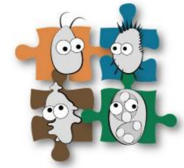
JSP Kobe2020

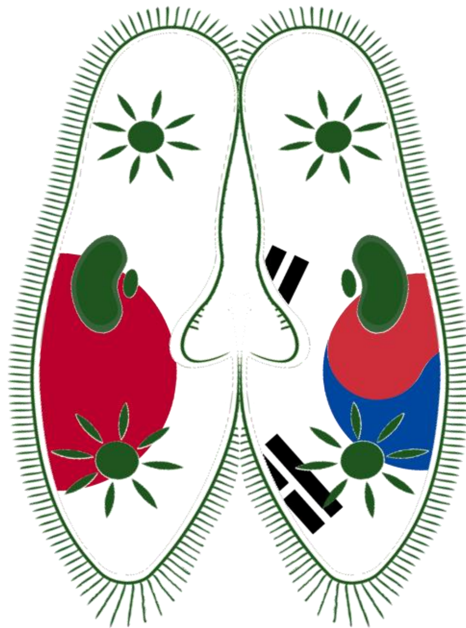
Japan Society of Protistology



한국원생생물학회
The Korean Society of Protistologists

JSP/KSOP Joint Online Meeting

Joint online meeting of the Japan Society of Protistology and Korean Society of Protistologists



22-23 November 2020

Program

Day 1 (22 November)

9:00-9:10	Opening remarks (Terue Harumoto, the president of JSP)
9:10-9:40	Plenary lecture 1 (Jong Soo Park) [PL-01]
9:40-9:50	Break
9:50-11:30	Oral session 1 (BPA selection session) [O-BPA01 – O-BPA06]
11:30-11:40	Break
11:40-13:10	Poster session 1 [Hall A: P-1A01 – P-1A07] / [Hall B: P-1B01 – P-1B06]
13:10-14:20	JSP & KSOP General assembly / Lunch break
14:20-16:45	JSP/KSOP Joint Symposium [S-01 – S-06]

Day 2 (23 November)

9:10-9:40	Plenary lecture 2 (Yuichiro Kashiyama) [PL-02]
9:40-9:50	Break
9:50-11:15	Oral session 2 [O-01 – O-05]
11:15-11:25	Break
11:25-12:55	Poster session 2 [Hall A: P-2A01 – P-2A07] / [Hall B: P-2B01 – P-2B07]
13:00-13:10	BPA award ceremony
13:10-13:20	Closing remarks (Young Ok Kim, the president of KSOP)
14:00-17:00	Association of young protistologists (This is an additional project specifically for young protistologists. Anyone who is actually young, or young at heart, is free to attend. See page 28 for more information.)

We never know what will happen in life. This joint meeting was originally scheduled to be held in Kobe, Japan, but due to COVID-19, we were forced to hold it online. It is extremely painful for us to not be able to invite Korean protistologists to Japan to deepen our research exchanges. However, we still realize that there are many good things about hosting online as well. For example, we have been able to get so many young students to attend the meeting, probably because there are no travel expenses. By taking advantage of the online features, we were also able to prepare poster sessions that would allow for more in-depth discussions. All of you are probably able to attend this conference from the comfort of your homes. Even in the midst of difficult circumstances, we can always see the light for tomorrow. I hope this conference will provide you with some inspiration for our new future.

On behalf of many Japanese and Korean friends who have worked hard to prepare this new academic endeavour with me, I sincerely hope that this conference will strengthen the academic ties between our two countries and with researchers in other countries as well.

Toshinobu Suzaki
22 November 2020, from Kobe, Japan.

Plenary Lectures

PL-01 (Day 1: 9:10-9:40)

The fate of eukaryotes in hypersaline environments

Jong Soo Park (Department of Oceanography, Kyungpook National University, Republic of Korea)

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Hypersaline environments where the dissolved oxygen concentration is low, are widely but sparsely distributed across Earth. Many biologists have believed that prokaryotes are abundant in high salinity waters (>25% salinity) whereas eukaryotes are rare in there for a long time. Since the 2000s, the inventory of eukaryotes in high salinity waters increases substantially. Culture-dependent approaches reveal that eukaryotes from hypersaline environments are mostly different from previous species and belong to Alveolates, Stramenopiles, and Heterolobosea. Furthermore, the larger creature may have geographically restricted distributions in comparison with the smaller one. Culture-independent Illumina analyses of the V4 and V9 regions of 18S rDNA show that eukaryotes in high salinity waters are more diverse than previously thought. Besides of fluctuation of salinity, the gene expression patterns of halophilic or halotolerant eukaryotes may be also affected by low dissolved oxygen in high salinity waters. Therefore, the evolutionary and physiological history of eukaryotes in hypersaline environments is influenced by salinity and dissolved oxygen concentration.

PL-02 (Day 2: 9:10-9:40)

The detoxification catabolism of chlorophylls that allowed protists to prosper on the oxygenated earth

Yuichiro Kashiyama (Fukui University of Technology, Japan)

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The establishment of a photosynthetic mechanism using chlorophyll as an essential cofactor in the Archean resulted in supply of large flux of energy to the Earth's biosphere, leading to the prosperity of modern life. The photochemistry of chlorophylls that realized an efficient photosynthesis is in fact a mixed blessing for lives. The phototoxicity of chlorophylls become manifest when they are photoexcited but not actively quenched, such as when the molecules are dislocated from degraded photosynthetic apparati, by transforming into their triplet excited state. Besides acting as potent oxidants, more importantly, the triplet-excited chlorophylls transfer the energy to molecular oxygen generating a highly cytotoxic reactive oxygen (ROS) so called “singlet oxygen”. This should lead a conclusion that any degradative process of photosynthetic cells under light is potentially risky to the surroundings. The risk is particularly of concern to protists such as when the predator digests algal material through phagocytosis, when the photoendosymbionts collapses, and when the chloroplasts need to be degraded. Therefore, the phototoxicity of chlorophylls must have been a cryptic yet a severe constraint on the evolution of protists. The present work, reported in a series of publications (Kashiyama *et al.*, 2012, *PNAS*; Kashiyama and Tamiaki, 2014, *Chem Lett*;

and Kashiyama *et al*., 2019, *ISME J*), demonstrate an ability of algivorous protists to safely degrade chlorophylls into non-phototoxic 13^2,17^3-cyclopheophorbide enols (i.e., CPE-accumulating chlorophyll catabolism; CACC) and its extremely widespread occurrences among diverse eukaryotic lineages, based on rigorous and extensive co-culture experiments with heterotrophs and algae and those with photoendosymbiotic organisms. Furthermore, many microalgae, particularly those possessing the secondary/tertiary chloroplasts, also conduct the same CACC upon occasional dismantling of their own chloroplasts. These metabolic functions are therefore likely to be plesiomorphic for all extant eukaryotes, thus suggesting algivorous ancestors, if not the last eukaryotic common ancestor (LECA) was likewise an algivore Indeed, the observed distribution of CACC protists possibly reflect that the catabolism would be traced back to the ancestral cells presented in the middle Proteozoic. Acquisition of CACC would have had dramatically modified the prey-predator associations and further phase-shifted the oceanic biogeochemical cycle of the late Proterozoic by intensifying direct consumption of the photo-synthetic producer in the illuminated water column. Furthermore, CACC must have allowed the algivorous ancestors to host the photoendosymbionts, hence evolution of eukaryotic algae.

Symposium

Diversity of Protists and Protist Scientists

(Day 1: 14:20-16:45)

S-01 (14:25-14:45)

Unveiling the hidden genetic diversity and chloroplast type of marine benthic ciliate *Mesodinium* species

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Ciliate *Mesodinium* species are commonly distributed in diverse aquatic systems worldwide. Among *Mesodinium* species, *M. rubrumis* closely associated with microbial food webs and red tide formation and is known to acquire chloroplasts from its cryptophyte prey for use in photosynthesis. For these reasons, *Mesodinium* has long received much attention in terms of ecophysiology and chloroplast evolution. *Mesodinium* cells are easily identifiable from other organisms owing to their unique morphology comprising two hemispheres, but a clear distinction among species is difficult under a microscope. Recent taxonomic studies of *Mesodinium* have been conducted largely in parallel with molecular sequence analysis, and the results have shown that the best-known planktonic *M. rubrum* in fact comprises eight genetic clades of a *M. rubrum*/*M. major* complex. However, unlike the planktonic *Mesodinium* species, little is known of the genetic diversity of benthic *Mesodinium* species, and to our knowledge, the present study is the first to explore this. A total of ten genetic clades, including two clades composed of *M. chamaeleon* and *M. coatsi*, were found in marine sandy sediments, eight of which were clades newly discovered through this study. We report the updated phylogenetic relationship within the genus *Mesodinium* comprising heterotrophic/mixotrophic as well as planktonic/benthic species. Furthermore, we unveiled the wide variety of chloroplasts of benthic *Mesodinium*, which were related to the green cryptophyte.

S-02 (14:45-15:05)/P-2A07

Interaction between centrohelid and actinophryid heliozoans in co-culture

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Centrohelid and actinophryid heliozoans are cosmopolitan, free-living aquatic protists that inhabit different biotopes. Heliozoans consume autotrophic and heterotrophic nanoplankton, and omnivorous/carnivorous species have to be considered as potential grazers on other protozoans [1)]. Interaction between different heliozoans taxa is not fully understood; however, some of them, such as actinophryid and centrohelid heliozoans, often occur in the same habitats [2)]. For studying the interaction between actinophryid and centrohelid heliozoans the co-cultured experiments were conducted. For this purpose, samples from different localities (Bangladesh, Japan, and Ukraine) were collected, specimens of actinophryid and centrohelid heliozoans were isolated and used for culturing. On the base of light and transmission electron microscopy species identification was performed. For some centrohelid species, the determination was confirmed by molecular genetic analysis. As a result, two species of actinophryid and four species of centrohelid heliozoans were registered. One of these species – *Choanocystis pantopoda* (Penard, 1904) which was recorded in pond (Kameyama city) is a new record for the Japanese fauna. In co-cultured two series of laboratory experiments was performed. First, for two species from brackish water (pond, Hiroshima, Japan) – *Actinophrys sol* and *Raphidocystis contractilis*. Second, for pairs of species from freshwater – *Actinosphaerium eichhornii* (pond, Kyiv, Ukraine) and *Choanocystis pantopoda* (pond, Kameyama, Japan), and also for *Actinosphaerium eichhornii* (pond, Kyiv, Ukraine) and *Raphidocystis ambigua* (pond, Bangladesh). It was shown that in co-culture experiments growth rate of centrohelid and actinophryid heliozoans

species are different. In all experiments, centrohelids demonstrated intensive development within the first 3-5 days, and after that its cell numbers remained at a constant level. In contrast to centrohelids, significant decrease in cell numbers of actinophryid heliozoans was observed. Moreover, the disappearing of actinophryid heliozoans was recorded in most experiments after 2 days (for brackish water species) and after 5-11 days (for freshwater species). In some experiments, we observed that several cells of centrohelids formed syncytium for the acquisition and ingestion of large food organisms such as *Actinophrys sol*. Thus, the results of our experiments show that inhibiting of growth of actinophryid heliozoan species which kept in mixed culture with centrohelids was observed. It is confirms the assumption that centrohelids have a negative impact on the development of actinophryid heliozoans.

1) Arndt, H. 1993. Mar. Microb. Food Webs, 7: 3-29.
2) Zimmermann, U., Miiller, H., Thomas Weisse, T. 1996. Aquat. Microb. Ecol., 11: 21-29.

S-03 (15:05-15:25)

Application of *Acanthamoeba*-specific antibodies for diagnosis of *Acanthamoeba keratitis*

Min-Jeong Kim, Hyun-Hee Kong, Eun-Kyung Moon* (Kyung Hee University School of Medicine)
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Acanthamoeba keratitis (AK) is a rare disease but its prevalence throughout the globe continues to grow, primarily due to increased contact lens usage. Since early-stage symptoms associated with AK closely resemble those from other corneal infections, accurate diagnosis is difficult and this often results in delayed treatment and exacerbation of the disease, which can lead to permanent visual impairment. Accordingly, developing a rapid *Acanthamoeba*-specific diagnostic method is highly desired. In this study, we described the identification and production of two *Acanthamoeba*-specific antibodies against secretory proteins of *A. castellanii* that can be used for the identification of *Acanthamoeba*. Among the secretory proteins of the pathogenic *Acanthamoeba* strain, inosine-uridine preferring nucleoside hydrolase (IPNH) and chorismate mutase (CM) genes were obtained, and polyclonal antibodies against them were generated. Western blot was performed using protein lysates and conditioned media of the human corneal epithelial (HCE) cells, non-pathogenic *Acanthamoeba*, pathogenic *Acanthamoeba*, and *Acanthamoeba* spp. isolated from a clinical sample, and other causes of keratitis such as *Fusarium solani*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. Polyclonal antibodies raised against *A. castellanii* IPNH and CM specifically interacted with lysates of *Acanthamoeba* origin and their culture media, while such interactions were not observed from other samples. *Acanthamoeba*-specificity of CM was also confirmed using immunocytochemistry after co-culturing *Acanthamoeba* with HCE cells. Specific binding of the CM antibody to *Acanthamoeba* trophozoites was observed, which were absent in the case of HCE cells. These results indicate that the IPNH and CM antibodies of *Acanthamoeba* may serve as a potential agent for rapid and differential AK diagnosis.

S-04 (15:35-15:55)

Multi-algae retaining protists (MARP): Exploring the diversity and role of photobionts

Ryo Hoshina (Nagahama Institute of Bio-Science and Technology)
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Many freshwater protists possess unicellular green algae within their cells. These protists typically contain hundreds of algae within a single host cell, and are collectively referred as multi-algae retaining protists (MARP)[1]. Photobionts in MARP usually exhibits so-called "*Chlorella*-like" morphology, which cannot be distinguished from free-living *Chlorella* and allies in water bodies. Therefore, it has been generally considered that the MARP host engulfed an alga from the outside and temporarily retains it. However, recent DNA sequence comparisons have indicated that MARP photobionts are, in most cases, different species from those free-living algae, and MARP symbioses are not temporal but rather persistent[1,2]. Furthermore, the phenomenon of sharing specific photobionts among various protists, that is, the existence of preferable algal partners, is becoming

apparent. What benefits does symbiosis with algae give to the host? It is known that the photobionts in *Paramecium bursaria* (model organism of MARP) leak out half of the photosynthate under certain conditions[3,4], which has been considered as the dedication to the host. Recently we found a MARP ciliate that seems to be the ultimate form of symbiosis. This ciliate stores starch granules inside the cells and generates blooms in oligotrophic highland marshes.

1) Hoshina, R. and Kusuoka, Y. 2016. Protist, 167: 174-184.
2) Hoshina, R., Iwataki, M. and Imamura, N. 2010. Phycol. Res., 58: 188-201.
3) Reisser, W., Vietze, S. and Widowski, M. 1988. Symbiosis, 6: 253-270.
4) Kamako, S. and Imamura, N. 2006. J. Eukaryot. Microbiol., 53: 136-141.

S-05 (15:55-16:15)

Paulinella micropora genome reveals dominant host contribution to plastid endosymbiosis

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Eukaryotic photosynthetic organelles, plastids, are the powerhouses of many aquatic and terrestrial ecosystems. The canonical plastid in algae and plants originated >1 billion years ago and therefore offers limited insights into the initial stages of organelle evolution. To address this issue, we focus here on the photosynthetic amoeba *Paulinella micropora* strain KR01 that underwent a more recent (ca. 124 Mya) primary endosymbiosis, resulting in a photosynthetic organelle termed the chromatophore. Analysis of genomic and transcriptomic data resulted in a high-quality draft assembly of size 707 Mbp and 32,361 predicted gene models. A total of 291 chromatophore targeted proteins were predicted in silico, 206 of which comprise the ancestral organelle proteome in photosynthetic *Paulinella* species with functions, among others, in nucleotide metabolism and oxidative stress response. Biggest portion of the chromatophore targeted proteins was derived from host, suggesting dominant host contribution to plastid endosymbiosis. Gene co-expression analysis identified networks containing known high light stress response genes as well as a variety of genes of unknown function.

S-06 (16:15-16:35)

A low vision scientist deeply loves microscopes

Katsuya Shimabukuro
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I am low vision, which means that I have serious problems with my eyes, but do not go blind yet. My right eye has gone a few years ago and my left only has a central vision with a tiny field of view. In spite of visual impairment, I am a microscopist and deeply love microscopes. Soon after starting my research project with microscopy as a master student, I was diagnosed with Retinitis Pigmentosa, a genetic disease causing loss of vision eventually. Since then, I have always had a dilemma, “Is it time to decide whether I give up or not?” and time has gone by. I keep postponing the decision for years. Unfortunately, I totally lack an ability to design a better life, which may not be so bad, now I suppose. My microscope experience is quite something. Dark field, phase contrast, DIC, fluorescence, TIRF, SEM, TEM and scanning probe microscope are some examples. Every single time I confront a new microscope, I tell myself “Am I sure that I will be able to handle this?”. My answer is always the same, “I will see”. People and technology have given a hand to me. Owing to a rapid development of sensors,

digital cameras capture images that I can not see through eye pieces. High spec computers can boost contrast in images, allowing me to see images. Students are my “eyes”. They carefully explain to me what they are looking at. Those are tricks why I manage to be a microscopist. It was not possible two decades ago. I was born in a right era and had a fortune to get a right job. These days, I often hear new exciting news about technologies that can be applicable to my research, making me feel that I will be able to be a microscopist for a while. Yes, I keep going. Nothing but my vigorous motivation can pave the way for my future.

Oral Session 1 (Day 1)

O-BPA01 (9:50-10:05)

Evolutionary history of mitochondrial genomes in Discoba, including the extreme halophile *Pleurostomum flabellatum* (Heterolobosea)

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Data from Discoba (Heterolobosea, Euglenozoa, Tsukubamonadida, and Jakobida) are essential to understand the evolution of mitochondrial genomes (mitogenomes), since this clade includes the most primitive-looking mitogenomes known, as well some extremely divergent genome information systems. Heterolobosea encompasses more than 150 described species, many of them from extreme habitats, but only six heterolobosean mitogenomes have been fully sequenced to date. Here we complete the mitogenome of the heterolobosean *Pleurostomum flabellatum*, which is extremely halophilic and reportedly also lacks classical mitochondrial cristae, hinting at reduction or loss of respiratory function. The mitogenome of *P. flabellatum* maps as a 57,829 bp long circular molecule, including 40 CDSs (19 tRNA, two rRNA, and 19 orfs). The gene content and gene arrangement are similar to *Naegleria gruberi* and *N. fowleri*, the closest relatives with sequenced mitogenomes. The *P. flabellatum* mitogenome contains genes that encode components of the electron transport chain similar to those of *Naegleria* mitogenomes. Homology searches against a draft nuclear genome showed that *P. flabellatum* has two homologs of the highly conserved Mic60 subunit of the MICOS complex, and likely lost Mic19 and Mic10. However, electron microscopy showed no cristae structures. We infer that *P. flabellatum*, which originates from high salinity (313‰) water where the dissolved-oxygen concentration is low, possesses a mitochondrion capable of aerobic respiration, but with reduced development of cristae structure reflecting limited use of this aerobic capacity (e.g., microaerophily).

O-BPA02 (10:05-10:20)

Single-cell DNA metabarcoding and *in situ* imaging reveal the symbiosis of Rhizaria (Phaeodaria and Radiolaria)

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**jasnakamura@gmail.com (Yasuhide Nakamura)*

The supergroup Rhizaria (including Phaeodaria and Radiolaria) are reported to have high biomass in the world oceans. Since these marine protists are supposed to play important roles in the material cycles[1], the ecological relationships between rhizarians and other organisms, which are still wrapped in mystery, would be indispensable to understand marine ecosystems. The DNA metabarcoding is becoming a standard approach to assess the plankton diversity. Whereas, *in situ* imaging techniques, such as Visual Plankton Recorder (VPR), allow us to observe the unknown ecological aspects of marine protists. By combining these new analytical techniques, this study attempt to clarify the symbiosis of rhizarians. Rhizarians were collected in 2015-2019 at 30 stations in the Northern Hemisphere. After the microscopic observation, collected rhizarians were fixed with 99% ethanol. Surveys by scuba diving and VPR were also conducted at eight stations. For a part of the ethanol-fixed individuals, the single-cell DNA metabarcoding (focusing on the V9 region of eukaryotic 18S

rRNA gene) was performed in order to clarify the taxonomic composition of the organisms contained in each rhizarian. The surveys by scuba diving and VPR revealed that large rhizarians are occasionally associated with (or held by) certain crustaceans. This phenomenon resembles "Jellyfish rider" (the symbiosis between jellyfish and crustaceans) and therefore, named "Rhizarian rider"[2]. The combination of Phaeodaria and Amphipoda was the most frequently observed, but the association between Radiolaria and Decapoda (phyllosoma larva) was also photographed. Rhizarians were possible to be used by crustaceans in order to reduce the swimming energy and to secure food sources. A total of 106 rhizarians were examined by the single-cell DNA metabarcoding. Possible symbionts, parasites and prey organisms were successfully detected, some of which were first discovered by this study. Possible symbionts (e.g., dinoflagellates, haptophytes and pelagophytes) were frequently detected from radiolarians, while parasitic organisms (e.g., *Massisteria, Dermocystidium*) were detected from phaeodarians. The composition of the intra-cellular organisms was obtained for 92% of the analyzed specimens, and consequently, the single-cell DNA metabarcoding would be an effective method to clarify the symbiosis and the predator-prey relationship of unicellular organisms.

1) Biard, T., Stemmann, L., Picheral, M., Mayot, N., Vandromme, P., Hauss, H., Gorsky, G., Guidi, L., Kiko, R. and Not, F. 2016. Nature, 532: 504-507.
2) Nakamura, Y., Minemizu, R. and Saito, N. 2019. Mar. Biodiv., 49: 2193-2195.

O-BPA03 (10:20-10:35) /P-1B07

Overview of the biodiversity of tintinnine ciliates (Protozoa: Ciliophora: Tintinnina) in coastal waters of China

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As planktonic model organisms for addressing microbial biodiversity, biogeography, ecophysiology, evolution, and energy transduction, Tintinnina ciliates are attractive by the possession of highly diverse architecture and dimensions of lorica. Up to now, at least 1000 tintinnine morphospecies have been discovered worldwide and their classification is based almost entirely on lorica features[1)]. However, the traditional taxonomies were challenged by the application of integrative new approaches in species delimitation that have limited our understanding of their true biodiversity and systematics, that is, the increasing evidences further demonstrate the lorica plasticity and cryptic species diversity in this species-rich group of ciliates[2, 3)]. In the past three years, more than 40 tintinnine ciliates collected from broad sampling sites in China, were investigated with modern methods, and sequences of SSU rRNA gene and LSU rRNA gene were phylogenetically analyzed. Among them, about 30 species were firstly revealed ciliary pattern and rRNA gene sequences, including two type species which represents an intermediate lineage between tintinnines (loricate form) and aloricate choreotrichids. In addition, we proposed a new genus based on the rediscovery of a synapomorphy from two poorly known species. Consequently, our data show the potential taxonomic relevance of details of the somatic ciliary patterns, cell features, as well as the lorica ultrastructure, and provide new sight in understanding the systematics and evolutionary relationships of tintinnines.

1) Zhang WC, Feng MP, Yu Y, Zhang CX, Xiao T. 2012. An illustrated guide to contemporary tintinnines in the world. Beijing: Science Press.
2) Xu DP, Sun P, Shin MK, Kim YO. 2012. Species boundaries in tintinnine ciliates: a case study? morphometric variability, molecular characterization and temporal distribution of Helicostomella species (Ciliophora, Tintinnina). Journal of Eukaryotic Microbiology 54: 351-358.
3) Xu DP, Sun P, Warren A, Noh JH, Choi DL, Shin MK, Kim YO. 2013. Phylogenetic investigations on ten genera of tintinnine ciliates (Ciliophora: Spirotrichea: Tintinnida), based on small subunit ribosomal DNA sequences. Journal of Eukaryotic Microbiology 60: 192-202.

O-BPA04 (10:45-11:00)

A Systematic Study of Two New Species of *Euplotes* (Ciliophora: Spirotrichea: Euplotida) with Morphological Description and Molecular Phylogeny

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The genus *Euplotes* is a species-rich genus in ciliated protozoa, and inhabitants of ubiquitous environments. Two *Euplotes* species collected from the algal mat and saline pond of saltmarshes in Korea were characterized as new species by morphological and molecular phylogenetic analysis. Both new species are similar in having ten frontoventral cirri, two caudal cirri, C-shaped macronucleus, and double eurystomus-type dargyrome pattern. However, *Euplotes* n. sp. 1 from the algal mat is different from *Euplotes* n. sp. 2 from the saline pond in terms of body shape (oval vs. elongated oval), number of left marginal cirri (2 vs. 2-3), number of dorsolateral kineties (8-9 vs. 7-8), the shape of each cortical granules (rounded vs. ellipsoidal), cell size (about 47-56 x 33-42 µm vs. 49-67 x 30-41 µm in vivo), number of adoral membranelles (25-30 vs. 25-33), and number of dikinetids in mid-kinety (12-14 vs. 12-15). The phylogenetic trees based on small subunit rRNA gene sequences for both new species and related species indicate that *Euplotes* n. sp. 1 is grouped with several populations of congener *Euplotes euryhalinus* with 97.18-99.22% similarity. While *Euplotes* n. sp. 2 clustered together with two populations of *Euplotes cf. antarcticus* with 99.17-99.37% similarity, and one population of *Euplotes trisulcatus* with 98.95% identity. The molecular data also support the different phylogenetic relationships of these new species in the phylogenetic tree.

O-BPA05 (11:00-11:15)/P-1A05

***Heterocapsa busanensis* n. sp. (Dinophyceae, Peridiniales): a new marine thecate dinoflagellate from Korean coastal water**

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A new species of the marine thecate dinoflagellate, *Heterocapsa busanensis* n. sp., was isolated from Yongho Bay off Busan, Korea, established in clonal culture, and used for light and electron microscopic examination of cell morphology. Cells were ellipsoid in shape, composed of a conical episome and a rounded hyposome, and exhibited thecal plate arrangement (Po, cp, X, 5', 3a, 7", 6c, 5s, 5"', 2"") consistent with most other *Heterocapsa* species. A large elongate nucleus was positioned on the left side of the cell, while a single reticulate chloroplast was located peripherally, with a single spherical pyrenoid situated in the episome or near the cingulum and surrounded by a starch sheath. Overall, morphological features of *H. busanensis* were very similar to those of *H. arctica*. TEM examination of negatively stained specimens revealed the organic body scales of *H. busanensis* to have three-dimensional fine structure different from all other *Heterocapsa* species. Molecular trees based on ITS and LSU rDNA sequences indicated *H. busanensis* to be genetically divergent from all established *Heterocapsa* species for which molecular data are available. Based on ITS rDNA, *H. busanensis* was placed in a separate branch within a clade of *H. horiguchii* strains. Taken together, morphological and molecular features supported the novelty of our Korean *Heterocapsa* species.

O-BPA06 (11:15-11:30)/P-1A01

Ultrastructure and function of the kinetocyst in the centrohelid heliozoon *Raphidiophrys contractilis*

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The kinetocyst of the centrohelid heliozoon *Raphidiophrys contractilis* is located beneath the cell surface of the axopodia and the cell body, and is known to participate in food capturing. The kinetocyst is an extrusive organelle with a size about 300 nm, which contains portentous substances

including a β-1,3-glucan binding protein that are discharged upon capture of food, recognizing and/or paralyzing prey organisms. In this study, we used *Tetrahymena thermophila* as food and examined the process of food capturing and kinetocyst discharge by electron microscopy. A complicated but well-organized filamentous structures in the kinetocyst of isolated and quick-frozen kinetocysts was also observed by both cryo-electron microscopy and electron tomography techniques. The central "core" structures were surrounded by a characteristic ladder-like ring structure that was composed of 96 unit structures, resembling a circular ladder with many steps. Filamentous materials are connected to the ring-like structure and are extended toward both inside and outside. When kinetocyst was ejected during food capturing, the structures contained in the kinetocyst became stretched out like a fishing net, and characteristic ring-like structure was also expanded. Localization of a prey-recognizing β-1,3-glucan binding protein (*Raphidiophrys* MVP) was examined by immuno-EM with an anti-MVP antibody. MVP signals were found to be localized on the fluffy filamentous structure, but not on the cores and the ring-like structure, suggesting that the filamentous materials function as antennae for recognizing and capturing β-1,3-glucan molecules on the prey cell surface.

Oral Session 2 (Day 2)

O-01 (9:50-10:05)

Synapomorphic Signature of Genus *Frontonia* (Ciliophora, Oligohymenophorea, Peniculida) for Inference of Evolution and Phylogeny from Molecular and Morphological Data

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The small subunit (SSU) rDNA secondary structure can explain the relatedness between problematic taxa and provide an independent clue to support morphological classification and molecular phylogeny. Genus *Frontonia* is one of the confusing taxa within Ciliophora because the relationship within genus *Frontonia* and related genera remain unclear. The tree topology based on SSU rDNA sequence showed previously that some members of genus *Frontonia* had more close to the members of other genera, i.e. *Paramecium*, *Apofrontonia*, *Stokesia*, and *Disematostoma*, than the rest members of genus *Frontonia*. The trees of SSU rDNA sequences are insufficient to solve the non-monophyly. In this study, the secondary structure of SSU rDNA was applied to analyze relationships within the members of the genus *Frontonia* and related genera. The secondary structure of V9 region in SSU rDNA shows significant characters to separate genus *Frontonia* from related genera. Genus *Paramecium*, *Apofrontonia*, *Stokesia*, and *Disematostoma* shared a similar structure of V9 region with two big asymmetrical loop on down part of helix, two small asymmetrical loop and six symmetrical loop on upper part of helix. Their secondary structure of V9 region also has smaller loop on the end of helix compare with secondary structure of V9 region of genus *Frontonia*. Secondary structure of V9 region on Genus *Frontonia* has two model, model I shared by all members of genus *Frontonia* except *F. terricola*, *F. acuminata*, and *F. atra*, these three species shared model II of V9 secondary structure. The differences between these two models are loop structure and number on down part of helix. Besides secondary structure analysis, 25 morphological characters were analyzed, and found that patterns in the structure of peniculi and their row numbers are essential characters for separation genus *Frontonia* and related genera. These characters can explain the unclear relationship of members between genus *Frontonia* and related genera.

O-02 (10:05-10:20)

The nuclear genome of Ochrosphaera neapolitana (Cocolithales, Haptophyta) with a study of

holobiont interaction

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Haptophyte is one of the predominantly distributed phytoplanktons in marine environments. As a primary producer, it plays an important role in a food web. Some species blooms annually in oligotrophic ocean (e.g., *Emiliania huxlyei*), and some species produces toxic compounds (e.g., *Chrysochromulina* spp. or *Prymnesium* spp.). As it has been reported that algae and bacteria impact each other in the natural environment, it is important to investigate the interaction between algae and symbiotic bacteria in a laboratory condition to better understand the interaction. Despite important role in ecocystem of this group, only six draft genomes (three genera, six species) were reported to date. To better understand our knowledge, we sequenced nuclear genome of *Ochrosphaera neapolitana*, which is commonly distributed in the littoral zones of North Atlantic, the Indian Ocean, the Mediterranean Sea and the North Pacific Ocean. The genome size was 176 Mb that encoded 36,585 predicted genes. For the *O. neapolitana* - holobiont interaction, we isolated 22 bacteria followed by co-culturing with *O. neapolitana*. From this co-cultivation test, we detected some bacteria enhance the growth of *O. neapolitana*. In this presentation, we will discuss evolutionary history and haptophyte-bacteria interaction.

O-03 (10:20-10:35)

Revised classification of the Cyanidiophyceae based on plastid genome data with descriptions of three new species

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Cyanidiophyceae is a class of unicellular red algae that thrives in acidic (pH 0.5-3.0), high temperature (50-55°C) and heavy-metal rich extreme environments in volcanic hot springs around world. These algae are primarily photo-autotrophic but some of these species (i.e. *Galdieria sulphuraria*) have mixotrophic growth. And, comparison between their morphological and physiological observation and recent molecular phylogenetic research have conflicts. To better understand its evolutionary history and clarify relationship within the Cyanidiophyceae class, five complete mitochondria and eight plastid genomes (*Cyanidiococcus yangmingshanensis* 8.1.23, *Cyanidium caldarium* ACUF 063, Mesophilic *Cyanidium* sybil cave, Mesophilic *Cyanidium* THAL 104, *Galdieria sulphuraria* SAG 108.79, *Galdieria sulphuraria* DBV 011, *Galdieria phlegrea* ACUF 629 and *Cyanidium* sp. OTU2) were constructed in this study. We compared them in terms of genome characteristic (CDS, GC contents and repeat frequency), gene synteny, different HGT pattern and genome rearrangement pattern among Cyanidiophyceae organelle genomes and their phylogenetic relationship that based on each concatenated organelle gene phylogeny. As a result, we found and propose several generic and genomic evidences of separation between the Galdieriales order and each three families of Cyanidiales order (*Cyanidiaceae,* Cavernicoliaceae*, Cyanidioschyzonaceae*). Based on these organelle genome data, we solve phylogenetic incongruence between species tree and gene tree and propose new classification system of Cyanidiophyceae.

O-04 (10:45-11:00)

Living euglenoids found in traps of an aquatic carnivorous plant, *Utricularia australis*

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Bladderworts (aquatic species of genus *Utricularia*, Letibulariaceae) are carnivorous plants capture microorganisms using suction traps, and get nutrition (e.g., nitrogen) by digesting prays. Lumens of *Utricularia* traps are completely sealed from outside. It has been thought that prey caught in traps die quickly due to low oxygen levels and low pH. However, protists surviving and reproducing in the traps has been reported. There are few studies examining trap contents and protists living in traps of *Utricularia* in Japan. This study aimed to examine the trap contents of *U. australis* the most abundant bladderwort species in the northern Japan, and to determine the identity of the euglenoids living in the traps. We collected *U. australis* from the pond in the water garden near Lake Izunuma (Miyagi Pref.) from 2018 to 2020, examined the contents of the traps of *U. australis* and identified them by the morphological observation under a right microscope. For the analysis of living euglenoids, we extracted total DNA from 3 traps including photosynthetic euglenoids in 2018. We also isolated 6 strains isolated from 5 traps collected in 2019 and extracted total DNA from them. Those samples were used for phylogenetic analysis. A total of 45 genera of Chlorophyta, 7 genera Euglenozoa, 36 genera of other microorganisms were identified from trap contents of *U. australis*. *Pediastrum*, *Scenedesmus* and photosynthetic euglenoids were frequently observed among them. Traps containing large amounts of living *Euglena* were found on several occasions. Furthermore, the presence of living *Euglena* in traps was confirmed at several other sampling sites. As a result of phylogenetic analysis for two plastid rRNA regions of *Euglena*, samples from 3 traps collected in 2018 were closely related to *E. hiemalis*, and 6 strains collected in 2019 were closely related to *E. gracilis*. Trap-fluid environment of *U. australis* may be suitable for the presence of euglenoids living in traps. *Euglena* generally can tolerate acidic conditions. In previous studies conducted in Europe, the ratio of living cells among all algal cells trapped by *U. australis* was higher than that of *U. minor*. Other photosynthetic euglenoids such as *Lepocinclis* and *Phacus* were also frequently captured, but were rarely found alive. *E. hiemalis* and *E. gracilis*, which are close to the euglenoids found in this study, are also close to each other. Living euglenoids found from other sites are need to be studied, but some euglenoids may be more likely to inhabit *U. australis*.

O-05 (11:00-11:15)/P-2A06

Validity of protists as live food for feeding brine shrimps larvae

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Freshly hatched fish and small crustaceans feed on small living organisms such as protists and bacteria. The purpose of this study was to search for protists suitable as live baits for the larvae of these animals. Brine shrimp (*Artemia salina*) larvae grow large enough to eat a common artificial diet in about a week. However, if they are not fed with proper food immediately after hatching, they die within a few days. Therefore, several kinds of protists were fed to the freshly hatched brine shrimp for 10 days, and the most suitable protist for the larvae was searched for based on the survival rate and growth rate of the brine shrimp. The protists used as baits were *Tetrahymena pyriformis* (ciliate), *Euglena gracilis* (Excavata), and *Chlorogonium capillatum* and *Chlorella variabilis* (Archaeplastida). Microscopic observation of the inside of the digestive tract showed that all of these protists were small enough to be predated on by the brine shrimp from the second day after hatching. As a result, this study showed that *Chlorogonium capillatum* was the best in terms of both survival rate and growth rate and was suitable as a diet for brine shrimp larvae. In addition, this study found that the survival rate of brine shrimp was significantly reduced when *Euglena gracilis* was given.

Poster Presentations

Day 1, Hall A (Structure 1, Environment, Simulation)

P-1A01 [English, Chinese] See O-BPA06

Ultrastructure and function of the kinetocyst in the centrohelid heliozoon *Raphidiophrys contractilis*

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P-1A02 [English, Japanese, Chinese]

Involvement of mitochondria in *Chlorella-Paramecium bursaria* endosymbiosis

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Peri-algal vacuole membrane (PVM) is regarded to be important for symbiosis in *Paramecium bursaria*. The PVM is originally derived from the phagosome membrane when symbiotic *Chlorella* is taken up by the host and always surrounds the symbiont even after the endosymbiosis is completely established. Symbiotic *Chlorella* cells are connected to the inner surface of the host *P. bursaria* cell cortex. Recent studies have shown that this connection is not direct but is mediated by a number of mitochondria rigidly attached to PVMs. Mitochondria are also densely attached to the cytoplasmic side of the cell surface of *P. bursaria*, and connections between adjacent mitochondria can also be found. A structural network is thus formed by these multiple mitochondria. PVMs are incorporated into this network, thereby constructing the cell surface layer of *P. bursaria*. In other words, mitochondria bind to PVMs, to the cell surface, and also to other mitochondria. The symbiotic relationship between *P. bursaria* and *Chlorella* is disrupted by treatment of the cells with cycloheximide (CHX); CHX treatment causes symbiotic *Chlorella* to detach from the cell surface of host cells and subsequently undergo intracellular digestion and disappear. As a result, *P. bursaria* cells lose their symbiotic *Chlorella*, finally forming "white" aposymbiotic cells. In this study, we investigated which of the mitochondrial-mediated connections (i.e., with the PVM, with the cell surface, or between mitochondria) are released by CHX treatment. Since *P. bursaria* mitochondria can be visualized by high-magnification DIC microscopy, mitochondrial dynamics were analyzed by video microscopy to examine the effects of CHX treatment. The results showed that, in control cells, mitochondria were firmly anchored to the cell surface and rarely detached or migrated. This situation was not changed by CHX treatment. In contrast, mitochondria attached to PVMs were strongly fixed to PVMs in control cells and showed no movement, but CHX treatment caused them to migrate heavily along the surface of PVMs and eventually detached from there. These results indicate that the detachment of mitochondria from the cell surface by CHX treatment is most likely due to reduced coupling between mitochondria and PVMs, which strongly suggests that the coupling between mitochondria and PVMs is important for the maintenance of the symbiosis.

P-1A03 [English, Japanese]

Cell division and conjugation processes in *Stentor pyriformis*

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Many aquatic organisms live in symbioses with photosynthetic algae. *Stentor pyriformis* contain green-algal symbionts within their cytoplasm. The habitats of *S. pyriformis* in Japan is restricted to only a few ponds and lakes in the Tohoku region. Of note, the detailed process of cell division and conjugation in *S. pyriformis* is unknown. In this study, we stained the nuclei of *S. pyriformis* and were able to observe the number of macronuclei, their division, cytokinesis, as well as the macronuclei in the process of conjugation. The number of macronuclei varied from 1 to 9. In the context of artificial

cultures, the most prevalent number of macronuclei was 4 and 5. Additionally, with respect to the division of the macronucleus, amitosis was seen, paralleling the observed in *Paramecium*. This said, the relationship between macronuclei division and cell division was not assessed in this study. On the other hand, cytokinesis occurred as transverse division, once more, similar to that in *Paramecium* and *Stentor coeruleus*; of note, we observed that a new membranellar band and oral pouch was formed near the division groove. This is a similar characteristic of *S. coeruleus*[1)]. In addition, conjugation was also studied in the context of artificial cultures. Two conjugating pairs mated for more than 72 hours; after this time, the macronuclei were stained with SYBR Green and observed with confocal laser scanning microscopy. Remarkably, the algal endosymbionts were aggregated in the center of the cells.

1) Tartar, V. 1961. The Biology of Stentor. PERGAMON PRESS, Oxford, pp.67-75.

P-1A04 [English, Korean]

Detection and quantification of the toxic marine dinoflagellate *Karenia papilionacea* using real-time qPCR assay in Korean coastal waters.

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Karenia papilionacea is known to produce a brevetoxin which has negative effect on human health. Often, *K. papilionacea* has been mistakenly identified as *K. brevis* in which they look very similar under bright field microscopy. In this study, we designed a set of primers specific to *K. papilonacea* within internal transcribed spacer (ITS) region and developed a real-time quantitative PCR (qPCR) method to detect *K. papilionacea* in Korean coastal waters. Standard curve was established with the threshold cycle (Ct) values against cell number of *K. papilionacea*. A linear relationship between the Ct and the log of cell number was always demonstrated (R^2>0.98) for all standard curves. The effeciency of the reaction (E) calculated by the formula: $E=10^{(1/m)}-1$ (where m is the slope of the standard curve: -3.295) was high. Field samples were collected in 17 stations along Korean coasts during September 2017. Using these assays, *K. papilionacea* was detected in 5 stations, ranged from 1.9 to 1938 cells/L with the maximum value at Bangjukpo harbor of Yeosu, Korea. The qPCR amplification products were sequenced and determined to be highly similar to *K. papilionacea*. Using the qPCR assay developed in this study, further monitoring and research are needed to evaluate the proliferation of *Karenia papilionacea* which contains a neurotoxin effects on humans.

P-1A05 [English, Korean] See O-BPA05

***Heterocapsa busanensis* n. sp. (Dinophyceae, Peridiniales): a new marine thecate dinoflagellate from Korean coastal water**

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P-1A06 [English, Japanese]

The mechanism of swimming ciliates, *Tetrahymena pyriformis*, resisting the flow

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The swimming unicellular organisms living in freshwater need to resist currents in the environment. If such organisms are unable to resist environmental currents, they cannot survive for long periods of time because their habitat changes with the flow and they cannot remain in the same

environment. *Paramecium* is well known as a swimming unicellular organism, and it was reported in 1904 that this organism swims upstream of a flow. However, since this report, there have been no reports on this kind of rheotaxis behavior of swimming ciliates. We aimed to investigate this mechanism in detail using *Tetrahymena pyriformis*. By observing the behavior of *T. pyriformis* in a flow field using a microfluidic device, we found that these cells swim against the flow near the wall. To investigate this mechanism, we performed numerical fluid calculations using the squirmer model, a fluid model commonly used to explain microorganism behavior. The movement of ciliate-like force-generating particles in a flow field was investigated, and no positive runnings to the flow near the wall were found. In other words, it was impossible to explain this behavior with existing models. We visualized the moving apparatuses in the flow field to solve this problem and found that the ciliary motion was suppressed near the wall. Therefore, we simulated the behavior by the squirmer model considering this condition and found that positive rheotaxis was realized near the wall when the suppression of cilia strike near the wall and the shape of the tetrahymena-like cells was taken into account. This suggests that the rheotaxis, which is essential for the survival of *T. pyriformis*, is realized by two factors: cell shape and inhibition of ciliation near the wall.

P-1A07 [English, Japanese]

The physical mechanism of behavioral change in the ciliate, *Stentor coeruleus* in narrow areas.

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A kind of ciliates, *Stentor coeruleus* has mainly three types of shape and two behaviors. During swimming, *S. coeruleus* shows a stable cone shape. Then the posterior region of the cell gradually elongates and anchors to bottom sediments or duckweed. Adhesive trumpet-shaped *Stentor* feeds bacteria using alimentary vortex caused by ciliary beating at the oral apparatus. When a mechanical stimulation is added to the trumpet-shaped or cone-shaped cells, *Stentor* exhibits all-or-none contraction, and it becomes droplet shape. However, these transitions haven't been studied, and the cause of the transition from cone to trumpet is not clear. Adhesive *Stentor* is observed at intricate place in nature and culture condition. Moreover, the cells form colonies. From these observations, it seems that *Stentor coeruleus* searches a narrow area during swimming, and then the behavior turns to adhesion there. Thus, we considered that *Stentor* prefers a narrow area. So, we report three topics, measurements of cellular length and swimming speed, a hydrodynamic model of the alimentary vortex, and spatial dependence of the swimming behavior. Firstly, we introduce that *S. coeruleus* shows switching behavior from swimming to adhesion with the shape transition from cone to trumpet. During the transition, swimming speed has a negative correlation to the body length. The increase of effective body surface area induces the deceleration due to hydrodynamic interaction. Thus, the shape transition contributes to the behavioral change. Secondly, the flow around the cell caused by ciliary beating is affected by the environmental wall. It is explained by a two-dimensional simple hydrodynamic model. The result indicates that spatial information around the cell translated spatial variation of velocity, shear flow. Thirdly, the swimming behavior also depends on the area around the cell. When *S. coeruleus* enters a narrow area during swimming, the cell frequently turns and stays there. From these results, we discuss our hypothesis and the possibility of spatial recognition in terms of hydrodynamics.

Day 1, Hall B (Biochemistry, Culture, Practical study)

P-1B01 [English, Japanese]

Excystment-inducing effect of soil extract in terrestrial ciliated protozoan *Colpoda cucullus*

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Cryptobiotic resting cysts enable some protists to survive under stressful conditions such as starvation and dehydration. The terrestrial ciliated protozoan *Colpoda cucullus* inhabits soil by forming a resting cyst. When habitat conditions become favorable (e.g., by rainfall), the vegetative cell of *C. cucullus* emerges from the cyst, known as excystment. *C. cucullus* culture has been established in our laboratory, where excystment is routinely induced with the addition of cereal infusion to cysts. However, the critical element that induces *C. cucullus* excystment in natural environments remains unknown. A water-soluble substance contained in soil is thought to be a candidate excystment-inducing factor, but the soil component that induces excystment remains unelucidated. Therefore, this study investigated the effect of soil extract on both wet and dried *C. cucullus* cysts to identify the environmental component that triggers the excystment process. The rate of excystment was 79% ± 3% when soil extract was added to wet cysts of *C. cucullus*, which was comparable with that of the control culture medium (74% ± 3%). Moreover, the excystment-inducing effect of the soil extract on wet cysts depended on the soil extract concentrations. Although the excystment-inducing effect was lower, similar results were obtained from the experiments using dried cysts, where excystment was induced by adding the soil extract in a concentration-dependent manner, and the maximum excystment rate was 46% ± 5%. We performed inductively coupled plasma mass spectrometry to identify and measure the elements necessary for excystment induction in *C. cucullus*. Our results revealed the presence of Si, Ca, Al, K, Fe, Na, and Mg in the soil extract, and Si was prominent among the elements detected (26.7 mg/L). However, these elements did not induce excystment in *C. cucullus* at all. Thus, we expected that in natural environments, the vegetative cells of *C. cucullus* emerge from cysts by sensing certain water-soluble organic substances in the soil extract.

P-1B02 [English, Japanese]

Mechanism of temperature-induced encystment in the ciliated protozoan *Colpoda cucullus*

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We had earlier demonstrated that temperature stimulation induced encystment in the terrestrial ciliated protozoan *Colpoda cucullus*. When the temperature of culture medium was increased, a vegetative cell of *C. cucullus* transformed immediately into a resting cyst. During this encystment process, Ca^{2+} that was stored in vesicles was released to the entire cytoplasm within a minute, thereby increasing the intracellular Ca^{2+} concentration. However, the molecular mechanism underlying Ca^{2+} release from vesicles after temperature stimulation yet remains unexplored. Therefore, we conducted the present study to investigate the existence of a transient receptor potential cation channel, subfamily V, member 1 (TRPV1), known as a temperature receptor in mammals, and its involvement in the molecular mechanism underlying Ca^{2+} release during the process of temperature-induced encystment. When temperature stimulation was applied to *C. cucullus*, the encystment was induced, and its rate was 73 ± 5%. In contrast, in the presence of a TRPV1 inhibitor (capsazepine, 5 μM), the encystment was significantly suppressed, and its rate was 14 ± 6%. Ca^{2+} imaging analysis with Fura-2 demonstrated that the temperature stimulation-induced Ca^{2+} release from vesicles was suppressed by the TRPV1 inhibitor. Western blot analysis with an anti-TRPV1 antibody (Alomone Labs, ACC-030) clearly revealed the expression of TRPV1 in the vegetative cells of *C. cucullus*. These results indicated that TRPV1 was involved in the Ca^{2+} dynamics after the temperature stimulation. Encystment was induced after the temperature stimulation (84 ± 6%) even

when the external liquid was replaced by ultrapure water (ion-free), indicating that Ca^{2+} influx was not necessary. Moreover, a ryanodine receptor (RyR) inhibitor (dantrolene, 10 µM) had no effect on the temperature stimulation-induced encystment (78 ± 6%), indicating that calcium-induced calcium release (CICR) did not occur after the temperature stimulation. Immunoelectron microscopy with the anti-TRPV1 antibody demonstrated that TRPV1 was localized not on the cell membrane but on the vesicle membrane. Based on these results, we concluded that when temperature stimulation was applied to vegetative cells of *C. cucullus*, Ca^{2+} that was stored in vesicles was released to the cytoplasm through TRPV1 localizing on the vesicle membrane, thus increasing the intracellular Ca^{2+} concentration followed by the formation of resting cysts.

P-1B03 [English, Japanese]

Semi-permeability assay of *Cryptosporidium* oocyst wall using saturated sodium chloride solution

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Cryptosporidium is a globally ubiquitous protozoa that infects the vertebrate digestive system. Especially in the case of water-borne diseases caused by tap water, it is important to take preventive measures. Although microscopic observation is usually done in order to detect this parasite and assess the risk of tap water transmission, it is valuable to detect whether it is a species that infects humans and also the viability of oocysts. Genetic research can tell about the species. On the other hand, as methods for determining the viability of oocysts, vital die (DAPI/PI), excystation test, mouse infection test, etc.(1,2) have been reported. For environmental samples such as river water, methods other than DAPI/PI are not practical due to the limited number of oocysts detection. This study is focused on the morphological changes of oocysts in saturated NaCl solution caused by the osmotic pressure difference between inside and outside of the oocysts. As a first step of the experiment, the oocyst was heat-treated, suspended in saturated NaCl, and its morphology was observed. In the control before heat treatment, the oocysts suspended in saturated NaCl solution were crushed However, the oocysts treated at 99°C for 10 minutes were not crushed even in saturated NaCl solution. Some of the oocysts treated at medium temperature were however, crushed. Next, fresh oocysts were stored at temperatures 20°C and 5°C and oocyst morphology in saturated NaCl solution was observed periodically. There was little change in the number of oocysts observed when stored at 5 °C but the number of crushed oocysts gradually decreased when stored at 20°C. Why are oocysts resistant to disinfectants and environmental pressures? This may be because the oocyst wall does not allow to pass even small ions other than water. The oocysts crushed in saturated NaCl solution which oocyst wall sustains semi-permeability are considered to be alive. This semi-permeability assay will be useful to assess the viability of individual oocysts under microscopic observations.

References

(1) Campbell, A.T., Robertson, L.J., Smith, H.V., (1992) Viability of *Cryptosporidium parvum* oocysts: Correlation of in vitro excystation with inclusion or exclusion of fluorogenic vital dyes. Journal of Applied Environmental Microbiology. 58:3488-3493.

(2) Jenkins, M. B., L. J. Anguish, D. D. Bowman, M. J. Walker, W. C. Ghiorse. 1997. Assessment of a dye permeability assay for determination of inactivation rates of *Cryptosporidium parvum* oocysts. Applied Environmental Microbiology 63:3844-3850.

P-1B04 [English, Japanese]

Effects of components in culture medium on growth and oil production of the marine thraustochytrid *Aurantiochytrium limacinum*

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Introduction: *Aurantiochytrium limacinum* is a marine microorganism in the group labyrinthula, Stramenopiles. This species has been the focus of attention because it has the ability to metabolize organic matter and produce and accumulate oil droplets. In this study, our aim was to optimize culture conditions for a higher yield of oil droplets. Thus, we investigated the effect of various components in culture medium on individual cell growth and oil droplet yield.
Materials and Methods: *A. limacinum* was cultured in a culture medium containing organic matter such as glucose, polypeptone, and yeast extract in 50% artificial seawater (ASW).In addition to ASW, we used three different types of seawater collected from Muroto, Kochi, Japan: deep seawater (DSW), surface seawater (SSW) and underground seawater (USW). To examine the effect of different seawater on cell growth, we reduced the amount of organic matter in the culture medium by half. The number of cells in the culture was determined using a spectrometer with optical density measurements at 650 nm. The number of cells and oil droplets stained with Nile red were estimated from 20 randomly selected cells in a micrograph. The organic composition of culture medium was modified to identify effective organic matter for oil droplet formation.
Results: *A. limacinum* grew well regardless of the type of seawater in culture medium. Although there was no significant difference among the types of seawater, the number of cells in the ASW-based medium tended to be higher than in the other seawater-based media. Conversely, the volume of oil droplets in cells cultured in DSW-based medium was higher than in other media. These results indicate that seawater type affects oil droplets formation. In the experiment in which organic matter in culture medium was modified, the number of cells increased, but the volume of the oil droplets did not. This indicates that the complete set of organic matter was essential for oil droplets formation.
Conclusions: The cell growth and oil production of *A. limacinum* might be improved by modifying the type of seawater and composition of organic matter in the culture medium.

P-1B05 [English, Korean]

Diversity of periphytic ciliates on vessel surface and effects of antifouling paint from the surface on the ciliate community

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Periphyton on vessel surface has a potential risk on endemic ecosystems through moving habitats on transportation routes. As ciliates can live on various natural and artificial substrates, they were targeted to investigate vessel biofouling organisms in this study. To know diversity of periphytic ciliates, biofilms were sampled from the surface of five vessels, and then ciliate species in the samples were identified by molecular analyses as well as microscopic observations of living and silver-impregnated cells. To monitor effects of antifouling biocidal agents painted on the vessel surface, non-treated and antifouling coated (Intersmooth 7475 Si) plates were deployed in a nearshore water for 7 weeks, and then ciliate species succession on the plates was weekly tracked. A periphytic ciliate species, *Diophrys appendiculata*, was isolated from the plates and cultured to be established as a toxicity test species for antifouling biocide Sea-Nine 211. Fourteen species of periphytic ciliates were found from the samples of vessel surface. Ciliate fauna was different on the non-treated and antifouling coated plates for earlier 4 weeks. *Aspidisca leptaspis* and *D. appendiculata* were attached on the non-treated plates while *Euplotes balteatus* on the paint treated plates. *D. appendiculata* exhibited toxic responses to Sea-nine 211 as follows; very low mortality at 1-250 ppb concentration range, encystment of ciliate cells at 500-1000 ppb range, and 100% mortality within 5 minutes at 10,000 ppb. Future studies need to address in detail survival and invasive strategies of periphytic ciliates on vessel surface.

P-1B06 [English, Japanese]

Culture and observation of green *Amoeba* sp. collected from Kamo-gawa River (Kyoto, Japan)

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In March 2020, we collected amoebae from Kamo-gawa River (Kyoto, Japan). The amoebae were morphologically *Amoeba* sp. (Class Tubulinea, Order Euamoebida), but the *Amoeba* cells had endosymbiotic green algae (zoochlorellae). In the *Amoeba* cells, one zoochlorella was contained in the single vesicle, which was the typical mode of endosymbiosis of zoochlorellae in freshwater protozoa (e.g. *Paramecium bursaria*). Besides, zoochlorellae were flowing along with the cytoplasmic streaming of the *Amoeba* cells. The green *Amoeba* could be cultured in Volvic with brown rice at 20°C under constant light conditions. Naked amoebae of Euamoebida are generally sensitive to the light and require small protists as foods. However, the green *Amoeba* could be survived under the light and did not require such food. Therefore, the green *Amoeba* might have light tolerance and utilize photosynthetic products of zoochlorellae as a nutrient source. As a naked amoeba of Tubulinea with zoochlorellae, *Parachaos zoochlorellae* was reported and described. However, except for *P. zoochlorellae*, naked amoebae of Tubulinea with zoochlorellae are little known. we would like to make it available as a model strain of *Amoeba* with zoochlorellae. As an amoeba with zoochlorellae, *Mayorella viridis* (Class Discosea, Order Dermamoebida) is well-known, and we cultured several *M. viridis* strains. The culture method of *M. viridis* were the same as that of the green *Amoeba*. we would like to discuss the green *Amoeba*, comparing with the characteristics of *M. viridis* and other colorless amoebae. In this study, the culture and observation of green *Amoeba* were conducted at home.

Day 2, Hall A (Structure 2, Taxonomy)

P-2A01 [English, Japanese]

Skeleton binding protein 1 (SBP1) of *Plasmodium falciparum* is localized in Maurer's cleft of the gametocyte infected erythrocyte

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Malaria is a mosquito-transmitted infectious disease caused by *Plasmodium*. During the intraerythrocytic stage is divided into ""asexual stage"" and ""gametocyte stage"". In malaria patient, immature asexual stage and mature gametocyte stage appear in the peripheral blood. During mature asexual stage, parasite adhesion molecules exported to the erythrocyte membrane and adhere to vascular endothelial cells. In the gametocyte stage, the immature stage adheres to the bone marrow, spleen, and other tissues, but mature stage, the adhesion breaks off and the cells are released in the peripheral blood. Thus, infected erythrocytes exhibit different adhesiveness during the asexual and gametocyte stages. This difference in adhesion considers that due to modification of the infected erythrocyte surface by protozoan proteins. In this study, we focused on the membrane structure called Maurer's cleft, which is involved during transport of the proteins to the erythrocyte surface. We investigate the distribution of Maurer's cleft in the gametocyte stage by indirect immuno-fluorescence assay (IFA) using polyclonal antibody of Maurer's cleft marker protein, Skeleton binding protein 1 (SBP1). By IFA using anti-SBP1 antibodies signal was detected as a patchy pattern in the cytoplasm of erythrocytes parasitized with immature gametocytes and light spots unevenly distributed near the membrane of mature gametocyte infected erythrocyte. SBP1 is involved in the transport of the adhesion molecule (PfEMP1) during asexual stage. These results suggest that SBP1 might be involved in the transport of adhesion molecules during gametocyte stage.

P-2A02 [English, Japanese]

A simple and quick method to prepare biological specimens for scanning electron microscopy by an ionic liquid

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We present a quick and easy technique to prepare a biological specimen for scanning electron microscopy (SEM) using ionic liquids (ILs). ILs are liquid salts with an electronic conductivity and extremely low vapor pressure. Because of these physical properties ILs have been considered useful for SEM sample preparation. In fact some applications of ILs to biological specimens have been reported so far, however, applications were limited to mainly large samples such as pollens and protists. High viscosity of ILs prevents formation of a thin layer on a sample surface, resulting in fine structures buried under ILs. To address this, we applied a spin-drying a method often employed in semiconductor field, to remove an excess ILs to facilitate formation of a thin IL layer with a few nm thickness. Consequently, we have successfully observed well preserved fine structures such as eukaryotic flagella under a conventional SEM. Because ILs have an electric conductivity, no metal coating is needed, which allows a quick and easy SEM sample preparations, whereas conventional methods such as critical point drying and t-butylalcohol are time consuming and require special equipment. However, one thing one has to be cautious is that a selection of IL. Because treatments with some ILs introduce structural change in samples, it is recommended that IL-treated samples are evaluated in another way like fluorescence microscopy.

P-2A03 [English, Korean]

A Contribution to Ciliate Diversity with Descriptions of Two Hypersaline Species of Genus *Schmidingerothrix* and Molecular Phylogeny

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Two *Schmidingerothrix* species isolated from hypersaline saltern ponds were identified as *Schmidingerothrix* n. sp. and *S. salinarum* Foissner et al., 2014, respectively. *Schmidingerothrix* n. sp. is mainly characterized by two prominent frontal cirri, absence of cortical granules, and prominently short distinct tail. The Korean population of *S. salinarum* is characterized by body size *in vivo* 122x22 µm on average, oblong to slightly sigmoidal shape, anterior end slightly convex and posterior end always acute (no distinct tail), contractile, highly flexible, and colorless. Adoral zone of membranelles separated by a gap, composed of three frontal membranelles and about 21 ventral membranelles. The right and left marginal rows are composed of 26 and 20 cirri, respectively. Four macronuclear nodules with a slender ellipsoidal shape and two micronuclear nodules with comma or ellipsoidal shape are attached. Compared to the original Portuguese population, Korean population of *S. salinarum* has a bigger size (122x22 µm vs 82x17 µm) and more loose arrangement of cortical granules. The small subunit ribosomal RNA gene sequence of *Schmidingerothrix* n. sp. was newly obtained with nucleotides 1743 bp and GC contents 44.92%. Based on phylogenetic analysis, the new species *Schmidingerothrix* n. sp. is clustered together with*S. salinarum* Foissner et al., 2014 and *S. salina* Shao et al., 2014 with similarity percentages 97.9% and 98%, respectively.

1) Foissner, W. 2012. Eur J Protistol., 48(3): 237-251.
2) Foissner, W., S. Filler, & T. Stoeck. 2014. J Eukaryot Microbiol., 61: 61-74.
3) Shao, C., L. Li, Q. Zhang, W. Song, & H. Berger. 2014. J Eukaryot Microbiol., 61: 371-380.
4) Lu, X., J. Huang, C. Shao, & H. Berger. 2018. Eur J Protistol., 62: 24-42.

P-2A04 [English, Korean]

Morphology and Molecular Phylogeny of Korean Population of *Strongylidium*

***wuhanense* (Ciliophora, Hypotrichia, Strongylidiidae)**

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A species of the genus *Strongylidium* Sterki, 1878, collected from the freshwater pond in Ulsan, Korea, was identified as *S. wuhanense* Luo et al., 2018. It is the first record of this species in Korea. The characterization was based on morphology and the molecular phylogeny of small subunit ribosomal RNA (SSU rRNA) gene sequence. The morphological characteristics, based on observations of live specimens and protagol impregnated preparations, are as following: body size in vivo 96 - 144 x 23 - 45 µm, fusiform outline, anterior part rounded and tapered in the posterior with a prominent tail. Cortical granules spherical and yellowish, numerous, and irregular arrangements. An inconspicuous gap dividing the adoral zone into two parts, distal and proximal parts comprising six and 17 membranelles, respectively; three frontal, one frontoventral, one buccal, one postoral ventral cirri; left and right marginal cirri on average 30 and 27, respectively; two long and oblique cirral rows on the ventral surface, with average 27 cirri in left ventral row, and 25 cirri in right row; three dorsal kineties and three caudal cirri. Multiple macronuclear nodules average 18 and two micronuclei. One contractile vacuole in left margin of body. The Korean population has some differences from the original Chinese population of this species: smaller body size (96 - 144 x 23 - 45 µm vs. 135 - 200 x 40 - 60 µm), the color of cortical granules (yellowish vs. colorless), adoral membranellar number of the distal part (six vs. five to nine). The topology of phylogenetic trees based on SSU rRNA gene sequence indicate that Korean population of *Strongylidium wuhanense* clustered with its congeners with full nodal support and nested together Chinese population with nodal support, and 99.94% of sequence similarity with 1 nucleotide difference.

P-2A05 [English, Korean]

A New Species Candidate of Pleurostomatid Ciliate, *Kentrophyllum* n. sp. (Ciliophora: Litostomatea: Pleurostomatida) Based on Morphology and Molecular Phylogeny

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A new species candidate of the genus *Kentrophyllum* Petz et al., 1995 was isolated from a freshwater pond in Haenam, South Korea, and analyzed its morphology (live observation and silver impregnation methods) and molecular phylogeny (SSU rDNA gene sequences). The new species candidate of *Kentrophyllum* is characterized morphologically by a leaf-shaped body without a conspicuous neck-like region, body size about 118 x 54 µm in vivo. The right side of the body is flattened while the left side is vaulted. It has two ellipsoidal macronuclear nodules without observable micronucleus and 17-33 left and 17-24 right somatic kineties. Two sutures present on both sides of body. Needle-like extrusomes are scattered in the cytoplasm and distributed along the entire margin of the cell, except the oral area. In contrast, the spine and wart are absent around the margin of the cell. One peripheral kinety consists of dikinetids and forms a complete circle around the margin of the cell. One contractile vacuole with satellite vacuoles around it located on the posterior dorsal side of the body. The SSU rDNA sequence of *Kentrophyllum* n. sp. was newly obtained with nucleotides 1649 bp and GC contents 42%. Based on the SSU rDNA sequence trees, systematic position of new species was inferred that *Kentrophyllum* n. sp. clustered together with its congeners but branched off separately from its congeners. The new species candidate shows 97.93%-97.01% of sequence similarities, and 34-46 nucleotide differences compared with its congeners.

P-2A06 [English, Japanese] See O-05

Validity of protists as live food for feeding brine shrimps larvae

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P-2A07 [English] See S-02

Interaction between centrohelid and actinophryid heliozoans in co-culture

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Day 2, Hall B (Motility, NGS analysis)

P-2B01 [English, Japanese]

Behavioral analysis and genome editing related to galvanotaxis of *Paramecium* in the microcurrent state

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Paramecium galvanotaxis is defined by the property that when a voltage is applied to a swimming paramecium, it swims forward and heads toward the cathode. We considered that the galvanotaxis depends on the magnitude of the current flowing through the solution, and investigated the galvanotaxis properties in deionized water with high electrical resistance. As a result, clear galvanotaxis was confirmed even in deionized water. Therefore, we investigated the factors that cause galvanotaxis by comparing swimming behavior in deionized water and a solution containing calcium and potassium ions (standard solution). We measured following three points: 1) the voltage at which galvanotaxis begins, 2) the voltage at which almost all cells show galvanotaxis, and 3) the swimming speed during the galvanotaxis. The taxis of *Paramecium* was clearly different between the standard solution and the deionized water. 1) The voltage at which galvanotaxis begins was lower in the deionized water. 2) The voltage at which all cells show taxis was lower in deionized water. 3) The swimming speed during the galvanotaxis was nearly twice as fast in deionized water. Next, we investigated the possibility that membrane voltage-gated ion channels are involved because galvanotaxis is caused by changes in ciliary movement. In this study, genome editing was performed using CRISPR-Cas9, which targets a membrane voltage-gated calcium channel gene. As a result, we obtained clones showing behavioral changes that occur when the activity of membrane voltage-gated calcium channels temporarily decreases. The galvanotaxis of these clones were shifted higher, with 1) the galvanotaxis starting voltage, and 2) the voltage at which all cells exhibited galvanotaxis. Furthermore, 3) the swimming speed during galvanotaxis was also faster than that of the untreated clones. These results suggest that *Paramecium* galvanotaxis may have a mechanism that responds not only to ions, but also to electron action that does not pass through voltage-gated ion channels.

P-2B02 [English, Japanese]

Analysis of cell behavior under hypoxic conditions in *Paramecium bursaria*

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The mechanism of the hypoxic response is well known in multicellular animals and plants, but it is not yet clear in unicellular protists. *Paramecium bursaria* is a unicellular protist, with *Chlorella* living symbiotically within its cytoplasm. Since symbiotic *Chlorella* is presumed to provide oxygen to host cells under the light condition, I hypothesized that symbiosis with *Chlorella* might confer hypoxia tolerance to *P. bursaria*. When *P. bursaria* was placed under a hypoxic condition in the dark, the swimming rate decreased gradually and after 24 h the swimming stopped completely. In contrast, there was no significant change in swimming speed under the hypoxic light condition. This study

reveals that *P. bursaria*, with its symbiotic chlorella, shows a clear tolerance to hypoxic environments, suggesting that this is one of the powerful survival strategies of this ciliate.

P-2B03 [English, Japanese]

Direct Force Measurement of a Swimming *Volvox* Spheroid by a High-sensitive Optical Lever System

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We report a new experimental system using modified atomic force microscopy to directly measure forces of swimming microorganisms. A cantilever is deflected upon collision of swimming microorganisms. The force generated by a single microorganism can be calculated from the vertical displacement and the spring constant of the cantilever. To evaluate the capability of this system, we measured forces generated by swimming in two *Volvox* species, *V. rousseletii* (~5000 cells) and *V. carteri* (~2000 cells) and found that their forces were 16.2 ± 9.0 nN and 6.6 ± 3.6 nN, respectively, which are relatively higher than that estimated by Stokes' law. This is the first demonstration of direct force measurement generated by swimming microorganisms.

P-2B04 [English, Japanese]

30 Hz Force Production Cycle Observed in a Swimming *Volvox carteri* Spheroid with Metachronal Waves

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Our direct force measurement of a swimming *Volvox carteri* spheroid has demonstrated that the spheroid can produce a force around several nN. The magnitude of the force, however, was not constant, oscillated at 30 Hz frequency, indicating that a swimming spheroid can change its velocity in the same manner as the force. To examine this, we observed the *Volvox* motility at 1 ms time resolution under a bright microscope with a LED illumination. High-speed imaging of swimming *V. carteri* spheroids demonstrated that the velocity oscillates at 30Hz, which well agrees with the results obtained from the fluid dynamics analysis by Goldstein and our direct force measurement. Detail analysis of relation between the flagellar beating and spheroid motility have indicated that the velocity oscillation is associated with the propagation of the flagellar beating along the Anterior-Posterior axis. At the maximum velocity, the flagella at the equatorial zone complete their effective strokes, whereas the flagella at the equator make their recovery strokes at the slowest velocity. Results here are still preliminary, therefore, further analysis is needed to verify our conclusion.

P-2B05 [English, Korean]

Illumina sequencing reveals under explored eukaryotic diversity in hypersaline environments

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Hypersaline environments (>40 ppt) are distributed globally and are attractive locations to investigate halophilic or halotolerant microorganisms. To find out the eukaryotic biodiversity in these extreme habitats using Illumina sequencing with two different primer sets for the V4 and V9 region of 18S rDNA, we collected seven subsamples of hypersaline waters (76 ppt to 300 ppt) from a Eui-Seong solar saltern (Taean, Korea) in April, June and August 2019. The biodiversity pattern of the V4 region along the salinity gradient is similar to that of the V9 region. The dominant eukaryotes belong to previously known ciliates, stramenopiles, *Dunaliella* spp., and *Artemia* spp. in high salinity waters. Interestingly, Heterolobosea (Excavata), which is a predominant group in cultured halophilic or

halotolerant protists, is only detected using the V9 biomarker. The heterolobosean OTUs (Operating taxonomic units) in this study are closely related to the halophilic or halotolerant *Percolomonas, Tulamoeba, Aurem, Euplaesiobystra, Selenaion* and *Pharyngomonas* groups, but some OTUs are related to non-halophilic Heterolobosea. This result suggests that the finding of protists in hyper saline environments is still far from complete. Alternatively, diverse non-halophilic groups have existed in cyst form or probably dead cells. Intriguingly, the highest number of OTUs is observed at 300 ppt, implying that the species richness does not decrease at this salinity. Thus, it is likely that salinity may not be a critical factor affecting the eukaryotic community structure in the saltern.

P-2B06 [English, Korean]

Exploring the benthic eukaryotic diversity at a deep-sea hydrothermal vent (Onnuri Vent Field) in the Indian Ocean based on Illumina sequencing

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The submarine hydrothermal vent is globally distributed on the seafloor in a deep-sea area where commonly produces hot springs. The deep hydrothermal vent communities on the seafloor have been adapted to extreme conditions and may represent a unique biota. However, the deep-sea hydrothermal eukaryote community remains poorly understood. Here, we investigate the eukaryotic community in sediments nearby the Indian Ocean hydrothermal vent known as the Onnuri Vent Field (OVF) using Illumina high-throughput sequencing. Seven subsamples are collected from sediments at >2000 m in depth using the three different sampling tools (TV Grab, Multiple Corers, and Box Corer). Compared with other methods, Multiple Corers appear to be a reliable method because the uncontaminated subsample can be collected. In total, 223 operational taxonomic units (OTUs) and 387 OTUs are detected using the V4 and V9 primer sets, respectively, at station MC1906 (2019 m in depth) nearby the hydrothermal vent. Interestingly, the most abundant sequence reads in both the V4 and V9 regions of 18S rDNA amplicons are the supergroup 'Opisthokonta' (V4: 93%, V9: 62%), and followed by the supergroups 'Rhizaria' and 'Alveolata'. However, the most abundant OTUs from the V4 and V9 primer sets belong to the supergroup 'Alveolata' including ciliates, apicomplexans, and dinoflagellates, but the sequence reads of the Alveolata are relatively low. These patterns of eukaryotes nearby the OVF are substantially different from those in another hydrothermal vent region (station MC1914, 4299 m in depth). In the present study, Opisthokonta is the most abundant supergroup in the OVF. Furthermore, protists are a highly diverse group and may play a critical role in the grazing food web of the OVF.

P-2B07 [English, Korean]

Genetic diversity of marine eukaryotic parasitoids (Alveolata) in Korean coastal water

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Marine Alveolate groups including the parasitoid Syndiniales, are widely distributed with great genetic diversity and abundance in the oceans. They infect and potentially control diverse hosts populations including dinoflagellates, ciliates, radiolarians, fish eggs, fish larvae, and crustaceans. By killing or reducing their hosts, Syndiniales may play a key role in maintaining ecosystem diversity. Despite the role, syndinean parasites remain enigmatic, particularly with respect to their temporal dynamics and parasite-host interactions. Here we employed 18S rRNA metabarcoding to analyze the genetic diversity and seasonal dynamics of Syndinales and network analyses were performed to reveal the interactions between syndinean parasites and hosts dynamics. Surface water samples were weekly collected from May 2018 to April 2019. Samples at three different depths (surface,

chlorophyll maximum, and bottom layers) were weekly collected from August to October 2019 in Yongho Bay of Busan, Korea. A total of 749,734 sequences and 3,241 OTUs were obtained from seasonal and depth samples. Among them, Syndiniales groups formed 8% in total sequences. In particular, they are genetically diverse, accounting for 17% of total OTUs. The groups exhibited higher relative abundance from October to December (5 to 18%) than other months (0.3% to 11%) and highest relative abundance reads (4~32%) in bottom layer then other depth (0.7~17%). In particular, Syndiniales Group I and II showed higher relative abundance and OTU richness than other Alveolate groups. Co-occurrence and network analyses showed 789 nodes and 311,656 edges. Among them, Syndiniales OTUs accounted for 6% nodes and 32% edges, suggesting that they had a lot of associations with other protist OTUs. Syndiniales Group I had significant positive associations with Spumellarida, Collodaria and Dinophyceae, while Syndiniales Group II had significant positive associations with Spumellarida, Pterocystida and Dinophyceae. The proportions, diversity and interactions of syndiniales sequences in microbial eukaryotes communities indicate that they are the important group as well as play potentially key roles in the microbial food webs in Korean coastal water.

Workshop

(More details will be announced on the LINC Biz channel "Young Protistologists Association")

Visualization Workshop:

The Life of Young Protistologists

若き原生生物学者たちの日常

젊은 원생 생물학자들의 일상

年轻原生生物学家的日常

(Day 2: 14:00-17:00)

Organizer: Community of Young Protistologists in JSP*
Facilitators: Syun Echigoya[1], Masashi M. Hayakawa[2] ([1]Hokkaido University, Japan [2]MicroLife Project, Japan)
**young.protistologists.jsp@gmail.com (Community of Young Protistologists in JSP)*

Part 1 (Day 2: 14:00-14:15) Introductory presentations (on Zoom)
Part 2 (Day 2: 14:15-17:00) Worktime (on SpatialChat, Hall A)

We are convinced that Kobe2020 has generated a wide range of research interactions among the participants through various presentations. However, it is quite difficult to get to know the personalities of the researchers in an online conference. So, the community of young protistologists in JSP has come up with a new additional idea. How about expressing our research life in a four-panel comic strip? By using them as materials for discussion, there will be opportunities for mutual exchange. By using illustrations, it will be possible to communicate across generations, positions and languages.

In Part 1, we will use Zoom to explain the project to the participants. There will also be introductions to Japanese and Korean laboratories at this time. In Part 2, you can use SpatialChat to have a conversation within your favorite group.

This project is designed to bring young people, especially students and postdocs, together to interact with each other. However, everyone is welcome to participate in this project, regardless of age or position. Being young is simply a matter of the heart. Let's overcome the language and age barriers and be brave enough to join this project.

Let's take off into a new future with the winds of online.

Kobe2020 では、様々な研究発表を通じて、参加者の皆さんの間に研究面での幅広い交流が生まれました。しかし、オンラインの会議では、研究者たちの人柄まで知ることはなかなか困難です。そこで、日本原生生物学会若手の会（community of young protistologists in JSP）は、新しい追加の企画を考えました。私たちの研究生活を 4 コマ漫画で表現してみてはいかがでしょうか？ それを素材として話し合うことで、お互いの交流のきっかけが生まれるでしょう。イラストレーションで表現することで、世代、立場、言語の垣根を超えたコミュニケーションが可能となります。

Part1 では、Zoom を使って私たちが参加者の皆さんに企画の内容を説明します。また、日本と韓国の研究室の様子を紹介し合うこともできるでしょう。Part2 では、SpatialChat を用いて、お好きなグループの中で会話を楽しんでいただけます。

この企画は、特に学生やポスドクといった若者同士がお互いに交流を深めるために企画されました。しかし、年齢や立場にかかわらず、どなたでもこの企画に参加していただけます。若さとは単に心の問題でしかありません。言葉や年齢の壁を乗り越えて、勇気をもってこの企画に参加してください。
オンラインの風に乗って、新しい未来に向かって飛び出しましょう。

Kobe2020 에서 다양한 연구발표를 통해 참가자 모든 분들의 광범위한 연구 교류가 이루어질 것을 저희들은 확신하고 있습니다. 그러나 온라인에서 이루어지는 학회에서 연구자 개개인들의 성격까지는 알기 어려울 것입니다. 그래서 일본원생생물학회-젊은 원생생물학자들의 모임（community of young protistologists in JSP）에서는 새로운 아이디어를 생각해 내었습니다. 각자의 연구 생활을 4 컷만화로 표현해 보는 것은 어떻습니까? 이를 소재로 서로 이야기한다면 서로 교류할 수 있는 계기가 될 수 있을 것입니다.　일러스트를 사용함으로 인해서 언어, 세대, 직위, 입장을 넘어 소통 할 수 있을 것입니다.
Part1 에서는 Zoom 을 사용하여 참가자 모든 분들에게 이 기획에 대해서 설명하겠습니다. 또한, 일본과 한국의 연구실 소개도 이 시간에 이루어질 것입니다.Part2 에서는 Spatial Chat 을 사용하여 원하는 그룹 내에서 대화를 즐길 수 있습니다.
이 프로그램은, 특히 학생이나 박사후연구원 등 젊은이들끼리 서로 교류를 깊게 하기 위해 기획되었습니다. 그러나, 연령이나 직위에 상관없이 누구라도 이 기획에 참가할 수 있습니다. “젊다”라는 것은 단지 마음의 문제일 뿐입니다. 언어와 연령의 벽을 넘어 용기를 가지고 이 기획에　참가해 주시기를 바랍니다.
온라인의 바람을 타고 새로운 미래로 향해 나아갑시다.

我们相信在 Kobe2020 的各种研究报告会在与会者中形成广泛的研究互动。然而，在在线会议中很难了解研究人员的个性。因此，JSP 的青年原生动物学家们提出了一个新主意。为什么不用四格漫画来表达我们的科研生活呢？把漫画作为一个讨论的媒介，我们得到一个相互交流的机会。通过插图，我们可以跨越世代、立场和语言的障碍进行交流。

在第一部分中，我们将使用 Zoom 向参与者解释我们的项目。在第二部分中，与会者可以使用 SpatialChat 加入有兴趣的群组中并参与交流。

这个项目是为了让年轻人，特别是学生和博士后在一起交流。当然，会议欢迎任何人参与这个项目，不分年龄和职位。青春只是心态的问题。让我们克服语言和年龄的障碍，勇敢地参与到这个项目中来吧。

让我们乘着在线会议的风口，走向新的未来。

Workshop Details —Its overview and our goals—

This workshop consists of two parts. In Part 1, using Zoom, the facilitator will explain the purpose and what we will actually do in the workshop. Details will continue to be uploaded in advance to LinC Biz channel “Young Protistologists Association”.

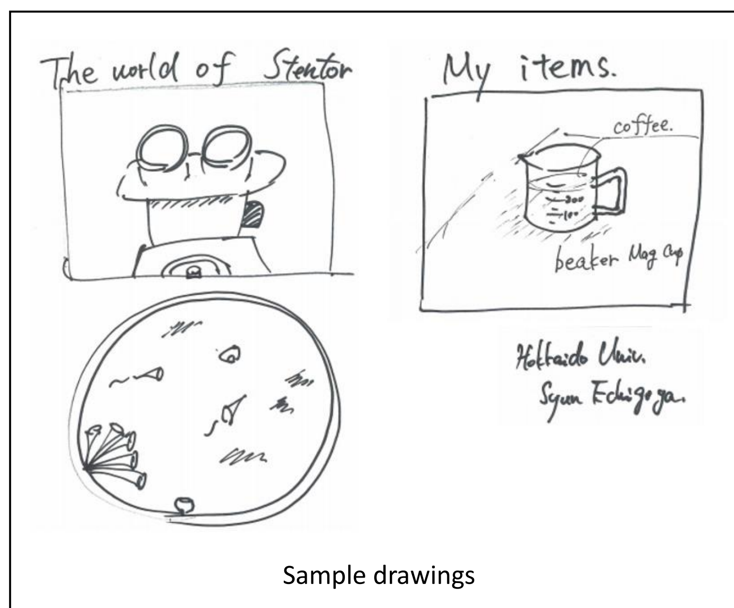
In Part 2, using SpatialChat, which is also used for Poster Session Hall A, the sketches drawn by the participants (see below) will be posted. All participants are divided into small groups of four people each and a group discussion begins.

Before the workshop begins, you will be asked to draw a simple picture of something (see sample drawings below). You can draw, for example, a favorite protist, a unique tool or method in the lab, a picture of a good research result, or even a picture of daily life in the lab or a slice of your personal life. You can draw whatever you want to express yourself best. During the workshop time, upload your drawings and explain them to each other. After the discussion, you will have time to draw again. You can draw a continuation of your drawing, either as a two-, three-, or four-frame comic, or as a more sophisticated single piece of art.

The most important thing for a scientist is to express himself or herself and share it with others. Artwork that visualizes your research life may replace your name card as a good icon or become a signpost to new human relationships.

What you need during the workshop

During the workshop time, you will be asked to upload your drawings to SpatialChat on the spot. Please prepare an environment for this. For example, prepare a piece of paper and a pen, take a picture of what you drew on the paper with your smartphone, and send it to your computer by e-mail. Alternatively, draw as data from the beginning using a drawing application on your smartphone or computer. Prepare in a way that is easy for you to do.



Sample drawings

List of participants by name

(Underlines indicate presenters)

Name	Affiliation	Presentations
AN, Jebak	Kyungpook National University, Korea	P-2B06, P-2B05
ANDO, Motonori	Okayama University, Japan	
ARIKAWA, Mikihiko	Kochi University, Japan	P-1B01, P-1B02, P-1B04
BAEK, Jun Hyeok	Advanced Institute of Convergence Technology, Korea	
BAI, Yang	Ocean University of China, China	O-BPA03
BHATTACHARYA, Debashish	Rutgers University, USA	S-05
BIBI, Rani	University of Ulsan, Korea	O-BPA04
CAHYANI, Novia	University of Ulsan, Korea	P-2A03
CHAN, Ya-Fan	Rutgers University, USA	S-05
CHI, Yong	Ocean University of China, China	
CHO, Chung Hyun	Sungkyunkwan University, Korea	S-05, O-03
CHO, Hyeonshik	Kyungpook National University, Korea	
CHO, Min Ji	Pukyong National University, Korea	P-1A04
CHOI, Hojoon	Pukyong National University, Korea	O-BPA05, P-1A05
CHOI, Jiwon	Sungkyunkwan university, Korea	
CHOI, Jung Min	Korea Institute of Ocean Science & Technology, Korea	P-1B05, P-2B06
CINIGLIA, Claudia	University of Campania, Italy	O-03
DO, Haerim	Kyungpook National University, Korea	
DO, Hye Rim	Kyungpook National University, Korea	P-2B05, P-2B06
DOHRA, Hideo	Shizuoka University, Japan	
ECHIGOYA, Syun	Hokkaido University, Japan	P-1A07
EMOTO, H.	National Institute of Technology, Ube College, Japan	P-2B03
ETTAHI, Khaoula	Sungkyunkwan University, Korea	O-BPA01, S-05
FENG, Xiaochen	Ocean University of China, China	FENG, Xiaochen
FUJISHIMA, Masahiro	Yamaguchi University, Japan	
FUKUDA, Yasuhiro	Tohoku University, Japan	
GABR, Arwa	Rutgers University, USA	S-05
GAPONOVA, Liudmyla Petrivna	National Academy of Sciences of Ukraine, Ukrine	S-02, P-2A07
GRAF, Louis	Sungkyunkwan University, Korea	O-03
HA, Ji-San	Sungkyunkwan University, Korea	S-05, O-02
HAGA, Nobuyuki	Ishinomaki Senshu University, Japan	P-2B01
HARADA, Yuya	Kochi University, Japan	P-1B04, P-1B01, P-1B02
HARUMOTO, Terue	Nara Women's University, Japan	
HASEGAWA, Aoi	Kaimei High School, Japan	P-2B02
HASEGAWA, Yuya	Kochi University, Japan	P-1B01, P-1B02, P-1B04
HAYAKAWA, Asahi	MicroLife Project, Japan	P-1B06
HAYAKAWA, Masashi Mark	MicroLife Project, Japan	P-1B06
HIGUCHI, Shinnosuke	Hiroshima University, Japan	
HIRONO, Masafumi	Hosei University, Japan	
HIROTA, Ryuichi	Hiroshima University, Japan	
HORI, Manabu	Yamaguchi University, Japan	
HORINAGA, K.	National Institute of Technology, Ube College, Japan	P-2B03
HOSHINA, Ryo	Nagahama Institute of Bio-Science and Technology, Japan	S-04
HOSOYA, Hiroshi	Kanagawa University, Japan	
HU Xiaozhong	Ocean University of China, China	O-BPA03
HYUNG, Junho	Advanced Institute of Convergence Technology, Korea	
ICHIKAWA, Masatoshi	Kyoto University, Japan	P-1A06
IIDA, Hitoshi	Chiba Institute of Science, Japan	P-1A03
IKEDA, Kenichi	Kobe University, Japan	
INOUE, Motomu	Kobe University, Japan	P-1B03
IRIKO, Hideyuki	Kobe University, Japan	P-2A01
ISHIDA, Hideki	Shimane University, Japan	
ISHIDA, Masaki	Nara University of Education, Japan	
ISHINO, Tomoko	Ehime University, Japan	P-2A01
ISLAM, MD Shafiqul	Ansar/VDP, Bangladesh	
IWAMOTO, Masaaki	Nihon University, Japan	
IZUMIYAMA, Shinji	National Institute of Infectious Diseases, Japan	P-1B03
JEON, Boo Seong	Chonnam National University, Korea	
JEONG, Dong Hyuk	Kyungpook National University, Korea	P-2B05, P-2B06
JEONG, Hwajung	Korea Institute of Ocean Science & Technology, Korea	P-1B05
JEONG, Yujin	Kyungpook National University, Korea	
JIANG, Limin	Ocean University of China, China	
JO, Jinseong	Chonnam National University, Korea	
JUNG, Minkyoung	Advanced Institute of Convergence Technology, Korea	
KAMIMURA, Ikuyo	Takarazuka Health and Welfare Office, Japan	
KANDA, Koki	Hokkaido University , Japan	
KANG, Jung-Hoon	Korea Institute of Ocean Science and Technology, Korea	P-1B05
KASHIYAMA, Yuichiro	Fukui University of Technology, Japan	PL-02
KATOH, Noriyuki	Kobe University, Japan	
KIM, Dongseok	Sungkyunkwan University, Korea	

KIM, Dongsung	Korea Institute of Ocean Science & Technology, Korea	P-2B06
KIM, Eunjoo	Advanced Institute of Convergence Technology, Korea	
KIM, Min-Jeong	Kyung Hee University, Korea	S-03
KIM, Miran	Chonnam National University, Korea	S-01
KIM, Sungwoo	Advanced Institute of Convergence Technology, Korea	
KIM, Sunju	Pukyong National University, Korea	O-BPA05, P-1A04, P-1A05, P-2B07
KIM, Young Ok	Korea Institute of Ocean Science & Technology, Korea	P-1B05, P-2B06
KIMURA, Yume	Yamagata University, Japan	O-04
KIRIMA, Junya	Asutamu Land Tokushima, Japan	
KITAKAWA, Madoka	Kobe University, Japan	
KOBAYASHI, Fumie	Azabu University, Japan	
KODAMA, Yuuki	Shimane University, Japan	
KOLOSUIK, Andrii	National Academy of Sciences of Ukraine, Ukrine	S-02, P-2A07
KONG, Hyun-Hee	Donga University College of Medicine, Korea	S-03
KRISTANTI, Nanda Dwi	University of Ulsan, Korea	P-2A05
KULAMAHAN, Jerusha	Kobe University, Japan	P-2A01
KUSUOKA, Yasushi	None	
KWON, Choonbong	University of Ulsan, Korea	
LEE, Eun Sun	Advanced Institute of Convergence Technology, Korea	
LEE, Hyeon Been	Kyungpook National University, Korea	P-2B05, P-2B06
LEE, Joon-Baek	JEJU National University, Korea	
LEE, Junmo	Kyungpook National University, Korea	S-05
LEE, Moojoon	Anyang University, Korea	
LEE, Yong Sung	Sungkyunkwan University, Korea	
LHEE, Duckhyun	Sungkyunkwan University, Korea	S-05, O-BPA01, O-02
LI, Ran	Ocean University of China, China	
LI, Tao	Ocean University of China, China	
MA, Mingzhen	Ocean University of China, China	
MAEDA, Marika	Nara Women's University, Japan	
MARUYAMA, Tadashi	Kitasato University, Japan	
MATSUOKA, Tatsuomi	Kochi University, Japan	P-1B01, P-1B02, P-1B04
MINEMIZU, Ryo	Ryo Minemizu Photo Office, Japan	O-BPA02
MITOME, N.	National Institute of Technology, Ube College, Japan	P-2B03
MIWA, Isoji	None, Japan	
MOON, Eun-Kyung	Kyung Hee university School of medicine, Korea	S-03
MORIYA, Shigeharu	RIKEN, Japan	
NAGAMUNE, Kisa	National Institute of Infectious Diseases, Japan	
NAKAGAKI, Toshiyuki	Hokkaido University , Japan	P-1A07
NAKAMURA, Yasuhide	Shimane University, Japan	O-BPA02
NGUYEN, Dung Quoc	University of Ulsan, Korea	P-2A04
NISHIDA, Yuki	Keio University, Japan	O-05, P-2A06
NISHIGAMI, Yukinori	Hokkaido University, Japan	P-1A06, P-1A07
NOSAKA, M.	National Institute of Technology, Ube College, Japan	P-2B04
NOWACK, Eva C. M.	Heinrich-Heine-Universität, Germany	S-05
NUMATA, Osamu	University of Tsukuba, Japan	
OHMURA, Takuya	Max Planck Institute for Terrestrial Microbiology, Germany	P-1A06
OKADA, Kaoru	Tokyo Gakugei University, Japan	
OMODA, Ayaka	Kobe University, Japan	P-2A01
OTA, Takahide	None, Japan	
PARK, Bum Soo	Korea Institute of Ocean Science and Technology, Korea	P-1A04
PARK, Jaeyeon	Advanced Institute of Convergence Technology, Korea	
PARK, Jemin	University of Ulsan, Korea	
PARK, Jong Soo	Kyungpook National University, Korea	PL-01, O-BPA01, P-2B05, P-2B06
PARK, Myung Gil	Chonnam National University, Korea	S-01
PARK, Seung In	Sungkyunkwan University, Korea	O-03
PRICE, Dana C.	Rutgers University, USA	S-05
SAGAWA, Yousuke	Ishinomaki Senshu University, Japan	P-2B01
SAITO, Nobuhiro	Suido-sha Co. Ltd, Japan	O-BPA02
SAKAMOTO, Hirokazu	Indiana University, USA	
SATO, Katsuhiko	Hokkaido University , Japan	P-1A07
SAWADA, Ken	Toho University, Japan	
SHIMABUKURO, Katsuya	National Institute of Technology, Ube College, Japan	S-06, P-2A02, P-2B03, P-2B04
SHIMADA, Yuto	Kochi University, Japan	P-1B02, P-1B01, P-1B04
SHIMANO, Satoshi	Hosei University, Japan	
SHIN, Mann Kyoon	University of Ulsan, Korea	O-BPA04, O-01, P-2A03, P-2A04, P-2A05
SHIN, Myeong Heon	Yonsei University College of Medicine, Korea	
SHIRATORI, Takashi	University of Tsukuba, Japan	
SIMPSON, Alastair G. B.	Dalhousie University, Canada	O-BPA01
SONG, Chihong	National Institute for Physiological Sciences, Japan	
SONG, Wenya	Ocean University of China, China	
SONOBE, Seiji	University of Hyogo, Japan	
STEPHENS, Timothy G.	Rutgers University, USA	S-05
SUEHIRO, Tatsuya	National Institute of Technology, Ube College, Japan	P-2A02, P-2B04
SUETOMO, Yasutaka	Iwakuni Microlife Museum, Japan	
SUGIURA, Mayumi	Nara Women's University, Japan	
SUNG, Ji Yeon	Kyungpook National University, Korea	O-BPA01

SUZAKI, Toshinobu	Kobe University, Japan	S-02, O-BPA06, P-1A01, P-1A02, P-1B03, P-2A06, P-2A07
SUZUKI, Toshikazu	Nagasaki University, Japan	O-BPA03
TABATA, Tetsuya	The University of Tokyo, Japan	
TACHIBANA, Mayumi	Ehime University, Japan	P-2A01
TAGUCHI, Saki	National Institute of Technology, Ube College, Japan	P-2A02
TAKAGI, Yumi	Nara Women's University, Japan	
TAKAHASHI, Kazutaka	The University of Tokyo, Japan	O-BPA02
TAKAHASHI, Shunichi	National Institute for Basic Biology, Japan	
TAKENAKA, Hiroki	Kobe University, Japan	
TODA, Narumi	Hiroshima University, Japan	
TORII, Motomi	Ehime University, Japan	P-2A01
UCHINOMIYA, Kouki	Central Research Institute of Electric Power Industry, Japan	
UEKI, N.	Hosei University, Japan	P-2B03
UEMURA, Naoki	National Institute of Technology, Ube College, Japan	P-2B05, P-2A02
UZMA, Sahr	University of Ulsan, Korea	O-BPA04, P-2A04, P-2A05
WAKABAYASHI, K.	National Institute of Technology, Ube College, Japan	P-2B03
WAKABAYASHI, K.	CLS, Tokyo Tech., Japan	P-2B03
WAKABAYASHI, Kaori	Hiroshima University, Japan	O-BPA02
WAN, Yumeng	Kobe University, Japan	O-BPA06, P-1A01
WANG, Congcong	Ocean University of China, China	
WANG, Rui	Ocean University of China, China	O-BPA03, P-1B07
WARDANI, Ratih Kusuma	University of Ulsan, Korea	O-01, P-2A03
WOO, Sang Yoon	Kyungpook National University, Korea	P-2B05, P-2B06
WU, Tong	Ocean University of China, China	
YABUKI, Akinori	Japan Agency for Marine-Earth Science and Technology, Japan	
YAGITA, Kenji	National Institute of Infectious Diseases, Japan	
YAJIMA, Yuka	Muroran Institute of Technology, Japan	
YAMADA, Kazumasa	Fukui Prefectural University, Japan	
YAMANE, Natsumi	Nara Women's University, Japan	
YAMASAKI, Nozomi	Kobe University, Japan	P-2A01
YANASE, Ryuji	Oxford Brookes University, UK	
YANG, Eun Chan	Korea Institute of Ocean Science and Technology, Korea	O-03
YANG, Xin	Kobe University, Japan	P-1A02
YAZAKI, Euki	RIKEN iTHEMS, Japan	
YIH, Wonho	Kunsan National University, Korea	
YIN, Taeyeon	Advanced Institute of Convergence Technology, Korea	
YOKOYAMA, Jun	Yamagata University, Japan	O-04
YOO, Jiae	Pukyong National University, Korea	P-2B07
YOON, Eunyoung	Advanced Institute of Convergence Technology, Korea	
YOON, Hwan Su	Sungkyunkwan University, Korea	S-05, O-BPA01, O-02, O-03
YOSHIMURA, Chisato	Kobe University, Japan	
ZELZION, Udi	Rutgers University, USA	S-05
ZHANG, Tengyue	Ocean University of China, China	
ZHANGGONG, Aote	Ocean University of China, China	
ZHUANG, Wenbao	Ocean University of China, China	

List of participants by affiliation

Japan

Name	Affiliation
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UCHINOMIYA, Kouki	Central Research Institute of Electric Power Industry
IIDA, Hitoshi	Chiba Institute of Science
WAKABAYASHI, K.	CLS, Tokyo Tech.
YAMADA, Kazumasa	Fukui Prefectural University
KASHIYAMA, Yuichiro	Fukui University of Technology
HIGUCHI, Shinnosuke	Hiroshima University
HIROTA, Ryuichi	Hiroshima University
TODA, Narumi	Hiroshima University
WAKABAYASHI, Kaori	Hiroshima University
ECHIGOYA, Syun	Hokkaido University
NISHIGAMI, Yukinori	Hokkaido University
KANDA, Koki	Hokkaido University
NAKAGAKI, Toshiyuki	Hokkaido University
SATO, Katsuhiko	Hokkaido University
HIRONO, Masafumi	Hosei University
SHIMANO, Satoshi	Hosei University
UEKI, N.	Hosei University
HAGA, Nobuyuki	Ishinomaki Senshu University
SAGAWA, Yousuke	Ishinomaki Senshu University
SUETOMO, Yasutaka	Iwakuni Microlife Museum
YABUKI, Akinori	Japan Agency for Marine-Earth Science and Technology
HASEGAWA, Aoi	Kaimei High School
HOSOYA, Hiroshi	Kanagawa University
NISHIDA, Yuki	Keio University
MARUYAMA, Tadashi	Kitasato University
OMODA, Ayaka	Kobe University
TAKENAKA, Hiroki	Kobe University
YOSHIMURA, Chisato	Kobe University
IKEDA, Kenichi	Kobe University
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Japan Society of Protistology:
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Korean Society of Protistologists:
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