

Phylogenetic evidence for the origin and diversification of whale-fall fauna

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The first discovery of a chemosynthetic faunal assemblage on a whale-fall in 1987 was significant not just in advancing our understanding of deep-sea ecology and succession, but in also generating new hypotheses for the evolutionary origin of the deep-sea fauna. Could these remarkable habitats also provide avenues for dispersal and speciation over evolutionary time? The early phylogenetic studies provided some support for this controversial hypothesis. But more recent and comprehensive research has shown that the picture is more complicated. Here I review evidence for what I see as three possible hypotheses: the ‘evolutionary stepping-stone hypothesis’ where whale-falls have actually created a pathway for radiation into other chemosynthetic habitats, the ‘ecological stepping-stone hypothesis’ in which whale-falls play a role only in aiding dispersal in ecological time, and the ‘hotspots of adaptive radiation hypothesis’ in which whale-falls act as a biodiversity pump for fauna that originally evolved at hydrothermal vents or seeps.

The decay process of and the biological community on sea turtle-falls at the Tsukumo Bay, Noto Peninsula, Jpan

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The whale-fall community is one of the unique biological community formed on decaying whale carcass on the sea floor. The community thought to be changed at least four stages of ecological successions, i.e. the mobile scavenger stage, the opportunist enrichment stage, the sulphophilic stage and the reef stage. Molecular studies on some organisms included in the community suggested that the origin of the vent- and/or seep-restricted animals have come from through environments formed around such organic-falls. In this point of view, the reptile-falls would be more important than the whale-falls because of its longer fossil record than the marine mammals. Although the ecosystem on the whale-fall has been studied well, the ecosystem on the reptile-fall hasn't been studied in details. Thus, we examined the decaying process of four sea turtles deployed on shallow sea floor (11 to 14 m in depth) in Tsukumo Bay, Noto Peninsula, Japan. The carcasses were observed by scuba, and some bones of the carcasses were recovered time to time to examined organisms which lived on/in the bones.

5 days after deployment, the sea turtle carcasses were covered by white microbial mat (*Beggiatoa* spp., indicator of sulphophilic stage). 21 days after deployment, the carcass was eaten by fish (indicator of mobile scavenger stage). *Zoothamnium* sp. (indicator of sulphophilic stage) was observed on the carcass. 36 days after deployment, dorvilleid and nereidid polychaetes (indicator of opportunistic stage) lived in the bones and barnacles (Crustacea; indicator of the reef stage) were attached to the bone. Thus, all four stages of ecological succession observed on whale-falls have also been observed on the sea turtle falls within 36 days after deployment. It is noteworthy that the sea turtle-fall sustained chemosynthetic community as same as whale-falls.

To examine sustainability of opportunistic and sulphophilic stages, we compared faunal assemblages in/on the recovered bones of the sea turtle carcasses with different body size and deployment period. Organic matrix was still remained in the bones of 2016ST-1 (green turtle, carapace length (CL) = 42 cm, 144 days after deployment) and 2013ST-L (loggerhead turtle, CL = 72 cm, 3 years after deployment). There were *Beggiatoa* microbial mats, *Zoothamnium* sp. and dorvilleid polychaetes in/on the bones. In contrast, there is no organic matrix in the bones of 2013ST-S (green turtle, CL = 36 cm, 3 years after deployment), and there were no *Zoothamnium* sp. and dorvilleids. Thus, the smaller sized carcass exhausted within 3 years, but the larger carcass sustained opportunistic and sulphophilic stages more than 3 years.

Keywords: sea turtle, carcass, whale-fall

Origin of methane at ancient seeps inferred from carbon isotopic signatures of biomarkers of methane-oxidizing archaea

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Methane is generated mainly by microbial or thermal degradation of organic matter, and the origin of methane can be estimated based on its stable carbon isotopic signature. Submarine methane seeps have been a major source of methane to the ocean, and knowing the origin of methane at the methane seeps can provide valuable insights into the subsurface biogeochemical processes and fluid circulation. Methane seeps in the geological past are archived as authigenic methane-derived carbonate rocks, which form via an alkalinity increase facilitated by microbially mediated anaerobic oxidation of methane. Although these carbonates inherit methane-derived carbon, they also incorporate non-methane-derived carbon such as seawater dissolved inorganic carbon, which masks original isotopic compositions of methane. Here we attempted to estimate origins of methane at ancient seeps based on carbon isotopic compositions of lipid biomarkers of methane-oxidizing archaea, which can more directly reflect isotopic signatures of methane. We examined methane-seep carbonate rocks in the Japan Sea region, collected from lower Miocene to middle Pleistocene sediments at 11 sites on land, and also carbonate nodules collected from the seafloor off Joetsu, where thermogenic methane is seeping. Early-diagenetic carbonate phases show various $\delta^{13}\text{C}$ values between -64.7 and -4.7% vs. VPDB, suggesting either biogenic or thermogenic, or both origins of methane. A lipid biomarker pentamethylcosane (PMI) extracted from the ancient carbonates showed $\delta^{13}\text{C}$ values mostly lower than -100% , whereas that from the modern methane-derived carbonate nodule showed a higher value (-80%). The $\delta^{13}\text{C}$ values of the seeping methane (-36%) and PMI in the modern Joetsu seep carbonate showed an offset of -44% . If this carbon isotope offset was similar at the ancient seeps, the $\delta^{13}\text{C}$ values of PMI indicate that methane at the ancient seeps in the Japan Sea region was mostly biogenic in origin, with $\delta^{13}\text{C}$ values lower than -50% . This suggests that methane was supplied mainly from the shallow subsurface, and the migration of thermogenic gases from the deep subsurface to the seafloor through pathways such as faults was minor at the studied ancient seeps. Further attempts to constrain original isotopic signatures of methane by extraction of residual methane from carbonates is also ongoing. Future works need to examine other proxies to know origins of seep fluids, such as rare earth elements in seep carbonates, which would also help to know origins of methane at ancient seeps.

Keywords: Methane, Cold seep, Biomarker, Cenozoic, Japan Sea

Mussel genomes provide insights into adaptation to deep-sea chemosynthetic environments

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The environment of deep-sea hydrothermal vents and methane seeps is characterised by darkness, lack of photosynthesis-derived nutrients, high hydrostatic pressure, variable temperatures and high concentrations of heavy metals and other toxic substances. Despite this, these ecosystems support dense populations of specialised macrobenthos such as giant tubeworms, mussels, limpets, clams and shrimps. Lack of genome information hinders understanding of the adaptation of these animals to such inhospitable environment. Here we report the genomes of a deep-sea vent/seep mussel *Bathymodiolus platifrons* Hashimoto & Okutani, 1994 and a shallow-water mussel *Modiolus philippinarum* (Hanley, 1843). Phylogenetic analysis shows that these mussel species diverged approximately 110.4 million years ago (MYA). Many gene families, especially those for stabilising protein structures and removing toxic substances from the cell, are greatly expanded in *B. platifrons*, indicating adaptation to extreme environmental conditions. The *B. platifrons* innate immune system is considerably more complex than that of other lophotrochozoan species including *M. philippinarum*, with significant expansion and high expression of gene families related to immune recognition, endocytosis and caspase-mediated apoptosis in the gill, revealing presumed genetic adaptation of the deep-sea mussel to the presence of its chemoautotrophic endosymbionts. A follow-up metaproteomic analysis of the gill of *B. platifrons* found methanotrophy, assimilatory sulfate reduction, and ammonia metabolic pathways in the symbiont, providing energy and nutrients to allow the host to thrive without dependent on photosynthesis derived food. Our study of the genomic composition allowing symbiosis in extremophile molluscs gives wider insights into the mechanisms of symbiosis in other organisms such as deep-sea tubeworms and shallow-water corals.

In addition, we would also like to take this opportunity to introduce a large-scale program on “U-Shape” area that targets at the vents along the Mid-Ocean Ridge of both South Atlantic and Western Indian Ocean, to be launched by China Ocean Mineral Resource Research and Development Association, for which we call for international collaboration in next 15 years.

Keywords: Genome sequencing, Bathymodiolus, Symbiosis

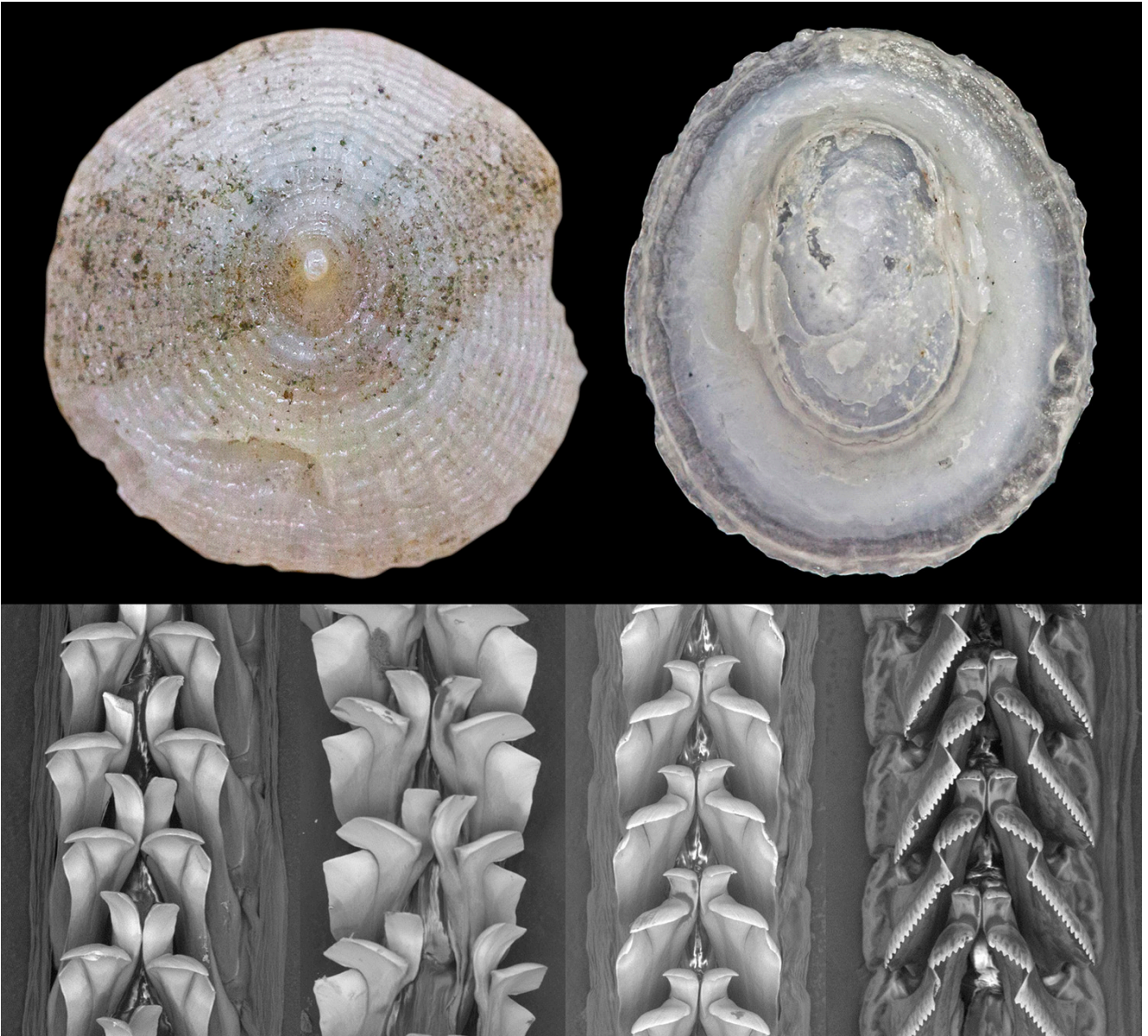
One, two, or many species? Morphological and genetic analyses of vent and seep pectinodontid limpets reveal extreme morphological plasticity

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Chemosynthetic ecosystems harbour rich megafauna communities, of which gastropod molluscs comprise a major component and have received considerable taxonomic efforts. Pectinodontid limpets, characterised by a white shell with cancellate sculpture and a radula with a single trifurcating lateral teeth on each side, are a common constituent of vents and seeps in the western Pacific. Thus far, two genera (*Bathyacmaea* and *Serradonta*) totalling eight species have been described based on shell and radula characteristics. However, no data on their intraspecific variation or genetic characterisation have been published. In the present study, numerous pectinodontid specimens from Sagami Bay and Okinawa Trough were investigated morphologically, revealing great disparity in both shell and radula in each locality, rendering existing species and even genera boundaries questionable. Changes in shell form according to substrate shape was confirmed through live-rearing in a controlled aquarium. Furthermore, phylogenetic and population genetic analyses of *Bathyacmaea* and *Serradonta* specimens from Sagami Bay to the South China Sea clearly demonstrated that most specimens belonged to one single molecular operational taxonomic unit regardless of their morphological affinity. Although a number of individuals from Minami-Ensei and Hatoma Knoll fields in Okinawa Trough were found to be genetically divergent, these did not exhibit obviously morphological differences and are likely cryptic. Altogether, these results strongly suggest that most (if not all) currently described vent and seep pectinodontids are actually mere forms of a single extremely morphologically plastic species. As a case study, the present study serves to raise awareness against splitting and describing species solely based on hard part morphology without carefully assessing the reliability of characteristics used.

Keywords: Hydrothermal vent, Cold seep, Mollusca, Morphological plasticity, Taxonomy, Population genetics



Locomotion and move tracking of "*Calytogen*" clams *in situ* and in patented H₂S controlling tanks

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Vesicomylid clams are one of the representative chemosynthetic animals, which have flourished in seep and vent sites during the Cenozoic. We report locomotion and behavior patterns of "*Calytogen*" clams to examine their adaptability to fluctuation of seep activity and disturbance of rapid burial. We analyzed time-lapse image data of *in situ* Real-time Deep Seafloor Observatory off Hatsushima Island in Sagami Bay, 1174 m in depth (3 min. interval) and patented H₂S controlling tanks in JAMSTEC (30 sec. interval) and Enoshima Aquarium (3 min. interval).

The "*Calytogen*" clams usually show semi-infaunal standing position with anterior side down and foot penetrating into underground sulfide layer, but sometimes crawl laying their shells with ventral side down. In standing position, the clams rearrange their postures swaying and obliquely rotating shells with foot as an axis. The clams sometimes push one another maybe quarreling over their favorite seep spots.

In crawling mode, they move forward by repeating the following steps: 1) laying shell down, 2) extending and penetrating foot in anterior direction, 3) maybe forming an anchor by foot expansion, and 4) pulling shell forward by foot contraction. In cases, the clams gathered into one spot and stranded one another, but could restore their postures into normal semi-infaunal position by extending and bending foot, which has wide movable range and high flexibility. During foot re-penetrating into the sediment, we observed that mass of sediment particles travels on whole surface of foot from its tip to root. Our SEM observation confirmed that the foot surface is densely covered by mucous cilia. The ciliary movement maybe enables the sediment conveying backward, which helps foot penetration.

In patented H₂S controlling tanks of Enoshima Aquarium, the "*Calytogen*" clams migrated keeping their track along the outer margin of bacteria mat. The average time and distance of one move action is about 15 min and 3.22 cm, so the average speed is about 0.38 cm/min. "*Calytogen*" clams have been reported to form a ring colony surrounding bacteria mat, and this ring colony is a result guided by best condition of chemical subbottom profile. The positions and areas of bacteria mats in the Enoshima Aquarium tank have been rather stable but the margin fluctuated. The Enoshima clam tracks seem to be a result of tracing the best spot for getting subbottom hydrogen sulfide. In contrast, the "*Calytogen*" clams of *in situ* the Hatsushima observation seem to move breaking bacteria mats, which were newly formed just after turbiditic rapid burial. In emergency where they lost their favorite seep spots just after such physical disturbance, the clams also repeated round trips from one position, back and forth in various directions, with maximum speed about 12 to 14 cm/min.

"*Calytogen*" clams have high ability of moving and restoring of their postures, as noted above. This ability is one of the keys of their adaptability to fluctuation of seep activity and disturbance of rapid burial, leading to their flourishing since the Cenozoic.

Keywords: chemosynthesis, methane seep, Vesicomylidae, locomotion