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 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 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 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, twelve have so far been reported to be photosymbiotic with eukaryotic algae (*Orbulina universa*, *Globigerinoides sacculifer*, *Globigerinoides conglobatus*, *Globigerinoides ruber*, *Globigerinella siphonifera*, *Turborotalita humilis*, *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 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 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 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 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 the size 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 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). 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 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 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 (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 is that many species possess non-functional chlorophyll (Fig. 5). For example, all specimens in *G. scitula* and *G. crassaformis* have a certain amount of chlorophyll inside, but it is always non-functional and likely derived from prey. The occurrence of fresh (fluorescent) chlorophyll in these species 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 when collected from shallower water depth (< 100 m). 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 possession of chlorophyll, yet they are non-functional (Table S1). This might indicate that possession of cyanobacterial symbionts may be a genotype-dependent, or regional or seasonal specific phenomenon.

Five species are newly confirmed as symbiotic based on the functionality of chlorophyll; *S. dehiscens*, *G. rubescens*, *G. tenella*, *G. calida*, and *G. uvula*. 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 (Spero and Parker, 1985, Fig. S4). The capability of cell divisions of symbionts cannot be 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 may indicate a persistent relationship of photosymbiosis through their lifetime. Moreover, *G. conglobatus*, *G. sacculifer* and *O. universa* (Cluster 1) should have the potential to support more photosynthesis due to the higher content of Chl *a* per protein biomass (Fig. 7).

The Cluster 1 and 2 include 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* 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 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.

The Cluster 3 (*P. obliquiloculata*, *G. inflata*, and *T. humilis*) 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 is common in the Cluster 1 and 2 is absent (Figs. 5 and 6). It indicates 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). We hypothesize that they can be temporarily symbiotic when foraminifera maintain certain algae for some time keeping them undigested and

keeping their photosynthetic capability to provide photosynthates. 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 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). This behavior is similar to what is known 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. A digestion experiment 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 (Fig. 6). In addition, the specimens 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. The F_v/F_m value of this species (0.51 in median) is clearly higher compared to the other two species in the Cluster 3 (0.36 for *P. obliquiloculata* and 0.33 for *G. inflata*). Besides, the possession of symbionts of this species 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 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. Therefore, we align 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 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 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 symbionts with host size, and a 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 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 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 shows 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. This kind of size scaling 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 has more Chl *a* compared to the non-spinose species (Fig. 10). For example, when the test size is ca. 300 μm, the macroperforate spinose group has almost five times more Chl *a* than the microperforate non-spinose group, and 10 times 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 may facilitate symbiosis or at least allows the harboring of a larger symbiont population. For example, efficient 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 contribute to the higher Chl *a* content in the spinose species. It may also be involved with their higher Chl *a*/biomass (Fig. 7). Moreover, clear clusters correspond to each morphogroup; macroperforate spinose, macroperforate non-spinose, and microperforate non-spinose (Fig. 10). 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. If such environmental/microenvironmental conditions surrounding the symbionts are measurable or numerically modeled, our understanding of the differences and the controlling factor of symbiont abundance would be improved.

4.3 Photophysiology and host-symbiont partnerships

When species are grouped according to symbiont type, 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 are significantly different between these groups. Chl *a*/biomass and F_v/F_m values are higher for dinoflagellate-bearing species ($p << 0.01$ and $p = 0.012$, respectively, Figs. 7 and 8a), and σ_{PSII} values 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 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 is the same tendency of photophysiology corresponding to the type of symbionts, regardless of the phylogenetic position the host.

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. 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 are compared, the Type I having haptophyte symbionts shows 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 constraining the habitat selection of the host species.

The dinoflagellate-bearing species, *G. ruber* (pink) shows high F_v/F_m with relatively small variation, and interestingly, it 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 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, 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, 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 extends our understanding of photosymbiosis in modern planktonic foraminifera. A thorough investigation of 30 foraminiferal species was performed using FRR fluorometry. Eleven species show no signal of photosynthesis, and are confirmed to be non-symbiotic. Nineteen species show 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 are regarded to show persistent symbiotic relationships. Especially, dinoflagellate-bearing *G. sacculifer*, *G. conglobatus*, and *O. universa* 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 no significant size scaling relationship in Chl *a* content. Moreover, their F_v/F_m values and the symbiont possession rates are comparatively low. Based on a PCA using the four features relating to photosymbiosis, we rank 30 species along with an integrated scale (the PC1 score scale). Finally, we propose a new framework of photosymbiosis in planktonic foraminifera as a continuous spectrum 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). Interestingly, photophysiology may be basically determined by the type of the symbiont, regardless of the phylogenetic position of the host and its test morphology. Physiological parameters, in particular σ_{PSII}, seem to correspond to the overall depth habitat of the host foraminifera. 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-
590 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 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]) / Type I	Unclassified Haptophyceae[10]	Obligate[6] / facultative[5]	0.81	Positive 0.504	0.47	515	2.56	2	Extracellular commensal

two different chrysophycophyte[4,5,8]								algae reported[8]
	Type II	*Pelagomonas calceolata* (Pelagophyte)[1]	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] / *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 significant −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] / barren[3] / chrysophyte[4]	Not reported		Facultative[6]	0.66	Not significant −0.135	0.36	518	0.07	3	
Globorotalia inflata										
Barren[3] / chrysophyte[4]	Not reported		Facultative[6]	0.69	Not significant 0.121	0.33	544	0.19	3	
Globorotalia menardii										
Prymnesiophyte[2] / barren[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
600 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

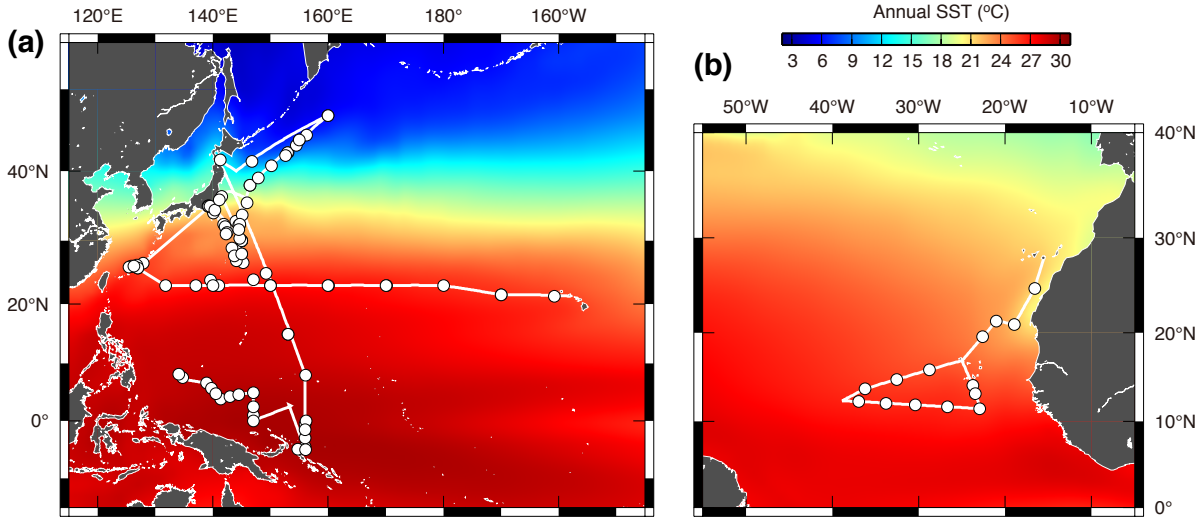


Figure 1. Maps showing the cruise tracks (lines) and the sampling points (circles). (a) Central and western Pacific area, and (b) tropical eastern Atlantic area. For detail sampling information, see Table S1. Annual sea surface temperature (SST) data was from World Ocean Atlas 2013 (Locarnini et al.,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

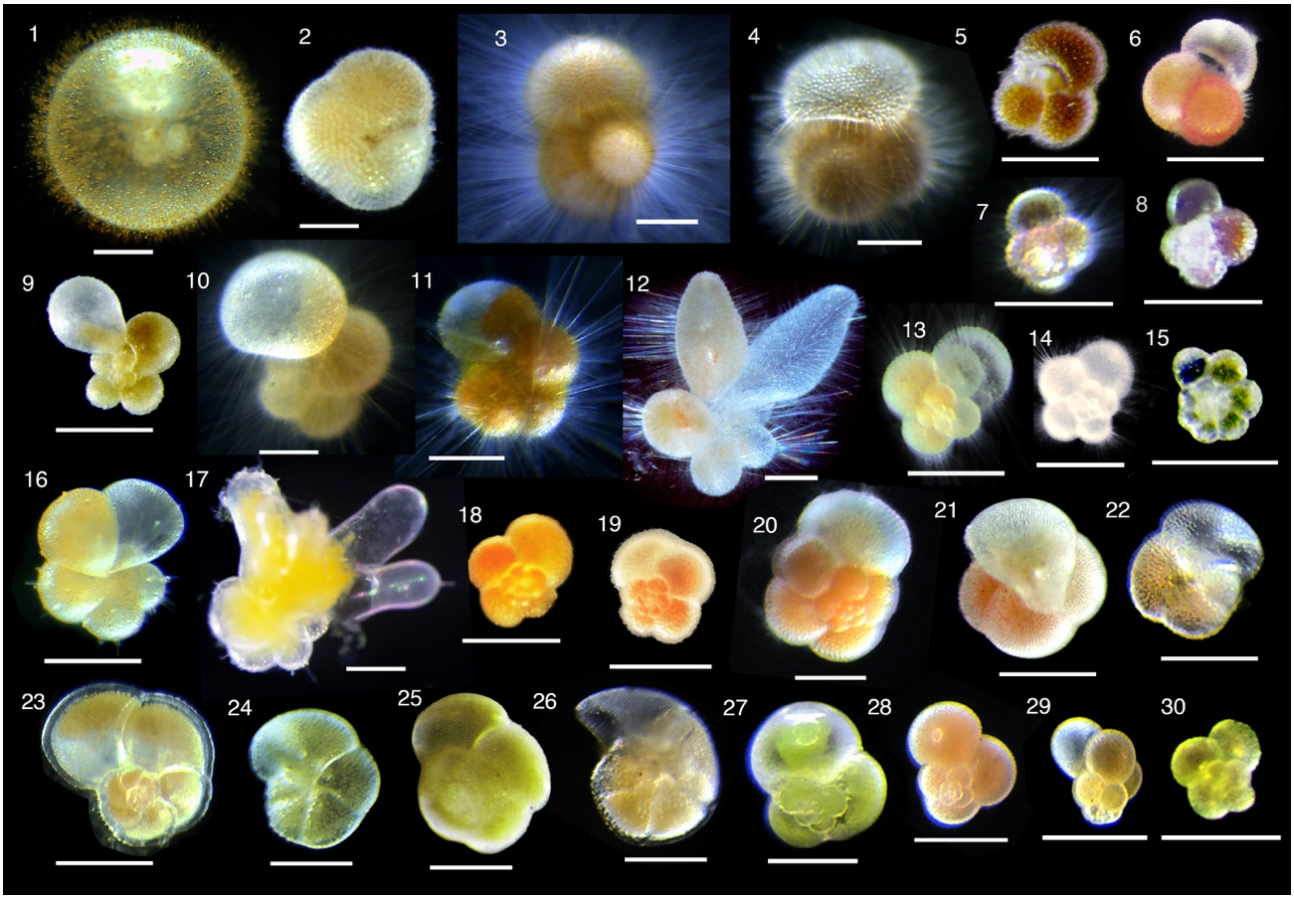
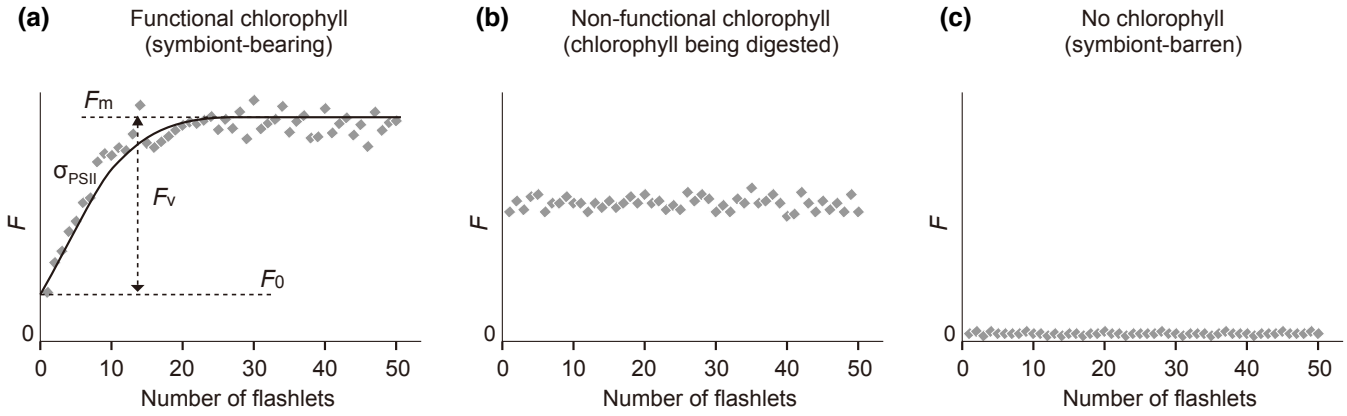


Figure 2. Photomicrographs of representative individuals for species analyzed. (1) *Orbulina universa*, (2) *Sphaeroidinella dehiscens*, (3) *Globigerinoides sacculifer*, (4) *Globigerinoides conglobatus*, (5) *Globigerinoides ruber* (white), (6) *Globigerinoides ruber* (pink), (7) *Globoturborotalita 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.

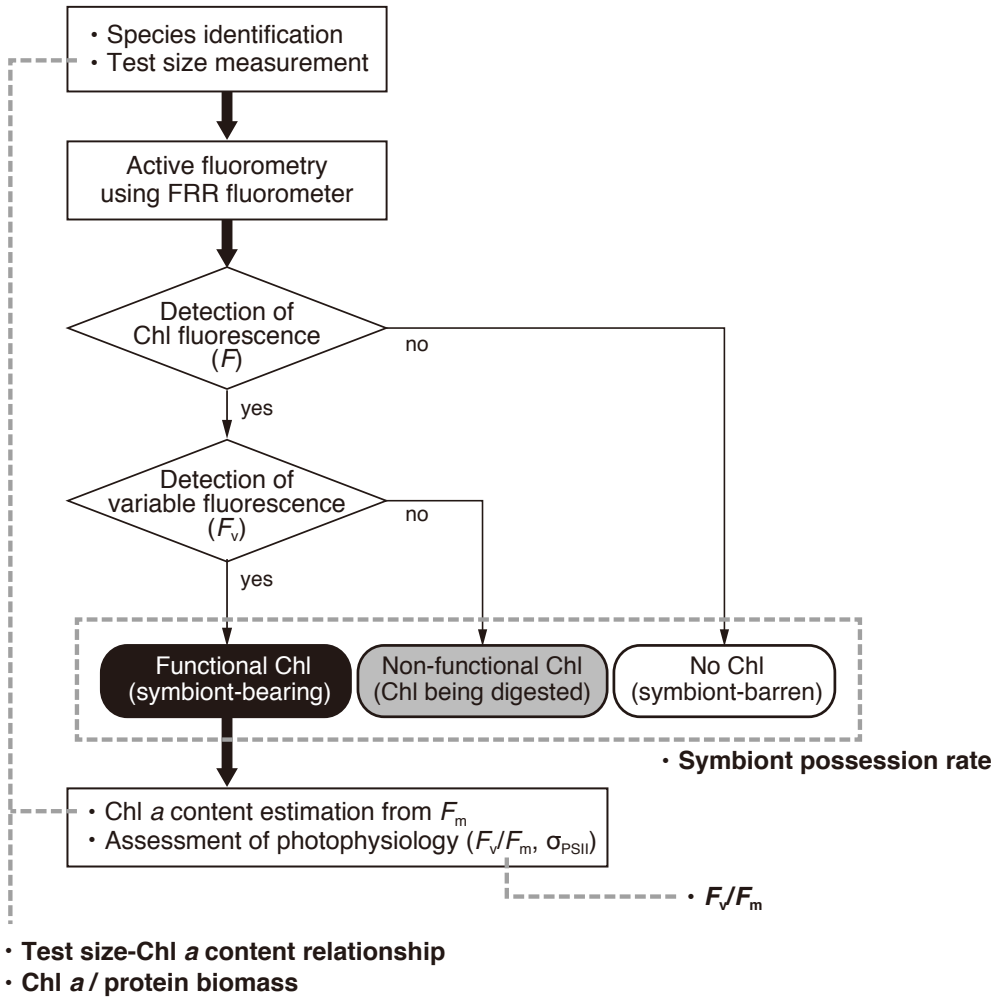
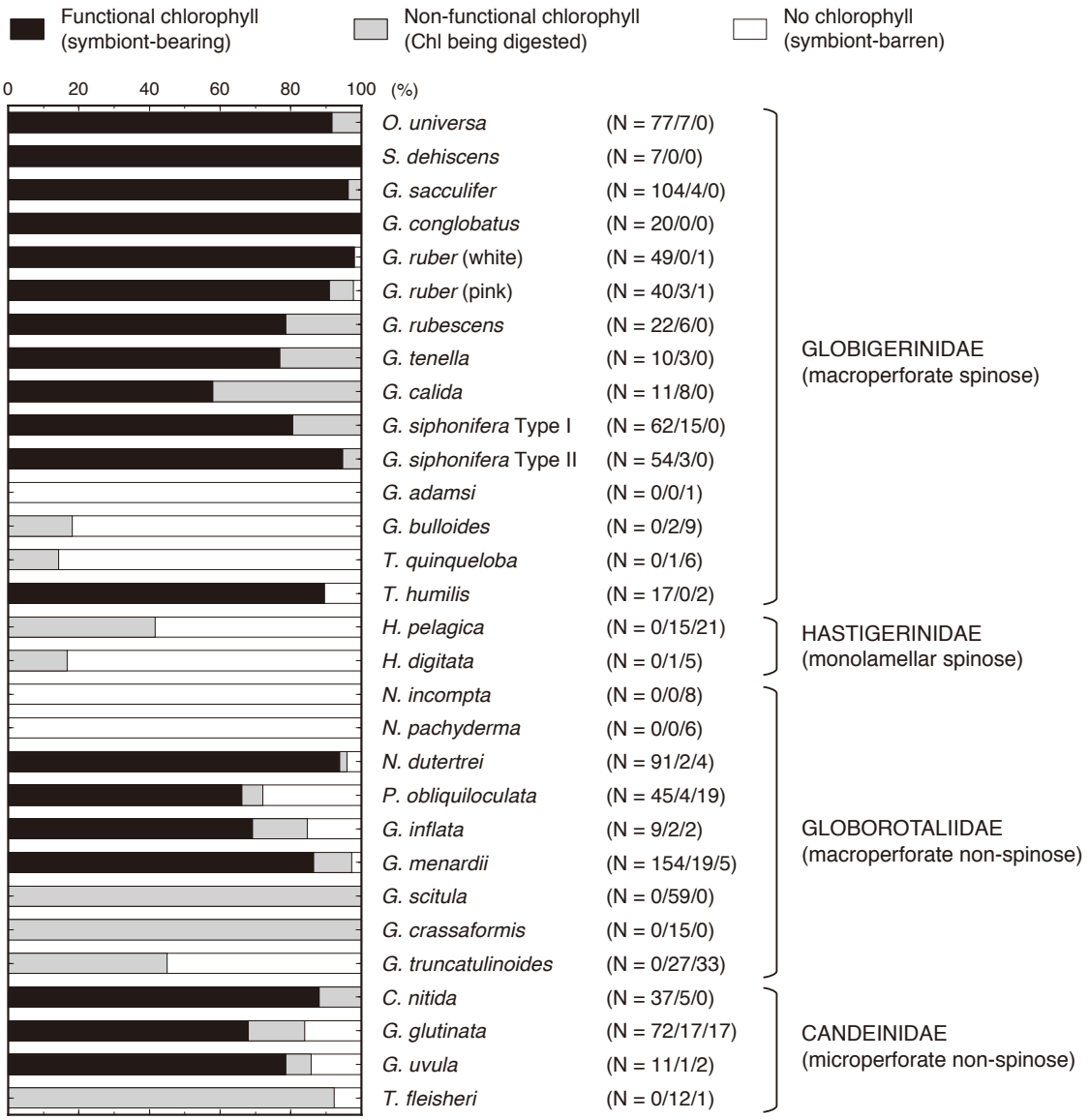


Figure 4. Workflow of this study and four indices used to characterize photosymbiosis. Firstly, individual specimens were identified to morphospecies level, measured for the test size, and analyzed with active fluorometry to check the functionality of chlorophyll. Based on the fluorescence results, intracellular 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).



670 **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).

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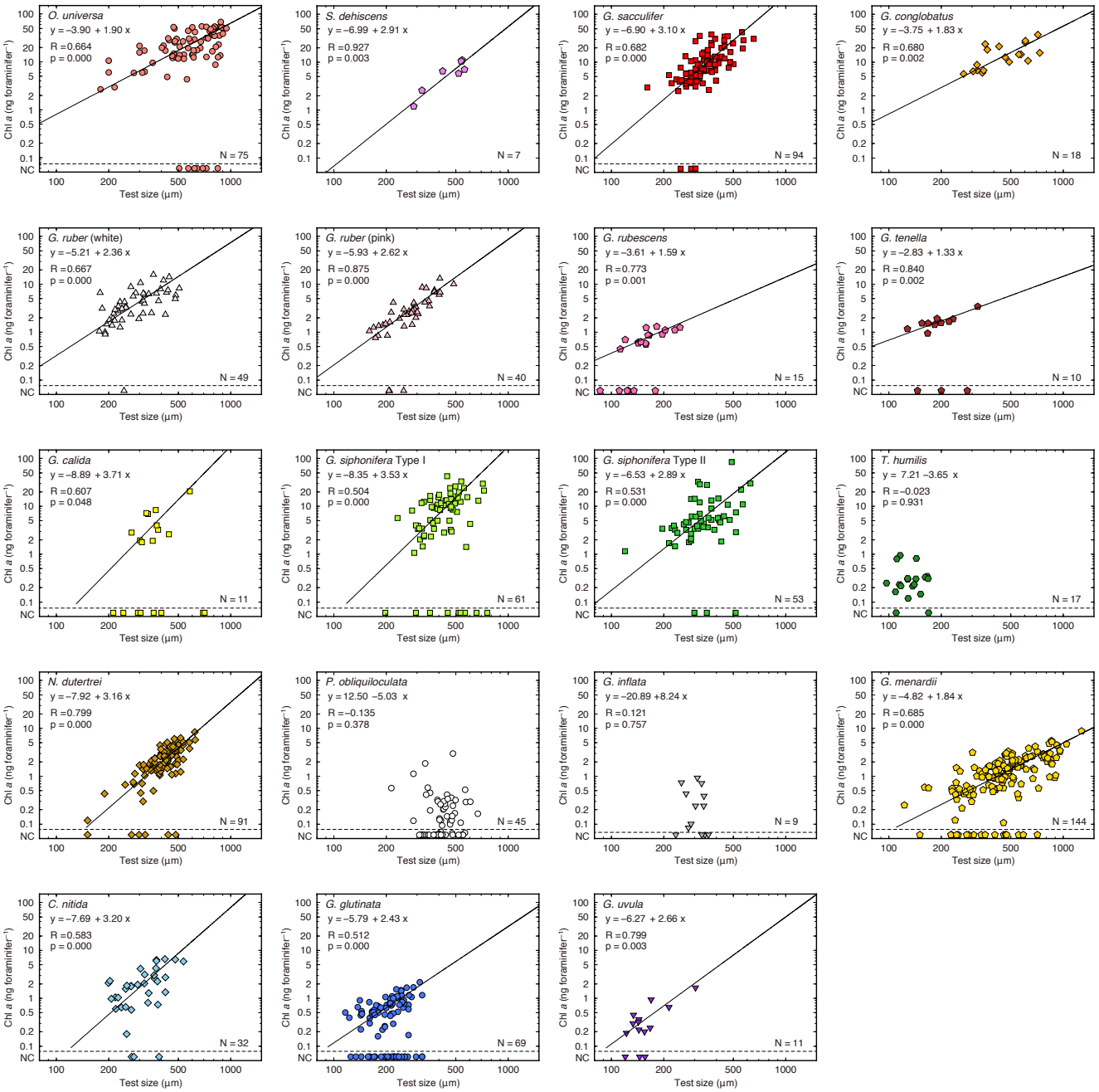

Figure 6. Relationships between test size and Chl *a* content for each species. Lines represent reduced major axis regression (y; log(Chl *a*), x; log(test size)). Specimens with no chlorophyll and non-functional chlorophyll (NC) are plotted at the bottom of each panel to show their test size information (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 S1).

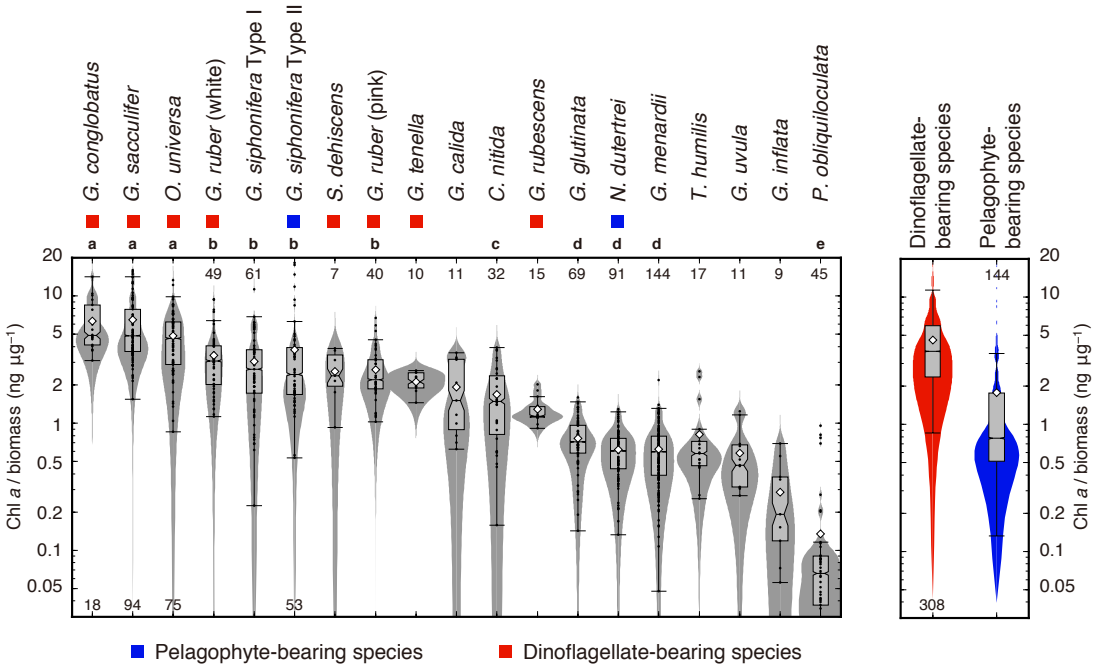

Figure 7. Ratios of Chl *a* content (ng foraminifer^{-1}) to protein biomass (µg foraminifer^{-1}) of 19 symbiont-bearing species. Dots represent individual data sampled from the upper 100 m water depth. Box plots represent first and third quartiles as hinges, and midlines as medians with notch representing 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.

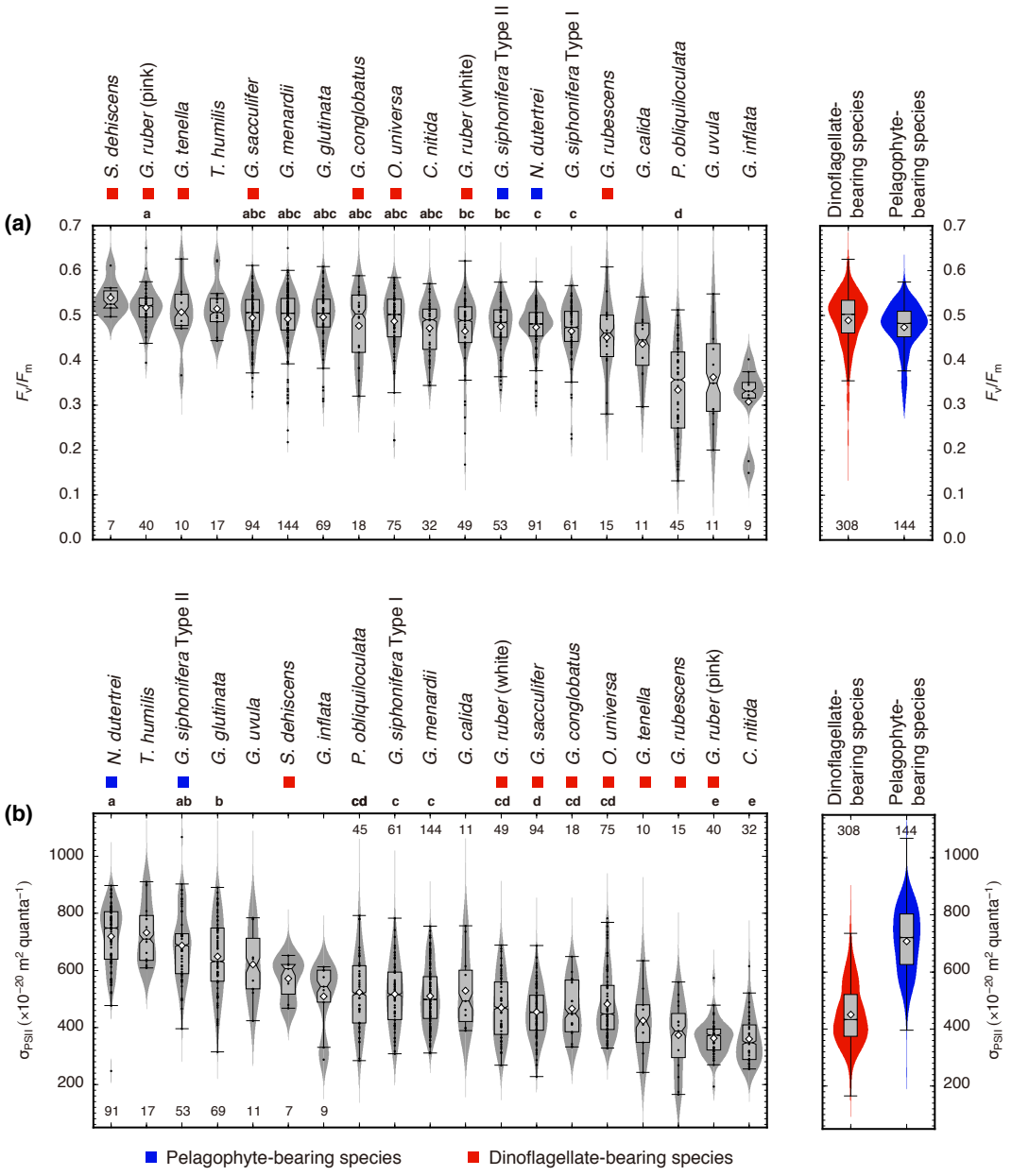

Figure 8. Photophysiological parameters of 19 symbiont-bearing species. (a) F_v/F_m, and (b) σ_{PSII}. Dots represent individual data sampled from the upper 100 m water depth. Box plots represent first and third quartiles as hinges, and midlines as medians with notch representing 95 % confidence interval 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).

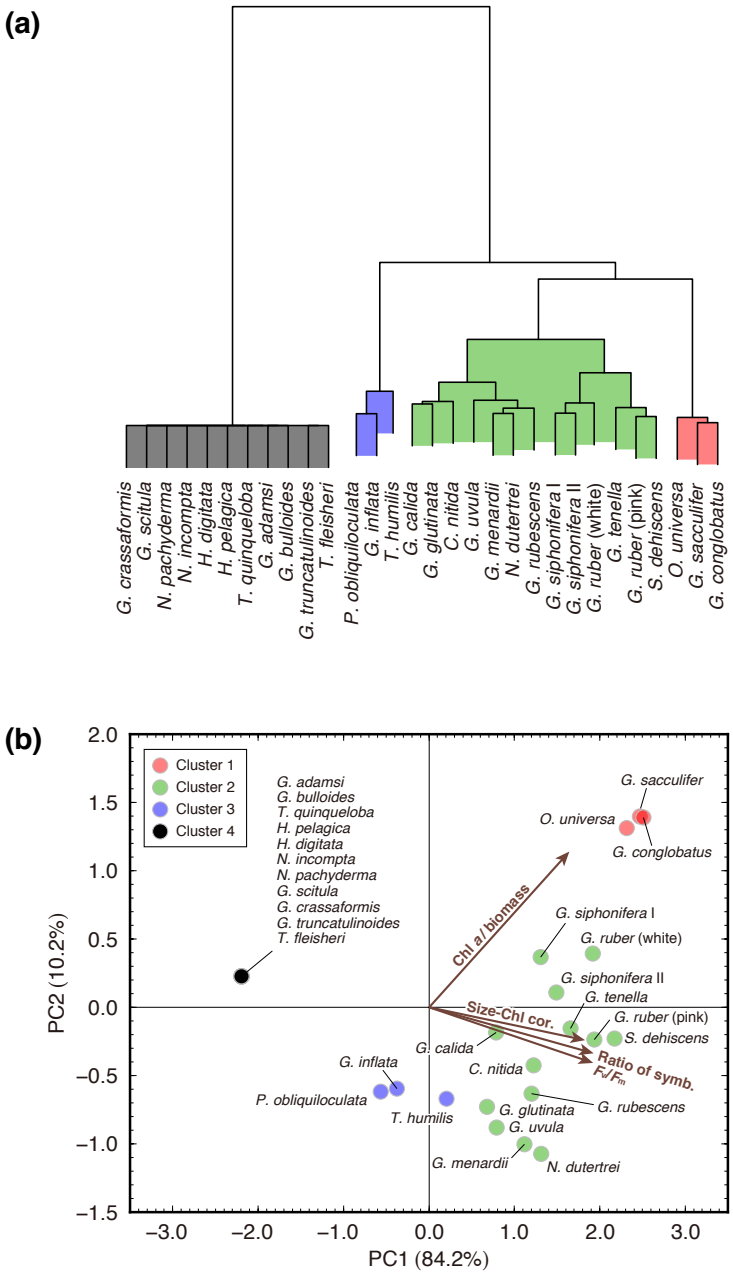

Figure 9. Results of cluster analysis and principal component analysis. (a) Cluster dendrogram obtained using Ward's method. (b) Biplot of principal component analysis. The colors of the symbols correspond to the four clusters. Vectors indicate the direction and strength of each variable to the overall 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.

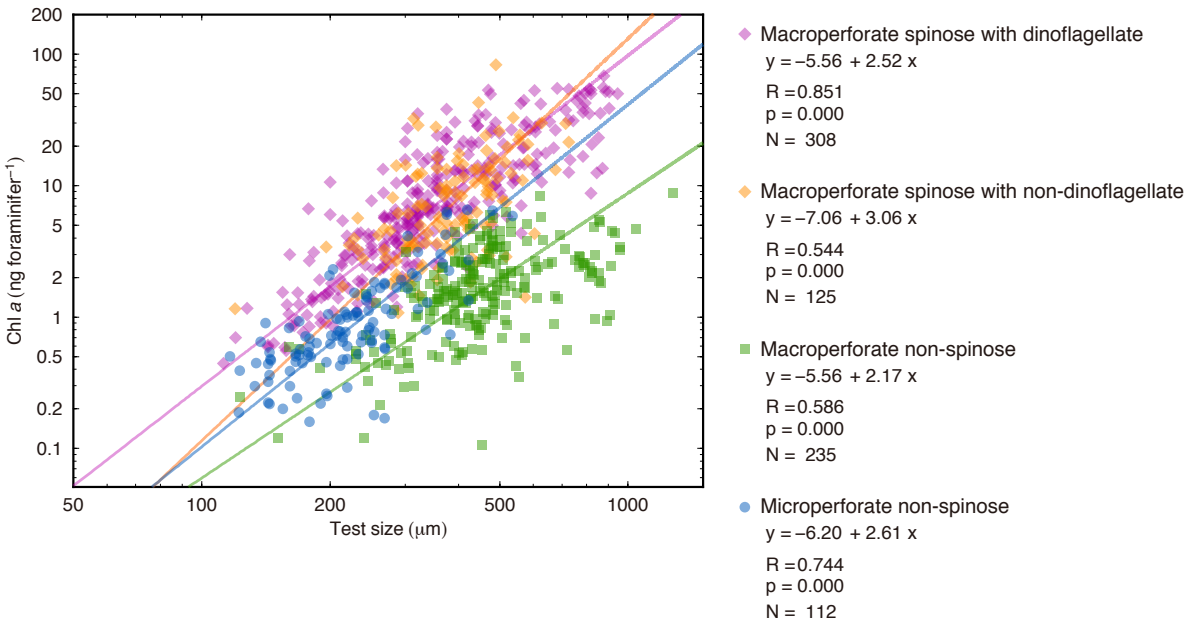

Figure 10. Relationships between test size and Chl *a* content for four groups. The 16 species with significant test size-Chl *a* correlation were used. Lines represent reduced major axis regression (y; log(Chl *a*), x; log(test size)). R, Pearson's correlation coefficient; p, p-value; N, number of specimens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

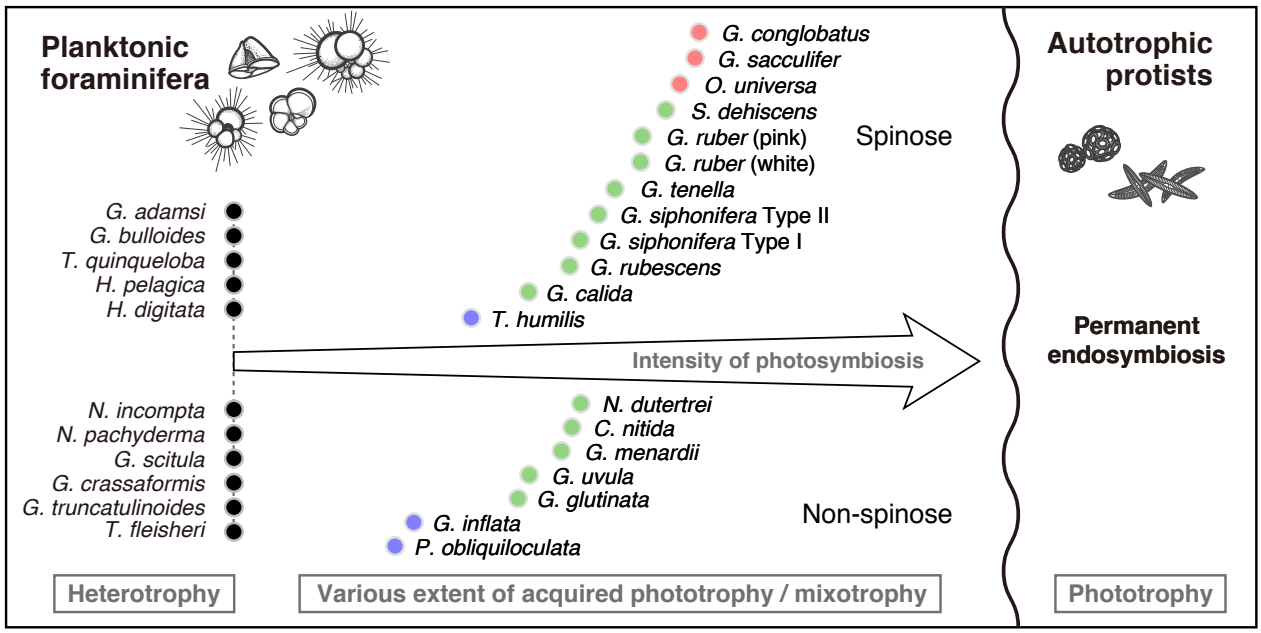

Figure 11. A conceptual diagram of the spectrum of planktonic foraminiferal photosymbiosis along the trophic gradient between permanent endosymbiosis (right) resulting in permanently integrated plastid (not found in planktonic foraminifera) and heterotrophy (left). Foraminiferal species are ordinated 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.