

## Chemotaxonomy of plants by resistant macromolecular analysis in charred mesofossils from the Cretaceous Futaba Group

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Resistant macromolecules composing living plant tissues tend to be preserved through degradation and diagenesis, hence constitute major parts of fossil plants or sedimentary plant-derived organic matter. And their monomer compositions vary widely among different plant taxa, organs and growth stages. Thus, analysis of such macromolecule may serve as new technique for paleobotanical evaluation distinctive from classical paleobotanical studies depends on morphological preservation of fossils. However, there have been few studies of the macromolecules, especially in ancient geological samples such as the Paleozoic and Mesozoic. In the present study, we analyzed plant fossils from the Cretaceous strata in Japan to examine chemotaxonomic characteristics of fossil macromolecules.

Charred mesofossils of angiosperms and gymnosperms were separated from carbonaceous sand stone of the Cretaceous Ashizawa Formation, Futaba Group. These mesofossils include fruit fossils of *Hironoia fusiformis* and *Archaeofagacea futabensis*, a flower fossil of *Esgueiria futabensis*, leaf fossil of *Juniperus*, a stem fossil of *Epfedra* and some fossils of fruits, seeds and woods. Powdered fossil samples were extracted with methanol and dichloromethane, and were subsequently refluxed under 110 °C to remove free compounds completely. The residues were hydrolyzed by KOH / methanol under 110 °C. These released compounds were analyzed by GC-MS. Additionally, multivariable analysis were calculated using SPSS software. We used hierarchical clustering to group fossils with similar lipid distributions among species or organs.

*n*-alkanes, branched isoprenoids, sterans, hopanes, and aromatic hydrocarbons were mainly present in solvent extract fraction. Aromatic hydrocarbons contained various higher plant derived diterpenoid and triterpenoid derivatives. These compounds are commonly considered as chemotaxonomic markers of gymnosperms and angiosperms respectively. Unexpectedly, triterpenoid derivatives were detected from gymnosperm fossils abundantly, indicating that free lipids may have moved in the coal bed, thus these lipids are not suitable for chemotaxonomic use in this study. On the other hand, as main hydrolyzed products (ester-bound molecular units) from all fossils, C6-C28 *n*-alkanoic acids and C8-C28 *n*-alkanols were detected. Multivariable analysis were calculated in lipid distribution for these released alkyl lipids from each fossils. Cluster analysis revealed a recognizable pattern in released alkyl lipid distribution. All five fossils of woody tissue were present in a cluster that excluded non-woody tissues. Additionally, exclusive of *Juniperus* fossil, the lipid signatures were similar among angiosperms or gymnosperms. From these results, we propose that it is likely to be realized that paleolipidomics-like detailed chemotaxonomy of fossil plants by making a comprehensive evaluation for various lipid components involve bond alkyl lipids.

Keywords: chemotaxonomy, alkyl lipid, plant fossil, Cretaceous, resistant macromolecule, multivariable analysis

## Foraminiferal psuedopodial observation during chamber formation

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Foraminifera, marine unicellular organism, have been considered as one of the major carbonate producer in ocean. Their calcareous tests are broadly utilized as paleo-environmental indicators in various studies of earth science because their tests have been archived as numerous fossil in sediment for long time and various environmental information are brought by population, morphology and geochemical fingerprints. The knowledge about the cytological process on carbonate precipitation has been described for couples of decade using by OM, SEM and TEM. Foraminiferal management of shell formation from ambient seawater are of great interest. Our study shows the potential to understanding the function of psuedopodial network for biomineralization by optical microscope.

Keywords: Foraminifera, Calcification

## Comparative anatomy of molluscs

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The phylum Mollusca is characterized by a diversity of body plan. Animals of extant molluscs are categorized into seven major types, namely (1) shell-less vermiform aplacoporans, (2) polyplacophorans with eight shell plates and repetition of internal organs, (3) monoplacophorans with a single shell and internal iteration, (4) bivalves with shells divided into right and left, (5) gastropods diagnosed by the operculum and ontogenetic torsion, (6) cephalopods with the arms/tentacles modified from the foot, (7) antero-posteriorly elongated tusk-like scaphopods. In addition, novel forms have been found in fossils, and they are regarded as ancestral molluscs connecting intermediate missing links or allegedly assigned to molluscs. One of keys to understanding of diversification of molluscan body structure is comparison of organogenesis in ontogeny. This viewpoint further needs investigation of development-controlling genes and leads into comparative genomic research.

Keywords: comparative anatomy, Mollusca

## Molecular basis of shell formation and shell evolution in gastropods

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The Molluscs constitutes one of the most diverse animal phyla, and they have evolved calcified exoskeletons called “shell” ever since the Cambrian. However, the molecular basis of molluscan shell development remains unclear. Thus we sought to understand the role of the homeotic gene *engrailed* in early shell development by focusing on retinoic acid signal pathway. We examined the expression patterns of RA metabolizing enzyme *cyp26* in the limpet *Nipponacmaea fuscoviridis* and found that *cyp26* is expressed around the edge of the shell field. As a result of gain or loss functional analysis of RA, shell deformation was observed in both gain and loss of RA analyses, and *engrailed* is down regulated. These results suggested that the common ancestor of Mollusca likely used RA signaling system to produce the novel phenotypic trait that is to be called “shell” by recruiting the homeotic gene.

Keywords: Shell evolution, RA pathway, Mollusca

## A possible coordinate system in the 3D coiling of molluscan shells

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A vast variety of forms have evolved in the molluscan shells since the Cambrian, all basing on the single and simple rules of growth, or the logarithmic spiral. Yet the biological realities underlying this mathematical regularity remained elusive except that the signal transduction protein Dpp has been demonstrated to be involved at least in the two-dimensional coiling of the shells. Here we show that another signal transduction protein is involved in the shell coiling, based on the results obtained from chemical treatments of the embryos of the pond snail *Lymnaea stagnalis*. We argue that those two 'morphogens' may form a coordinate system, which grows like a moving frame of the theoretical 'growing tube', enabling the mantle epithelial cells to form secretory three-dimensionally coiled structures.

## Genetic mechanisms of shell growth and shell coiling

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Molluscan shells have been a focus of study in the global environmental changes and morphological evolution, because they have a rich and continuous fossil record throughout the Phanerozoic. The knowledge of development and growth of the shells is important to reveal their morphological evolution, which may well be related to global environmental changes. Although theoretical aspects of the shell growth have been explored extensively, the biological reality of shell growth, such as the molecules and genes related to the theoretical parameters, remained largely unexplored. However, a clue has been found in recent years, that is, Dpp. It is the transcription product of *dpp*, which is the homologous gene of *bmp 2/4* in vertebrates. It has been shown that *dpp* is an important factor for shell formation and shell coiling. In this study, we focused on another signaling protein important in development. To investigate the function of this protein to *Lymnaea stagnalis*, we used an inhibitor and an activator. We found that these signals are heavily associated with the development and growth of embryos. In particular, the phenotypes of the shell form observed when the embryos were activated at the veliger stage demonstrated that the activity of these signals likely control the speed of the shell growth and the extent of shell coiling.