# 浮遊性有孔虫１個体による同時３要素（遺伝子・形態・同位体）分析法の新規開発

# New analytical method of triple combination: gene, morphology, and isotopes, for a single planktonic foraminifer

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Stable oxygen and carbon isotopes of planktonic foraminiferal shells are the most important proxies for paleoceanographic studies. This is because that each (morpho)species of planktonic foraminifers is distributed in a certain area/depth in the world oceans, and their shells are formed under an influence of ambient water condition (i.e., temperature). However, this commonly accepted theory needs improvement, according to the classification of genetically incompatible species (biological species). Molecular phylogeographic studies have revealed that multiple biological species found in a single morphospecies of planktonic foraminifers are differently distributed in the oceans. This improved species concept (biological species) encourage ecological study, and is able to provide novel environmental proxies combing with other basic methods (i.e., morphology and isotope). Although the foraminiferal shells can be preserved after DNA extraction by using the guanidium isothiocyanate buffer, no study has examined the impacts of the chemicals and incubation step with 65–70˚C on the shells. In this study, we carefully tested whether the process of DNA extraction physically and chemically damage to the shells of *Globigerinoides ruber*, one of the most useful planktonic foraminifers, or not. First, we checked the changes of the shell densities in pre- and post-DNA extraction by using the micro-focus X-ray CT (MXCT) scanning. The simultaneous measurement of a sample and the standard material enable us to calculate the accurate CT number, which indicates the density of the shell. As the result, the shell densities showed no significant differences. Second, we prepared three sample sets with: (a) no chemical and incubation as control, (b) incubation in the DNA extraction buffer at 65–70˚C for 40 minutes as standard way, and (c) incubation in the DNA extraction buffer at 65–70˚C for 120 minutes. Stable oxygen and carbon isotopes were measured one by one from these three samples sets by using the microscale isotopic analytical system (MICAL3c). Although the isotope values largely varied among specimens, there were no significant differences among the three sample sets. These data of MXCT scanning and isotopic measurements clearly certified that we define morphological and geochemical features from same specimens after genetic identification. Utilizing our developed method, we compared stable oxygen and carbon isotopes between two different genetic types of *G. ruber*, which were phylogenetically distant. All examined specimens were collected at the same place in the same season. We demonstrated that the isotopic signatures between biological species. Thus, our challenge provide future studies to establish the paleoceanographic proxies in higher-resolution based on the biological species of planktonic foraminifers.

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