Approach to comprehensive analyses of molecular phylogeny, morphometrics, and geochemistry of planktonic foraminifera

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Planktonic foraminifers have been widely used for the studies of paleontology and paleoceanography. Stable oxygen and carbon isotope ratios of planktonic foraminiferal shells are the major proxies for paleo-environment, as these isotopes are affected by inhabiting water temperature and the chemical components. Most of previous studies have been conducted with the traditional species concept based on the morphological differences of their calcareous shells. However, molecular phylogenetic studies have unveiled the presences of multiple cryptic species in a single morphospecies of planktonic foraminifers. Such a high diversity of planktonic foraminifers suggests that the current paleoceanographic proxies are underestimated due to the mixed information of multiple biological species. Therefore, it is the urgent task to re-assess the ecological and geochemical characters at each biological species. For this purpose, we need to work out the implementation method, which combines the multiple analyses: DNA, morphological, and geochemical analyses, for a single individual. The DNA extraction method by using the buffer based on the guanidium isothiocyanate now enables us to preserve the calcareous shells after the extraction. By using this method, we can detect the morphological and geochemical characters on a same individual, which is identified by the molecular technique. However, the thermal and chemical reactions of this DNA extraction method to the calcareous shells are still unknown. In this study, we test whether or not the method of the molecular experiment physically and chemically damage the calcareous shells. We collected the living specimens of planktonic foraminifers and divided them into three experiment sets. In the first set, the specimens were applied to the DNA extraction with incubation process at 70 °C for 40 minutes as usual. In the second set, the time for incubation was three times longer than the first one. Through the comparison between these two patterns, the effect of the incubation time to the calcareous shells can be detected. We also prepared the specimens just collected from the sea-water but without the process for the DNA extraction, as a control. The densities of the calcareous shells were measured by the micro-focus CT scanning, and then their stable oxygen and carbon isotopes were analyzed one by one. The comparisons of physical and geochemical characters of the planktonic foraminiferal shells showed that the heat and chemical treatments concerning the DNA extraction never changed the shell component. Thus, we succeed to establish the experimental methods, which investigate the morphological and geochemical features for each biological species from single individual.

Keywords: planktonic foraminifers, Molecular phylogeny, micro-focus CT scan, Stable isotopes

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