incorporate such materials as food, resulting in possession of non-functional chlorophyll. *Hastigerina pelagica* are known to show vertical depth segregation among the genotype (Weiner et al., 2012). It has been speculated that such segregation might be related to their possession of symbionts (e.g., Huber et al., 1997; Seears et al., 2012). Though our study did not identify their genotype, we revealed that this species never possessed symbionts even ~~they were~~when collected from shallower water depth (< 100 m). ~~The~~ A recent study showed that *G. bulloides* type IId possessed cyanobacterial symbionts (Bird et al., 2017). By using our fluorescence technique, chlorophyll fluorescence of cyanobacteria should also be detectable although the most effective wavelength of the fluorescence is slightly different. In fact, two specimens of this species show~~ed~~ possession of chlorophyll, yet they ~~were~~ are non-functional

(Table S1). This might indicate that possession of cyanobacterial symbionts may be a genotype-dependent, or regional or seasonal specific phenomenon.

~~On the other hand, f~~Five species are ~~were~~ newly confirmed as symbiotic based on the functionality of chlorophyll~~in this study~~; *S. dehiscens*, *G. rubescens*, *G. tenella*, *G. calida*, and *G. uvula*. ~~All species in the~~ Cluster 1 and 2 including the above five species showed relatively high rates of possession of symbionts, and exclusively showed significant positive correlations between the test size and Chl *a* content (Figs. 6 and 10). It was previously revealed that *G. sacculifer* and *G. siphonifera* Type II showed positive correlations between test size and Chl *a* content (Takagi et al., 2016). Similarly, *O. universa* has been demonstrated to have a positive relationship between test size and symbiont number in logarithmic scale ~~itself~~ (Spero and Parker, 1985, Fig. S4). The capability of cell divisions of symbionts cannot be ~~directly clarified~~determined from our active fluorescence-based study, but the significant positive correlation can be a strong indication for the growth of the symbiont population inside the host foraminifera. Hence, in addition to the high percentage of symbiont-bearing individuals in a species, such strong positive correlation ~~should be a specific diagnostic of~~may indicate a persistent relationship of photosymbiosis through their lifetime. Moreover, *G. conglobatus*, *G. sacculifer* and *O. universa* ~~species in the~~ (Cluster 1) should have the potential to ~~perform~~ support more ~~effective~~ photosynthesis due to the higher content of Chl *a* per protein biomass (Fig. 7). ~~It may imply more phototrophic nature of these species.~~

The Cluster 1 and 2 include~~d~~ well-studied symbiotic species like *O. universa*, *G. ruber*, *G. sacculifer*, and *G. siphonifera* previously reported to be in "obligate" symbiosis (Hemleben et al., 1989). Amongst "facultative" symbiotic species inferred in previous studies, *N. dutertrei*, *G. menardii*, *C. nitida* and *G. glutinata* ~~were~~ are revealed to have the persistent symbiotic relationships based on our test size-Chl *a* correlation analysis. In this study, not only so-far called "facultative" symbiotic species, but also most of the species were sometimes found without symbionts (all species except for *S. dehiscens* and *G. conglobatus* includes specimens with non-functional chlorophyll, Fig. 5). It was repeatedly observed that *G. sacculifer* and *G. siphonifera* digest their symbionts prior to gametogenesis (e.g., Bé et al., 1983; Faber et al., 1988; Takagi et al., 2016). Thus, symbiont-barren individuals could be present in the adult stage. However, the size of such symbiont-barren specimens recognized in this study was not necessarily large (Fig. 6). We speculate that ~~they~~ these small specimens were in an unhealthy condition and going to die. In any case, the percentage of symbiont-barren individuals in this group was small. We think the presence of symbiont-barren specimens in symbiont-bearing species, unless it is dominant, is not critical to describe the nature of photosymbiosis (i.e., conventional categorization of obligate or facultative symbiosis). Rather, the presence of such symbiont-barren individuals in these groups has led to the confusion in earlier works who placed some of these species into the category "facultative". Nevertheless, the ratio of symbiont-bearing individuals may overall reflect the ecological differences among species like the persistence of symbiosis or the dependence on phototrophy that can quantitatively represent photosymbiosis~~represent a "strength" of photosymbiosis~~.

The Cluster 3 (*P. obliquiloculata*, *G. inflata*, and *T. humilis*) ~~had~~ has intermediate features between persistent symbiosis (Cluster 1 and 2) and non-symbiosis (Cluster 4). Species do possess symbionts and can be called symbiotic species, but the significant correlation in test size-Chl *a* relationship which ~~was~~ is common in the Cluster 1 and 2 ~~was~~ is absent (Figs. 5 and 6).

~~Contrary to the interpretation of the significant positive correlation in test size-Chl *a*, the absence of such a relationship indicates that the algae in the examined species were not able to increase their biomass as the host grew. It~~It indicates ~~can be said~~ that larger sized host does not necessarily require more algae, or the algae could not persistently reside in their host to increase their biomass, in other words, the symbiosis is transient. *Pulleniatina obliquiloculata* and *G. inflata* are non-spinose species whose eating habits are reported to be primarily herbivorous (Anderson et al., 1979; Spindler et al., 1984). ~~Therefore, the lack of the relationship with test size and Chl *a* content may reflect the residence time of algal prey.~~ We ~~speculate~~ hypothesize that ~~all individuals that are capable to host symbionts~~they can be temporarily symbiotic ~~as long as the foraminifera incorporates a certain algal species. W~~when ~~a~~ foraminifera maintain~~s~~ ~~the~~ certain algae for some time keeping them undigested and keeping their photosynthetic capability to provide photosynthates~~, the algae can serve as symbionts~~. Regardless of the role of the algae, i.e., symbionts or preys, when the algae are all digested, the foraminifera becomes temporarily chlorophyll-barren. ~~If these are the case, possession of symbionts should be temporal, resulting in no size correlation in the Chl *a* content.~~ If the symbionts do not increase inside the host, the Chl *a* content of a specimen is regulated by the incorporation frequency/rate of algal cells and their residence time inside the host (i.e., a balance between incorporation and digestion). ~~They could potentially serve as symbionts if the residence time is long.~~ This behavior is similar to what is known ~~of the behavior of~~for the benthic species with kleptoplasts (e.g., Bernhard and Bowser, 1999; Pillet et al., 2011); these are actively harvested and are functional, but wear off with time and have to be replenished (e.g., Bernhard and Bowser, 1999; Pillet et al., 2011). A ~~D~~digestion experiment~~s~~ for these species, therefore, is an interesting subject in the future to test the hypothesis, and the FRR fluorometry can also serve such culturing studies. Together with *P. obliquiloculata* and *G. inflata*, *T. humilis* which was previously inferred as "obligate" symbiotic species (Hemleben et al., 1989) falls into the Cluster 3 representing such transient symbiosis. However, caution should be paid for the narrow size range of *T. humilis* we analyzed, which might cause the low correlation in test size-Chl *a* relationship~~(97–168 μm)~~ (Fig. 6). In addition, the specimens ~~we analyzed~~ were mostly with 13–15 chambers, probably in their adult stage. In this respect, since a sufficient size range of specimens with a variety of ontogenetic stages were not covered, it is difficult to strongly conclude that symbiosis in *T. humilis* is not persistent. ~~Considering t~~The F_v/F_m value of this species (0.51 in median)~~, it~~ is~~was~~ clearly higher compared to the other two species in the Cluster 3~~,~~ *P. obliquiloculata* and *G. inflata* (0.36 for *P. obliquiloculata* ~~and *G. inflata*~~and 0.33 for *G. inflata*~~, respectively~~). Besides, the possession of symbionts of this species ~~was~~ is 89 %, and higher than the other two as well (66 % for *P. obliquiloculata* and 69 % for *G. inflata*). We, therefore, interpret that *T. humilis* has established more persistent symbiosis compared to *P. obliquiloculata* and *G. inflata*.

Here, considering the above characterization of photosymbiosis, we propose a new framework of planktonic foraminiferal photosymbiosis (Fig. 11). As suggested in Stoecker et al. (2009), we think photosymbiosis can be regarded as a spectrum from absolute non-symbiosis (heterotrophy) to more robust symbiosis (higher extent of acquired phototrophy/mixotrophy) which ends with a permanent plastid endosymbiosis seen in ~~utter~~ autotrophs. Each foraminiferal species that possesses symbionts can be located somewhere in-between phototrophy and heterotrophy (a certain extent of mixotrophy, Fig. 11). Since the PC1 score well represents the photosymbiotic characteristics, it is suitable as a quantitative indicator of the level of photosymbiosis ~~in species which harbor active chlorophyll~~. Therefore, we align~~ed~~ the species along with the PC1 score scale in the conceptual

diagram (Fig. 11). In this diagram, we do not consider the necessity of photosymbiosis; i.e., whether the relationship is essential for the host survival since we cannot go into a detailed interactional relationship from our method. A recent study using a ^{13}C pulse-chase experiment of *O. universa* and subsequent subcellular microimaging and elemental analysis revealed the fate of assimilated carbon by the symbionts (LeKieffre et al., 2018). They showed a line of evidence of substance transfer from the symbionts to the host and their tight interrelationship. Considering their results for *O. universa*, it is speculated that *G. conglobatus* and *G. sacculifer* with higher PC1 score than *O. universa* should have a similar or even tighter interaction in their symbiotic system. If the similar experiment can be conducted for species with low PC1 score, especially for *G. inflata* and *P. obliquiloculata* whose mode of symbiosis is expected to be something different, the information of the internal phenomena can be added, which will provide us an insight of the necessity of photosymbiosis.

An important point here is that this spectrum allows~~ed~~ us to gain an overview of the relative strength of photosymbiosis among species and across various families of planktonic foraminifera Globigerinidae, Globorotaliidae, and Candeinidae. The relative ordination may be amended by further exploration in the future, but we believe our thorough investigation ~~could~~ can shed light on the species-specific difference in the nature of photosymbiosis in planktonic foraminifera. This would be a solid basis to help us to think about evolutionary aspects of photosymbiosis, its role in the earth system history, and possible effects on test geochemistry.

4.2 Size scaling of Chl *a* content in symbiotic foraminifera

The significant positive correlation between test size and Chl *a* content (Figs. 6 and 10) shows the increasing number of ~~is not just showing the growing nature of~~ symbionts with host size, and a~~, but also has information on a kind of~~ quantitative relationship in the host and symbionts based on their scaling exponent (Table 2). In theory, the size scaling exponent of 3 means that the dependent variable increases proportionally to the volume development. If the test shape is ~~flatter~~less spherical, as is the case of *G. menardii*, the exponent should be smaller and approaching 2. Alternatively, when the test volume does not reflect the cytoplasm volume (the increase in~~growth rate of~~ the cytoplasm is less than that of the test volume) like adult spherical specimens of *O. universa*, the scaling exponent results in relatively small values. The fact that all 16 species show~~ed~~s scaling exponent in the range of 2 to 3 (95 % confidence intervals overlap with this range, Table 2) indicates that the Chl *a* content, indirectly reflecting the symbiont biomass, increased in nearly proportional to the host's test volume~~ increased~~. This kind of size scaling ~~theory~~ across different species of planktonic foraminifera suggests a robust relationship between the host and symbionts.

The other notable point in the test size-Chl *a* relationship is that the spinose species, irrespective of their symbiont type, commonly ~~had~~ has ~~higher contents of~~more Chl *a* compared to the non-spinose species (Fig. 10). For example, when the test size ~~was~~ is ca. 300 µm, the macroperforate spinose group ~~had~~ has almost five times ~~higher~~ more Chl *a* than the microperforate non-spinose group, and 10 times ~~higher~~ more than the macroperforate non-spinose group. The light-dark rhythm of symbiont deployment along the spines was commonly observed in *Globigerinoides*, *Orbulina* and *Globigerinella* species (Anderson and Bé, 1976; Bé et al., 1977; Hemleben and Spindler, 1983; Takagi et al., 2016)~~,~~ Considering this phenomenon, the presence of

spines ~~could be seen as a character that~~may facilitate~~s~~ symbiosis or at least allows the harboring of a larger symbiont population. For example, efficient ~~lighting~~ illumination for each symbiont cell and maximizing total photosynthetic rates can be achieved due to the spherical distribution of symbionts along the radiating spines. The distribution would also enhance their availability of nutrients or dissolved inorganic carbon for photosynthesis which should be quickly exhausted when symbionts are sequestered inside the test. These photosynthetic advantages derived from spine possession may ~~This may~~ contribute to the higher Chl *a* content in the spinose species. It may also be involved with their ~~lower~~higher Chl *a*/biomass ~~observed in non-spinose species~~(Fig. 7).

Moreover, ~~the distribution of the plots in Figure 10 showed three~~ clear clusters correspond~~ing~~ to each morphogroups~~;~~, macroperforate spinose, macroperforate non-spinose, and microperforate non-spinose (Fig. 10). ~~It is also an interesting feature firstly revealed in this study.~~ In addition to the possession of spines, the overall ecology such as depth habitat and the type of prey differ among the groups. Therefore, the light availability as a function of depth and the internal nutrient supply from the host to the symbionts (i.e., preys of the host) can differ among the groups, which would affect the distribution of the plots. ~~The largest morphological difference among these three groups is the test wall architecture including the density of pores, the presence of spines, and smoothness of test surface. Hence, these features might be related to the light penetration through the test wall. When the symbionts are sequestered within the test as seen in the non-spinose species, the test wall architecture is expected to affect the efficiency of photosynthesis.~~ It may also be involved with lower Chl *a*/biomass observed in non-spinose species. If such environmental/microenvironmental condition~~s~~ surrounding the ~~intracellular~~ symbionts are measurable or ~~theoretically~~ numerically~~-~~ modeled, ~~it will enhance~~ our understanding of the differences and the controlling factor of symbiont ~~density~~abundance would be improved.

4.3 Photophysiology and host-symbiont partnerships

When species are grouped ~~based on the~~according to symbiont type ~~they possess~~, dinoflagellate (*O. universa*, *G. sacculifer*, *G. conglobatus*, *S. dehiscens, G. ruber, G, tenella,* and *G. rubescens*)~~,~~ or pelagophyte (*G. siphonifera* Type II and *N. dutertrei*) (Table 1), photophysiological parameters ~~were all~~are significantly different between these groups. Chl *a*/biomass and F_v/F_m values ~~were~~ are higher for dinoflagellate-bearing species ($p << 0.01$ and $p = 0.012$, respectively, Figs. 7 and 8a), and σ_{PSII} values ~~were~~ are higher for pelagophyte-bearing species ($p << 0.01$, Fig. 8b). As far as the species whose symbionts are known are compared, it seems that the symbiont photophysiology ~~was~~ is overall related to the type of symbiont rather than the host size or the host morphological groups. In fact, we previously published experimental results on photophysiology of cultured *G. sacculifer* (dinoflagellate-bearing) and *G. siphonifera* Type II (pelagophyte-bearing), and reported lower F_v/F_m and higher σ_{PSII} in *G. siphonifera* Type II than in *G. sacculifer* (Takagi et al., 2016). In this study, what we observed ~~was~~ is the same tendency of photophysiology corresponding to the type of symbionts ~~they have~~, regardless of the phylogenetic position the host ~~belongs to~~.

Previous studies revealed high-light adapted photophysiology of dinoflagellate symbionts in *O. universa* and *G. sacculifer* (Jørgensen et al., 1985; Spero and Parker, 1985; Rink et al., 1998) based on the parameters in photosynthesis-irradiance (P-I)

curves. They reported high saturation irradiance (I_k = 386 μmol photon m^{-2} s^{-1}, Spero and Parker, 1985), and no photoinhibition at as high as 4000 μmol photon m^{-2} s^{-1} (Jørgensen et al., 1985). By definition, a saturation irradiance (I_k) is inversely proportional to the extrapolated initial slope (α) in a P-I curve. Since the slope α takes into account that the light absorbed by the algal cell is proportional to the functional absorption cross-section of PSII (σ_{PSII}), I_k should be inversely related to σ_{PSII} (Falkowski and Raven, 2007). Therefore, the high I_k reported for dinoflagellate symbionts is consistent with the low σ_{PSII} in our results. Although I_k or α of pelagophyte-bearing species has not been reported, the high σ_{PSII} for pelagophyte-bearing species, *vice versa*, indicates low-light acclimated photophysiology. ~~The higher σ_{PSII} indicates a higher acclimation potential to a low-light environment.~~ This observation is consistent with the living depth of the involved species. In general, dinoflagellate-bearing species like *G. ruber* and *G. sacculifer* prefer shallower habitat, and pelagophyte-bearing species like *N. dutertrei* and *G. siphonifera* Type II prefer relatively deeper water (Rebotim et al., 2017). Moreover, when *G. siphonifera* Type I and Type II ~~were~~ are compared, the Type I having haptophyte symbionts shows~~ed~~ significantly lower σ_{PSII} than the Type II (Fig. 8b). The previous report on the difference in pigment content of these types also implied deeper habitat in *G. siphonifera* Type II (Bijma et al., 1998). The σ_{PSII} difference revealed in this study supports their arguments. Moreover, even in the time before the type difference of this species was recognized, *G. siphonifera* was often reported to have a bimodal vertical distribution (Tolderlund and Bé, 1971). It possibly reflected the difference of the light preference of their associating symbionts. The current knowledge on σ_{PSII} in foraminifera is still limited, but the observed consistency to their known depth preferences indicates that the symbiont acclimation potential may be one of the factors ~~controlling~~ constraining the habitat selection of the host species.

The dinoflagellate-bearing species, *G. ruber* (pink) shows~~ed~~ high F_v/F_m with relatively small variation, and interestingly, it ~~was~~ is significantly higher than that of *G. ruber* (white) (Fig. 8a). In general, F_v/F_m values vary depending on the nutrient availability (Kolber et al., 1988; Parkhill et al., 2001); i.e., the higher F_v/F_m may be achieved by the higher nutrient supply to the symbionts. A recent study showed that the inorganic nutrients in ~~the~~ ambient seawater do not affect the F_v/F_m of *G. sacculifer*, suggesting that it is the internal supply of nutrients from the host to symbionts that can influence on the F_v/F_m (Takagi et al., 2018). In this context, it can be assumed that among the species having the same symbionts, the higher F_v/F_m possibly reflects the higher level of host-symbiont interaction. If it is the case, among the species used for the statistical analysis, it can be said that the strongest symbiotic relationship has been established in *G. ruber* (pink). ~~,~~ In fact, ~~however,~~ the interspecific comparison may not be suitable because the other environmental factors which might affect the physiology of the host-symbiont consortia, such as seawater temperature, salinity, light intensity, and prey abundance, ~~were~~ are not considered in this study. *Globigerinoides ruber* (pink) was collected only from the Atlantic cruise, whereas *G. ruber* (white) was collected from various oceanic realms (Table S1). It may also be involved with relatively constrained F_v/F_m values in *G. ruber* (pink) and contrastingly large variability in *G. ruber* (white). In order to discuss more detail on interspecific photophysiological differences, comparison of the photophysiological parameters for specimens cultured under controlled condition, or the compilation of individual data collected from a similar environmental condition is needed. Besides, since various potential factors are affecting the photophysiology (e.g., host taxonomy, symbiont taxonomy, light, nutrient, etc.), statistical modeling

approaches such as generalized linear/additive mixed models would be useful to elucidate which factor is important to determine the photophysiology.

5 Conclusion and future perspectives

The present study ~~was aimed to extend~~extends our understanding of photosymbiosis in modern planktonic foraminifera. A thorough investigation of 30 foraminiferal species was performed using FRR fluorometry. Eleven species show~~ed~~ no signal of photosynthesis, and ~~were~~ are confirmed to be non-symbiotic. Nineteen species~~, in contrast,~~ show~~ed~~ the functionality of photosynthesis which is convincing evidence of photosymbiosis. Of these species, we found significant positive correlations in test size-Chl *a* content relationship in 16 species, which ~~were~~ are regarded to show persistent symbiotic relationships. Especially, dinoflagellate-bearing *G. sacculifer*, *G. conglobatus*, and *O. universa* ~~had~~ have higher Chl *a* density, probably reflecting the higher potential of photosynthesis. The rest of three species, *T. humilis*, *P. obliquiloculata*, and *G. inflata* show~~ed~~ no significant size scaling relationship in Chl *a* content. Moreover, their F_v/F_m values and the symbiont possession rates ~~were~~ are comparatively low. Based on a PCA using the four features relating to photosymbiosis, we rank~~ed~~ 30 species along with an integrated scale (the PC1 score scale). Finally, we propose~~d~~ a new framework of photosymbiosis in planktonic foraminifera as a continuous spectrum ~~implying a strength~~ of photosymbiosis. In the context of nutrition, this concept represents a varying degree of mixotrophy which is commonly seen in marine planktonic organisms (Stoecker et al., 2017). ~~Besides~~Interestingly, ~~an interesting finding in this study was that the~~ photophysiology ~~was~~ may be basically determined by the type of the symbiont ~~they have~~, regardless of the phylogenetic position of the host and~~or~~ its~~their~~ test morphology. ~~The p~~Physiological parameters, in particular~~especially~~ σ_{PSII}, seem~~ed~~ to correspond to the overall depth habitat of the host foraminifera. It might imply that the habitat of the host foraminifera is partly governed by the symbiont type. However, what is missing in our study is the taxonomy of the symbionts. Combining the information of FRR fluorometry, DNA, as well as microscopic evidence on their ultrastructure will provide a more comprehensive understanding of photosymbiosis in planktonic foraminifera.

Author contribution. HT conceived the project. KK, TF, and KM advised on methodology. HT, KK, HS, CS, and MK participated sampling and collected planktonic foraminifera. HT carried out the on-board lab work with help of KK, TF, CS, and MK. TF and CS contributed to photophysiological measurement and data analysis. HT carried out data analysis and statistical analysis. HT wrote the manuscript and KK, TF, HS, CS, MK, and KM provided critical discussions and editions to the manuscript.

Data availability. All data obtained in this study is in Supplementary materials.

Competing interests. The authors declare that they have no conflict of interest.

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Table 1. Summary of species symbiotic ecology. [1]Spindler and Hemleben (1980); [2]Taylor (1982); [3]Hemleben and Spindler (1983); [4]Gastrich (1987); [5]Faber et al. (1989); [6]Hemleben et al. (1989); [7]Gast and Caron (1996); [8]Huber et al. (1997); [9]Shaked and de Vargas (2006); [10]Gast et al. (2000); [11]Fujiki et al. (2014); [12]Bird et al. (2017); [13]Schiebel and Hemleben (2017); [14]Bird et al. (2018). *Based on microscopic observations of living specimens in this study. Comparison with the other dinoflagellate-bearing species revealed almost identical features of the symbionts (e.g., cell size, shape, color, see Figure S3).

Species	Previous studies			This study						Remarks
	Algal type		Obligate / facultative / none	Ratio of symbiotic individuals	Test size-Chl *a* correlation~~Correlation~~ (correlation coefficient R)	F_v/F_m	σ_{PSII} ($\times 10^{-20}$ m^2 quanta^{-1})	Chl *a* /biomass (ng µg^{-1})	Cluster	
	Microscopy-based	Molecular-based								
Orbulina universa										
	Dinoflagellate[1,4]	*Pelagodinium béii* (Dinoflagellate)[7,9]	Obligate[6]	0.95	Positive 0.664	0.50	448	4.65	1	
Sphaeroidinella dehiscens										
	Not reported	Not reported	Not reported	1.00	Positive 0.927	0.53	606	2.36	2	Presence of dinoflagellate symbionts inferred[13]
Globigerinoides sacculifer										
	Dinoflagellate[1,4]	*Pelagodinium béii* (Dinoflagellate)[7,9]	Obligate[6]	0.96	Positive 0.682	0.51	453	4.78	1	
Globigerinoides conglobatus										
	Dinoflagellate[1,4]	*Pelagodinium béii* (Dinoflagellate)[7]	Obligate[6]	1.00	Positive 0.680	0.50	449	4.80	1	
Globigerinoides ruber										
	Dinoflagellate[1,4]	*Pelagodinium béii* (Dinoflagellate)[7,9]	Obligate[6]	white 0.98	Positive 0.667	0.49	469	3.09	2	
				pink 0.91	Positive 0.875	0.52	374	2.28	2	
Globoturborotalita rubescens										
	Not reported	Not reported	Not reported	0.79	Positive 0.773	0.46	388	1.13	2	Probably dinoflagellate-bearing*
Globoturborotalita tenella										
	Not reported	Not reported	Not reported	0.77	Positive 0.840	0.51	421	2.12	2	Probably dinoflagellate-bearing*
Globigerinella calida										
	Not reported	Not reported	Not reported	0.58	Positive 0.607	0.44	492	1.33	2	
Globigerinella siphonifera										

Haptophyte[1] (Prymnesiophyte[2]) / two different chrysophycophyte[4,5,8]	Type I	Unclassified Haptophyceae[10]	Obligate[6] / facultative[5]	0.81	Positive 0.504	0.47	515	2.56	2	Extracellular commensal algae reported[8]
	Type II	*Pelagomonas calceolata* (Pelagophyte)[11]	Obligate[6] / facultative[5]	0.95	Positive 0.531	0.49	689	2.42	2	Extracellular commensal algae absent[8]
Globigerinella adamsi										
Not reported		Not reported	Not reported	0.00	–	–	–	–	4	
Globigerina bulloides										
Barren[3,4,6] / ~~*echococcus*~~[12] *Synechococcus*[12]		*Synechococcus*[12]	None[3,4,6]	0.00	–	–	–	–	4	
Turborotalita quinqueloba										
Not reported		Not reported	Not reported	0.00	–	–	–	–	4	
Turborotalita humilis										
Dinoflagellate[1] / haptophyte[3] / chrysophyte[4]		Not reported	Obligate[6]	0.89	Not s~~S~~ignificant −0.023	0.51	710	0.58	3	
Hastigerina pelagica										
Barren[1,3]		Not reported	None[3,4,6]	0.00	–	–	–	–	4	
Hastigerinella digitata										
Not reported		Not reported	Not reported	0.00	–	–	–	–	4	
Neogloboquadrina incompta										
Not reported		Barren[14]	None[14]	0.00	–	–	–	–	4	
Neogloboquadrina pachyderma										
Not reported		Not reported	None[6]	0.00	–	–	–	–	4	Absence of symbionts inferred[6]
Neogloboquadrina dutertrei										
Barren[3] / chrysophyte[4] / pelagophyte[14]		Pelagophyte[14]	Facultative[6]	0.94	Positive 0.799	0.48	749	0.60	2	
Pulleniatina obliquiloculata										
Prymnesiophyte[2] / b~~B~~arren[3] / chrysophyte[4]		Not reported	Facultative[6]	0.66	Not ignificant~~Signif ant~~ −0.135	0.36	518	0.07	3	
Globorotalia inflata										
Barren[3] / chrysophyte[4]		Not reported	Facultative[6]	0.69	Not ignificant~~Signif cant~~ 0.121	0.33	544	0.19	3	

Globorotalia menardii								
Prymnesiophyte[2] / bBarren[3] / chrysophyte[4]	Not reported	Facultative[6]	0.87	Positive 0.685	0.50	498	0.58	2
Globorotalia scitula								
Not reported	Not reported	Not reported	0.00	–	–	–	–	4
Globorotalia crassaformis								
Not reported	Not reported	Not reported	0.00	–	–	–	–	4
Globorotalia truncatulinoides								
Barren[1,3,4]	Not reported	None[3,4,6]	0.00	–	–	–	–	4
Candeina nitida								
Chrysophyte[4]	Not reported	Facultative[6]	0.88	Positive 0.583	0.49	347	1.48	2
Globigerinita glutinata								
Barren[3] / chrysophyte[4]	Not reported	Facultative[6]	0.68	Positive 0.512	0.50	632	0.71	2
Globigerinita uvula								
Not reported	Not reported	Not reported	0.79	Positive 0.799	0.35	618	0.47	2
Tenuitella fleisheri								
Not reported	Not reported	Not reported	0.00	–	–	–	–	4

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Table 2. Scaling exponents (slopes in Figs. 6 and 10) for relationships between test size and Chl *a* content. Reduced major axis regression was used to estimate the scaling exponents after logarithmic transformation of the two variables. CI; confidence
625 interval. When the correlation was not significant, the values are not shown. N; the number of specimens used for the analysis.

Species / morphogroup	N	Scaling exponent		
		Best estimate	2.5 % CI	97.5 % CI
Orbulina universa	75	1.90	1.60	2.26
Sphaeroidinella dehiscens	7	2.91	1.92	4.43
Globigerinoides sacculifer	94	3.10	2.66	3.60
Globigerinoides conglobatus	18	1.83	1.25	2.68
Globigerinoides ruber (white)	49	2.36	1.90	2.93
Globigerinoides ruber (pink)	40	2.62	2.24	3.07
Globoturborotalita rubescens	15	1.59	1.09	2.30
Globoturborotalita tenella	10	1.33	0.87	2.04
Globigerinella calida	11	3.71	2.10	6.56
Globigerinella siphonifera Type I	61	3.57	2.85	4.47
Globigerinella siphonifera Type II	53	2.89	2.28	3.66
Turborotalita humilis	17	–	–	–
Neogloboquadrina dutertrei	91	3.16	2.79	3.59
Pulleniatina obliquiloculata	45	–	–	–
Globorotalia inflata	9	–	–	–
Globorotalia menardii	144	1.84	1.63	2.08
Candeina nitida	32	3.20	2.37	4.31
Globigerinita glutinata	69	2.43	1.97	2.99
Globigerinita uvula	11	2.66	1.71	4.12
Macroperforate spinose with dinoflagellate	308	2.52	2.37	2.67
Macroperforate spinose with non-dinoflagellate	125	3.06	2.64	3.55
Macroperforate non-spinose	235	2.17	1.95	2.41
Microperforate non-spinose	112	2.61	2.30	2.95

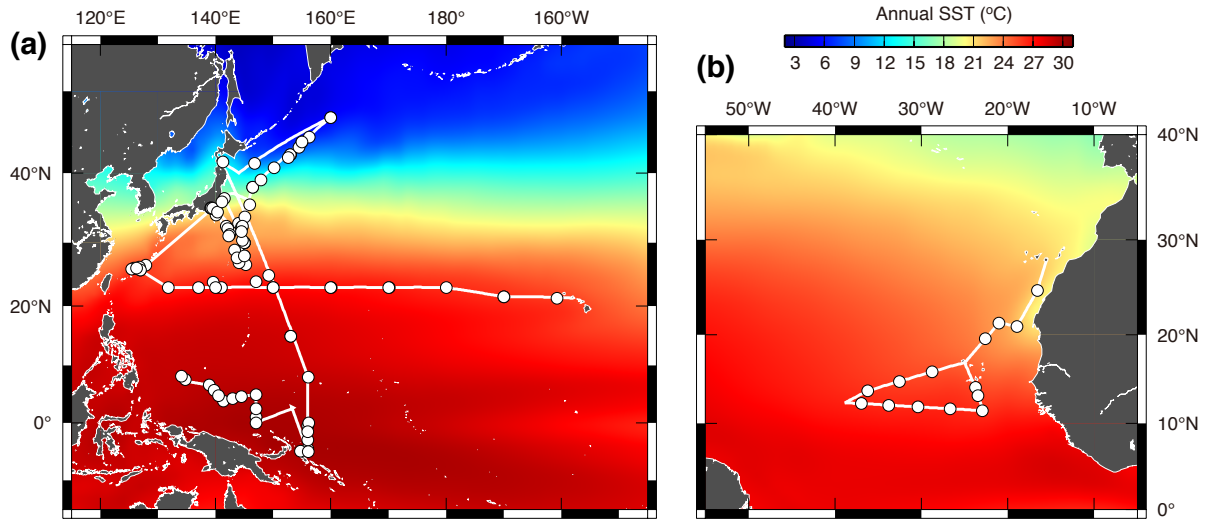


Figure 1. Maps showing the cruise tracks (lines) and the sampling points (circles). (a) Central and western Pacific area, and (b) ~~northeastern~~ tropical eastern Atlantic area. For detail sampling information, see Table S1. Annual sea surface temperature (SST) data was from World Ocean Atlas 2013 (Locarnini et al., 2013).

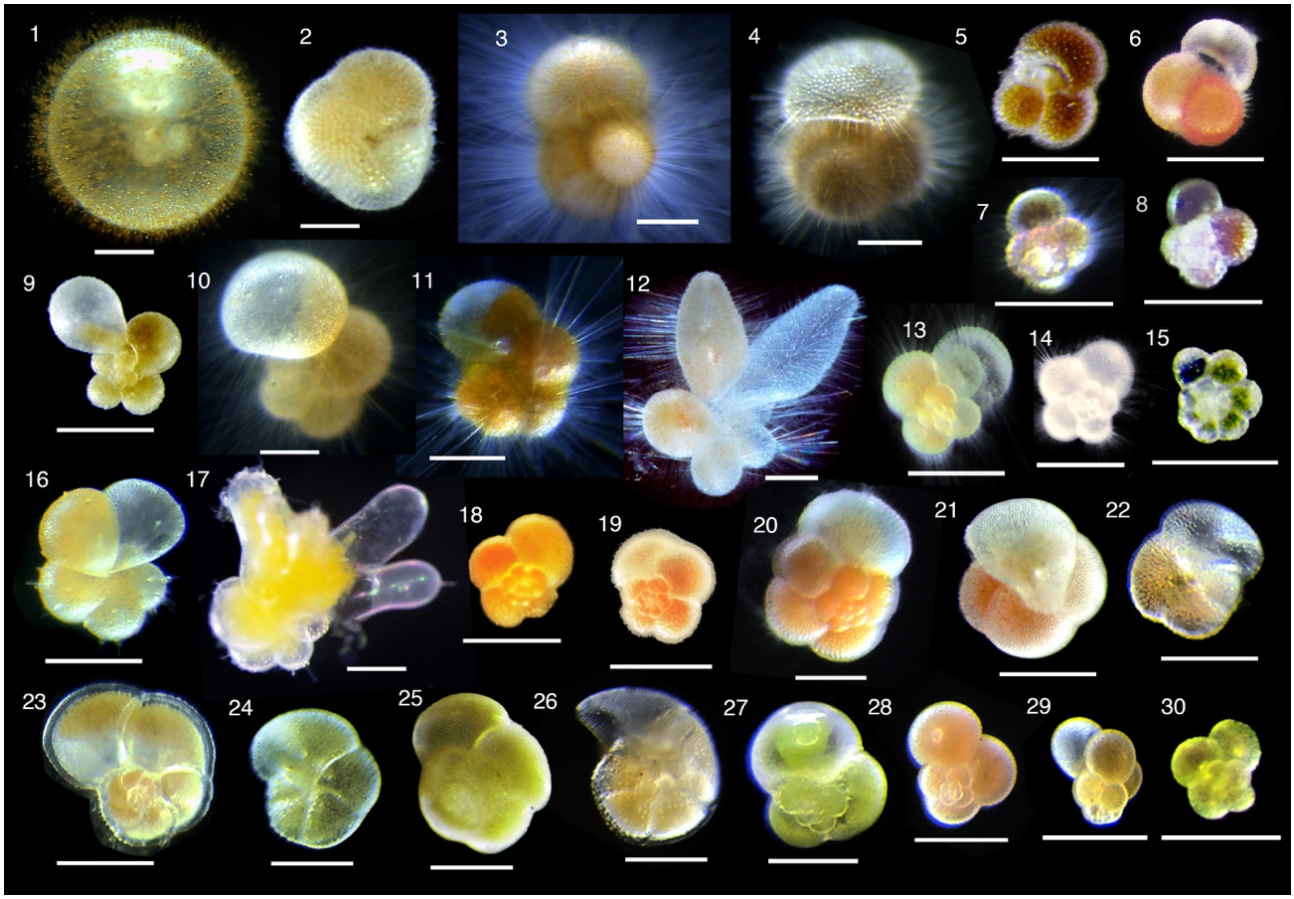
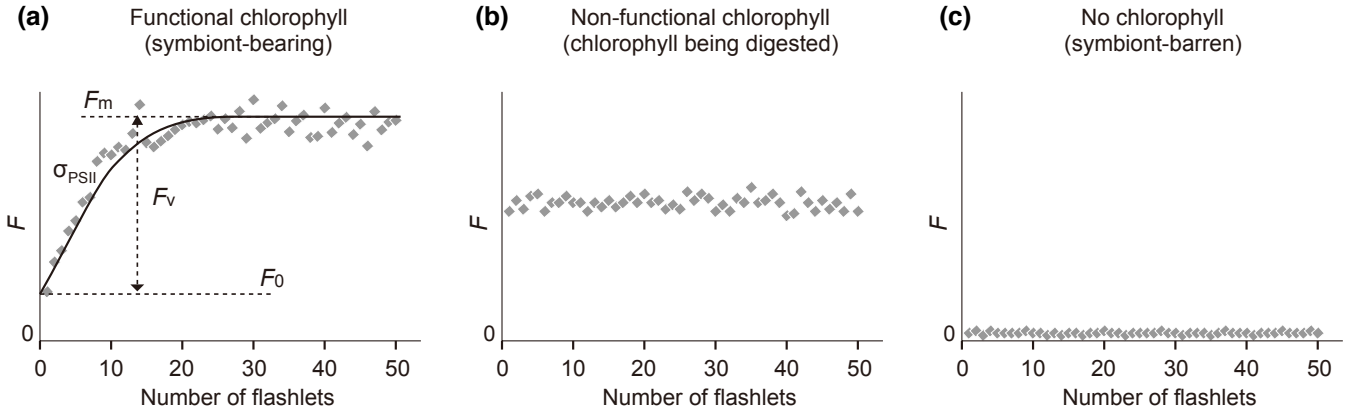


Figure 2. Photomicrographs of representative individuals for species analyzed. (1) *Orbulina universa*, (2) *Sphaeroidinella dehiscens*, (3) *Globigerinoides sacculifer*, (4) *Globigerinoides conglobatus*, (5) *Globigerinoides ruber* (white), (6) *Globigerinoides ruber* (pink), (7) *Globoturborotalita rubescens*, (8) *Globoturborotalita tenella*, (9) *Globigerinella calida*, (10) *Globigerinella siphonifera* Type I, (11) *Globigerinella siphonifera* Type II, (12) *Globigerinella adamsi*, (13) *Globigerina bulloides*, (14) *Turborotalita quinqueloba*, (15) *Turborotalita humilis*, (16) *Hastigerina pelagica*, (17) *Hastigerinella digtata*, (18) *Neogloboquadrina incompta*, (19) *Neogloboquadrina pachyderma*, (20) *Neogloboquadrina dutertrei*, (21) *Pulleniatina obliquiloculata*, (22) *Globorotalia inflata*, (23) *Globorotalia menardii*, (24) *Globorotalia scitula*, (25) *Globorotalia crassaformis*, (26) *Globorotalia truncatulinoides*, (27) *Candeina nitida*, (28) *Globigerinita glutinata*, (29) *Globigerinita uvula*, (30) *Tenuitella fleisheri*. Scale bars are 200 μm.



Term	Definition	Indication
F	Chlorophyll fluorescence (arbitrary unit)	Presence of chlorophyll
F_m	Maximum fluorescence (arbitrary unit)	Chl *a* content
F_0	Minimum fluorescence (arbitrary unit)	–
F_v	Variable fluorescence $F_m - F_0$ (arbitrary unit)	Presence of functional chlorophyll indicating the presence of symbionts
F_v/F_m	Potential photochemical efficiency (dimensionless)	Photosynthetic activity indicating vitality of symbionts
σ_{PSII}	Functional absorption cross section of PSII photochemistry ($\times 10^{-20}$ m^2 quanta^{-1} /Å^2 quanta^{-1})	Light absorption efficiency

Figure 3. Schematic diagram of fluorescence induction curves by fast repetition rate fluorometry and their interpretation. (a) Profile of a symbiotic individual. (b) Profile of a non-functional chlorophyll-bearing individual. (c) Profile of a non-symbiotic individual. Photosystem II parameters used in this study are also listed. All parameters are obtained in dark-adapted states.

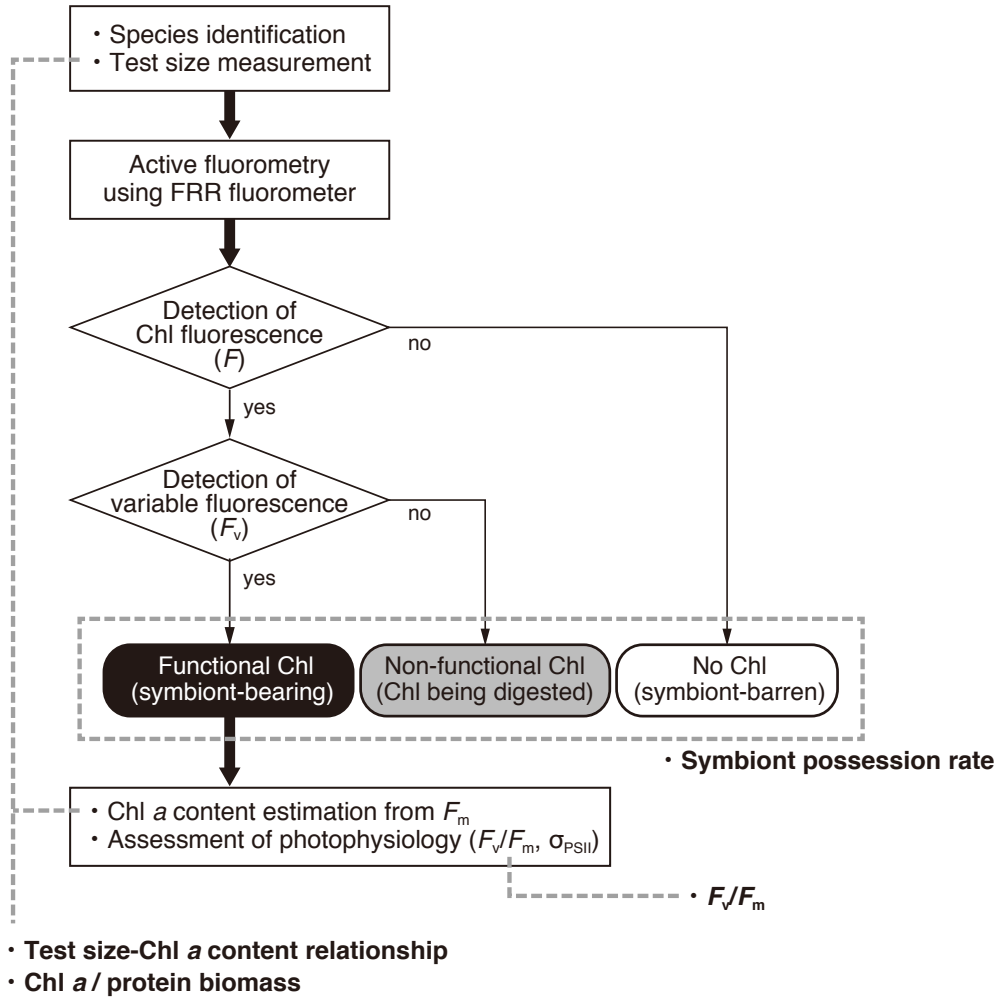


Figure 4. Workflow of this study and four indices used to characterize photosymbiosis. Firstly, individual specimens were identified to morphospecies level, measured for the test size, and analyzed with active fluorometry to check the functionality of chlorophyll. Based on the fluorescence results, intracellular chlorophyll types (status) were categorized into three groups; functional chlorophyll, non-functional chlorophyll, and no chlorophyll. When chlorophyll was functional, the content of Chl *a* per individual and the photophysiological parameters were analyzed. Finally, four indices in bold (symbiont possession rate, test size-Chl *a* relationship, Chl *a*/biomass, and F_v/F_m) were derived and used for characterization of photosymbiosis (see text for detail).

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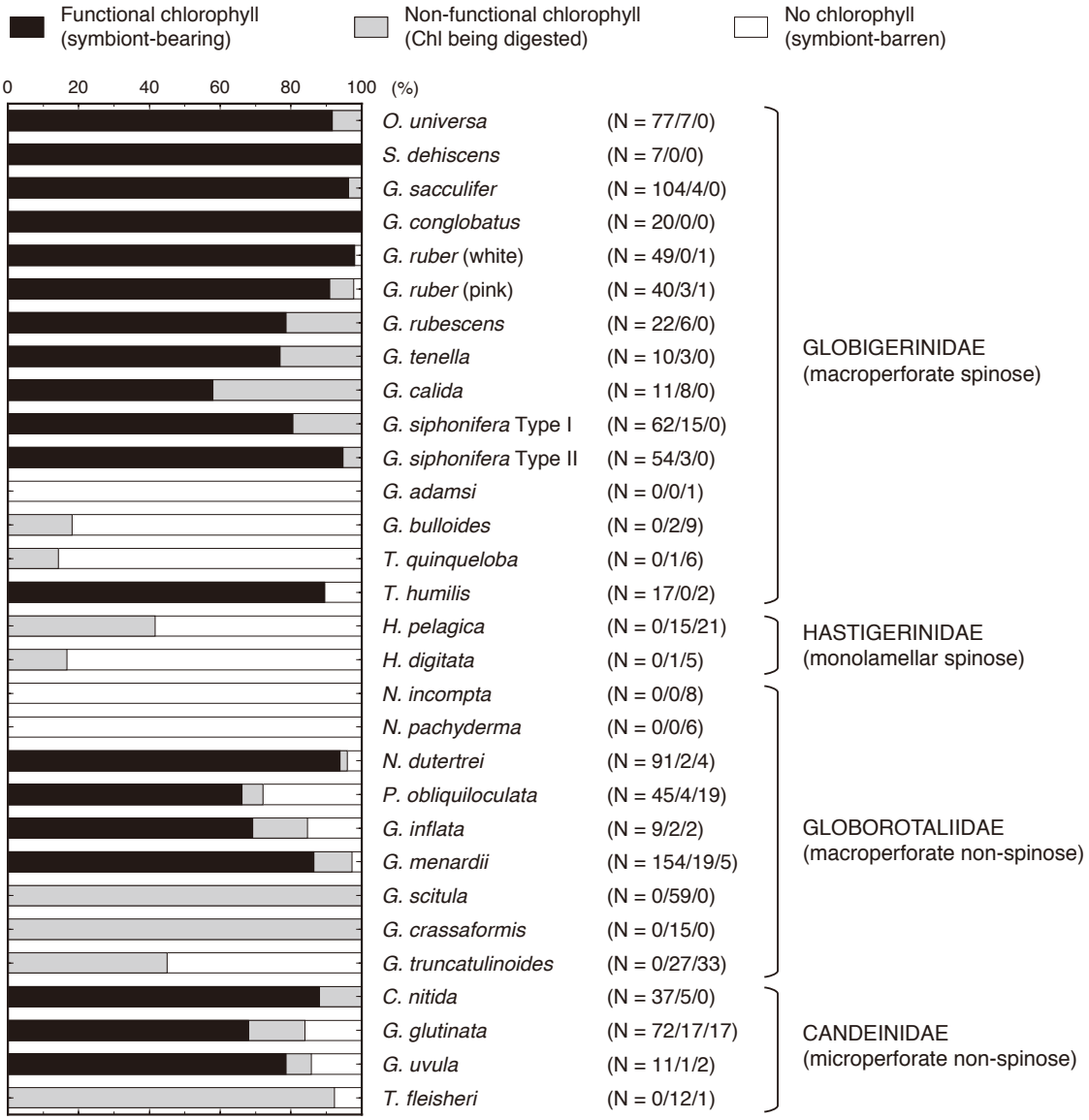
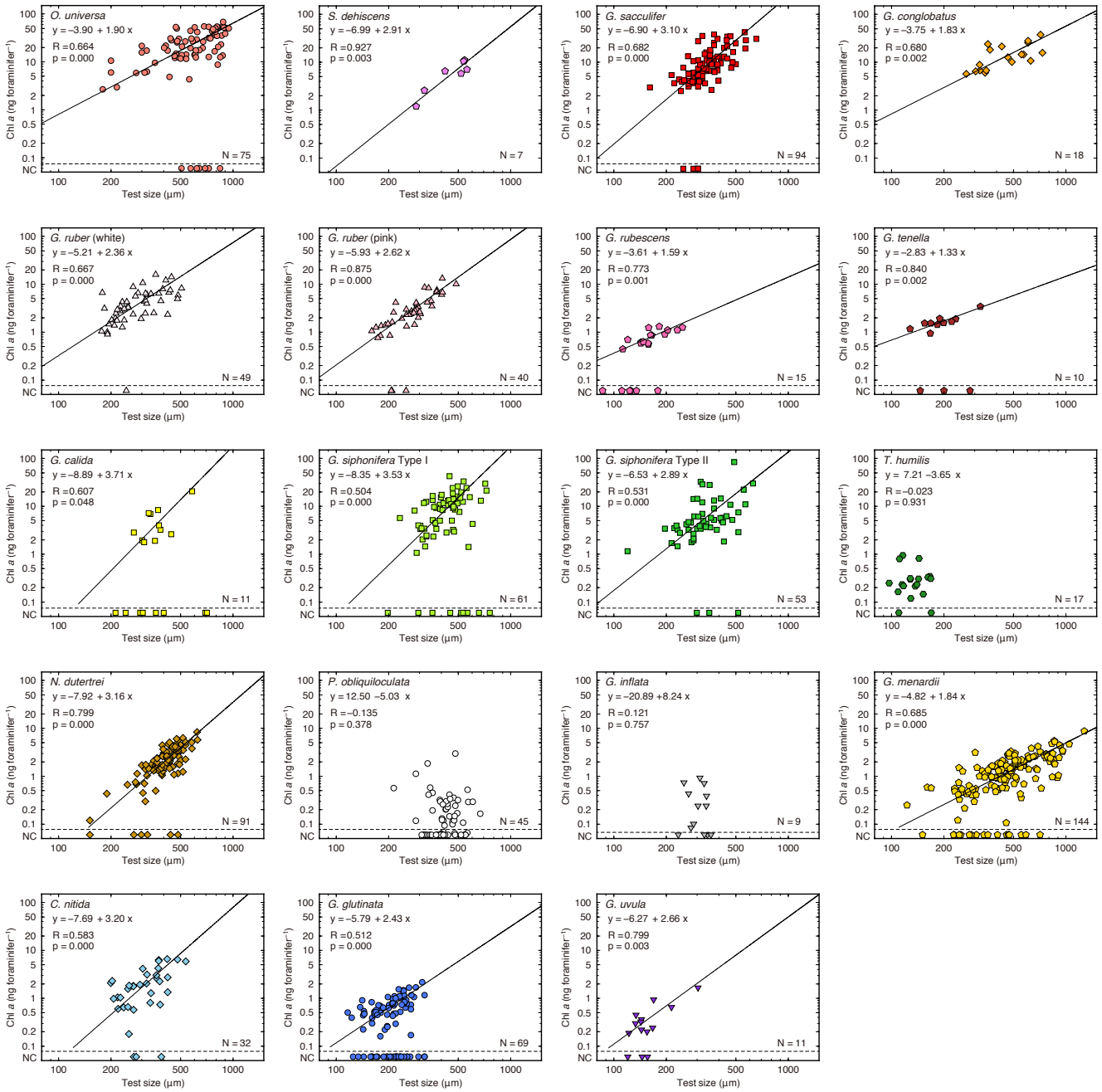


Figure 5. Summary of categorization of intracellular chlorophyll. The functionality of chlorophyll indicates the presence of symbionts. Numbers of specimens for three categories are represented in parentheses (functional chlorophyll / non-functional chlorophyll / no chlorophyll). The percentage of functional chlorophyll are essentially the same as the symbiont possession rate used as a variable to characterize photosymbiosis (see text for detail).



715 **Figure 6.** Relationships between test size and Chl *a* content for each species. Lines represent reduced major axis regression (y; log(Chl *a*), x; log(test size)). Specimens with no chlorophyll and non-functional chlorophyll (NC) are plotted at the bottom of each panel to show their test size information (these data are not used for the regression analysis). R, Pearson's correlation coefficient; p, p-value; N, number of specimens with functional chlorophyll (i.e., with symbionts). For *O. universa*, specimens smaller than 400 µm are pre-spherical trochospired test diameter, and those larger than 400 µm are sphere diameter (see Table
720 S1).

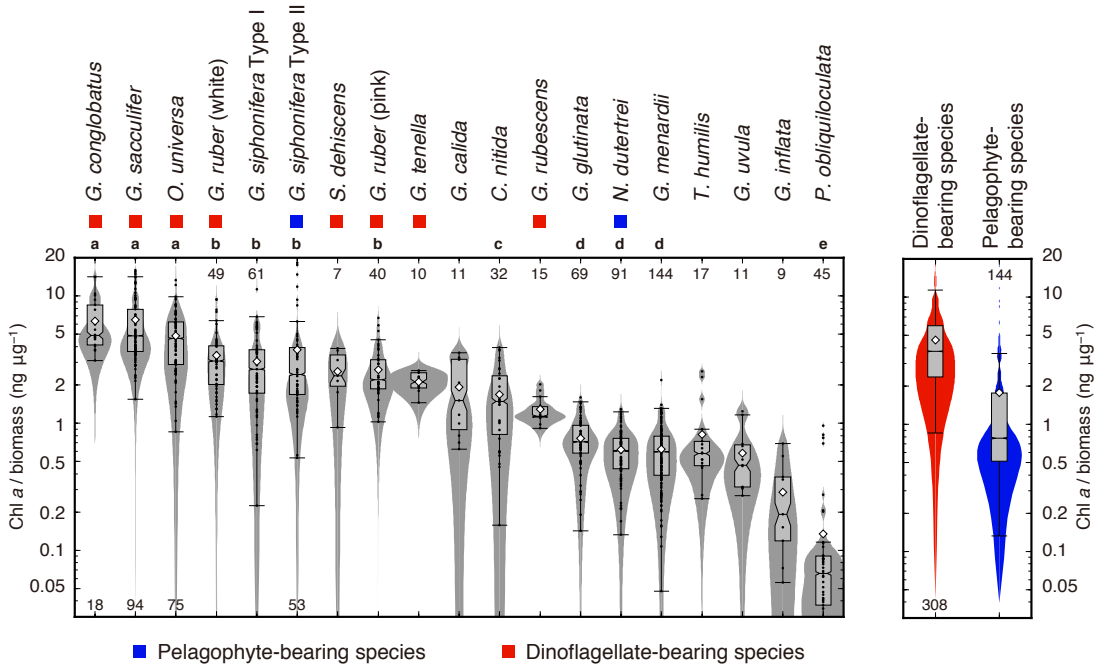


Figure 7. Ratios of Chl *a* content (ng foraminifer^{-1}) to protein biomass (µg foraminifer^{-1}) of 19 symbiont-bearing species. Dots represent individual data sampled from the upper 100 m water depth. Box plots represent first and third quartiles as hinges, and midlines as medians with notch representing 95 % confidence interval of the medians. Means are also represented with open diamonds. Whiskers are extended up to 1.5 times interquartile ranges from the end of each box to the furthest datum within that distance. Violin plots show the distributions as Kernel density estimation. Numbers at either end of the panel are the sample size for each species. Species with more than 20 specimens were used for statistical testing (Kruskal-Wallis test for comparison of differences in medians, and post-hoc Steel-Dwass test for multiple comparison, $p < 0.05$). Species with the same letter were not significantly different. Color symbols represent the difference of symbiotic algae (see Table 1). Note that the data are represented on a logarithmic scale.

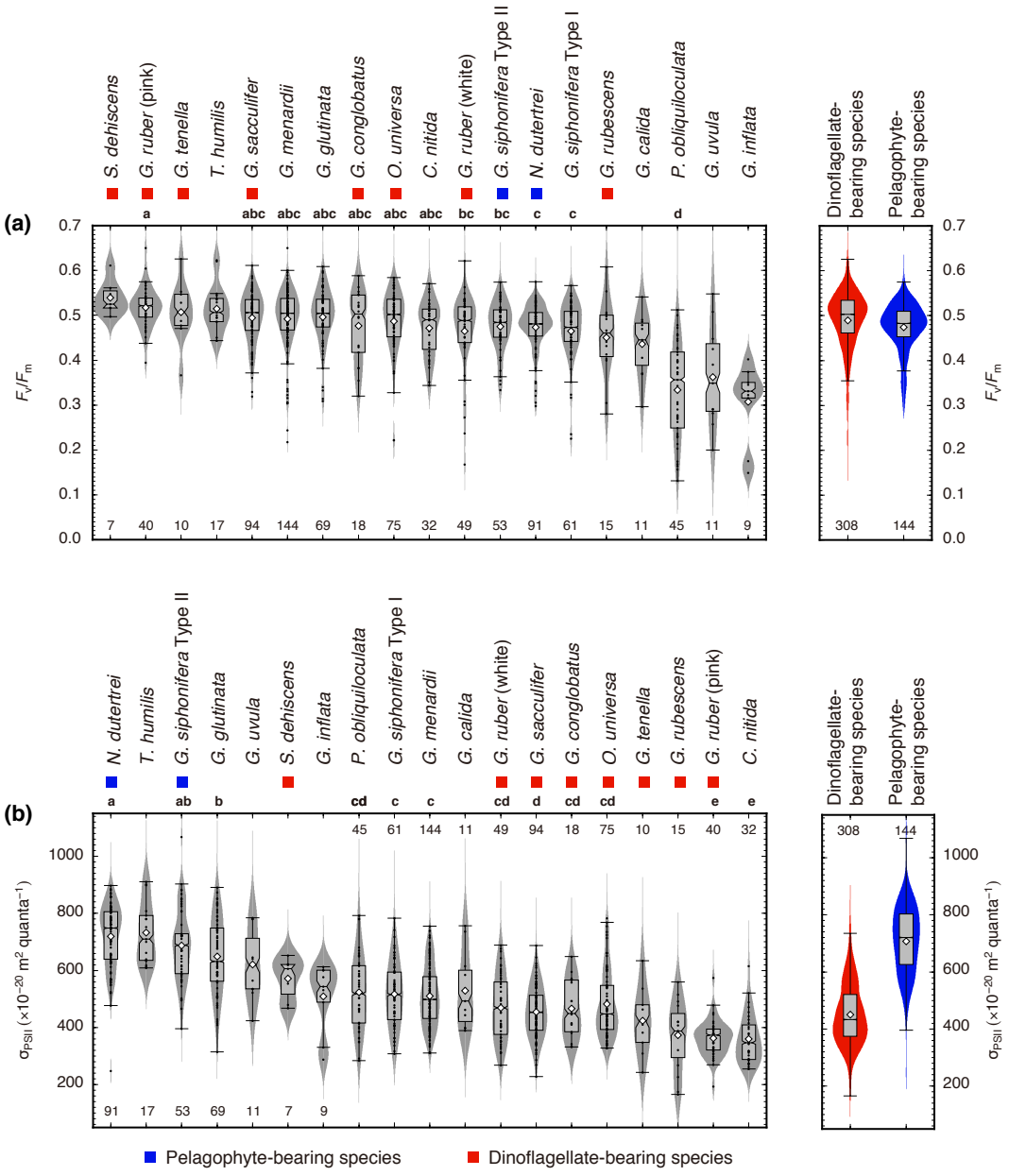


Figure 8. Photophysiological parameters of 19 symbiont-bearing species. (a) F_v/F_m, and (b) σ_{PSII}. Dots represent individual data sampled from the upper 100 m water depth. Box plots represent first and third quartiles as hinges, and midlines as medians with notch representing 95 % confidence interval of the medians. Means are also represented with open diamonds. Whiskers are extended up to 1.5 times interquartile ranges from the end of each box to the furthest datum within that distance. Violin plots show the distributions as Kernel density estimation. Numbers at either end of the panels are the sample size for each species. Species with more than 20 specimens were used for statistical testing (Kruskal-Wallis test for comparison of differences in medians, and post-hoc Steel-Dwass test for multiple comparison, $p < 0.05$). Species sharing the same letter were not significantly different. Color symbols represent the difference of symbiotic algae (see Table 1).

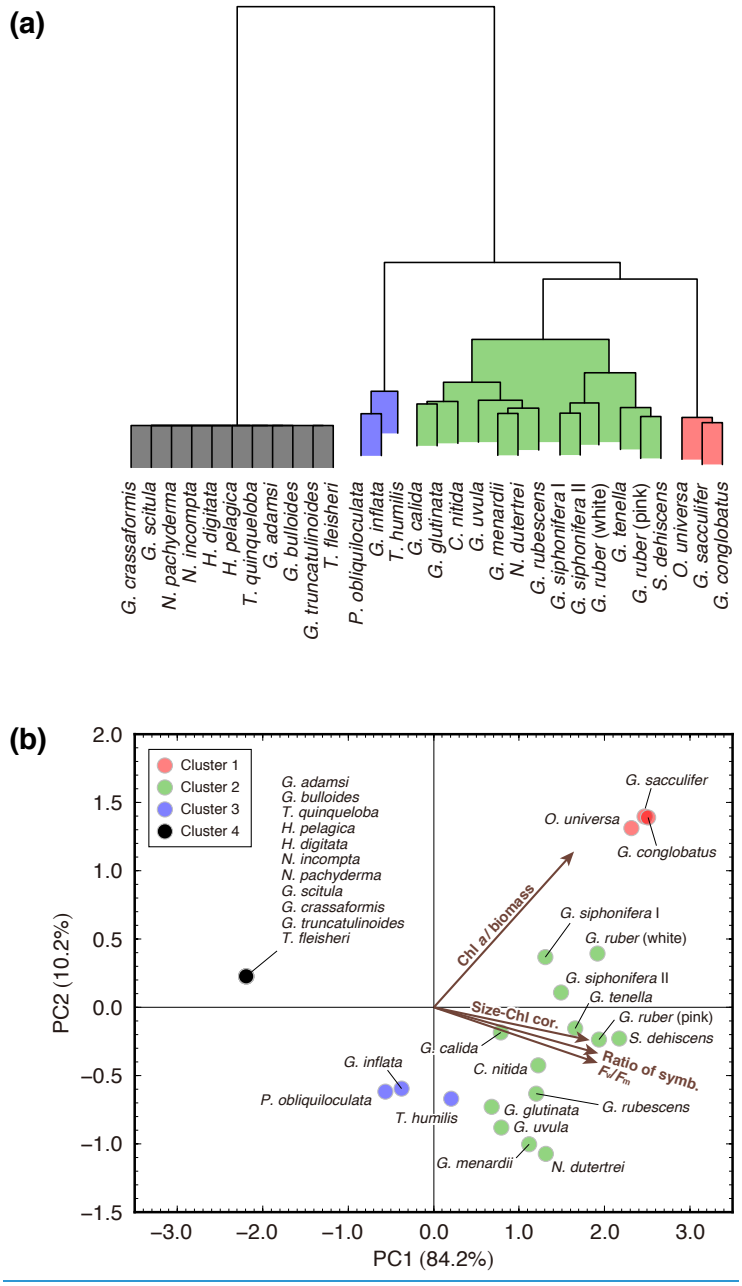


Figure 9. Results of cluster analysis and principal component analysis. (a) Cluster dendrogram obtained using Ward's method. (b) Biplot of principal component analysis. The colors of the symbols correspond to the four clusters. Vectors indicate the direction and strength of each variable to the overall distribution. The first axis explains 84.2 % of the variation, and the second axis 10.2 %. Chl *a*/biomass; Chl *a* content per protein biomass estimated from test size of individuals, Size-Chl cor.; correlation coefficient of test size-Chl *a* content relationship as an indicator of the persistence of symbionts, Ratio of symb.; ratio of symbiotic individuals, F_v/F_m; median F_v/F_m value.

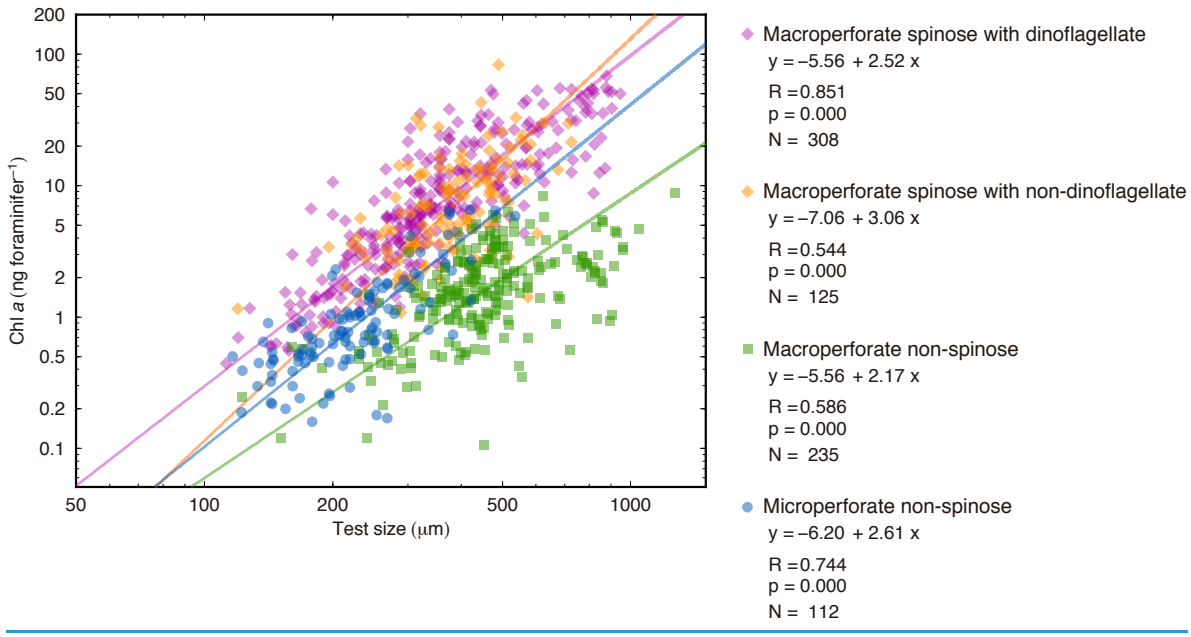


Figure 10. Relationships between test size and Chl *a* content for four groups. The 16 species with significant test size-Chl *a* correlation were used. Lines represent reduced major axis regression (y; log(Chl *a*), x; log(test size)). R, Pearson's correlation coefficient; p, p-value; N, number of specimens with functional chlorophyll (with symbionts). Note that the groups do not correspond to the clusters in Figure 9.

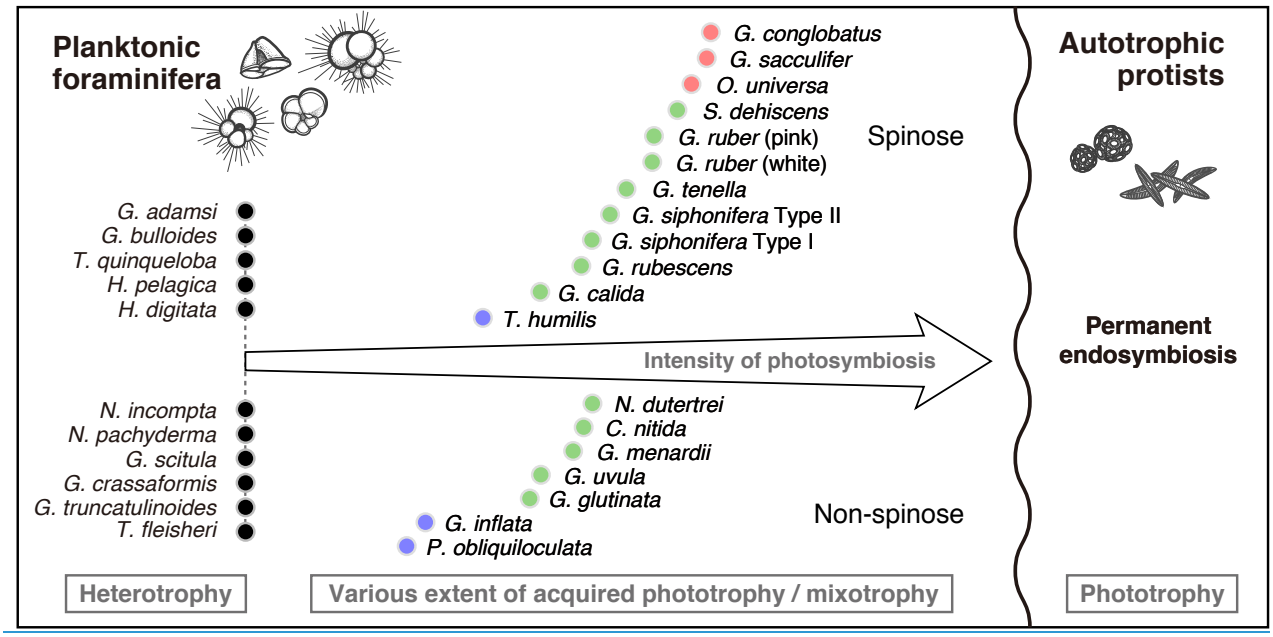


Figure 11. A conceptual diagram of the spectrum of planktonic foraminiferal photosymbiosis along the trophic gradient between permanent endosymbiosis (right) resulting in permanently integrated plastid (not found in planktonic foraminifera) and heterotrophy (left). Foraminiferal species are ordinated on the basis of their PC1 score of the principal component analysis conducted in this study (Fig. 9). The symbol colors correspond to those in Figure 9. Foraminiferal photosymbiosis has been acquired regardless of their morphological features (i.e., spinose or non-spinose, macroperforate or microperforate). Please note that in planktonic foraminifera, sexually reproduced new generation must acquire symbionts from the environment.