

Magnetite formation through thermophilic anaerobic nitrate-depending Fe(II) oxidization bacteria of Tibetan hot spring

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The Tibetan Plateau hosts thousands of hot springs, which are inhabited by various thermophilic microorganisms. For example, anaerobic nitrate-depending Fe(II) oxidization bacteria (NDFOB) are known to contribute to iron biogeochemical cycling and mineralization. However, little is known about the NDFOB community composition and the process of nitrate-depending Fe(II) oxidization. In this study, sediments were collected from three neutral thermophilic hot springs of QZM-1, QZM-2 and QZM-16, with temperatures higher than 80 °C. NDFOB enrichment experiments were established with the collected hot spring sediments by supplementing ferrous iron [Fe(II)], nitrate and lactate, followed by construction of 16S rRNA gene clone library. Phylogenetic analysis showed that the NDFOB population was mainly affiliated with phyla of *Betaproteobacteria*, *Alphaproteobacteria* and *Firmicutes*. The kinetics of nitrate-depending Fe(II) oxidization by the three NDFOB enrichments were investigated by adding Fe(II), NO₃⁻ and lactate in growth medium. During growth, visible black precipitation was produced within one week. The consumed NO₃⁻ and produced Fe(III) was approximately in the ratio of 1:4 and NO₂⁻ was detected as the intermediate product but did not accumulate, indicating that the NO₃⁻ may be reduced to N₂O or N₂ in the Fe(II) oxidization process. The amount of the consumed Fe(II) was higher than the produced Fe(III), indicating that part of Fe(II) was involved in the mineralization. X-ray diffraction (XRD) and scanning electron microscopy (SEM)-energy dispersive spectrometry (EDS) analysis showed the resulted precipitation mainly consisted of magnetite crystals with different morphology from nanoball to mature rhombic dodecahedrons or regular hexahedrons. These results together increased our understanding on NDFOB involved in the process of nitrate-depending Fe(II) oxidization and their roles in promoting iron and nitrate cycling and mineralization in geothermal ecosystems.

Keywords: Tibetan hot spring, anaerobic Nitrate-depending Fe(II) oxidization bacteria, magnetite ,
Betaproteobacteria, Alphaproteobacteria, Firmicutes

Abundance and diversity of Sulfate-Reducing Bacteria in Tagejia and Quzhuomu geothermal zones of Tibet, western China

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Sulfate-reducing bacteria (SRB) play an important role in the sulfur cycles of hot springs. However, little is known about the distribution of SRB and their response to temperature in terrestrial hot springs of the Tibetan Plateau. In this study, sediments from eight hot springs and two geothermal channels in the Tagejia (TGJ) and Quzhuomu (QZM) geothermal zone. These sampled hot springs possessed temperature ranging from 32 °C to 82°C and circumneutral pH. The TGJ hot springs contained lower (< 100 mg/L vs. > 250mg/L) sulfate content than the QZM hot springs. The QZM hot springs were inhabited by thick green or red microbial mats, while the TGJ hot springs contained green/grey/pink microbial mat. The abundance and diversity of SRB in the collected hot springs sediment were investigated by using quantitative polymerase chain reaction (QPCR) and cloning-based phylogenetic analysis on the *dsrB* gene (beta subunit of dissimilatory sulfite reductase in SRB), respectively. The QPCR data showed *dsrB* gene abundance ranged from 1.75×10^6 to 0.96×10^8 copies per gram of sediments in the studied hot springs and *dsrB*-containing microorganisms comprised ~ 8% of the total bacteria in the studied hot springs. The relative abundance of *dsrB* gene to total bacterial 16S rRNA gen was positively correlated with temperature ($R=0.4$ and $R=0.82$ for the two geothermal channels, respectively). The phylogenetic analysis showed that SRB populations in the studied Tibetan hot springs were diverse and were mainly composed of *Desulfobacterales*, *Desulfovibrionales*, *Syntrophobacterales*, *Clostridia* and *Nitrospirales*, and unclassified *dsrB* gene. The molecular diversity analysis showed that *Syntrophobacterales*-related *dsrB* gene clones were dominant in the high-sulfate hot springs (Fe^{2+} up to 2.39 mg/L), in contrast with dominance of *Desulfobacterales*-related clones in the low-sulfate hot springs. The *dsrB* gene diversity at the OTU level (97% cutoff) was negatively correlated with increasing temperature ($R=0.98$ and $R=0.7$ for the two geothermal channels, respectively). These results suggested that temperature and sulfate concentration played an important role in affecting the *dsrB* gene distribution in the studied Tibetan hot springs.

Keywords: sulfate-reducing bacteria, *dsrB* gene, hot spring, temperature, Tibetan Plateau

Paleoceanographic proxy and skeletal records of sclerosponges from the Ryukyu Islands, Japan

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Hypercalcified sponges (hereafter, “sclerosponges”), living in dark environments of tropical to subtropical shallow oceans, precipitate calcium carbonate skeleton with growth bands. They grow slowly at an approximate rate of <1 mm/year unlike corals (ca. 1 cm/year) but can be so long-lived for several decades to hundred years like corals (e.g., Benavides and Druffel, 1986). Skeletal oxygen isotopic ratios in sclerosponges can reflect variations in sea surface temperature and seawater with the latter being potentially related to salinity reflecting the precipitation–evaporation balance at the sea surface and changes in water mass transport (e.g., Grottoli et al., 2010). In contrast to zooxanthellate corals, which occasionally show positive correlations between skeletal oxygen and carbon isotopic ratios, it is considered that there do not exist vital effects in the secretion of sclerosponge skeleton. Previous studies showed significant decrease trends in the carbon isotope records toward the present, which is probably a result of isotopically-light carbon dioxide added into the atmosphere/ocean from fossil fuel burning (e.g., Swart et al., 2010). Therefore, sclerosponges are shown to provide annually resolved time series of proxy records of ocean environments since the Industrial Revolution. However, few evaluation studies on the environmental proxy of sclerosponges are reported and temporally longer (>100 year-continuous-long) records from sclerosponges were derived only from the Atlantic Ocean.

Here we present oxygen and carbon isotope records from sclerosponges (*Astrosclera willeyana* and *Acanthochaetetes wellsii*) collected from Kume-jima, Okinawa-jima and Miyako-jima, Ryukyu Islands in the North Pacific. In order to evaluate the utility of sclerosponge as an environmental proxy, within-skeleton and intraspecific variations in stable isotopic records of 37 samples were investigated. Soft X-ray images of large-size sclerosponges showed highly developed skeletal growth bands with >100 high/low density layers. The secular changes in radiocarbon-dated time series of the sclerosponge carbon records were consistent with previously reported data from Atlantic and Pacific corals and sclerosponges. The long-term oxygen isotopic trends of the samples are characterized by slight depletions throughout their living periods, indicative of an overall trend toward warmer ocean environment around the Ryukyu Islands. Our sclerosponge-based estimates of sea surface temperature and salinity may document thermal and hydrologic variations in the Ryukyu Islands, furthering a better understanding of northwestern tropical-subtropical Pacific climate change for the last several centuries in conjunction with coral-based long proxy records.

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Keywords: sclerosponge, skeleton, proxy, oxygen isotope composition, carbon isotope composition, paleocean

Coral barium/calcium record of sediment load in Sumiyo Bay, Amami Oshima

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Massive coral skeletons (*e.g. Porites sp.*) which distributed through sub-tropical and tropical regions provide the archives of various proxies for environmental and climatic reconstructions with high-temporal resolution owing to their rapid extension rate. Barium (Ba) is incorporated into the coral skeletons in close proportion to seawater concentration, therefore coral skeletal Ba/Ca ratio reflects the variation of Ba concentration, which is associated with oceanic upwelling, river discharge and terrestrial input. Additionally, high sediment resuspension and water turbidity caused by coastal runoff will lead the disturbance of coral growth and reef ecosystem.

In Amami region, south-west part of Japan, the localized heavy rainfall has occurred frequently in autumn season (Sep to Nov) during recent years. Remarkable heavy rainfall events have occurred on 18th-20th Oct 2010, 20th Sep and 2nd Nov 2011 in Amami Oshima, which have been reported devastating damages on the coastal ecosystem by the river flood. Understanding the influences of the environmental stress such as heavy rainfall and sediment input on reef corals (and their responses) is important for predicting inhabit environmental changes in the future. In this study, we reconstruct the heavy rainfall event in Amami Oshima using coral skeletal growth parameters analysis (annual extension rate (mm/yr); annual average density (g/cm³); annual calcification rate (g*cm⁻²/yr)) and skeletal geochemical records (stable isotope ratios and trace elements) with weekly resolution. Our coral record of seawater Ba concentration during last 50 years also provide the coral responses to habitat environmental changes by sediment load after heavy rainfall events.

We collected a living *Porites* coral core at 5.1 m depth in Sumiyo Bay (nearby Sumiyo river mouth; eastern area of Amami Oshima) on Oct 2014. Annual bands of the coral skeletons were observed by X-radiographs. We analyzed stable oxygen and carbon isotope ratios ($\delta^{18}\text{O}$ and $\delta^{13}\text{C}$) using a mass spectrometer coupled a carbonate reaction device, and trace element ratios (Sr/Ca, Mg/Ca and Ba/Ca) using inductively coupled plasma atomic emission spectroscopy (ICP-AES) with ultrasonic nebulizer under pre-treatment with ultra-pure water. $\delta^{18}\text{O}_{\text{seawater}}$ was calculated skeletal Sr/Ca and skeletal $\delta^{18}\text{O}$. Seawater Ba concentration was calculated from skeletal Ba/Ca.

Low density bands were observed in winter season and/or rainy season (including Typhoon season). This result suggests that low density bands of our specimen would be produced by environmental stress such as low sea surface temperature, low salinity and sediment input by rain fall.

During 2010 heavy rainfall event, estimated SST from skeletal Sr/Ca and Mg/Ca were lower than those for averaged during last 50 years. $\delta^{18}\text{O}_{\text{seawater}}$ calculated from $\delta^{18}\text{O}$ and Sr/Ca ratio in coral skeletons were corresponding with precipitation records, with negative peak after the flood events. On the other hand, skeletal $\delta^{13}\text{C}$ did not show large variation during heavy rainfall events. Skeletal Ba/Ca tracked the changes in sediment load by heavy rain in 2010 and 2011 with increasing trend during several months. The estimated seawater Ba concentration in Sumiyo Bay was increased two- or three-fold than averaged background during last 50 years. In addition, skeletal extension rate decreased dramatically in the aftermath of 2010 heavy rainfall, suggested that the skeletal growth was influenced by river runoff (lower salinity and sediment input).

The baseline of estimated seawater Ba concentration was characterized by increasing trend toward the present day. In addition, there were significant negative correlations between the annual baseline of

estimated seawater Ba concentration and annual extension rate and calcification rate. It may be due to the influences of increasing sediment input from Sumiyo river by land development and the frequent localized heavy rainfall nearby Sumiyo Bay.

Benthic foraminiferal response to sedimentary disturbance in the Capbreton canyon (Bay of Biscay, NE Atlantic)

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Living (Rose Bengal stained) and dead benthic foraminifera were investigated at 6 deep-sea sites sampled in the Capbreton canyon area (Bay of Biscay, France). Three sites were located along the canyon axis at 301 m, 983 m and 1478 m and 3 stations were positioned on adjacent terraces at 251 m, 894 m and 1454 m. Sedimentary features indicate that frequent sedimentary disturbances of different magnitudes occur along the Capbreton canyon axis and adjacent terraces. Such environmental conditions cause the presence of very particular benthic environments. Along the 6 studied sites, different foraminiferal responses to various sedimentary patterns are observed revealing the complexity of this canyon environment. Some sites (Gitan 3 (canyon axis), Gitan 5 (canyon axis) and Gitan 6 (terrace)) are characterized by moderate to low standing stocks and low diversity and are mainly dominated by pioneer taxa such as *Fursenkoina brady*, *Reophax dentaliniformis* and *Technitella melo* suggesting a recent response to turbidite deposits recorded at these sites. Others sites (Gitan 1 and Gitan 2) show extremely high standing stocks and are mainly dominated by the opportunistic *Bolivina subaenariensis* and *Bulimina marginata*. Such faunal characteristics belonging to a more advanced stage of ecosystem colonization indicates strongly food-enriched sediment but extremely unstable conditions. Moderate standing stocks and diverse assemblage composed of species such as *Uvigerina mediterranea* and *U. peregrina* has only been observed at the terrace site Gitan 4. More stable sedimentary conditions recorded at this terrace seem to be suitable to the development of a dense and diverse foraminiferal community. Numerous neretic allochthonous species were observed in the dead foraminiferal fauna. These allochthonous species mainly originate from shelf areas (< 60 m).

Keywords: Benthic foraminifera, Capbreton canyon, Habitat disturbance, Turbidite, Submarine canyon

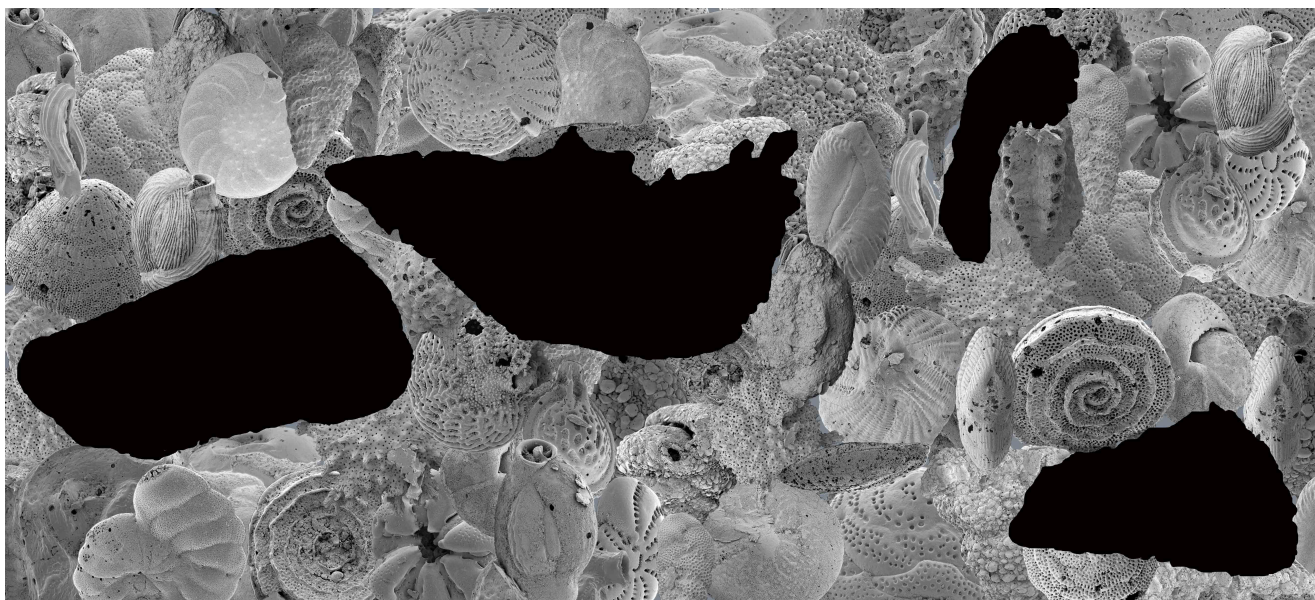
Benthic foraminiferal baselines for the southern Great Barrier Reef: a foundation for future ecological research

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Effective environmental management and monitoring has become increasingly important as anthropogenic processes increasingly impact natural ecosystems. One locality that is under direct threat due to human activities is the Australian Great Barrier Reef (GBR). Marine benthic foraminifera represent an abundant and readily applicable tool that can be used in environmental studies to investigate an array of ecological parameters and assist in understanding ecosystem dynamics and influence management protocols. Initially, baseline knowledge of the taxonomic composition within the region must be established to facilitate comparative studies and monitor change to maximise understanding and management efficacy. A detailed taxonomic assessment is provided of 133 species of benthic foraminifera in 76 genera from Heron Island, One Tree Island, Wistari and Sykes Reefs, which form the core of the Capricorn Group (CG) at the southern end of the GBR. Of these 133 species, 46% belong to the order Miliolida, 34% to Rotaliida, 7% to Textulariida, 5% to Lagenida, 3% to Lituolida, 3% to Spirillinida, 1% to Loftusiida and 1% to Robertinida. Samples were collected from a variety of shallow shelf reef environments including reef flat, lagoonal and channel environments. This work establishes a platform from which future investigations can stem.

Keywords: Foraminifera, Benthic, Carbonate, Reef



Temporal variability of the environmental conditions in Hiuchi-Nada Bay, Seto Inland Sea, Japan for the past 100 years as recorded by diatoms, ostracodes, and heavy metals.

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Diatoms are important primary producers in coastal ecosystems, however, the complex interactions with both abiotic and biotic variables are not enough clarified. Stratigraphic analysis of sediments to reconstruct the past environments is an effective method to further understand how different environmental conditions have varied in the past and its effect on primary productivity. This study demonstrates the temporal variations of primary producers in Hiuchi-Nada Bay in Seto Inland Sea approximately over the past 100 years by using fossil diatom assemblages. The relationship between diatom assemblages and other ecosystems in anthropogenic coastal area is also discussed by comparing with the data from other site in Seto Inland Sea.

Diatom analysis demonstrated that almost all of recorded taxa were marine or marine-brackish taxa which could be regarded as autochthonous. Two biozones, termed diatom zones DA (80-34 cm depth) and DB (34-1 cm depth), were identified on the basis of cluster analysis for diatom assemblages. The transition of these two zone represents the period of 1960' s high economic growth after WW2 in Japan.

Neodelphineis pelagica, small *Thalassiosira* spp., resting spores of *Chaetoceros* spp., which were reported as eutrophic taxa in Osaka Bay (Hirose et al., 2015, INQUA), markedly increased in concentration and in relative abundance in this phase.

Among autochthonous taxa, the increase of concentrations in planktonic taxa from 1960' s is the common trend in the Seto Inland Sea, but their absolute concentrations are different. That is, the average valve contents in Hi-2C is 1/2 of Suo-Nada Bay, 1/3 of Harima-Nada Bay, 1/5 of Osaka-Bay (Sako, unpublished data; Hirose et al, 2016, JpGU). This result demonstrates the difference of the primary productivity due to eutrophic levels between each area. Meanwhile, although concentration of autochthonous benthic taxa decreased in Osaka Bay after 1960' , increased in Hiuchi-Nada Bay. These results indicate that the productivity of phytoplankton increased under the influence of human-induced eutrophication, but water transparency and bottom environment was relatively sustained in condition to a sufficient degree also for benthic taxa in Hiuchi-Nada Bay than other area which are neighboring the metropolises. We will further discuss ostracode assemblages and chemical components (TOC, TN, TS and heavy metals) from the area.

Keywords: diatom, coastal ecosystem, anthropogenic environmental change, eutrophication, Hiuchi-Nada Bay, Japan, past 100 years

Macrofauna Activity in Quaternary Bottom Water Environments off Western Australia: Fecal Pellets Evidence

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Fossil macrofauna are difficult to assess quantitatively compared with micro- and meiofaunas because of their large body size and rare occurrence, particularly in fine-grained sediments. To estimate the quantitative activity of the macrobenthos, the abundance of the fecal pellet in the Quaternary sediments can be considered. In this investigation, we used a sediment core (Site U1461) recovered off Western Australia in the eastern Indian Ocean during IODP Exp.356.

We focused on horizons that clearly exhibited sedimentary cycles. The upper part of the core mainly comprised of alternating beds of dark-colored packstone/wackestone and light-colored wackestone/mudstone. Well-preserved molluscan fossils and peloids occurred in the light-colored wackestone/mudstone in the upper part of the sedimentary sequence.

The morphological character and size of the peloids within the studied sediments are similar to modern fecal pellets of shallow water polychaetes. The abundance of fossil fecal pellets shows fluctuating trends similar to those of macrofossils (e.g., bivalve, gastropod, scaphopod, and echinoderm) and it is likely that the fecal pellet abundance is an indicator of paleo-macrobenthos activity. This activity was compared to ostracode abundance and temporal changes in fossil fecal pellet abundance and an inverse correlation was found.

In the other intervals, the peloids are replaced by superficial ooids and occurred in conjunction with larger benthic foraminifera, indicating deposition within the photic zone. Thus, these intervals may indicate a shallow water environment during deposition.

In this research, we reveal that the abundance of fossil fecal pellets shows macrobenthic activity, reflecting the evolutions of bottom water environments during the Quaternary.

Keywords: Macrobenthos, Fecal pellet, Ooid, Quaternary

Temporal size change of the middle Miocene planktonic foraminiferal species *Paragloborotalia siakensis* (LeRoy)

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The extinction of Miocene planktonic foraminiferal species *Paragloborotalia siakensis* (LeRoy) defines the uppermost boundary of planktonic foraminiferal Zone N.14 (Blow, 1969). Many workers have examined the taxonomy of the morphospecies, however it has still been controversy. Bolli and Saunders (1982) proposed that *Globorotalia siakensis* (= *P. siakensis* of this study) should be a junior synonym for *Globorotalia mayeri* Cushman. The geologic time scale of Berggren et al. (1995) is also based on this taxonomic criterion, and the extinction of “*Neogloboquadrina mayeri*” was used for his zonal boundary. Zachariasse and Sudijono (2012) conducted morphological analyses using a scanning electron microscope (SEM) for *P. siakensis* collected near the type locality. They also examined holotypes of both species and concluded that *P. siakensis* could be distinguished from *G. mayeri* by its suture and surface structure. Okada and Hayashi (2013) carried out taxonomical examinations for *P. siakensis* obtained from IODP Site U1338 in the eastern equatorial Pacific, which is located in the central part of the distribution area. Through their SEM analysis, most specimens could be correlated with the holotype of *P. siakensis* with few exceptions. In addition, their diagrams of morphological analyses indicate that holotypes of *P. siakensis* and *G. mayeri* should be contained within the same morphological space of the specimens from Site U1338. They also reported the size distribution pattern of *P. siakensis* from approximately 15 to 11 Ma and pointed out that the size distribution pattern would have a good potential for global correlation and needs more study. The purpose of this study is to refine the temporal size distribution of *P. siakensis* and to establish global correlation based on the size distribution pattern of the species. We conducted size measuring of this species at Site U1338 from approximately 16 to 11 Ma. At the same time, we performed X-ray microcomputed tomography (XMCT) analyses and thin section observations at selected horizons. Seventy-five samples at an interval of approximately 0.05 Ma from the site were used for this study. These samples had been already examined for planktonic foraminiferal assemblage (Hayashi et al. 2012). Then, total 6895 specimens of *P. siakensis* were measured in maximum diameter. The size distribution was discussed with respect to previous geochemical and paleontological data. In the next step, we are examining the size distribution at the Site U1337 near the Site U1338. And some three-dimensional images of specimens collected from characteristic horizons were acquired by XMCT. The CT images enable us to visualize the inner structure such as the form of chambers, ontogenetic growth pattern, and density distribution in each test. Based on CT images, we can estimate the three-dimensional morphologic comparison, degree of maturation and obesity of each test. For mineralogical approaches, thin sections of foraminiferal tests were observed by a polarization microscope. In a result, we detected twice giantisms and twice dwarfings in the size distribution pattern. The cycle of size change was approximately 2 Ma. One of the dwarfing events could be correlated with Mi3 event (Miller et al., 1991). Therefore, this dwarfing event could be caused by cooling of sea surface water. According to 3D profiles of foraminiferal chambers, tests of relatively small specimens were composed of both high and low CT value layers. In contrast, the larger tests were generally composed of a pair of low CT value layers. Considering previous CT studies, we assume that the difference in the inner layers might reflect the growth the rate for each individual: pair of low CT value layers might mean relatively rapid growth rate.

Keywords: planktonic foraminifera, temporal size distribution, global correlation , Miocene, IODP

Marine ostracode valve weight (preliminary report)

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Ostracoda is a crustacean taxon to have two calcified valves. They grow from juveniles to adults with 6-8 moltings. Ostracodes form low-magnesium calcite valves, using ambient water carbonate. Their valves are calcified rapidly for several hours to days during a molting. For understanding ostracode calcification, the process of calcification has been observed and valve chemistry has been analyzed. Calcified mass has been seldom measured. In this study, I weighed marine ostracode valves to discuss variety of the valve mass.

From Paleocene to Holocene marine sediments, 82 ostracode valves were collected. I identified 20 taxa, that are composed of 2 orders (Platycoida and Podocopida), 7 families, and 14 genera. For cleaning valve specimens, particles were removed from a valve inside using a wet fine brush. The fossil specimens were sonicated with 2% sodium hexametaphosphate solution for two seconds, whereas the subfossils were penetrated in 2% sodium hypochlorite solution for three hours. After the cleaning, they were weighted with a microbalance. Valve length, height, and width of all the specimens were measured, using a digital microscope. Under the assumption of ellipsoid-shaped valves, valve volume (μm^3) and density ($\mu\text{g } \mu\text{m}^{-3}$) were calculated.

The valve weight ranges from 3.9 to 100.6 μg . The valve length and volume show ranges from 384 to 1304 μm and from 2.07×10^7 to $8.96 \times 10^8 \mu\text{m}^3$, respectively. The valve density indicates a range between 3.48×10^{-7} and $5.72 \times 10^{-8} \mu\text{g } \mu\text{m}^{-3}$.

Comparing with the valve density, platycopids have more densely valves than podocopids. In the mean density, platycopids and podocopids show 2.35×10^{-7} and $1.45 \times 10^{-7} \mu\text{g } \mu\text{m}^{-3}$, respectively. The permutation test with 1000 iterations indicates significant difference in the mean density at 0.01 level ($p = 9.9 \times 10^{-4}$; n of platycopids = 24; n of podocopids = 58).

Applying power regression into the plot of valve volume vs valve weight, the exponential scaling indicates 1.0244 for platycopids and 0.9397 for podocopids. The difference in exponential scaling suggests that calcified mass through ontogeny are different between the two taxa. Platycopids form more densely calcified-valves than podocopids. The result indicates that the calcified mass and density are different in taxa.

Keywords: Body size, Ostracoda, Valve weight

Magical Proton Usage on Calcification of Ammonia "beccarii" -Acidify Environment to Realize Favorable pH at Calcification Site-

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The physiological processes responsible for calcification in foraminifera are poorly understood despite their contribution to oceanic CaCO₃ production. Here we show that calcification is driven by rapid transformation of bicarbonate to carbonate inside the cytoplasm, achieved by active outward proton pumping. We furthermore show that a V-type H⁺ ATPase is responsible for the proton flux and thereby, calcification. External transformation of bicarbonate into CO₂ due to the proton pumping implies that biomineralization does not rely on availability of carbonate ions, but total dissolved CO₂ in perforate foraminifera may not reduce calcification, thereby potentially maintaining the current global marine carbonate production.

Keywords: Biomineralization, Calcium carbonate, pH