

有孔虫：環境と生物進化の語り部

Foraminifera: Story teller of both Earth's environments and biotic evolution

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原生生物有孔虫類は、海洋生態系の重要な一員である。生元素循環とくに炭素循環、窒素循環に深く関わっている。また、真核生物進化の初期に現れ、現在に至るまで栄えている。有孔虫はさまざまな殻を持っている事から化石記録が豊富であり、海洋環境、生物進化双方の情報を読み出す事が出来る。しかし、生物としての有孔虫の実態は十分に理解されているとは言えない。講演では、生物としての有孔虫に焦点を当て、そのユニークな生態と生物像を紹介する。

キーワード：有孔虫、地球史、生物進化

Keywords: Foraminifera, Earth's history, biotic evolution

有孔虫のチャンバー形成時のpH変化

The variable ambient pH during chamber formation process of benthic foraminifera

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Some of major studies recently showed that calcifying marine organisms respond differently to ocean acidification (OA). Prediction of OA impact is getting tangled by this variability. Moreover, this complicates modelling future ecological cycles. Carbon usage is key to understanding calcification and hence understanding impact of OA. Using fluorescent techniques to visualize pH gradients, this shows foraminifera actively pump protons to promote passive CO₂ uptake. This appearance the basis of calcification and effectively support carbon uptake independent from seawater pH. The resulting fundamentally new calcification model has major implications for understanding past changes in atmospheric CO₂ as well for predicting future CO₂.

キーワード：有孔虫、石灰化

Keywords: Foraminifera, Calcification

Living (stained) benthic foraminifera from the Mozambique Channel (eastern Africa):
Exploring deep-sea biodiversity and biogeochemistry of unicellular fossilizing meiofauna

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This study was done in the framework of the PAMELA project ("Passive Margin Exploration Laboratories") funded by TOTAL and Ifremer. Live (Rose-Bengal stained) deep-sea foraminiferal faunas have been studied at four stations between 530–3200-m depth in the Mozambique Channel (eastern Africa) to understand how complex environmental conditions (e.g., organic matter, oxygenation) control (1) their ecological structure (i.e., diversity, standing stocks, and microhabitats) and (2) their geochemical signatures (stable isotopes, and trace elements). Two upper-slope stations, located at 530- and 780-m depth off Madagascar, are bathed by well-oxygenated bottom waters. They are characterized by fine sediments enriched in highly degraded organic matter (low amino-acid bio-availability and reduced chlorophyllic freshness). Mineralization of organic compounds results in relatively moderate oxygen penetration depth (i.e., 15 and 30 mm) in sediment. Interestingly, foraminiferal simple diversity (S) is exceptionally high at both sites. The higher standing stocks are observed in the 780-m deep station, where peculiar sedimentary facies of organic matter focusing are recorded (OC >2.0% DW). Redox conditions and sedimentary organic matter control the composition and the vertical distribution (i.e. microhabitat) of benthic faunas at both upper-slope sites. *Bolivina alata*, *Bulimina marginata*, *Haplophragmoides bradyi* and *Nouria compressa* are relevant bio-indicators of enhanced burial of organic matter prevailing at the 780-m deep station (i.e., eutrophic settings), whereas *Uvigerina hispida* and *Uvigerina semiornata* are dominant at the 530-m deep station (i.e., relatively mesotrophic settings). Two other stations are located on well-ventilated terraces from the deep-sea canyons of Tsiribihina and Zambezi (>3000-m depth). They are characterized by carbonate ooze, which is depleted in degraded organic matter and, where oxygen penetration depth is relatively deep (i.e., > 80 mm). Because of food scarcity, foraminiferal simple diversity (S) and standing stocks are relatively low, and agglutinated and organic-walled taxa dominate foraminiferal faunas. *Hospitella fulva*, a foraminifera belonging to Allogromiida, occupy very deep infaunal microhabitat, what disrupts the classical scheme of microhabitat patterns in oligotrophic settings.

Keywords: Mozambique Channel, Foraminifera, Diversity, Microhabitat, Sedimentary Organic Matter, Stable Isotopes and Trace Elements

小規模データを有効活用した有孔虫石灰化プロセス動力学の再構築

Reconstruction of foraminiferal calcification process by effective use of restricted data

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有孔虫の石灰化プロセスの動力学の詳細を理解することは、古環境を再構築する手段としての有孔虫の役割をより明確に確立するために必要である。しかしながら石灰化過程における周辺カルシウムの挙動やそれと関わる殻表面成長など、重要なプロセスをリアルタイムで詳細に観測できていない。本公演では、このような石灰化過程における重要なプロセスを、カルシウムプローブなどから得られる少ないカルシウム挙動情報から予測・再構築する情報処理手法を提案し、提案手法を実際に適用した結果について報告する。

アコヤガイ貝殻稜柱層カルサイト結晶内の有機物ネットワークと結晶欠陥の関係

Relation between the organic network and the crystal defects in the calcite of the prismatic layer of *Pinctada fucata*

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Biominerals are biogenic mineralized tissues containing not only inorganic components but also a small amount of organic matrices that play an important role in formation of biominerals. Molluscan shells, which are typical biominerals consisting of calcium carbonate and organic matrices, have various kind of microstructures. The shell of a pearl oyster, *Pinctada fucata*, has two different layers. The outer layer of the shell is a prismatic layer consisting of calcite crystals. Each prism, which is surrounded by the organic framework, is composed of some single crystals of calcite. The single crystal has the small distortion of crystal orientation because this crystal has some small-angle grain boundaries inside of the crystal which cause the minim lattice distortion or defect and divide the crystal into subgrain units of a few hundred nanometers. Such minim lattice distortion and defects may increase the toughness of the shell by inhibiting the cleavage and fracture of the crystals in the prismatic layer. The small-angle grain boundaries were observed by TEM along with the localized organic matrices like networks, indicating that the organic networks may cause the small-angle grain boundaries. TEM observations showed that the organic networks are a few dozen nanometers in thickness and divide the crystal into subgrain units of a few hundred nanometers. However, what the organic networks consist of or how they cause the small angle grain boundaries has not been reported yet.

To reveal the components of the organic networks and formation mechanism of the small angle grain boundaries in the calcite crystal of the prismatic layer, we extracted the organic network from the prismatic layer and tried to identify the components. The IR spectrum of acetic acid-insoluble materials from the prisms revealed that the major component of the organic network was chitin. LC-MS/MS analysis of acetic acid-insoluble and SDS/DTT-soluble fractions showed that the chitinolytic enzymes such as chitinase and chitobiase were involved in the intracrystalline organic matrices of the prismatic layer. These results suggested that the chitinase and chitobiase regulate the formation of chin fibers that interact with the calcium carbonate to make the small-angle grain boundaries. To understand the function of the chitinolytic enzymes in the prismatic layer, calcium carbonate was precipitated in the chitin hydrogel after being treated with the commercially available enzyme. The calcite crystals precipitated in the chitin hydrogel appeared to contained larger crystal defects as the chitinolytic enzyme concentration increase and crystal defects similar to those in the prismatic layer were observed as to some extent concentration. In addition to observation, a variance of lattice spacing was calculated from peak broadening in powder X-ray diffraction and compared among the these calcite crystals. As a result, a variance lattice spacing tended to increase depending on chitinolytic enzyme concentration, implying that chitinolytic enzyme decrease the thickness of the chitin fiber to increase the interaction between chitin fibers and calcium carbonate. Such interaction is probably important to produce the small-angle grain boundaries in the calcite crystal and strengthens the toughness of shell.

キーワード：バイオミネラル、貝殻、キチナーゼ

Keywords: biomineral, shell, chitinase

有孔虫の殻における水平および垂直なテクスチャー

Lateral and Vertical Textures in Shells of Calcitic and Aragonitic Foraminifers

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Although shells of various foraminifers were reported to be composed of unique layered structures, details of the crystallographic structure have not been clarified sufficiently. Here, we investigated the shells of foraminifers, calcitic *Ammonia beccarii* and aragonitic *Hoeglundina elegans*, using scanning electron microscopy (SEM), transmission electron microscopy (TEM), and selected area electron diffraction (SAED). Despite the difference in their crystal polymorph and shapes, they exhibited similar textures in micro- and nano-scales. The shells were revealed to consist of single-crystalline micrometric domains with their c axes perpendicular to the shell surface from SAED patterns of platy samples 100 nm thick prepared using a focused ion beam (FIB) technique. Cross-sectional SEM and TEM observations showed the presence of lateral organic layers ~10-20 nm thick with ~200-400 nm intervals in the single-crystalline domains. Whereas a micrometric layered structure has previously been reported as a periodic growth pattern, we discovered finer lateral textures. On the other hand, a columnar structure ~100-400 nm wide derived from vertical textures was observed on the cross-sections after mild etching of the shells in a diluted acetic acid solution. We also found the vertical textures which originated from strains of the crystal lattice as a difference of contrast in the TEM images of the FIB-cut samples. The columns were produced through selective dissolution of the strained area. Therefore, the foraminifer shells of the two species were deduced to consist of micrometric single-crystalline domains having lateral and vertical textures with organic thin layers and strains, respectively, regardless of the difference of polymorph.

Morphospecies, cryptic species or biological species...? Searching for the evolutionary significance of diversity in foraminifera

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Many groups of foraminifera are characterized by the formation of elaborate shells. These shells provide detailed morphological features that can be used for species classification. Since the majority of works on foraminifera focuses on their fossil record and their application as proxies in micropaleontological studies, a comprehensive morphotaxonomy has been established, describing tens of thousands of extant and extinct morphospecies. On the other hand, genetic analyses of the group revealed an even higher diversity on the molecular level, hidden within the traditional morphospecies. These cryptic species are usually marked by large genetic distances, differentiated biogeographic distribution patterns and ecological adaptations, implying that cryptic species rather than morphospecies represent the level of biological species.

Biological species are defined as reproductively isolated groups of organisms and their identification forms the basis for all studies on the biodiversity, biogeography and ecology of any group of organisms. Yet so far in foraminifera the evolutionary significance of both the morphological as well as genetic diversity and their relation to reproductive isolation remain uncertain, even though this knowledge is indispensable to improve the application of foraminifera as paleoceanographic proxies. E.g. the amount of genetic variation that represents species level divergence instead of intra-population variability is not yet known. In addition, it cannot be objectively stated which morphological features represent species-level differences and which are the result of environmental adaptations. Thus, in order to achieve an objective identification of the level of biological species in foraminifera, culturing experiments are needed to observe reproductive isolation.

We apply a single cell approach to survey the extent of cryptic diversity within foraminifera morphospecies and to examine their distribution and ecological adaptations. In addition, we conduct morphometric analysis in order to establish a connection between morphological and genetic diversity. Furthermore, we carry out mating experiments in order to determine the degree of genetic divergence that corresponds to reproductive isolation and thus represents the level of biological species in foraminifera. In addition, we will try to investigate the existence of different mating types, in order to elucidate the influence of the mating system on the evolution and diversification of the group.

Keywords: foraminifera, genetics, morphology

軟体動物 *Lymnaea stagnalis* の貝殻プロテオーム解析

Proteomic analysis of shell matrix proteins in the pond snail *Lymnaea stagnalis*:
identification of potentially functional proteins

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Matrix proteins have important roles in molluscan shell formation, and their amino acid sequences have been characterized for some species. However, the mechanisms of shell formation have barely been clarified. In order to setup a platform for a systematic functional analysis of shell matrix proteins, we performed a combined transcriptome and proteomic analysis of the shell matrix proteins for the pond snail *Lymnaea stagnalis*. We found a total of 203 shell matrix proteins from the shell matrix of *L. stagnalis*. A total of 161 amino acid sequences of them showed sequence similarities to known proteins, including four paralogs of dermatopontin, which was previously reported from the shell matrix of *L. stagnalis*, when searched against public databases, while the remaining 42 showed no similarity to the proteins in the current databases. Next, in order to discriminate 'functional' shell matrix proteins from those that were accidentally entombed in the shells, we compared the levels of expression of these shell matrix proteins between the right side and the left side of the mantle, which makes the shell, underlying assumption being that genuine functional shell matrix protein genes would be more strongly expressed in the right hand side of the mantle in the dextral shells, while there would be no such differential expression pattern for the proteins which were accidentally trapped within the shells. Actin is the most abundant shell matrix protein found in the shell of *L. stagnalis*, but the expression patterns of the actin gene showed no difference between right and left of the mantle. On the other hand, the second most abundant shell matrix protein called Pif-like protein, which is an acidic shell matrix protein identified also in *Pinctada fucata* and *Crassostrea gigas*, showed that its gene is more strongly expressed in the right hand side than the left hand side of the mantle. Our results suggest that Pif-like protein is a functional shell matrix protein, while actin is a protein occluded in the shell accidentally. Finally, we searched for conserved domains of these amino acid sequences. We found various domains and classified them into six categories. Those are extracellular protein, enzyme, cation-interaction protein, polysaccharide interaction protein, proteinase inhibitor, and others. Conserved domains allow us to estimate possible functions of novel shell matrix proteins. Characterization of those novel proteins, and functional analyses of all those proteins identified in this study for this 'model organism' will help understand the mechanisms of biomineralization as well as the evolutionary processes of shell formation in molluscs.

キーワード：トランスクリプトーム解析、プロテオーム解析、生体鉱物、貝殻形成、基質タンパク質

Keywords: Transcriptome, Proteomic analysis, Biomineralization, Shell formation, Matrix protein

琉球列島完新世サンゴ礁コアにおけるマイクロビアライト（礁性微生物岩層）の発見

Reefal microbial crusts found in a Holocene reef sediment core, off Okinawa Island, the Ryukyu Archipelago

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Reefal microbialites are fine-grained, non-skeletal crusts, locally common in late Quaternary coral reef deposits. They are interpreted as microbial carbonates produced by heterotrophic bacterial communities. The carbonate crusts are typically up to 10 cm thick and form the late stage of reef developments, covering on framework skeletons. Major questions still remains unanswered concerning their uneven distributions in space and time, formation process and controlling factors. Here we first report fine-grained, non-skeletal carbonate crusts in a Holocene reef core drilled at Naha New Port area, off west of Okinawa Island, the Ryukyu Archipelago, which are similar in texture and fabric to reefal microbialites reported from deglacial reef deposits from other coral reef regions. The carbonate crusts are up to several centimeters in thickness, and mainly developed in a particular stratigraphic horizon (depth: 5~6 mbsl, age: ca. 7 ka), from which the crust thickness decreased downward and upward. Based on their external surfaces and internal sections, these crusts are classified mostly as a digitate type (thrombolite) and partly as a weakly layered type (stromatolite). Surface elemental analysis showed that crusts are composed mainly of Ca and Mg (Mg calcite). Microscopic observations clearly showed a biological succession from in situ bioeroded corals, overlain by coralline algae and encrusting foraminifers, finally to fine-grained crusts. Silt-sized grains were mainly made of peloids, with subordinate bioclastic and siliciclastic grains. SEM-EDX analysis recognized four different elemental spectrum patterns in the carbonate crusts: sulfur (S)-rich pattern (interpreted as trapped skeletal Mg calcite grains), poor S pattern (precipitated Mg calcite crystals with Mg/Ca ratio of ~0.14), Si and Al common pattern (precipitated Mg calcite crystals with trapped siliciclastic grains or clay minerals), and no Mg pattern (trapped coral aragonite grains). These petrographic and geochemical features are very similar to reefal microbialites found in deglacial reef deposits from other coral reef regions. Some differences in thickness and elemental compositions are possibly related to environmental settings (a volcanic hinterland vs. a mixed carbonate-siliciclastic hinterland).

キーワード：微生物岩、琉球列島、完新世

Keywords: microbialite, Ryukyu Archipelago, Holocene

Syn depositional formation of calcareous nodules on muddy sea floor: elucidating depositional history by C, O, S isotope characterization

Syn depositional formation of calcareous nodules on muddy sea floor: elucidating depositional history by C, O, S isotope characterization

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Calcareous nodules are commonly observed in mudstone sequences, however, paleodepositional condition including paleothermometry have never been discussed based on its chemistry without exceptional case such as methane seepage. It partly owes to their complicated depositional history. We characterized dark grey muddy carbonate nodules collected from Cretaceous strata in Hokkaido, Japan with carbon, oxygen, sulfide sulfur and sulfate sulfur isotopes, and demonstrated the syn depositional formation of some nodules. It is notable that initial formation of carbonate "precursor" for all muddy nodules studied here were syn depositional near oxic/anoxic boundary at shallower depth of muddy sea floor.

Some outcrop observations demonstrate calcareous nodules can be formed just below the sea bottom (JBSB). Structure suggesting consolidation JBSB includes burrows that eject calcareous precursor material from nodule. JBSB origin of the nodules consolidated associated with anaerobic oxidation of methane (identified by carbon isotope ($d^{13}C$) values) with sulfate reduction are also demonstrated with oxygen isotope ($d^{18}O$) and sulfur isotope ($d^{34}S$) values. Such nodules show the exactly same $d^{18}O$ values with that of benthic foraminifers. A bivalve fossil found on one of the methane seep nodules preserved aragonite of the shell and yielded close $d^{18}O$ paleotemperature with that of host nodule.

The cross-plot of the $d^{13}C$ and $d^{18}O$ data can emerge "upper limit line" of $d^{18}O$ values showing syn depositional formation. On the other hand, nodules from the Oyubari area characterized with $d^{34}S$ (sulfate) lower than -18 permil indicating JBSB formation had conflicting $d^{18}O$ values. It can be ascribed to the difference of burial depth between sediments of the Haboro and Oyubari areas. Even if precursor of the nodules initially crystallized JBSB, strong compaction during burial would have caused permeation of pore water into the incomplete nodules. Carbon dioxide or bicarbonate ions derived from decomposed organic matter would have caused recrystallization of calcite with $d^{18}O$ as low as -10 permil in the nodule.

キーワード：石灰質ノジュール、酸素同位体比、硫黄同位体比、炭素同位体比

Keywords: calcareous nodule, oxygen isotope, sulfur isotope, carbon isotope

A novel approach in utilising marine methane seep derived bivalve shells for developing local ΔR corrections in deep sea environments

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Shells and corals are commonly used as proxies for sea surface temperature reconstructions in paleoclimatology. These species utilise the dissolved inorganic carbon (DIC) in seawater making it suitable for analysing ocean circulation through the measurement of radiocarbon ^{14}C . It is also possible to estimate the age of the shell through analysing the growth increments within the shells. Yet calibration models in deeper marine environment remains complicated due to the multiple variables that must be considered which include problems such as carbon sinks, ocean current flux and dead carbon effect from seafloor vents.

This study proposes a novel approach in ^{14}C dating shells found in a deeper marine environment (600m) located near methane vents off Tokai using a Single-Stage Accelerated Mass Spectrometer (AMS), to develop a suitable calibration model with a greater goal of observing local marine environmental changes in the deep sea. This method may allow bivalves living in deeper marine environments to be used as future tools for measuring samples located in complicated environments such as deep hydrothermal vents and cold seep vents, possibly leading to assessing local shallow fault movements occurrences in the past.

Sclerochronological analysis of the shells proved difficult since growth increments of deep sea shells are not determined according to simple changes in temperature such is the case with most surface marine shells which often reveal daily, fortnightly or annual bands during seasonal temperature extremes. The radiocarbon ^{14}C age of these bivalve shell measurements ranged between three age groups of 1396 ± 36 - 1448 ± 34 , 1912 ± 31 - 1938 ± 35 and 5975 ± 34 . The ^{14}C age of shells that were alive upon collection and the dissolved inorganic carbon (DIC) in seawater show little difference (around 100 ^{14}C age) indicating that shells are not heavily affected by the dead carbon effect from cold seeps that is of biogenic or thermogenic origin, which can make the age of the shells to become considerably older than their actual age. The novel calibration model used was therefore based on the seawater DIC collected above the *Calyptogena* spp. colony site (1133 ± 31), resulting in the dead shells to be clustered around 1900 Cal AD. This age group proves to be interesting as the Ansei-Tokai earthquake (M 8.4) in 1854 is extremely close to the bivalve colony site. Based on the theory that the bivalve shells formed sometime after the venting of methane fluids, it may support the validity for applying such novel calibrations methods in complicated deep sea environments. Using geological data obtained using visual analysis and sub-seafloor structural analysis that show multiple shallow faults and chaotic sediment structure below the colony site, the *Calyptogena* spp. shells have a strong connection to the coseismic faulting activity and could show potential for radiocarbon dating to be applied on marine samples providing the necessary calibration tools are available.

Keywords: Radiocarbon dating, Active fault, Cold seep, Bivalves, Methane

大型底生有孔虫の高マグネシウム方解石の殻における水温プロキシとしての酸素同位体比および Mg/Ca比

Oxygen isotope and Mg/Ca ratio of high magnesium calcite of benthic foraminifera as a proxy for water temperature

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Although metal/Ca or oxygen isotopes ($\delta^{18}\text{O}$) of organic precipitated calcium carbonate (calcite and aragonite) of marine species have been used to estimate paleotemperature, the relationships between components of large benthic foraminiferal shells (high-magnesium calcite) and seawater temperature has yet to be established. We investigated the possibility as a proxy for seawater temperature through culture experiments of three species of reef-dwelling large benthic foraminifera in a laboratory. Three species found commonly in the western Pacific were selected including perforate species *Calcarina gaudichaudii*, and imperforate species *Amphisorus kudakajimensis*. All of them are host to algal symbionts. They grew sufficiently during culture experiments and showed a maximum in terms of shell length and weight at 27°C and 29°C while they were significantly small at 30°C. Mg/Ca ratios of three species in similar range showed high correlation to water temperature, suggesting Mg/Ca ratios as a precise proxy for paleo-temperature in shallow-reef environment. In terms of $\delta^{18}\text{O}$, *C. gaudichaudii* showed strong correlation versus temperature while $\delta^{18}\text{O}$ of *A. kudakajimensis* showed less significant correlation possibly caused by poor growth of unhealthy individuals. The trends in the temperature and oxygen isotope ratios were similar for both species, suggesting the potential of oxygen isotope ratios in the tests of reef-dwelling foraminifera as a paleo-thermometer. Species-specific calibration may be necessary for the use of $\delta^{18}\text{O}$ of reef-dwelling large benthic foraminifera as a proxy of paleo-temperature.

キーワード：高マグネシウム方解石、水温プロキシ、大型底生有孔虫

Keywords: high magnesium calcite, proxy for water temperature, large benthic foraminifera

Culture experiments to better understand biomineralization under varying geochemical conditions

--Zombie factory in Japan: the first trial on Scandinavian foraminifera--

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Ocean acidification is a consequence of ongoing global climate change, and it may have severe impacts on calcifying organisms. This process may be amplified in coastal regions where erosion, land run-off and eutrophication contribute in lowering the pH.

The aim of our study is to define how bottom-dwelling foraminifera will respond to pH changes in the Skagerrak/Baltic Sea region in addition to other environmental stressors such as temperature and salinity changes.

First results demonstrate variations in shell preservation of the living foraminiferal fauna. The more marine foraminifera have pristine shells, while the low salinity Baltic ones are more or less dissolved and only inner organic linings are still visible. However, these "zombie" foraminifera are still alive, as determined from the CellTracker Green labelling. The dissolution can be linked to the lower pH in the Baltic.

In order to investigate the zombie foraminifera further, we set up culture experiments on healthy foraminifera under controlled geochemical parameters. We discovered that lowering the pH is not sufficient to create zombie foraminifera and that abrasion between foraminifera and sediment is probably involved in the shell loss. Those results highlight the multiple factors affecting the balance of benthic ecosystems subjected to environmental stressors.

Keywords: benthic foraminifera, ocean acidification, laboratory experiment

浮遊性有孔虫に対する分子系統・形態計測・地球化学の複合分析

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

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Planktonic foraminifera 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 foraminifera. Such a high diversity of planktonic foraminifera 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 foraminifera 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.

キーワード：浮遊性有孔虫、分子系統、CTスキャン、安定同位体

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

放散虫アカンタリア目骨格中のシリカ (SiO₂) 含有についての検討

A study on chemical composition of living acantharian (Radiolaria) shell

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放散虫とは、オパール(SiO₂+nH₂O)やSrSO₄からなる鉱物質の内殻を持つ海洋性動物プランクトンのことである。現生の放散虫類はオパールの殻をもつコロダリア目、ナセラリア目、スプメラリア目、SrSO₄から成る骨格をもつアカンタリア目、そしてタクソポディア目の5目に分類されている。本研究では、放散虫の内骨格形成過程の観察のため、黒潮表層海域の放散虫を採取し室内における飼育実験を実施した。殻の成長観察については、蛍光試薬を用いオパールの付加成長部位を発光させる手法(Ogane et al., 2010)を用いた。その結果、従来SiO₂成分が含まれていないと考えられてきたアカンタリア目の生体骨格にも Siが含有される可能性が示されたのでそれについて発表する。

検討に用いた現生放散虫は、黒潮海域であり放散虫が多種生息する高知県柏島近海にて採取した。プランクトン採集は2015年の7月12日、11月30日、2016年1月11日の全3回行い、採取した放散虫を目別に数個体ずつピックアップし、専用の飼育装置に入れて飼育を行った。飼育の際、水温を常に27℃に保ち、12時間LEDライトの照射、消灯を相互に行なった。飼育装置にて24時間静置した後、HCK-123蛍光試薬を投薬し、24時間~30時間経ったものをスライドに封入した。蛍光試薬を入れてから生体内に試薬が取り込まれその部分を蛍光発光させる事で、SiO₂を含む骨格の付加成長が起きた部分と生体内でのSi元素分布を知ることができる。作成したスライドは愛媛大学理学部設置の共焦点レーザー स्क্যান顕微鏡 (Carl Zeiss LSM510) を用いて観察した。スライドには波長488nmのArレーザー光を照射し、HCK-123蛍光試薬投薬後の蛍光発光を観察した。また、Siの含有についての真偽を検討するため、さらにアカンタリア骨格のFE-SEMおよびWDS分析を行った。

採集した放散虫類の内、今回は4個体のアカンタリア目の結果について報告する。蛍光試薬投薬後

Acanthometra muelleri (個体番号: 20150712A-1)、*Amphilonche complanata* (個体番号: 20151130A-1)は24時間飼育、*Acanthostaurus conacanthus* (個体番号: 20160111A-1)、*Acanthometron pellucidum* (個体番号: 20160111A-2)は30時間飼育し、スライドに封入した。各個体を共焦点レーザー स्क্যান顕微鏡にて観察したところ、*Acanthometra muelleri*は表層の骨針と中央部、*Amphilonche complanata*は骨針の根元と中央の一部、*Acanthostaurus conacanthus*は骨針の根元の表層部、*Acanthometron pellucidum*は折れた骨針と新しく成長中の骨針の一部にSiO₂の付加が確認できた。更に、アカンタリア目*Amphilonche complanata*の異なる個体でFE-SEMおよびWDS分析を行った。アカンタリア目の主成分はSrSO₄なので、今回はSr、S、Oと、今回注目しているSiについて元素マッピング分析を行った。主成分であるSr、S、Oは骨格全体に広く含まれていたが、その中に部分的にSiの分布も確認できた。殻周辺の付着物以外でSiの反応が確認できた箇所を拡大して分析したところ、骨針の先端表層部分に集中してSiが含まれていることが確認できた。

以上の蛍光試薬を用いた飼育実験結果および生体骨格の元素マッピング分析により、従来骨格にSiO₂を含まないと考えられてきたアカンタリア目は、生きている状態においては部分的にSiO₂を含有する事が示された。またそれは、殻の成長活発な部位において顕著で、アカンタリア目の骨格形成においてSiO₂成分が重要な役割を果たす事を示唆している。

キーワード: 放散虫、アカンタリア、骨格組成

Keywords: Radiolaria, Acantharia, skeletal composition

アワビ類2種の殻・軟体部成長、貝殻の安定同位体組成への海洋酸性化影響

Effects of ocean acidification on shell and somatic growth, and stable isotopes of shell carbonate of two species of abalones

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Ocean acidification is now one of the important issue to appreciate the impact on marine calcifiers (IPCC, 2013), and potentially affects their survival, calcification, growth, physiology and development. To evaluate the effects of elevated CO₂ levels on shell and somatic growth, and stable isotope compositions of two species of abalones (*Haliotis discus discus*, *Haliotis gigantea*), we conducted culture experiments at three treatment levels of seawater pCO₂ (400, 750, and 1200 μatm), at approximately 23 °C.

The effects of seawater pH on calcification (shell width, shell weight) was non-significant in both species. On the other hand, the positive relationships between pH and wet weight of soft tissue of two species were observed. Their adjusted wet weight of soft tissue at 1200 μatm was significantly greater than that at 400 μatm. These results suggest that elevated pCO₂ affected their metabolism (e.g. higher metabolic rates to maintain homeostasis).

Stable oxygen isotope compositions of outer (calcite) and inner (aragonite) shell layers of two species showed non-significant relationships with pH. The negative correlations between carbon isotope compositions and pH of both layers appeared in both species, and the slopes of these relationships of shells were lower than that of dissolved inorganic carbon (DIC) of seawater. We estimated the equilibrium values of carbon isotope compositions at each pCO₂ treatment, and the difference between the carbon isotope compositions of shell and equilibrium values showed gradual increases in shell carbon isotope compositions with decreasing pH. Thus, the pCO₂-induced change in metabolism of abalones might appear in carbon isotope compositions of shells as the metabolic effect.

キーワード：海洋酸性化、飼育実験、安定同位体、アワビ類、代謝、バイオミネラリゼーション

Keywords: ocean acidification, culture experiment, stable isotopes, abalone, metabolism, biomineralization