

## Solar energy and life: the diversity of phototrophic processes in the environments

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The evolution and recruitment of phototrophic processes that convert solar radiation into biochemical energy are among the most critical issues for the life on Earth. Recent works has revealed mechanisms of the phototrophy that contribute to ecosystem and geochemical cycles are rather diverse beyond the well-known oxygenic photosynthesis is. In addition, recent works also revealed diversity and quantitative significance of pico-phytoplanktons in the aquatic environment, hence the microbial energy/material flow founding on them. Understanding of microbial ecology is now refreshing a basis for biogeochemical processes in the ocean.

Keywords: phototrophy, photosynthesis, chlorophyll, aquatic ecosystem, biogeochemical cycles

## Examination for lipid biomarker compositions in culture samples of *Palmalles*.

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*Palmalles* is very small marine microalga, in which cell size is 2-5  $\mu\text{m}$ , and is classified as picoplankton. It is pointed out that this alga is one of main primary producer in restricted subarctic regions. *Palmalles* has siliceous tests, and may be closely related to diatom, which is a main important primary producer in the Cenozoic ocean. In 2008, Kuwata's research group can succeed in isolation of the *Palmalles* collected from the Oyashio region. In the present study, we try to search lipid biomarkers of the *Palmalles*, and to give understanding for first appearance and first processes of evolution of diatom. There have been no reports for siliceous fossil of *Palmalles*. It is known to well preserve siliceous diatom fossil in ancient sediment, and however, such fossil is frequently lost through its dissolution by diagenesis during postdeposition. Therefore, very small siliceous tests of *Palmalles* must be easily dissolved by diagenesis, and it cannot evaluate the timing of first appearance and reconstruct productivity of *Palmalles* by using its siliceous fossil. Thus, we clarified the *Palmalles* biomarkers and their compositions, and these biomarkers are used as molecular fossils for giving understanding evolution processes and historical variations of productivity of this alga.

We use a cultural strain NIES-2565(TOY-0807) of *Palmalles* *Triparma* sp. (*Triparma laevis*) for analysis of lipid biomarker. We can identify unsaturated alkene, unsaturated alkenoic acids, C27-C29 sterols as *Palmalles* biomarkers, which have detected from culture samples of diatoms. In particular, this strain is found to be characterized by overwhelmingly abundance of C29 beta-sitosterol. In addition, we can detect a number of unknown polar lipids with higher molecular weight. In the present study, we report preliminary results for *Palmalles* biomarker study and discuss its geoscientific significance as molecular fossil.

Keywords: *Palmalles*, lipid biomarker, evolution of diatom, chemotaxonomy, culture, steroid

## Photosynthesis of fern species having different habitats and frond morphologies

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### Introduction

Ferns are known to live in various habitats. Many species live in the shady and humid sites under tree canopy but some species live in the open or xeric sites. Their sporophyte fronds have quite different morphologies between species. The morphology of fern frond is considered to reflect the adaptation strategies to their environments. To understand the evolution of ferns and their adaptation to the various habitats, the information of morphological and ecophysiological trait of ferns are needed. Recent studies showed the relationship between habitats and morphological traits or photosynthetic traits separately. The integration of frond morphological traits, photosynthetic traits and their habitats or life forms are essential, since the frond morphology of ferns reflects the adaptation for effective photosynthesis and water relations.

The aim of this study is to integrate the frond morphological traits and photosynthesis for understanding the adaptation strategy of ferns to the various habitats.

### Materials and Methods

4 species of temperate fern such as shady terrestrial deciduous, *Adiantum pedatum* (kujakushida), Open terrestrial deciduous, *Pteridium aquilinum* (warabi), shady terrestrial evergreen, *Dryopteris erythrosora* (benishida) and Epiphyte evergreen, *Lepisorus thunbergianus* (nokishinobu) in sporophyte stage were grown in a glasshouse. Leaf mass per area (LMA), stomatal density (SD), leaf water content (LWC), relative water content (RWC) and photosynthetic potentials ( $V_{max}$  and  $J_{max}$ ) were measured before stress treatment, and then they were subjected to drought stress to investigate the photosynthetic response.

### Result

Stomata were on the abaxial side of fronds only in the all the ferns investigated. SD of the Open site fern, *P. aquilinum* was twice of the other ferns. Evergreen fern had significantly higher LMA than the deciduous ferns. Epiphytic fern, *L. thunbergianus* showed the highest LMA among the ferns. Photosynthetic potentials ( $V_{max}$  and  $J_{max}$ ) of *D. erythrosora*, *L. thunbergianus* and *P. aquilinum* were similar. However, their photosynthetic rate ( $A_{max}$ ) and stomatal conductance ( $g_s$ ) were lower than *P. aquilinum*.

Some fronds of the deciduous ferns under drought stress were dead. Tip of fronds were withered partly in *D. erythrosora*. In *L. thunbergianus*, all of the fronds were withered, but they were recovered after reirrigation. In all ferns,  $A_{max}$  and  $g_s$  decreased concurrently under drought stress. There were positive correlations between  $A_{max}$  and  $g_s$ . Epiphyte fern, *L. thunbergianus* showed negative transpiration in severe drought stress. The rubisco content of each ferns were not affected by drought stress.

### Discussion

In well watered condition, open site fern, *P. aquilinum*, have similar photosynthetic potentials ( $V_{max}$  and  $J_{max}$ ) to *D. erythrosora* and *L. thunbergianus*, but showed the highest  $A_{max}$  among the fern species. High SD and  $g_s$  of *P. aquilinum* may cause their high  $A_{max}$  in the open sites.

The evergreen ferns showed higher WC than the deciduous ferns. The higher construction cost may be higher for water storage structure in the evergreen ferns. In stress conditions, some fronds of deciduous ferns were dead, but evergreen ferns maintained the frond.

All ferns decreased photosynthetic rate and  $g_s$  concurrently but maintained the similar rubisco content in drought stress compared to the control. These results suggest that low stomatal conductivity may be a major factor reducing the photosynthetic rate in drought stress.

*L. thunbergianus* did not dead below 40% of RWC and showed negative transpiration in drought stress. These results suggest that *L. thunbergianus* is poikilohydry. Additionally, *L. thunbergianus* has the extremely higher LMA and WC than the other ferns, indicating that *L. thunbergianus* has succulent. *L. thunbergianus* live on the tree, where water resources may be limited. In such a prolonged drought environment, poikilohydry as well as succulent type morphology may be favorable.

Keywords: fern, pteridophyte, photosynthesis, adaptation, evolution, habitat

## Acquisition of kleptoplast in *Planogalbratella opercularis* (foraminifer) and its putative function

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A rocky-shore benthic foraminifera, *Planogalbratella opercularis*, constructs specific host-symbiont relationships that has chloroplast as kleptoplast. The "kleptoplast" means "stolen chloroplast" or "symbiotic chloroplast", and refers to a chloroplast originating from a separate organism. Host organisms may have some benefit from kleptoplast, such as organic matters, or amino acids. To understand the functions of kleptoplast, we conducted molecular phylogenetic analyses of kleptoplasts, culture experiment and stable isotope analyses. The trophic position of individuals with or without kleptoplast, we measured stable isotopic composition of amino acid to understand whether their nutritional requirements come from kleptoplast or not. As a result, trophic position (TP) of the individual with kleptoplast shows 1.2. In contrast, TP of cultured individual specimens that digested kleptoplast shows 2.0. It is possible that *P. opercularis* behave as a primary producer, "phyto-benthos", in nature.

Keywords: Kleptoplast, benthic foraminifera, nitrogen isotope of amino acid

## Intra- and interspecies variations in intercellular concentrations and compositions of alkene and alkenone in Haptophyte

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Haptophyte algae are one of the major primary producers in the ocean. Long-chain alkenones, unsaturated linear methyl and ethyl C<sub>37</sub>-C<sub>40</sub> ketones, are synthesized by few species of haptophyte algae (*Emiliana huxleyi*, *Gephylocapsa oceanica*, *Isochrysis galbana* and *Chrysolida lamellosa*). Alkenones have frequently been used for estimating the paleotemperature in geological samples, since the number of double bonds change in response to the growing temperature. Along with alkenones, these haptophyte species also produce polyunsaturated long-chain alkenes. Long-chain alkenes are more susceptible to diagenetic process (i.e. photochemical and bacterial degradations), hence are regarded less important to apply in geological past. Therefore, compositions and distributions of long-chain alkenes among its producers have not been systematically examined. Recently, algal biomass is expected to be a new energy resource. We look for the use of haptophyte algae for biorefinery, based on their high rate of reproduction, high content of long-chain lipids, and some other positive features. Alkenes gain importance in this context because of its chemical nature as hydrocarbon. In this study, we analyzed alkene and alkenones in the haptophyte algae in order to gain suite of concentration and compositional data covering wide range of Haptophyte algae.

More than 50 strains of haptophyte algae were obtained from stock culture of Shiraiwa's laboratory and the other culture collections (e.g. NIES, NCMA, RCC). These strain were grown at 17°C & 20°C for 10-21days. Extraction and separation of lipids were performed based on Sawada and Shiraiwa (2004). After extraction, the lipids were separated by silica gel column, and fraction 1, 2 and 3 (hexane, hexane / ethyl acetate (95/5 v/v), hexane / ethyl acetate (9/1 v/v)) were analyzed by gas chromatography with flame ion detector (GC-FID) and gas chromatography / mass spectrometer (GC/MS).

Alkenes (C<sub>29</sub>-C<sub>38</sub>) and alkenones with alkenoates (C<sub>37</sub>-C<sub>40</sub>) were detected from strains of four species: *E. huxleyi*, *G. oceanica*, *I. galbana* and *C. lamellosa*. Other species lack all of these compounds. The total concentrations of alkenones and alkenes were 0.02-1.96 pg/cell (0.09-11.1 ug/ml) and 0.001-0.57 pg/cell (0.01-1.58 ug/ml), respectively. Both intercellular concentrations and compositions of these compounds showed significant differences between strains. A majority of strains mainly contain C<sub>31</sub> and C<sub>33</sub> alkenes, while some others contain C<sub>37</sub> and C<sub>38</sub> alkenes in significant proportion. Furthermore, some strains of *E. huxleyi* contained significant amount of C<sub>29</sub> alkadienes. Rieley et al. (1998, Lipids 33, 617-625) reported that C<sub>37</sub> and C<sub>38</sub> alkenes have *trans* double bonds resemble to those of C<sub>37</sub> and C<sub>38</sub> alkenones while C<sub>31</sub> and C<sub>33</sub> alkenes have *cis* double bonds, suggesting distinct biosynthetic pathway for these two groups of alkenes. However, consistent occurrence of alkenes and alkenones highlights close biochemical relationship between these two groups of compounds, as well as importance of the four alkenone producing species as potential hydrocarbon resource.

Keywords: Haptophyte, alkenone, alkene, biorefinery

## Microbial eukaryotes living in the coastal marine of Tokyo bay insights from the metagenome

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Progresses in sequencing technique makes it possible to reveal the existence and to detect the diversity of pico-planktonic organisms, and also decide the taxonomic position of uncultured organisms, rapidly and easy way. Metagenomic approaches show that the huge amounts of pico-planktonic organisms are living in various environments. It means that eukaryotic microorganisms play important roles as a producer, consumer or reducer in the biosphere such as microbial loop or biogeochemical cycle. There are so many taxonomically unknown organisms, even though their phylogenetic positions have been determined. It would not be an exaggeration to say that we just reached at the start line to reveal the eukaryotic diversity. Furthermore, there are less physiological or ecological information about them. In this study, we determined the 18S rDNA sequences using filter captured microbial eukaryotes collected every month from the sea water in Tokyo Bay.

Throughout the investigation period, the sequences belonging to Opisthokonta, Stramenopiles and Alveolata, were respectively obtained in each quarters of the total. The residual one quarter were the mixture of Rhizaria, Cryptophyta, Chlorophyta, Rhodophyta, Katablepharid, Telonemia and Haptophyta. When *Heterosigma akashiwo* made the bloom, the half of the determined sequence comes from the surface bloom water were originated from this alga, and the one from the lower layer under the bloom was mainly composed by Ciliophora and/or parasitic Dinophytes. In summer, centric diatoms, and the Opisthokonta in sometime, became dominant taxa. Most of the Opisthokonta sequences were originated from Metazoa. Notably, Chytridiomycetes were the dominant taxa in August. The sequences identified as Picobiliphyta or MAST(MARine STRamenopiles) were infrequently detected. These findings indicate the existence of many unidentified or missing link organisms in the universal aquatic ecosystems, and the importance of our studies. For the further understandings of the unknown organisms, we are developing the technique to get the both profiles of the phylogenetic position and morphological features together.

Keywords: Metagenome, Environmental sequences, Algae, Protist, aquatic ecosystem

## Coping with toxicity of chlorophylls: a biochemical strategy

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Chlorophylls in aquatic environments have been known to be transformed into various derivatives; a portion of chlorophylls are degraded via a so-called Type I process in which tetrapyrrole macrocycles are preserved intact with a variety of defunctionalization, hence surviving into sediments. These survived chlorophyll derivatives could be further altered chemically to be fossil porphyrins and red pigments extracted from sedimentary rock as old as the Proterozoic. We improved HPLC methods that carefully excluded analytical artifacts. We thus revealed that a major component of the chlorophyll derivatives is derived from eukaryotic microbe, which is produced along chlorophyll detoxification catabolisms.

Keywords: chlorophyll, eukaryotic microbes, detoxification, chlorophyll catabolism



## Vertical distribution and abundance of *Acaryochloris* spp. at the deepest area of Lake Biwa

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The genus *Acaryochloris* is the only organism which contains Chlorophyll *d* (Chl *d*) as their predominant light harvesting pigments. This cyanobacteria can absorb and use far-red light (FR), ranging from 700 to 750 nm, as well as visible light (400-700 nm) for oxygenic photosynthesis. Therefore, the ecological and the critical role of Chl *d* have been thought to utilize FR effectively, avoiding the competition for visible light with other chlorophyll *a* (Chl *a*) containing algae and providing niche to the genus *Acaryochloris*.

In our successive works, we have detected 16S rRNA gene of *Acaryochloris* spp. from the water samples collected at more than 30 m depth in Lake Biwa. At the depths, no FR was thought to be remaining. This suggested that the detected planktonic *Acaryochloris* spp. could not utilize FR for photosynthesis.

The purposes of this study were to reveal the niche of planktonic *Acaryochloris* spp. and the adaptive significances of Chl *d* in that depth. For these purposes, we established a real-time PCR method to quantify the copy of *Acaryochloris*-16S rRNA gene and investigated the vertical distribution of planktonic *Acaryochloris* spp. at the deepest area of Lake Biwa. Additionally, we compared the solar radiation spectra at the depth where *Acaryochloris* spp. were detected and the excitation spectrum of *Acaryochloris* cells. We would like to present the results of our investigations and discuss about the adaptive significances of Chl *d* for the planktonic *Acaryochloris* spp. in Lake Biwa.

Keywords: *Acaryochloris* spp., chlorophyll *d*, vertical distribution, real time PCR, Lake Biwa