The 4th Asian Congress of Protistology -*i*nternet

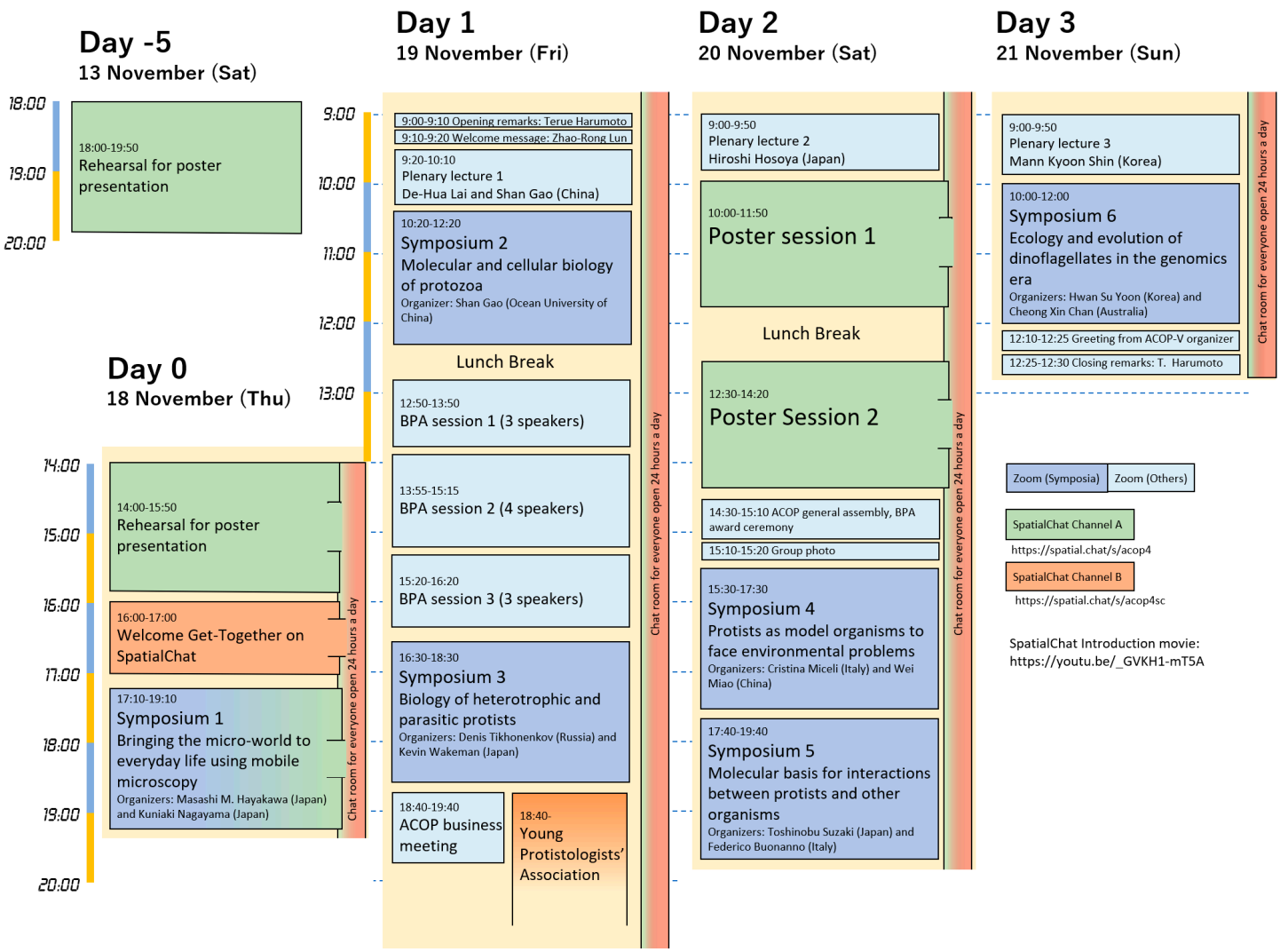
2021, November 19th–21st

Welcome Get-Together and Symposium 1 was held on the 18th.

Nov. 21st, 2021 (Website Ver.)

Conference Timetable

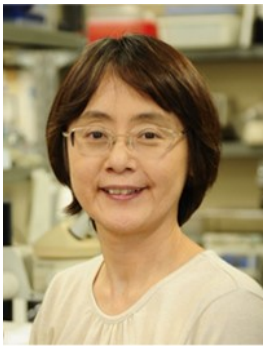
All times are Japan Standard Time (JST, UTC+9 hours).



Zhao-Rong Lun
President of Asian Congress of Protistology
School of Life Sciences, Sun Yat-Sen University, China

Dear Colleagues,

Welcome to the *i*ACOP. The Asian Congress of Protistology (ACOP) is an international conference in Asia for people who are interested in protistology to share results and deepen exchanges with each other. Previous meetings have been held in Jeju, South Korea (2011), Kalyani, India (2014), and Guangzhou, China (2018). Although we will not be able to meet face to face this time due to the pandemic of Covid-19, we can share our research results with the participants via the internet. As a matter of fact, this way is much easier, more effective, and more economic for the participants. I would like to thank our Japanese friends who are working so hard to make this meeting a success. I would also like to thank the presenters who will share their exciting results to our participants. I sincerely hope that all participants will enjoy the meeting and that it will be very successful.



Terue Harumoto
Conference Convener of ACOP-IV
President of Japan Society of Protistology
Nara Women's University, Japan

Dear fellow protistologists,

Welcome to *i*ACOP-IV!
Virtual meetings are not just a substitute for real meetings but have great potential to open up scientific interactions to a larger number of researchers in a more comfortable situation. It is a pity that we cannot invite all of you to the on-site conference in Japan because of the COVID-19 pandemic. However, we have decided to hold the ACOP online. We have received registrations for participation not only from Asia, but also from many other countries. This time, the plenary lectures and symposia will be conducted orally by ZOOM, and the poster sessions will be presented by SpatialChat. Many of you may have not experienced SpatialChat before, but the technology of online conferencing is constantly evolving, and you will probably be surprised how easy it is to use. This kind of poster presentation may become a new form of international conference in the future, which also allow us to meet and discuss personally with the presenters and other participants. All this can be done at your favorite place, sitting in a familiar chair, drinking tea or coffee. Since this is an online conference, anyone can easily join from anywhere in the world. Please join us from anywhere and at any time of your choice.
We hope that this conference will be one of the opportunities to advance research in the field of protistology.

We look forward to your participation in *i*ACOP-IV Online.

Plenary lectures

P1: 19th, 09:20–10:10 (Chair: Zhao-Rong Lun)

P2: 20th, 09:00–09:50 (Chair: Toshinobu Suzaki)

P3: 21st, 09:00–09:50 (Chair: Weibo Song)

Plenary lecture 1 (19th Nov. 09:20–10:10)

P1-1

Trypanosome mitochondrial genome: the mechanism of inheritance

De-Hua Lai[1], Zhao-Rong Lun[1]
[1]: Sun Yat-Sen University, China

Trypanosomes are haemoflagellates found in various vertebrates. They represent a group of early branched eukaryotes, with numbers of diverged characteristics from mammalian cells. The most obvious one is that they contain a specialized mitochondrial genome, termed a kinetoplast. The kinetoplast forms a disc shape of interlocking DNA circles of two types, maxicircles and minicircles. Maxicircle is generally over 20 kb, and encoding some typical and more encrypted mitochondrial genes, which are decrypted only using hundreds of keys from minicircles. Concerning this structure, there are some very interesting questions to be answered. 1) How do they maintain this complex mitochondrial genome? 2) How does this complex mitochondrial genome work? 3) How does this complex mitochondrial genome evolve from and to? To address these questions, the authors investigated the model organisms of *Trypanosoma brucei*, *T. evansi*, *T. equiperdum* and *T. lewisi* and found that the maintenance of kinetoplast was via basal body, and identified novel protein of BBLP. The authors revealed that the transcriptome patterns of kinetoplast, and identified new structure of minicircles. In addition, the authors discribed the distinct features of various kinetoplasts, and proposed evolutionary scenery of kinetoplast. Altogether, kinetoplast is a distinct and complex structural mitochondrial genome, which is also an excellent example to show the diversity of eukaryotic cells and stupidity of designer if presents.

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P1-2

The cell cycle-dependent oscillation of histone methyltransferase TXR1 is implicated in regulating replication-transcription conflicts

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[2]: Department of Molecular and Cellular Medicine, Texas A&M University, USA
[3]: Department of Biochemistry and Molecular Medicine, University of Southern California, USA

With replication origins and transcription cis-regulatory elements frequently in juxtaposition, eukaryotic cells need to coordinate replication and transcription. TXR1 (*Tetrahymena Trithorax*-related 1), the histone H3 lysine 27 monomethyltransferase in *Tetrahymena thermophila*, was reported to cause replication stress in mutation strains. In this study, we analyzed TXR1's role in regulating replication-transcription conflicts. 1) The protein level of TXR1 oscillates during cell cycle and cell cycle was a prerequisite to this oscillation. The active degradation of TXR1 in S-phase is mediated by its interaction with PCNA. 2) Precise regulation of TXR1 level is required for normal DNA replication. Under- and over-expression of *TXR1* lead to DNA damage, but the underlying triggers may be different. 3) Transcription profile was dramatically changed in both Δ*TXR1* and over-expression (TXR1 OE) cells, manifest as transcripts in non-transcribed regions. We propose that TXR1 functions as a switch between replication and transcription.

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Plenary lecture 2 (20th Nov. 09:00–09:50)

P2

Studies of symbiotic association between green paramecia and their symbiotic algae using feedless culture strain of *Paramecium bursaria*

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The green paramecium, *Paramecium bursaria*, is a unicellular protist, which harbors hundreds of symbiotic algae in its body. It is widely known as a research target for eukaryotic cell-cell symbiosis. Many experiments have been conducted and their results have been also reported for the purpose of clarifying phototaxis, photosynthesis of symbiotic algae and cell proliferation, using *Paramecium bursaria*. However, in each researcher, various kinds of microorganisms including bacteria are used as food when culturing the ciliate. Further, lettuce infusion is used as a medium, but the composition of lettuce leaf is not always constant. Due to these multiple reasons, common culture conditions have not been established among *Paramecium bursaria* researchers. This gives a significant problem on the reproducibility of the results of various experiments using *Paramecium bursaria*. Therefore, in our laboratory, it has been investigated whether *Paramecium bursaria* collected from the field can be cultivated without feeding. As a result, a "feedless culture strain (KUNY-2)" was established. This strain was isolated from the field in 2015, cultivated with feeding until 2017, and then has been cultivated without feeding until now. Interestingly, it was revealed that bacteria were always present in the culture medium of KUNY-2, even under the condition that the prey bacteria were not fed. Therefore, the composition of bacteria in *Paramecium bursaria* was analyzed at each time point before and after start of culture without feeding. As a result, it has been clarified that a certain type of bacteria is always detected in the body of *Paramecium bursaria*. Symbiotic algae in *Paramecium bursaria* can be easily isolated from the host and cultured. In addition, by co-culturing this cloned symbiotic algae with the host, the endosymbiotic relationship can be simply re-established. Then, Liquid culture of isolated symbiotic algae was carried out, and bacteria in the symbiotic algae were also examined. As a result, it became clear that the types of bacteria detected in each of the host and the symbiotic algae are different. Here, we discuss the role of bacteria in establishment of symbiosis between *Paramecium bursaria* and symbiotic algae.

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Plenary lecture 3 (21st Nov. 09:00–09:50)

P3

Molecular phylogeny and species delimitation of heterotrich ciliates (Alevolata, Ciliophora, Heterotrichea)

Shahed UA Shazib[1,2], Peter Vdacny[3], and Mann Kyoon Shin[1]
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[2]: Department of Biology, Smith College, USA
[3]: Department of Zoology, Comenius University, Slovakia

Ciliates (Ciliophora) are single-celled heterotrophic eukaryotic group of protozoans that emerged more than one billion years ago, and characterized by the hair-like organelles called cilia, and sexual reproduction of conjugation, and having dimorphic nuclei called macronuclei and micronuclei presenting unique genetic phenomena. They are widely distributed in diverse aquatic and terrestrial habitats, regarded as important trophic linkers in food webs of many ecosystems. Ciliates are currently divided into two subphyla and 11 classes, among these, heterotrich ciliates, i.e., class Heterotrichea, are characterized by somatic dikinetids bearing postciliodesmata, oral apparatus consisting of a paroral membrane and an adoral zone of membranelles, as well as features of nuclear division involving extramacronuclear microtubules. Although phylogenetic relationships among heterotrichs have been analysed previously multiple times, but deeper nodes of the tree remain poorly resolved. To resolve the phylogenetic relationship of heterotrichs, we have accumulated morphological data since Lee & Shin (2009), and acquired the nucleotide sequences of small-sized genetic markers (18S, ITS1-5.8S-ITS2 and 28S rRNAs genes) based on Sanger sequencing. By utilizing these data, we performed a family-level phylogenetic study with establishing two new families and reorganizing the widely used scheme of Lynn (2009). This study has been received attention and accepted by the community (Adl et al., 2019). After this study, several investigators repeated phylogenetic study of heterotrichs. However, the instability of some high-level lineages was not resolved. To resolve it, we have adopted a transcriptome (next-generation sequencing)-based study. Consequently, most nodes in the phylogenetic tree were relatively stable. However, family-level relationships using small-sized genetic markers and transcriptome sequence data are inconsistent and are still resolved partially. Therefore, a more comprehensive study for the phylogeny and evolution of heterotrichs will be needed in future. Moreover, among the heterotrichs, we are interested in the species of the genus *Spirostomum* because they occur at high frequencies in natural environments and are commonly used as biological indicators for assessing water quality and serve as model organisms for applied ecology and symbiosis research. However, some *Spirostomum* species may still be difficult to be identified due to their morphological plasticity and the existence of cryptic species. The delimitation of the species in *Spirostomum* congeners has been started by us (Shazib et al., 2016) by using both primary and secondary gene sequence-structure. In this study, we have focused on the secondary structure of the ITS2 molecule. Our analyses of the ITS region revealed a complex genealogical structure within the genus *Spirostomum*. However, boundaries among *Spirostomum* species could not be unambiguously determined. Therefore, we continued further research on this topic, by adding two more marker genes of CO1 and α-tubulin and applying Bayseian coalescent method. As a result (Shazib et al., 2019), the species complex of *Spirostomum minus* might have a different origin with *S. teres*, and tentatively concluded that *S. teres* had ancestral traits, suggesting that *S. yagiui* and *S. dharwarensis* were derived from *S. teres*. However, *Spirostomum minus* and *S.*

teres have different origins, and it is suggested that more comprehensive data should be supplemented to solve the problem. On that account, we have performed transcriptome analysis of 24 populations of six morphospecies of *Spirostomum*. Phylogenomic trees have been reconstructed by using the curated gene families, and the transcripts diversity was estimated by using their paralogs present in the gene trees. We have assessed the genetic structure of species by analyzing ~1,000 gene trees for the species boundaries and evolutionary relationships among *Spirostomum* species using transcriptome data. By applying this state-of-art approach may unambiguously resolve existing taxonomic problems and suggests a new horizon of phylogenetics to understanding biological species.

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Symposia

S1: 18th, 17:10–19:10

S2: 19th, 10:20–12:20

S3: 19th, 16:30–18:30

S4: 20th, 15:30–17:30

S5: 20th, 17:40–19:40

S6: 21st, 10:00–12:00

Symposium 1 (18th Nov. 17:10–19:10)

Bringing the micro-world to everyday life using mobile microscopy

Organizers: Masashi M. Hayakawa[1], Kuniaki Nagayama[1]
[1]: Life is small. Projects, Japan

Synopsis: A mobile microscope[1] is built on a simple mechanism: a single lens is attached to the digital camera of a smartphone or tablet device. With the mobile microscope, the sample image magnified by the single lens are photographed with the camera, making it easy to observe the detailed structure of microorganisms such as protists. The mechanism of mobile microscope is the same as that of a simple optical microscope which was invented as a hand-made microscope about 300 years ago by Leeuwenhoek. While Leeuwenhoek observed samples through his own eyes, the mobile microscope uses the digital camera of mobile devices instead. And then, the enlarged sample image is displayed on the monitor screen of smartphones or tablets. Therefore, our eyes never get tired, and the same sample image can be shared among a group of people at once, leading a microscopic world sharing. In the past, Leeuwenhoek-type simple optical microscopes has gone extinct, and compound optical microscopes became the mainstream of tool for microscopy. With the advent of mobile devices such as smartphones and tablets, nowadays spread to every corner of this globe, the 21st century Leeuwenhoek-type microscope has been revived as a new tool for inviting people in the micro-world. Interestingly, Leeuwenhoek was also the first person in human history to discover protists using his simple microscope. Just as Leeuwenhoek once observed protists with his microscope, let us play with protists[2] and micro-world as well using mobile microscopy[3]. In order to raise the new culture of microscopes, we, Life is small. Projects, have been managing the science communication using mobile microscopes. In this symposium, we are going to introduce the basic ideas of mobile microscopes, methods of observing micro-world including protists, and our science communication activities in general along with. We would like to share this new tool to access the micro-world with everyone who likes everyday enjoyment with the tool including one who specializes in protistology.

[1] The mobile microscope in Japan was invented by K. Nagayama (co-chair) and T. Ito in 2013, and various variants have since been developed by S. Shirane at the Institute for Science Communication. [2] Diverse protists movies observed using mobile microscopy are exhibit at MMH (chair) YouTube channel: https://www.youtube.com/channel/UCbRUUtQl1oZ1PcB9LnivpNg [3] Maeda, Nagayama et al., Microscopy Today., 2020 July, 54-59.

S1-1

Watching the micro world with a mobile microscope

Kuniaki Nagayama[1], Junto Shirane[2]
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[2]: Science Communication Research Institute LLC.

What is a mobile microscope?

A mobile microscope is a microscope with a minimum configuration consisting of a mobile device and a single lens that is attached externally to the camera of the mobile device. The lens can be attached to either the camera on the screen side of the mobile terminal (in-camera) or the camera on the back side of the screen (out-camera), depending on the purpose. Since the physical magnification of a mobile microscope is given by the ratio of the focal length of the camera lens to that of a single lens, a mobile terminal with a lens having a focal length of 2-4 mm will have a physical magnification of around x 1 if a 3 mmφ ball lens is used. This guarantees theoretically a resolution of 2 μm, since the pixel size of the mobile terminal photosensor is about 1 μm. The mobile microscope has following features; 1) small, lightweight, portable, easy to operate and maintain, 2) 3D printer manufacturing from a single item at a low price according to the purpose, 3) possible to produce a small quantity of a wide variety of microscopes and able to deliver them directly to users anywhere in the world through internet distribution without going through distributors.

Types of mobile microscopes and their potential applications

Various types of microscopes such as reflective/transmission microscopes, polarized light microscopes, underwater microscopes, and centrifugal microscopes have been realized to date (see Figure). 1) These microscopes look toys only for simple observation but now they have moved into the practical phase, such as improving the efficiency of dissection practice in medical schools2), professional observation in biology3), and use in citizen sciences. In this presentation, we would like to share with you some examples of micro-world observation using hitherto developed mobile microscopes.

Figure. Examples of various mobile microscopes

References

1) The mobile microscope in Japan was invented by K. Nagayama and T. Ito in 2013, and various variants have since been developed by S. Shirane of Science Communication Research Institute.
2) M. Maeda, N. Usuda, M. Kokubo, S. Shirane, M. Fukasawa and K. Nagayama, “A Leeuwenhoek-Type Mobile Microscope for Histology Education”, Microscopy Today, 2020 July, 54-59.
3) M. Hattori, S. Shirane, T.Matsuda, K. Nagayama and Takeharu Nagai, Smartphone-Based Portable Bioluminescence Imaging System Enabling Observation at Various Scales from Whole Mouse Body to Organelle, Sensors 2020, 20, 7166-1~11.

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S1-2

Using mobile microscope in high school biology "Cell Observation" class in Cambodia

Kim Sothary[1], Isao Tsuzuki[2]
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[2]: Tokyo University of Science

Microscopes are indispensable for observing living things in schools. In Cambodia, there are at most several and many schools do not have any microscopes in rural areas. We thought that the use of a mobile microscope would be effective under such conditions. In this study, we report the practice of observing cells using a mobile microscope in a biology class at a high school in Cambodia, and discuss the effectiveness of its use and the ideas for development of biology education. An 80-minute class was held on February 7th, 2020, for 36 students of Grade 10 at Preah Sisowath High School in Phnom Penh. 36 students were divided into 9 groups. Mobile microscope enabled students think about a method of observation and discuss with other students, instead of conducting observations and experiments restricted in the textbook and confirming the results. Students were very excited to experience using mobile microscopes. The microscopes are easier to set up and to use. In this research, I could expect the usefulness of the mobile microscope in the class in Cambodia. In the near future, I would like to use it in other unit and conduct classes at other schools. In addition, we aim to realize the supply to each school and conduct teacher training. I will continue to study the development of teaching materials and teaching methods with people involved in education of Cambodia.

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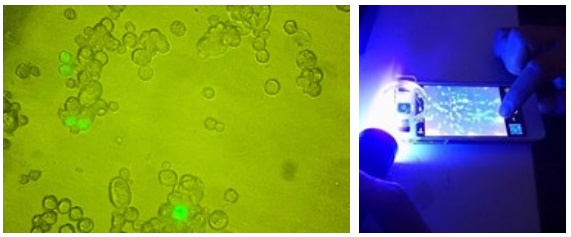
S1-3

Performance improvement in mobile microscopes

Kazumasa Sato[1]
[1]: Lasalle middle and high school



Since the invention of the mobile microscope, I have been interested in improving its performance. First, I explored the applications of the mobile device, which can increase the capability of the microscope. In general, microscopes used in molecular and cellular biology research (such as CLSM) are controlled by a computer, so I made the mobile device as a display and computer unit. Combination of interbal shooting application and produce animation application enabled advanced microscopic observation such as time-lapse imaging. Second, I modified mobile microscope. For example, I made “mobile fluorescence microscope” just by using UV LED. And I made polarlizing microscope by adding polarlizing filter. These methods can be applied to observe GFP fluorescence. These methods have been basic technology for mobile microscopy. I’m going to introduce these methods and show you beautiful pictures and movies.

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S1-4

The workshop of mobile microscopes for children

Yoko Takeshita[1]
[1]: Science Communicator, Ochanomizu University

In the spring of 2020, I held a workshop for children how to use and enjoy “mobile microscopes” in a small village at Kochi prefecture, Japan. A mobile microscope is a small Leeuwenhoek-type microscope that can be made by attaching a single lens to a mobile device such as a tablet device. Due to the COVID-19 pandemic, I had to apply an online meeting system and to remotely ask my colleague scientist who could join to help me. I introduced a picture book “Water Ball Lens” to the participated children and performed several experiments together with them. First, I explained the lens functions to the children by using a drop of water. Then, they moved to the garden from the workshop room to observe plants, animals and microorganisms with the tablet microscopes at several magnifications between x10 and x100. Lastly, a photo contest was held based on the micrographs taken by children and the colleague scientist gave comments to each of the children works through the internet. In this talk, I will present the details of my experience in the unforgettable workshop.

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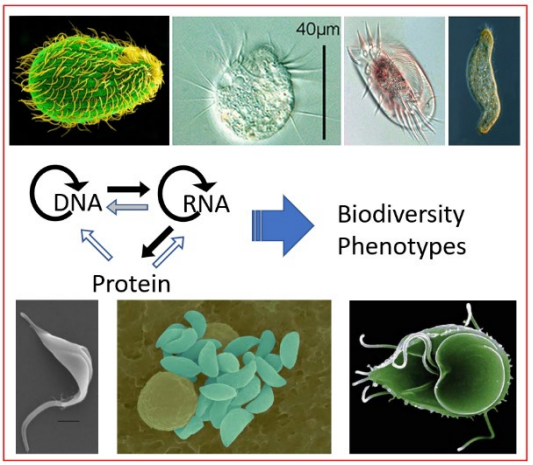
Symposium 2 (19th Nov. 10:20–12:20)

Molecular and cellular biology of ciliated protozoa

Organizers: Shan Gao[1], De-Hua Lai[2]
[1]: Ocean University of China, China
[2]: Sun Yat-Sen University, China



Synopsis: Parasitic and free-living protozoans are eukaryotic pathogens and/or biomass of medical and ecological importance. Numerous features of protozoa, such as nuclear dualism, specified cell structure or organelle, distinct cell cycles and phenotypic/genotypic strain diversity, account for their contribution to molecular and cellular biology. Historical highlights of discoveries include telomere and telomerase, ribozyme, histone modifications, microtubule motors, RNA editing, small RNA-directed DNA elimination, and so on. Nonetheless, mechanisms that govern the accuracy of DNA elimination, synaptonemal complex-independent meiosis, stage specific metabolism and regulation, stage differentiation and species diversification remain elusive. This symposium will bring together a diverse group of young researchers interested in revealing molecular and cellular biological machinery of both parasitic and free-living protozoans. A combined tool of conventional and molecular genetics, biochemistry, cytology, and bioinformatics was applied to address how protozoans maintain the genome integrity, survive in competition, and diversify. Many of their findings have much to offer for future studies of protozoans themselves and consequently human-related basic and medical biology.

S2-1

Multi-HP1-like protein containing complex regulates DNA elimination in *Tetrahymena*

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S2-2

Rab family small GTP in autophagy and life cycle differentiation in *Trypanosoma brucei*

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Cellular differentiation is important for the life cycle and development of both single-cellular and multi-cellular organisms. Hallmarks of cellular differentiation include activation/inactivation of various signaling pathways and remodeling of transcriptional and translational activities. *Trypanosoma brucei* subspecies are causative agents for African Sleeping Sickness in humans and nagana in cattle. Its life cycle alternates between mammalian hosts and tsetse fly vector, with the parasites taking on different forms adapted to different environmental niches. Within the complex life cycle, the slender-to-stumpy differentiation step occurs in mammal blood is of particular interest as a main point of therapeutic intervention for control of these deadly pathogens. At least three pathways have been found to induce slender-to-stumpy differentiation. The best characterized is the most physiological, stumpy inducing factor (SIF)-induced stumpy formation through a quorum sensing mechanism mediated by the uptake of oligopeptides via GPCR homologue GRP89. Activation of *T. brucei* adenosine monophosphate kinase (TbAMPK) via AMP analogs or oxidative stress also induce stumpy formation, possibly via concomitant inhibition of an unconventional Target of Rapamycin homolog, TbTOR4. Recent studies on the expression of variant surface glycoproteins (VSGs) that are crucial for parasite immune evasion also found a link between VSG expression suppression and stumpy formation. While each of these pathways could function during parasite differentiation *in vivo* and together ensure stumpy formation and parasite transmission, it is not clear if there is crosstalk among these pathways, and whether or how these different pathways all converge to the same cell fate determinants leading to stumpy formation. In our recent studies to examine the role of small GTPases in autophagy, we identified TbRab2B as an autophagy regulator, functioning late at the autophagosome degradation step. TbRab2B is normally present at the Golgi apparatus in both procyclic and slender form cells under fed conditions, but relocates to the lysosomes under amino acid-starvation conditions, possibly as activated GTP-bound form. Further characterization revealed a role of TbRab2B in lysosome biogenesis and function in both procyclic and slender form cells. Most intriguingly, depletion of TbRab2B in the monomorphic slender cells induced cell differentiation to the stumpy form, which could be further differentiated to stable procyclic cells. Together these results strongly supported a novel function of TbRab2B as a negative regulator of slender-to-stumpy differentiation. Whether TbRab2B is part of the known pathways or represents a new differentiation mechanism awaits further studies.

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S2-3

RNAi-dependent Polycomb repression controls transposable elements in *Tetrahymena*

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[7]: J. Craig Venter Institute, USA

[8]: These authors contributed equally to this work.

RNAi and Polycomb repression play evolutionarily conserved and often coordinated roles in transcriptional silencing. Here, we show that, in the protozoan *Tetrahymena thermophila*, germline-specific internally eliminated sequences (IESs)—many related to transposable elements (TEs)—become transcriptionally activated in mutants deficient in the RNAi-dependent Polycomb repression pathway. Germline TE mobilization also dramatically increases in these mutants. The transition from noncoding RNA (ncRNA) to mRNA production accompanies transcriptional activation of TE-related sequences and vice versa for transcriptional silencing. The balance between ncRNA and mRNA production is potentially affected by cotranscriptional processing as well as RNAi and Polycomb repression. We posit that interplay between RNAi and Polycomb repression is a widely conserved phenomenon, whose ancestral role is epigenetic silencing of TEs.

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S2-4

Adaption and applications of plant auxin inducible degron systems in *Toxoplasma gondii*

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[1:] China Agricultural University, China

Toxoplasma gondii is an obligate and intracellular parasite, which infects almost all warm-blooded animals. Though *T. gondii* is a good model for studying apicomplexan parasites due to its ease of culturing and genetic traceability, the genetic modification and downregulation of proteins are not as efficient and prompt as expected for better dissection of proteins function, using the bacterial tetracycline operator/regulator system and dimerizable Cre-recombinase system. In recent years, we introduced the plant-derived auxin-inducible degron (AID) system I and II into *T. gondii*, by applying the rational in which plant auxin (IAA) can bind TIR1 and activate the ubiquitin proteasome system to degrade AID degron fusion proteins. We efficiently dissected the function of conoid hub protein 1 (CPH1) on conoid stability and parasite motility by combining CRISPR technology and the AID system I. In the second AID system (AID II), we adapted the upgraded system in which a mutation at residue 74 (F74G) of TIR1 makes the TIR1 to use a bump-and-hole approach to improve the performance of protein degradation system. A bumped-IAA analogue 5-Ph-IAA showed high efficiency of induction using a much lower level of inducer (1 nM), comparing to the IAA (500 nM). The AID II system offers another advantage of using a 1/3 length of the original AID degron – miniAID. Recently we have successfully applied the AID II system to study a key phosphatase possibly regulating parasite cytoskeleton, and a novel protein involved in parasite division. The AID I and II system will be highly useful for studying *T. gondii* and other related parasites, to promote basic study and pharmaceutical development.

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S2-5

A feedback mechanism controls meiotic DNA double-strand break formation in *Tetrahymena*

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Meiosis is a special cell division programme for producing gametes (i.e. eggs and sperm). Successful meiotic recombination is ensured by the programmed induction of DNA double-strand breaks (DSBs), which are the most dangerous form of DNA damage. Thus, the DSB number is strictly controlled because they are potentially harmful. We found a novel protein, Pars11, which is required for Spo11-dependent DSB formation in the protist *Tetrahymena*. Pars11 localizes to chromatin early in meiotic prophase in a Spo11 independent manner and is removed before the end of prophase. Pars11 removal depends on DSB formation and ATR-dependent phosphorylation. In the absence of the ATR, a DNA damage sensor kinase, Pars11 is retained on chromatin and excess DSBs are generated. Similar levels of Pars11 persistence and DSB overproduction occur in a non-phosphorylatable pars11 mutant. We conclude that Pars11 supports DSB formation by Spo11 until enough DSBs are formed; thereafter, DSB production stops in response to ATR-dependent degradation of Pars11 or its removal from chromatin. A similar DSB control mechanism involving a Rec114-Tel1/ATM-dependent negative feedback loop regulates DSB formation in budding yeast. However, there is no detectable sequence homology between Pars11 and Rec114, and DSB numbers are more tightly controlled by Pars11 than by Rec114. The discovery of this mechanism for DSB regulation in the evolutionarily distant protist and fungal lineages suggests that it is conserved across eukaryotes.

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S2-6

Cyclic nucleotide signaling in the obligate intracellular pathogen *Toxoplasma gondii*

Nishith Gupta[1,2]
[1]: Humboldt University, Berlin, Germany
[2]: Birla Institute of Technology & Science Pilani, Hyderabad Campus, India

Infection, pathogenesis and transmission of the intracellular parasitic protist, *Toxoplasma gondii*, depend on cAMP, cGMP and calcium signaling. Cyclic nucleotide cascades in this widespread model pathogen show a remarkable parasite-specific divergence compared to mammalian host cells. Our group has implemented wide-ranging methods including genome engineering and mutant phenotyping to determine the physiological importance of cAMP and cGMP during lytic cycle (acute infection) of *T. gondii*. In extended work, we deployed optogenetics in conjunction with phosphoproteomics and gene mutagenesis to identify novel signaling mediators and decipher the hierarchical topology of cyclic nucleotide cascades. Not least, our research has pioneered the utility of light-activated proteins for dynamic, reversible, specific and spatiotemporal control of cAMP and cGMP in genetically-tractable pathogens.

Gupta.Nishith@hu-berlin.de (Nishith Gupta)

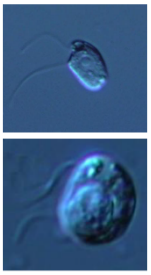
Symposium 3 (19th Nov. 16:30–18:30)

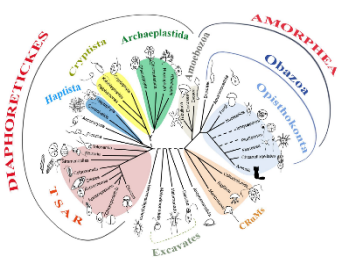
Biology of heterotrophic and parasitic protists

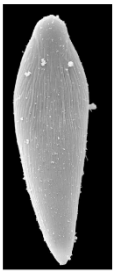
Organizers: Denis Tikhonenkov[1], Kevin Wakeman[2]

[1]: Russian Academy of Science, Russia

[2]: Hokkaido University, Japan







Synopsis: Protists represent diverse phylogenetic lineages, and contain a large number of species that possess ancestral cellular and genomic characteristics in relation to their multicellular relatives. Phylogenetic data on heterotrophic and parasitic protists are extremely important in terms of reconstructing the universal tree of life. The basal or intermediate evolutionary positions occupied by these organisms make them particularly important for elucidating the origin, diversity, and evolution of model organisms. Within the framework of the proposed symposium, we will be looking at (1) new data on novel, deep-branching lineages of heterotrophic protists and (2) phylogenomic reconstructions uniting Archaeplastida and Cryptista supergroups. Also, protists are a compulsory linked in microbial food webs and provide effective pathways for the transformation of matter and energy in aquatic ecosystems. They possess a full range of trophic and life strategies seen across eukaryotes, albeit on a microscopic scale. Many protists are secondary-heterotrophic and non-photosynthetic. They have descended from photosynthetic ancestors and reverted to solely heterotrophic lifestyles. In this context (3), an investigation of secondary-heterotrophic protists and their peculiar metabolism and genomic organization will be presented. Finally, a considerable number of protists have evolved independently into a parasitic lifestyle, many of which are notorious pathogens that have an impact on public health and ecology. Here, current views on (4) patterns of evolution and diversity of marine Apicomplexa and parasitic dinoflagellates will be presented. One of the major aspects of protozoan biology is symbiotic relations with prokaryotes, which date back at least two billion years ago to the origin of mitochondria. A broad view of (5) bacterial and archaeal symbioses associated with protist hosts, focusing on their evolution, ecology, and cell biology will be present.

S3-1

Novel deep-branching lineages of heterotrophic protists and their evolutionary significance

Denis Tikhonenkov[1]
[1]: Papanin Institute for Biology of Inland Waters, Russian Academy of Sciences, Russia

Recent investigations and findings of new species, high-level taxa and even previously unknown phylogenetic lineages of eukaryotes demonstrates that heterotrophic (especially predatory) flagellates remain extremely poorly studied, but often represent the most important deep-branching lineages of eukaryotes. They often form a sister groups to giant eukaryotic clusters on phylogenetic trees, and illustrate an ancestral state of one or another supergroup of eukaryotes. Recent studies of predatory flagellates, have provided new data revealing the early stages of evolution of Opisthokonta, Alveolata, and Archaeplastida supergroups; leading to the revision of the eukaryotic tree and ideas on mitochondrial evolution and the root of the tree of all eukaryotic organisms; demonstrating the ways of the emergence and development of unique cellular and genomic innovations that led to the formation of multicellularity, photosynthesis, and parasitism. Further investigations of predatory flagellates are important for understanding the role of phagotrophy in the origin of symbiogenetic organelles – plastids and mitochondria, and will also help to clarify the evolution of major eukaryotic supergroups and resolve their relationships.

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S3-2

Microheriella maris units Archaeplastida and Cryptista in phylogenomics

Euki Yazaki[1], Akinori Yabuki[2], Ayaka Imaizumi[3], Keitaro Kume[4], Tetsuo Hashimoto[5,6], Yuji Inagaki[6,7]
[1]: RIKEN iTHEMS
[2]: Japan Agency for Marine-Earth Science and Technology
[3]: College of Biological Sciences, University of Tsukuba
[4]: Faculty of Medicine, University of Tsukuba
[5]: Faculty of Life and Environmental Sciences, University of Tsukuba
[6]: Graduate School of Life and Environmental Sciences, University of Tsukuba
[7]: Center for Computational Sciences, University of Tsukuba

There are still many "orphan" protists of which phylogenetic affiliation has not yet been determined by previous studies in the eukaryotic tree. Determining their phylogenetic positions has the potential to fill the gaps in eukaryotic diversity and it contributes to reveal true phylogenetic relationships among major lineages that still remain to be resolved well. *Microheliella maris* was originally described as a member of the phylum Heliozoa, but previous large-scale phylogenetic analyses have not been able to confidently place this organism within any previously described species or lineages. In this study, we analyzed the alignment of 319 genes and demonstrated that *M. maris* represents the basal lineage of Cryptista. Here, we propose a new clade name "Pancryptista", which includes *M. maris* and Cryptista. In addition, the results of the analysis of 319 genes showed that *M. maris* is an important taxon to recover the monophyly of Archaeplastida and the sister relationship between Archaeplastida and Pancryptista, so we call this assemblage "CAM clade" here. Cryptista tends to be attracted to Rhodophyta depending on the taxon sampling in phylogenomic alignments, and this phylogenetic "signal" may have prevented the stable recovery of Archaeplastida monophyly in previous studies. Based on our detailed molecular phylogenetic analyses using 319 genes, we hypothesize that many genes in Cryptophyceae, the internal lineage of Cryptista, have been partly recombined with homologous genes transferred from red algal endosymbionts during secondary endosymbiosis and have a faint phylogenetic affinity with the genes of Rhodophyta.

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S3-3

To be algae or not to be algae: losses of photosynthesis in diatoms

Ryoma Kamikawa[1]
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S3-4

Patterns of evolution and diversity of marine apicomplexans and parasitic dinoflagellates

Kevin C. Wakeman[1]
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The alveolates are a diverse group of singe celled eukaryotes (protists) that have been traditionally defined by three major groups: apicomplexans, dinoflagellates, and ciliates. More contemporary work on select alveolate lineages (i.e., early diverging parasitic lineages) has let researchers in this field revisit some of the earliest stages of alveolate evolution. In this talk, I will give a brief introduction to alveolate lineages, covering some of the recent advances in the field that have been brought about in this age of genomics. In particular, I will discuss on-going themes related to the early diversification and host specificity of parasitic alveolates within the genera Lankesteria, Haplozoon, and Platyproteum. Here, I will present some preliminary work that highlights coevolutionary patterns (host specificity) between these parasites and their hosts. I will also cover some of the character evolution of these groups that make them intriguing models for understanding the early evolution of alveolates, in particular, fundamentally understanding the repeated and independent evolutionary transition of alveolates from a free-living (photosynthetic) ancestor to an obligate parasitic niche.

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S3-5

Bacterial and archaeal symbioses with protists

Filip Husnik[1]
[1]: Okinawa Institute of Science and Technology, Japan

Most of the genetic, cellular, and biochemical diversity of life rests within single-celled organisms — the prokaryotes (bacteria and archaea) and microbial eukaryotes (protists). Very close interactions, or symbioses, between protists and prokaryotes are ubiquitous, ecologically significant, and date back at least two billion years ago to the origin of mitochondria. However, most of our knowledge about the evolution and functions of eukaryotic symbioses comes from the study of animal hosts, which represent only a small subset of eukaryotic diversity. In this talk, I will take a broad view of bacterial and archaeal symbioses with protist hosts, focusing on their evolution, ecology, and cell biology, and also explore what functions (if any) the symbionts provide to their hosts. With the immense diversity of protist symbioses starting to come into focus, we can now begin to see how these systems will impact symbiosis theory more broadly.

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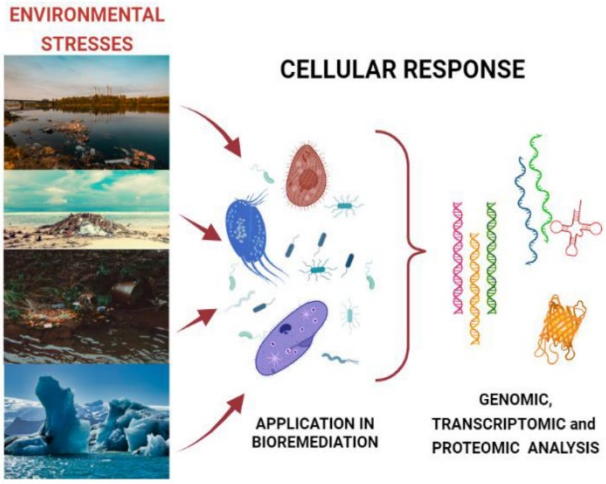
Symposium 4 (20th Nov. 15:30–17:30)

Protists as model organisms to face environmental problems

Organizers: Cristina Miceli[1], Wei Miao[2]
[1]: University of Camerino, Italy
[2]: Chinese Academy of Sciences, China



Synopsis: Despite general progress in environmental research, the impact of environmental changes on living organisms and human health remains deeply worrying Monitoring of water contamination, air pollution, exposure to metals and global climate change can be faced with the contribution of modern omics techniques applied to key model organisms/systems. Genomics and transcriptomics are used to identify marker genes involved in environmental responses, to analyze differential gene expression under environmental stress, to study the relationship between genotype and phenotype, including possible epigenetic control Response to stresses and environmental changes is a relevant issue to which protists can provide a strong contribution, due to their wide distribution in many different environmental conditions The proposed workshop is planned with the ambition to gain insight into practical environmental problems using protists model organisms This means that the focus will include applications of genetics/genomics/transcriptomics to contribute to deliver solutions to relevant environmental issues We expect to open a discussion about applications to identify new marker genes, to better understand the adaptation to environmental changes, and to use quantitative genetics and RNAseq to measure cell response to different toxicants and environmental contaminants The progress in technologies is producing new environmental contaminants for which the effect is so far underestimated Just to provide a simple example, the effect of metals has been largely investigated However, the effect on biological processes produced by aggregation of metals in nanoparticles appears a more relevant issue to be unraveled Therefore, it is useful to discuss new practical approaches in a large audience, where the knowledge and the best practices will be shared.

S4-1

Microbial community in inland waters in the Anthropocene

Jun Yang[1]
[1]: Institute of Urban Environment, Chinese Academy of Sciences, China

Microeukaryotic plankton and bacterioplankton are critical components of aquatic microbial food webs and play essential roles in the structure and function of aquatic ecosystems. Understanding the processes and mechanisms that community dynamics and assembly of these microorganisms is one of major goals in both pure and applied microbial community ecology. Plankton communities normally consist of few abundant and many rare species, yet little is known about the ecological pattern and role of rare planktonic species. We found the rare bacterioplankton subcommunity had a distinct biogeographical pattern in inland waters that was reasonably similar to the abundant bacteria. However, local processes and factors play the most important role in structuring rare bacterial subcommunity, with regional factors explaining more variation in abundant bacteria. Both deterministic and stochastic processes significantly influenced eukaryotic plankton community assembly, and the stochastic pattern was particularly pronounced for rare taxa. Stochastic processes are sufficient in shaping substantial variation in rare plankton metacommunity in a river-reservoir system across different hydrographic regimes. Co-occurrence network analysis revealed that keystone taxa mainly belonged to rare species, which may play fundamental roles in network persistence following a cyanobacterial bloom event. Both warming and decline in water level can boost cyanobacterial dominance in subtropical reservoirs. The long-term observations revealed that the cyanobacterial biomass cycle created distinct niches between persistent bloom, non-bloom, decrease and increase of cyanobacteria, and therefore associated with distinct eukaryotic plankton patterns. These findings provide a new perspective for the ecological significance of rare plankton in changing aquatic ecosystems, clarifying the contribution of microbial interactions in plankton food web theory.

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S4-2

Genomic biomonitoring of protistan communities to assess environmental impacts of coastal marine aquaculture

Thorsten Stoeck[1]
[1]: Technische Universität Kaiserslautern, Ecology Group, Germany

Traditionally, the backbone of environmental biomonitoring in finfish aquaculture is the collection and identification of benthic macrofauna, which react predominantly to organic enrichment and oxygen (depletion). However, this monitoring strategy with the microscopic identification of macroinvertebrates as biological indicators is very tedious and expensive. Therefore, concerted efforts are to develop alternative monitoring tools. One very promising approach is environmental DNA metabarcoding, which relies on the identification of short DNA fragments amplified from bulk sediment samples. We have used this molecular approach to evaluated the indicator qualities of protists along organic enrichment transects in the vicinity of salmon farms in Scotland and Norway. Significant changes in protistan community compositions, above all in ciliates and diatoms, were typical along these transects. These changes correlated significantly with organic enrichment and corroborated well with benchmark reference data obtained from traditional macrofauna monitoring of the same sampling sites. We then used supervised machine learning (SML) to train an algorithm, which successfully predicts ecological quality in marine sediments of aquaculture sites based on DNA metabarcodes of protistan communities as features and macrofauna-derived biotic indices as reference data. In conclusion, the genomic biomonitoring of benthic protistan communities as a source of new biosensors in combination with supervised machine learning is an extremely powerful tool for environmental biomonitoring in marine aquaculture. The costs of this technology are notably lower than for macrofauna-based monitoring and results are available within days rather than within months (as for macrofauna monitoring). Hence, we propose the integration of protistan metabarcoding in future compliance biomonitoring regulations in aquaculture.

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S4-3

The Antarctic ciliate *Euplotes focardii* and its associated bacterial consortium: insights in environmental adaptation and responses to stress from genomics analysis

Sandra Pucciarelli[1], Matteo Mozzicafreddo[1], Angela Piersanti[1], Cristina Miceli[1]
[1]: School of Biosciences and Veterinary Medicine, University of Camerino, Italy

Euplotes focardii is an Antarctic ciliate classified as an obligate psychrophilic stenothermal organism. As all ciliates, *Euplotes focardii* is characterized by the presence of cilia on its surface and by nuclear dimorphism: a micronucleus (MIC) that represents the germ line, and a macronucleus (MAC) serving as the somatic line involved in the gene expression during the vegetative stage. We compared *Euplotes focardii* MAC genome with those available from mesophilic Euplotes species to characterize differences that may be consequent to cold adaptation and defense to oxidative stress, the main constraints of the Antarctic marine microorganisms. We focused on the comparison of antioxidant enzymes and heat shock protein (HSP) 70 families, molecules which possess peculiar characteristics correlated with cold adaptation. We found that SODs and CATs antioxidant enzymes are more numerous in *Euplotes focardii* than in the mesophilic *Euplotes* species. In contrast, there are fewer hsp70 genes in *Euplotes focardii* compared to mesophilic *Euplotes* and these genes respond only to oxidative stress, suggesting a loss of response to heat stress in this Antarctic ciliate adapted to a constant cold water. We also characterized the *Euplotes focardii* associated bacterial consortium. We isolated five members of the consortium and we found that these bacteria can transform chromium, copper, and silver into harmless nanoparticles, that may be a mechanism of heavy metals resistance. Our results suggest that environmental adaptation rely on molecular changes such as peculiar amino acid substitutions and gene duplication. However, a role of associated bacteria cannot be excluded.

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S4-4

Heavy metal bioremediation by sulfide nanoparticle synthesis using *Tetrahymena thermophila*

Jiawei Tu[1], Tian Li[1,2], Zihan Gao[1,2], Wenjun Xiong[1,2], Jie Xiong[1], Wei Miao[1]

[1]: Key Laboratory of Aquatic Biodiversity and Conservation, Institute of Hydrobiology, Chinese Academy of Sciences, China

[2]: University of Chinese Academy of Sciences, China

Heavy metal pollution is increasingly becoming a problem and has become of great concern due to the adverse effects it is causing around the world. An emerging way to decrease the toxicity of heavy metals is bioremediation using microorganisms. Reasonably, transforming harmful heavy metal ions into stable and less toxic metal sulfide precipitates becomes a possible way to repair the heavy metal pollution *in situ*. *Tetrahymena thermophila*, as a eukaryotic model organism, was widely used in toxical studies. It is easy to culture at a large scale, and the genome has been sequenced. Various genetic manipulation methods were established in this organism. These advantages make *Tetrahymena thermophila* to be a powerful tool for remediation of heavy metal ions polluted water. Herein, intracellular synthesis of HgS and CdS was achieved using *Tetrahymena thermophila* by adjusting the activity and concentration of metal precursors, the type of sulfur source and the reaction time. Furthermore, the distribution and morphology of heavy metal elements and nanoparticles in *Tetrahymena thermophila* were analyzed at different time points of the growth process of HgS and CdS nanoparticles to investigate the possible mechanism of this intracellular metal sulfide synthesis. These studies lay a foundation for further identifying the key factors involving in heavy metal ions metabolism, and provided a basis for bioremediation of heavy metals pollution using Synthetic biology approaches in *Tetrahymena thermophila*.

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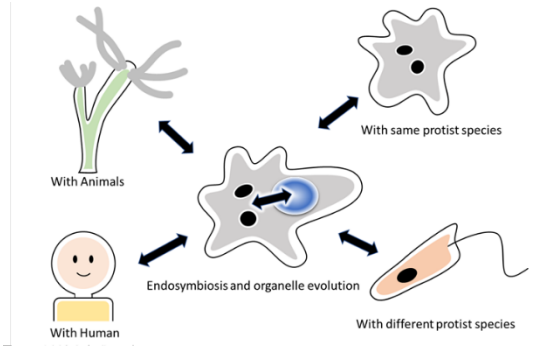
Symposium 5 (20th Nov. 17:40–19:40)

Molecular basis for interactions between protists and other organisms

Organizers: Toshinobu Suzaki[1], Federico Buonanno[2]
[1]: Kobe University, Japan
[2]: University of Macerata, Italy



Synopsis: Since protists are single-celled organisms, one might think that these organisms live a solitary life, independent of other organisms. In reality, however, many protists develop complex interrelationships with other unicellular and multicellular organisms. For example, predatory protists recognize and capture other organisms as prey. On the other hand, to escape from predators, they need to recognize their enemies correctly. Some protists recognize cells of the same species of different cell types for sexual reproduction. Some are symbiotic with other eukaryotes or prokaryotes in their cells, and some are symbiotic in the bodies of other larger organisms. There are also protozoa that can infect animals and cause disease. Recent studies have revealed the mechanisms by which protists recognize other organisms. As a result, we have gained many unique insights into the molecular mechanisms of cellular interactions between protists and other organisms, and the aspects of biological evolution driven by these interactions. This symposium will highlight the cell-cell interactions of protists, especially from their molecular and evolutionary perspectives, and will present the latest research results.

S5-1

The offensive-defensive strategies adopted by ciliated protists

Federico Buonanno[1], Claudio Ortenzi[1]
[1]: Laboratory of Protistology and Biology Education, Department of ECHT, University of Macerata, Italy

In the last 30 years, a lot of studies have been devoted to describe the predator-prey interactions among unicellular eukaryotic organisms like protists. Especially in ciliates, a particular attention has been focused on the significant role of specialized ejectable membrane-bound organelles, generally called extrusomes, used in the immobilization and capture of prey, and in defense from predators. Essentially, two types of strategies are adopted by ciliates in predator–prey interactions: the first is mediated by mechanical mechanisms involving some subpellicular non-toxic extrusive organelles (for example the trichocysts), while the second is mediated by toxic secondary metabolites (contained in different kinds of chemical extrusomes) used for offense or defense by a number of ciliate species. These interactions are mainly studied in unicellular predator-prey models but, recently, some researches have also focused their attention on analyzing predation or defensive strategies against metazoans. With regard to these strategies, the interactions between ciliates and microinvertebrates seem to indicate that the evolution of chemical-behavioral machinery in micro-ecosystems can be compared, in terms of variations and complexity, with those characterizing macro-ecosystems.

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S5-2

Genome analysis reveals interactions and evolution in *Hydra-Chlorella* symbiosis

Mayuko Hamada[1]
[1]: Ushimado Marine Institute, Okayama University, Japan

Symbiosis with microalgae is a general biological phenomenon found in various organisms. In Cnidarian, it can be observed in many species in corals, jellyfish, sea anemones and hydras. In particular, the symbiosis between green hydra and its symbiotic algae has been the subject of research since decades. To understand principles of symbiogenesis and their links to evolution at the molecular- and the genome-level, we decoded genomes of the green hydra *Hydra viridissima* A99 and its symbiotic algae *Chlorella* sp. A99, and focused on the specific features in their genomes. In the symbiosis of green hydra, the symbiotic alga requires nitrogenous amino acids derived from the host, and the host acquires photosynthetically fixed carbon from the algae in the form of maltose. The alga is unable to grow outside the host, indicating loss of autonomy during establishment of the dependent relationships. In the symbiotic *Chlorella* genome, degeneration of inorganic nitrogen assimilation system and duplication of amino acid transporter genes were observed, reflecting metabolic dependency of the symbiont on the host. On the other hand, in the green hydra genome, innate immune genes are specifically increased and their domain structures are further complicated, compared to non-symbiotic hydra. These characteristics are also seen in corals, and may contribute to defence and maintenance of the symbiotic environment in common. In this symposium, I will present the findings obtained from the comparative genome analyses of green hydra and its symbiotic algae, and discuss the generality and diversity of animal-algal symbiosis.

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S5-3

Paulinella micropora KR01 genome reveals dominant host contribution and role of novel genes in primary plastid endosymbiosis

Hwan Su Yoon[1], Duckhyun Lhee[1], Debashish Bhattacharya[2]
[1]: Department of Biological Sciences, Sungkyunkwan University, Korea
[2]: Department of Biochemistry and Microbiology, Rutgers University, USA

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 (hereafter, KR01) that underwent a more recent (ca. 124 Mya) primary endosymbiosis of a photosynthetic organelle, termed the chromatophore. Phylogenetic analyses using four gene markers revealed three distinct lineages of photosynthetic *Paulinella* species. We generated the complete chromatophore genome sequences from *P. longichromatophora* and *P. micropora* KR01/NZ27. Our analysis suggests that when a basal split occurred among photosynthetic *Paulinella* species ca. 60 Mya, only 35% of the ancestral orthologous gene families from the cyanobacterial endosymbiont remained in chromatophore DNA. Analysis of genomic and transcriptomic data resulted in a high-quality draft assembly of size 707 Mbp and 32,358 predicted gene models. A total of 287 chromatophore targeted long-proteins were predicted in silico, 206 of which comprise the ancestral organelle proteome in photosynthetic *Paulinella* species with functions in nucleotide metabolism and oxidative stress response. Gene co-expression analysis identified networks containing known high light stress response genes as well as a variety of putative “dark” genes of unknown function. We characterized diurnally rhythmic genes in this species and found that over 51% are dark. Our results demonstrate the massive amount of genetic innovation needed to domesticate a photosynthetic organelle and identify a storehouse of novel genes implicated in the transition from a heterotrophic lifestyle to photoautotrophy.

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S5-4

Protein trafficking and parasite-derived membrane structures in *P. falciparum*-infected erythrocytes.

Hideyuki Iriko[1]

[1]: Division of Global Infectious Diseases, Department of Public Health, Graduate School of Health Sciences, Kobe University, Japan

Malaria is caused by *Plasmodium* parasites that are transmitted to humans through the bite of infected Anopheline mosquitoes. In the human body, malaria parasites undergo repeated cycles of erythrocyte invasion, proliferation within the cell and egress. Of the five species that infect humans, the most pathogenic species is *Plasmodium falciparum*. *P. falciparum* modify infected erythrocyte membrane by the export of parasite proteins. The modifications in cell adhesion, deformability, and permeability properties of infected erythrocytes contribute to parasite survival and immune evasion. Malaria parasites export hundreds of proteins into the erythrocytes. In infected erythrocyte of *P. falciparum*, the individual steps of protein export are associated with parasite-derived membranes. The intra-erythrocytic parasites are surrounded by a lipid bilayer membrane referred to as parasitophorous vacuole membrane (PVM). To reach the erythrocyte cytosol, all parasite-exported proteins should cross PVM through a protein translocon called *Plasmodium* Translocon of EXported proteins (PTEX). Then the proteins are transported to the Maurer's clefts. Maurer's clefts are parasite-derived structures within the host cell cytoplasm that are thought to function as a sorting compartment between the parasite and the erythrocyte membrane. In this symposium, I will present an overview of the protein trafficking and the parasite-derived membrane structures in *P. falciparum*-infected erythrocytes.

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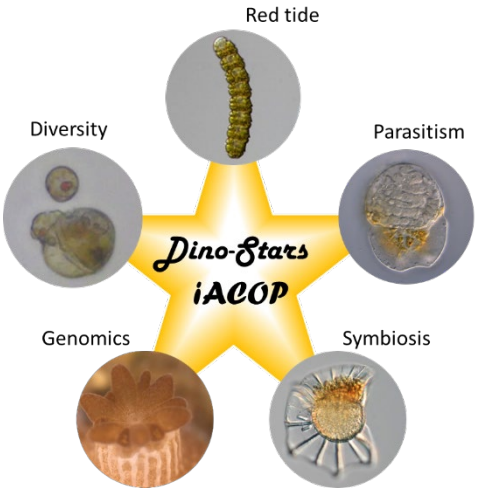
Symposium 6 (21st Nov. 10:00–12:00)

Ecology and evolution of dinoflagellates in the genomics era

Organizers: Hwan Su Yoon[1], Cheong Xin Chan[2]
[1]: Sungkyunkwan University, Korea
[2]: University of Queensland, Australia



Synopsis: Dinoflagellates are protists that are ubiquitous in marine and fresh waters. They critically sustain global aquatic ecosystems via primary production and cycling of organic carbon and nitrogen. Estimated at ca. 2,500 species, dinoflagellates are highly diverse, covering a broad spectrum of trophism (heterotrophy, photoautotrophy, and/or mixotrophy), environment (tropics, temperate, or polar), and lifestyle (free-living, symbiotic, or parasitic). Bloom-forming species may cause “red tides”, which produce toxins that pose serious human health risks. Symbiotic species of family Symbiodiniaceae are crucial symbionts in corals and other coral reef organisms. Parasitic species can cause death in economically important crustaceans such as crabs and lobsters. Although dinoflagellates are ecologically and economically important, they pose many challenges in modern research. Their taxonomy can be confounded by subtly different morphology, ecology may involve distinct modes of symbiosis and multiple interacting species in a complex ecosystem, chromosomes are permanently condensed in crystalline structure, nuclear genomes are large (up to 70-fold larger than a human genome), and organellar genomes are atypical of eukaryotes. Their evolutionary history is also highly intricate; some photosynthetic lineages harbor tertiary plastids derived from haptophyte, diatom and/or green algal sources. In this symposium, we will discuss current understanding of dinoflagellate ecology and evolution, with perspectives of taxonomy/diversity, red tide, parasitism, symbiosis, and genomics, and how we can use this knowledge to drive future research.

S6-1

Morphological and physiological diversity of dinoflagellates

An Suk Lim[1]
[1]: Division of Life Science, Gyeongsang National University, Korea

Dinoflagellates are primarily unicellular flagellates having two distinctive flagella. However, the types of dinoflagellate are various such as a single cell without flagellum or cells in a chain-form. The dinoflagellates cell surface is covered by thecal plates, which are major morphological features that have been widely used in taxonomy. The numbers, sizes, and shapes of thecal plates vary in the genera and species of dinoflagellates. Also, some intercellular structures of dinoflagellate are often used in taxonomy. Physiologically, dinoflagellates have diverse trophic modes such as phototrophic, mixotrophic, and heterotrophic. Such a variety of trophic modes may allow dinoflagellates to play an essential role in the marine environment and habitat a wide range of environments. Dinoflagellates are found in both low-nutrient pelagic and highly eutrophic waters and benthic habitats as well. Complex cellular structure and physiological features help to explain the essential ecological roles of dinoflagellates and their adaptation in a wide variety of environments. I will discuss the diversity in the morphological and physiological features of dinoflagellates, and it might provide an insight into dinoflagellate ecology.

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S6-2

Effect of marine heatwaves on bloom formation of the harmful dinoflagellate *Cochlodinium polykrikoides*

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In 2018, the bloom of harmful dinoflagellate *Cochlodinium polykrikoides* occurred under abnormally high water temperature (WT) conditions caused by heatwaves in Korean coastal water (KCW). To better understand *C. polykri*koides bloom at high WTs in 2018, we conducted field survey and physiological and genetic experiments in laboratory using two different strain of CP2013 and CP2018. The heatwave increased the WT from 24.1℃ to 29.2℃ for two weeks, leading to strong stratification even in mid-July ($p < 0.01$, Chi square = 94.656, Kruskal–Wallis test). Under early stratification conditions, patch blooms formed more earlier than the average outbreak in the last 17 years in KCW, despite high WT reaching 30℃. In laboratory experiments, although there were no genetic differences in the LSU rDNA, both strains showed a significant different growth response to high WTs; above 28℃, CP2013 did not survive, but CP2018 was able to grow, suggesting that CP2018 had potential growth capacity at high WTs. However, the growth rate of CP2018 was low at 30°C. In addition, the blooms of *C. polykrikoides* in 2018 lasted only 3 weeks, an exceptionally short compared to average duration of past 20 years. The negative correlation between the average WT and duration of *C. polykrikoides* bloom in previous 17 years ($R2=0.52$, $p<0.01$) supports that high WT approaching 30℃ is not favorable for *C. polykrikoides* in KCW. Thus, in relation to heatwaves, our findings indicated that early stratification condition plays a critical role in developing *C. polykrikoides* blooms, but maintaining bloom are negatively affected under high WT conditions.

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S6-3

Diversity and distribution of marine parasitic dinoflagellates

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Approximately 150 species (35 genera) of dinoflagellates are known as parasites of a variety of marine organisms including protists, larvaceans, crustaceans, annelids, and fish. Many of these species are parasitoids, which kill the hosts to complete their life cycle. Thus, epidemics by parasitic dinoflagellates causing host mortality could influence host population dynamics and cause significant loss to fisheries. A majority of literatures for infections of parasitic dinoflagellates have been mainly recorded from temperate coastal habitats. During the past decade, molecular studies exploring in marine picoeukaryote diversity using a high-throughput sequencing discovered the novel eukaryotic lineages (i.e. Marine AVLeolate; MALV). These enigmatic new lineages represent up to 50% of sequences retrieved in all marine environments, from temperate coastal waters to polar regions, to anoxic environments, even to deep hydrothermal vents. Now, the MALV sequences are believed to be attributed to parasitic dinoflagellate Syndiniales, a group of composed of obligate parasites. Evidences for the great diversity and widespread distribution of syndiniales parasites suggest that these parasites could have an important role in host population regulation and further studies are required to better evaluate the functional role of these parasites and their contribution to carbon flow in marine food webs.

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S6-4

Diversity of symbiotic cyanobacteria seen in pelagic dinoflagellates

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Symbiosis is one of the most important topics among the diverse phenomena exhibited by dinoflagellates. While some dinoflagellates are widely known to live in the bodies or cells of other organisms as symbionts, some of them are also known to host a variety of unicellular organisms in their cells. The symbioses hosted by dinoflagellates have attracted attention as a key to understanding symbiotic evolution, yet the details of many of these relationships remain to be explored. Pelagic species of Dinophysiales have long been known to host cyanobacteria as symbionts. These dinoflagellate species possess an extracellular chamber per cell and cyanobacterial symbionts are residing in the specialized space. Despite the existence of the symbionts being first recorded over 100 years ago, detailed characteristics of the cyanobacteria as well as the nature of the symbiosis had remained poorly understood. In the presentation, I will introduce features of symbiotic cyanobacteria isolated from two Dinophysiales species, *Ornithocercus magnificus* and *Histioneis depressa*, revealed by single-cell genomic analysis. The genome information suggested contrasting natures between two symbionts from the different hosts. The cyanobacterial symbiont from *O. magnificus* was unveiled to be of lineage lacking nitrogen-fixing ability and its genome has been reduced compared to genomes of close relatives. In addition, a metagenomic analysis suggested an obligate symbiotic relationship between the cyanobacteria and dinoflagellates. On the other hand, all the genes necessary for nitrogen fixation were identified in the genome of *H. depressa* symbiont, indicating that the symbiont has nitrogen-fixing ability in addition to photosynthetic ability. Furthermore, the estimated distribution pattern in the environment suggests that the symbiosis between *H. depressa* and the cyanobacterium would be facultative. These results illuminate the diversity of symbiotic relationships seen in Dinophysiales, in terms of the lineage and roles of the symbionts, as well as the strength of the relationship.

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S6-5

Dinoflagellates in the genomic era: what do genomes tell us about their ecology and evolution?

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Dinoflagellates range from free-living, parasitic to symbiotic species in a broad spectrum of trophism. Genome data from dinoflagellates offer clues to how molecular and evolutionary mechanisms contributed to their diversification to occupy distinct ecological niches. Genomes of dinoflagellates are large (some over 200 Gbp), complex and exhibit idiosyncratic features compared to other eukaryote genomes. They had long been out of reach of academic researchers until recently, due in part to the time and cost of generating high-quality assemblies. Dinoflagellate research is entering a new genomic era, with the recent availability of chromosome-level genome assemblies, and more genome data from more-broadly sampled taxa are expected. To date, we have generated genome data from 14 taxa of dinoflagellates: 12 from family Symbiodiniaceae (known for their symbiotic associations with corals and other marine organisms), two from the sister lineage *Polarella*. Our comparative genomic analyses thus far have uncovered extensive genomic divergence among Symbiodiniaceae, reflecting the rapid evolution of these dinoflagellates and their phylogenetic diversity hidden behind subtly different morphology. Our results also reveal conserved gene functions related to symbiosis and meiosis, tendency of genes encoded in unidirectional clusters, and tandemly repeated single-exon genes in the genomes. In this presentation, I will discuss how our knowledge of genomes is elucidating the molecular and evolutionary mechanisms that underpin the ecological and genetic diversity of dinoflagellates and their adaptation to changing environments, and how other genomic technologies can help address key question of how dinoflagellates evolved to become some of the most ecologically successful organisms.

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Best Presentation Award (BPA)

Session 1: 19th, 12:50–13:50 (Chair: Mikihiko Arikawa)

B-01~03

Session 2: 19th, 13:55–15:15 (Chair: Yasuhiro Fukuda)

B-04~07

Session 3: 19th, 15:20–16:20 (Chair: Hwan Su Yoon)

B-08~10

The best presentation award (BPA) are given to the most outstanding young researchers of the conference.

A. BPA applicants must be under 40 years of age as of August 31, 2021.
B. In addition to the usual abstract submission, each applicant should also submit an application document for BPA with a written statement (maximum of 300 words) of the importance of the research and his/her date of birth. The application form can be downloaded here. Figures and References can be added to the statement. The statements should clearly describe the background and significance of the research to help understanding by researchers in other fields of protistology. The document should be sent in PDF format on a single A4 page to the ACOP-IV Organizing Committee at acop4@protistology.jp.
C. The BPA Committee of JSP will conduct an initial screening of the applications and select about eight candidates.
D. The selected candidates must give a 20-minute oral presentation (15 minutes for presentation and 5 minutes for discussion) in addition to the poster presentation (optional).
E. The final review based on the oral presentation will be conducted by the members of the International Committee (excluding the members who conducted the preliminary review).
F. For the final review, each committee member chooses two presentations and votes on them by email with a short evaluation comment.
G. If there are more than two candidates with the same number of votes, the decision will be left to the ACOP president and the congress organizer.

B-01

Morphological changes in *Paramecium bursaria* associated with algal endosymbiosis

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Paramecium bursaria is a type of ciliated protozoan characterized by the presence of many symbiotic green algae in its cells. Since the symbiotic algae can be removed from normal *P. bursaria* (green cells), it is easy to artificially obtain non-symbiotic *P. bursaria* (white cells). This organism is considered to be a good experimental model of the process of endosymbiosis, as white cells can be fed with algae isolated and cultured separately to produce green cells again. Previous comparative studies have reported various functional and genetic changes between green and white cells, but the morphological changes were rarely reported. In this study, we quantitatively observed morphological differences between green and white cells using optical and transmission electron microscopy. The first difference we found was the structure of nucleoli in the macronucleus: significantly larger nucleoli were observed in white cells in the stationary phase than in green cells. Since nucleoli are the site of ribosome biogenesis, they are regarded as a barometer of metabolic activity. Therefore, this difference indicated that the dependence of *P. bursaria* on symbiotic algae may have reduced the overall metabolic activity of the cell. In other words, the symbiotic algae may truly contribute to the host *P. bursaria* as functional organelles. The second difference was the arrangement of organelles in the subcortical region. White cells had a higher density of trichocysts than green cells. In addition, in white cells, no mitochondria were observed in the cell surface area, whereas in green cells, many mitochondria were present, fused with each other and attached to the cytoplasmic side of the cell cortex, as well as to the plasma membrane, trichocysts, and peri-algal vacuole membranes. This difference indicates that the mitochondria are the key structures for the symbiotic algae to be moored in the correct position below the host cell surface. In the process of endosymbiotic evolution, the host cell is thought to have been both structurally and functionally altered by the presence of the symbiont. The present observation on *P. bursaria* may provide clues to the early evolutionary process of endosymbiosis.

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B-02

Attachment plaque development in the haptomonad form of *Leishmania*

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Leishmania are protozoan parasites that cause disease in millions of people worldwide. The parasite is transmitted by sand flies in which *Leishmania* have multiple developmental stages and understanding this process is critical to understanding disease spread. Within the sand fly *Leishmania* has two major morphologies, a promastigote morphology with an elongated cell body from which a long motile flagellum extends and a haptomonad morphology with a cell body that is attached to the insect through its flagellum, which has shortened and enlarged its tip. The role of the haptomonad form is cryptic but this attachment process, generating an attachment plaque connecting the flagellum to the insect is conserved across the kinetoplastid parasites and is likely to be important for the development of the parasite.

To gain an in-depth understanding of the *Leishmania* haptomonad, we have established an *in vitro* differentiation system. To validate our system we compared our *in vitro* generated haptomonads with those *in situ* in the sand fly, using serial block face-scanning electron microscopy (SBF-SEM). This generated high resolution 3D models of attached haptomonads and importantly confirmed that our *in vitro* haptomonad mimics the haptomonad in the sand fly – both *in vitro* and *in situ* haptomonads were attached through the tip of the shortened flagellum, and the entire attachment interface was filled with an electron-dense plaque. We next followed haptomonad development using light and electron microscopy and observed that this process has three steps – i) exploration of the surface by the flagellum, ii) establishment of the initial attachment, and iii) disassembly of the flagellum and maturation of the attachment. Using these approaches we have revealed the dynamics of flagellar and cellular morphological changes during haptomonad development. Currently, we are now trying to identify proteins involved in the haptomonad attachment, which will provide insights into breaking the *Leishmania* transmission cycle.

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B-03

Intrinsic rhythm of ciliary reversal acts as spatial memory, in the ciliate, *Stentor coeruleus*

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A kind of ciliates, *Stentor coeruleus*, is a blue-green colored and single-celled microorganism, which lives in freshwaters. *S. coeruleus* shows mainly two types of behaviors, free swimming and adhering to a substratum. When adhering, *Stentor* feeds bacteria using alimentary vortex caused by ciliary beating. It is important where the cell adheres. Adhesive *Stentor* is observed at intricate places in nature and culture conditions. Moreover, the cells form colonies. However, the mechanism of selection of adhesive place is not clear. So we focus on how the organism recognizes spatial information around the cell.
In this presentation, we report two findings about (1) *S. coeruleus* repeatedly stopped at same locations in the ring-shaped arenas with a different diameter and (2) it showed periodic temporal stopping during free swimming and the period was distributed in some wide range. These results indicate that the intrinsic period of stopping could be adjusted by specific locations of swimming arena.
A possible trigger at the specific location is a chemical substance released from an organism itself when stopping. But no substance is detected yet. *S. coeruleus* has been well studied in photoresponses. When the cell encounters a sudden increase light intensity, the organism performs ciliary reversal, stops and turns. Then the cell swims to dark region. However, our findings do not accompany the light or periodic external stimuli. And it implies that the intrinsic rhythm of ciliary reversal acts as spatial memory. Future problems are to study what an external trigger is and what the spatial memory plays a role as an exploration of better feeding environment.

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B-04

New insights into N^6-adenine DNA methylation in the unicellular eukaryote *Paramecium*

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As a rediscovered epigenetic mark, N^6-adenine DNA methylation (6mA) has been revealed with essential functions in eukaryotes. It diversifies across species in abundance, distribution and function, necessitating the study of 6mA in more species. It has been known for decades that the model ciliate *Paramecium* contains 6mA. However, the absence of detailed distribution about 6mA in *Paramecium* impedes further investigation into its function and regulation. In this study, we select *Paramecium bursaria* to reveal the representative 6mA characteristics in the *Paramecium* genus and provide an invaluable resource for investigating the role of 6mA in endosymbiosis as it harbors hundreds of endosymbiotic *Chlorella variabilis* in the cytoplasm. By reanalyzing the published SMRT sequencing results, we present the first detailed map of 6mA genomic distribution with single base pair resolution in *P. bursaria*. Locally, 6mA is preferentially located in the AT motif, mostly as symmetrically and highly methylated sites. Globally, 6mA exhibits a bimodal distribution at the 5' end of RNA polymerase II (Pol II)-transcribed genes. Functionally, 6mA is weakly associated with transcription, and more intriguingly, may also act as a mark facilitating alternative splicing. Evolutionarily, 6mA co-evolved with gene age and may be a mark of endosymbiosis-related genes. The 6mA methyltransferase PAMT1 of *Paramecium* is identified and its importance in cell fitness is confirmed by RNA interference. This study demonstrates potential roles of 6mA in *Paramecium*, offering new insights for the functional and evolutionary diversification of 6mA as an important epigenetic landmark in eukaryotes.

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B-05

Characterization of a novel encystment-inducing pheromone in the ciliated protozoan *Colpoda cucullus*

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The terrestrial ciliated protozoan *Colpoda cucullus* transforms into a resting cyst that is resistant to dehydration and high temperature when the habitat conditions become unfavorable. In laboratory, the encystment can be induced by the addition of Ca^{2+} into the overpopulated cells. Recently, we found that, when the encystment was induced, the vegetative cell of *C. cucullus* releases a certain pheromone-like substance that has an ability to induce the encystment of other vegetative cells. We named the substance encystment-inducing pheromone (EnIP) and characterized it in this study. By incubating vegetative cells of *C. cucullus* at high density in ultrapure water (ion-free), the encystment could be induced without the addition of Ca^{2+}. When the external solution of the cell suspension was added to the vegetative cells at low density, the encystment was markedly induced. These results indicate that EnIP was released from the encystment-induced cells and induced encystment to other cells. Further investigation revealed the characteristics of EnIP as follows; 1) EnIP was released within a couple of hours after cells were incubated at high density. 2) The release of EnIP was inhibited by the addition of an exocytosis inhibitor. 3) EnIP induced the encystment of other cells in a concentration-dependent manner. 4) EnIP lost the encystment-inducing activity after heating treatment at 70°C for 20 min and the treatment with proteolytic enzymes. 5) The encystment-inducing activity of EnIP appeared in suspension after being fractionated with a 100 kDa ultrafiltration membrane. Judging from these results, we concluded that encystment-induced vegetative cell of *C. cucullus* releases 100 kDa protein that acts as a pheromone inducing the encystment to other cells.

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B-06

Patterns of abundant and rare eukaryotes across a broad range of salinities in a solar saltern

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Solar salterns are excellent artificial systems for examining species diversity and succession along salinity gradients. Here, the eukaryotic community in a Korean solar saltern (30 to 380 practical salinity units) was investigated from April 2019 to October 2020 using Illumina sequencing targeting the V4 and V9 regions of 18S rDNA. A total of 926 operational taxonomic units (OTUs) and 1,999 OTUs were obtained with the V4 and V9 regions, respectively. Notably, the high-abundance groups (>5% relative abundance (RA), Alveolata, Stramenopila, Archaeplastida, and Opisthokonta) usually accounted for >90% of the total cumulative read counts and >80% of all OTUs. Moreover, the high-abundance Alveolata (larger protists) and Stramenopila (smaller protists) groups displayed a significant inverse relationship, probably due to predator-prey relationships. Most of the low-abundance (0.1–5% RA) and rare (<0.1% RA) groups remained small across different seasons and years. Taxonomic novelty (at <90% sequence identity) was high in the Amoebozoa, Cryptista, Haptista, Rhizaria, and Stramenopila groups (69.8% of all novel OTUs), suggesting the presence of a large number of hidden species in hypersaline environments. Remarkably, the high-abundance groups had little overlap with the other groups, implying the weakness of rare-to-prevalent community dynamics. The low-abundance Discoba group alone temporarily became the high-abundance group, suggesting that it is an opportunistic group. Overall, the composition and diversity of the eukaryotic community in hypersaline environments may be persistently stabilized, despite diverse disturbance events.

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B-07

Response of 6mA to environmental stressors in *Tetrahymena thermophila*

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Rediscovered as a potential epigenetic mark, N6-methyladenine DNA modification (6mA) was recently reported to be sensitive to environmental stressors in several multicellular eukaryotes. As 6mA distribution and function differ significantly in multicellular and unicellular organisms, whether and how 6mA in unicellular eukaryotes responds to environmental stress remains elusive. Here, we characterized the dynamic changes of 6mA under starvation in the unicellular model organism *Tetrahymena thermophila*. Single-molecule, real-time (SMRT) sequencing reveals that DNA 6mA levels in starved cells are significantly reduced, especially symmetric 6mA, compared to those in vegetatively growing cells. Despite a global 6mA reduction, the fraction of asymmetric 6mA with a high methylation level was increased, which might be the driving force for stronger nucleosome positioning in starved cells. Starvation affects expression of many metabolism-related genes, the expression level change of which is associated with the amount of 6mA change, thereby linking 6mA with global transcription and starvation adaptation. The reduction of symmetric 6mA and the increase of asymmetric 6mA coincide with the downregulation of AMT1 and upregulation of AMT2 and AMT5, which are supposedly the MT-A70 methyltransferases required for symmetric and asymmetric 6mA, respectively. These results demonstrated that a regulated 6mA response to environmental cues is evolutionarily conserved in eukaryotes.

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B-08

Comparative genomics of peritrich ciliates

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In his famous letter to the Royal Society dated in October 9, 1676, Antonie van Leeuwenhoek described a peritrich ciliate, *Vorticella*, and its movement in his first sort of discovery. *Vorticella* is a genus of peritrich ciliate[1)]. *Vorticella* cells could contract ultrafast in a few milliseconds with a great reduction of their length and then extended a little bit slower. Besides the *Vorticella*, many peritrich ciliates could contract using the stalk, such as species in the *Carchesium* and *Zoothamnium*. The ultrafast contraction of the stalk of these ciliates is fascinating, and many researchers have done a lot of work to elucidate the structures, biophysical, and contraction mechanism of the stalk[2)]. However, no high-quality genome sequences of peritrich ciliates were obtained. By incorporating the sequence features (e.g. GC content) and large-scale fast homology searches, we established a custom pipeline, which could efficiently exclude various contaminations of high-throughput DNA sequencing reads. Based on this, we assembled the MAC genomes of six peritrich ciliates (including *Vorticella microstoma, Vorticella convallaria, Carchesium polypinum, Zoothamnium arbuscula, Epistylis chlorelligerum* and *Epistylis plicatilis*) from the wild samples. The genome size of these peritrich ciliates range from 35-66 Mb, and harbor 17-39K genes, respectively. These high-quality MAC genomes of peritrich ciliates will provide a good resource to study the fascinating features of these species, for example, the contraction and extension of the stalk.

1) Van Leewenhoeck, A. 1676. Philosophical Transactions (1665-1678) 12, 821–831.
2) Ryu, S., Pepper, R.E., Nagai, M., and France, D.C., 201, Micromachines (Basel) 8, 4.

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B-09

The first draft genome of obligate extremohalophilic Heterolobosea, *Pleurostomum flabellatum*, and dynamic transcriptome response to temperature and salinity fluctuations

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Heterolobosea belongs to the superphylum Discoba and is a major heterotrophic protist group including at least 150 described species. They contain many extremophilic species such as halophiles, acidophiles, thermophiles, and anaerobes. *Pleurostomum flabellatum* is a representative extreme halophilic heterolobosean inhabiting saturated brines, and its optimal salinity for growth was the highest among eukaryotes on Earth. They can optimally thrive in more than 300‰ salinity water at 40°C with a three times lower oxygen saturation level than ordinary natural seawater. However, almost nothing is known about the nuclear genome and transcriptome of this halophilic protist.

In this study, we generated a draft genome of *P. flabellatum*, and also investigated transcriptomes under temperature and salinity fluctuations to explore the underlying genomic adaptations to extreme environments. In addition, we assembled 73 indigenous prokaryotic genomes, which coexist in the monoeukaryotic culture of *P. flabellatum* to infer the genomic interaction in this harsh condition. As a result, we obtained ≈22 Mb of the draft genome with 7,771 genes including 81.8% of eukaryote's universal single-copy orthologs. We demonstrated that the stress-responsive regulatory pathway and osmolyte-associated genes are dynamically regulated with diverse fluctuations. Furthermore, the assembled prokaryotic genomes mainly consisted of halophilic Archaea, Halobacteriaceae (47%, 34/73), and several halophilic Bacteria. Hence, genome data of *P. flabellatum* and indigenous prokaryotes shed light on the ecological and/or evolutionary interactions in extreme hypersaline habitats. Our sequencing data from the transcriptome analysis provide essential information for the environmental adaptation of this halophilic protist.

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B-10

Syndinean dinoflagellates of the genus *Eduboscquella* are paraphyletic

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The genus *Eduboscquella*, one of several described genera within the syndinean dinoflagellates, exhibits considerable morphological and developmental diversity. Of the 10 formally described *Eduboscquella* species, seven infect tintinnid ciliates, one aloricate ciliates, and two athecate dinoflagellates. Thus far, rRNA gene sequences have been reported only for *Eduboscquella* species that infect tintinnids, with phylogenies incorporating environmental clone-library sequences placing the known tintinnid parasites in a specific subclade within clade 4 of Group I syndinean dinoflagellates. We determined the general morphology and rRNA gene sequences of four *Eduboscquella* species that infected free-living dinoflagellates from coastal waters of Busan, Republic of Korea during summer to fall of 2019-2021. The four parasite species were morphologically distinct from one another, with each infecting one of four host species (*Cucumeridinium coeruleum*, *Gyrodinium* cf. *ochraceum*, and two unidentified species of *Gyrodinium*). Three of the parasites (*Eduboscquella* ex *Cucumeridinium coeruleum*, *Gyrodinium* cf. *ochraceum*, and *Gyrodinium* sp. 2) were morphologically similar to species previously described from athecate dinoflagellates, while the fourth (*Eduboscquella* ex *Gyrodinium* sp. 1) more closely resembled species that infect tintinnids. In our SSU tree for known syndinean taxa, *Eduboscquella* ex *Gyrodinium* sp. 1 located in close proximity to *Eduboscquella* species that parasitize tintinnds. *Eduboscquella* ex *Cucumeridinium coeruleum* and *Gyrodinium* cf. *ochraceum* grouped together, forming a poorly supported sister lineage to *Ichthyodinium* species, while *Eduboscquella* ex *Gyrodinium* sp. 2 branched basal to *Ichthyodinium* and its poorly supported sister lineage. In a SSU tree for syndinean Group I dinoflagellates, our four parasites also failed to group together, instead being distributed across two, perhaps three Group I clades. Our findings show that the genus *Eduboscquella* has much broader sequence divergence than previously recognized and indicate that the genus, as currently recognized, is paraphyletic.

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Poster presentation

Session 1: 20th, 10:00–11:50

R1-01, -03, -05, -07, -09

R2-01, -03, -05, -07

R3-01, -03, -05, -07, -09

Session 2: 20th, 12:30–14:20

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Categories

R1: Cell Biology, Genomics, and Molecular biology
R2: Ecology, Environmental microbiology, and Epidemiology
R3: Evolution and phylogeny, and Taxonomy

R1-01

Protists as live food to feed brine shrimp 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 franciscana*) 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* (Ciliophora, Alveolata), *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. 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, it was found that the survival rate of brine shrimp was significantly reduced when *Euglena gracilis* was given, suggesting that *Euglena* contains substances that are harmful to *Artemia*. On the other hand, *Chlorogonium* was digested and decomposed in the digestive tract of *Artemia*. These results indicate that among the five protists studied, *Chlorogonium capillatum* is the most suitable food for *Artemia* larvae, while *Euglena gracilis* is harmful. This raises a question about the safety of *Euglena*, which has been highly regarded in recent years as an excellent healthy food with high nutritional value.

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R1-02

Actinophryids–centrohelids interactions and their behavior in field and laboratory experiments

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Despite free-living protists being a major factor in modifying aquatic autotrophic biomass, interactions between different taxa of unicellular organisms have been poorly described. However, some of them, such as actinophryid and centrohelid heliozoans, are very common in aquatic ecosystems. For studying cell-to-cell interactions between actinophryids and centrohelids, live observation for behavior of species which were previously involved in co-culture experiments, was performed. Larger actinophryid heliozoan species (*Actinophrys sol*), were captured and ingested by *Raphidocystis contractilis*. When capturing, several individuals of *R. contractilis* gathered around the cell of *A. sol*. At the same time, the axopodial contraction of the *A. sol* occurred, and all axopodia disappeared entirely, making it impossible for *A. sol* to escape from the attack by *R. contractilis*. Subsequently, *R. contractilis* gradually fused with neighboring individuals, and the resulting internal space became a food vacuole containing *A. sol* that was being digested. Another interesting cellular behavior was observed between *Choanocystis pantopoda* (centrohelida) and *Actinosphaerium eichhornii* (actinophryida). Two species of heliozoans just after they have made contact with each other, where their axopodia were closely entwined with each other. The position of *C. pantopoda* remained unchanged, but *A. eichhornii* gradually moved away from the smaller size centrohelid heliozoa. This escape response was always observed when *A. eichhornii* came into contact with *C. pantopoda*, suggesting that *A. eichhornii*recognized *C. pantopoda* as a predator and actively avoided contact with it. In actinophryids, it is known that the induction of cell motility is mediated by some cell surface receptors[1)]. Therefore, it is possible that actinophryid heliozoans perceive some signal on the axopodial surface when they come into contact with the attacking centrohelid and respond by changing the axopodial movement. In actinophryid heliozoans, captured prey is known to swim around in the food vacuole for a long time without being paralyzed. On the other hand, the present results are consistent with previous observations that *R. contractilis* seems to release a specific substance in the process of capturing prey that has the effect of paralyzing the prey and rapidly weakening the prey cells[2)]. This would probably give the centrohelid heliozoans an advantage in the race for survival, making it impossible for even the much larger actinophryid heliozoans to prey on them.

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R1-03

Tolerance of *Colpoda cucullus* Nag-1 wet resting cysts to extreme pH (pH 1 and 13): Implications of less permeability of the cyst membrane to H^+ and OH^-

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Acid tolerance of parasitic unicellular eukaryotes to the low pH of gastric acid is a crucial survival strategy, so that they can proliferate in the gastric intestinal tract. In the present study, we found that the resting cysts of non-parasitic soil ciliate *Colpoda cucullus* Nag-1 showed a strong tolerance to both extremely low and high pH. The purpose of this study was to explore the tolerance mechanism of resting cysts of *Colpoda* to extreme pH. Resting cyst formation in *Colpoda* was induced by addition of Ca^{2+} to overpopulated vegetative cells. The cyst wall which is composed of several layers of endocyst, ectocyst and mucus layer containing lepidosomes was constituted gradually over several days after the encystment induction. The tolerance of the resting cysts to 0.1 M HCl (pH 1) and 0.1 M NaOH (pH 13) increased gradually passed through the days (1 day to 4 days). Moreover, most of mature (1-week-old) resting cysts were alive after exposure to 0.1 M HCl (pH 1) for 4 h, or 0.1 M NaOH (pH 13) for 3 h. These results suggest that the tolerance of resting cysts to both extremely low and high pH was acquired gradually for several days after the encystment induction. The resting cysts were reversibly dehydrated and rehydrated by osmotic pressure. When the resting cysts were transferred from water to 0.1 M HCl or 0.1 M NaOH, they shrank in diameter and recovered to the original state after soaking in water. These results suggest that H^+/Cl^- and Na^+/OH^- may diffuse through the cyst wall to reach the plasma membrane. When mature resting cysts were exposed to 0.1 M HCl for 1 h in the presence of protonophore (CCCP), acid tolerance of the resting cysts was reduced in a CCCP-concentration dependent manner. This suggests that less permeability of the resting cyst plasma membrane to H^+may be responsible for acid tolerance.

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R1-04

Feedless culture strain of *Paramecium bursaria*

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The green paramecium, *Paramecium bursaria* harbors hundreads of symbiotic algae in its body. These symbiotic algae can be easily isolated from the host and cultured. In addition, by co-culturing this cloned symbiotic algae with the host, the endosymbiotic relationship can be simply re-established. Therefore, *Paramecium bursaria* is thought as an ideal system to investigate symbiotic systems established among unicellular eukaryotic cells. When culturing *Paramecium bursaria*, it is common to feed various microorganisms including bacteria. However, it has been pointed out that the culture conditions are not common among researchers in the world because the kinds of microorganisms fed are different. This has a significant problem on the reproducibility of the results of various experiments using *Paramecium bursaria*. Therefore, in our laboratory, it has been investigated whether *Paramecium bursaria* collected from the field can be cultivated without feeding. As a result, a "feedless culture strain (KUNY-2)" was established. This strain was isolated from the field in 2015, cultivated with feeding until 2017, and has been cultivated without feeding until now. Interestingly, it was revealed that bacteria were always present in the culture medium of KUNY-2, even under the condition that the prey bacteria were not fed. Therefore, the composition of bacteria in *Paramecium bursaria* was analyzed at each time point before and after the start of culture without feeding. As a result, it has been clarified that a certain type of bacteria is always detected in the body of *Paramecium bursaria*. Further, liquid culture of isolated symbiotic algae was carried out, and bacteria in the symbiotic algae were also examined. As a result, it became clear that the types of bacteria detected in each of the host and the symbiotic algae are different. We discuss the role of bacteria in establishment of symbiosis between *Paramecium bursaria* and symbiotic algae.

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R1-05

Symbiotic chlorella controls swimming speed of the host cell in the photo-accumulation response of *Paramecium bursaria*

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Photo-accumulation response of photosynthetic organisms is considered as a reaction that enables the efficient photosynthesis. *Paramecium bursaria* is a ciliate which bears endosymbiotic chlorella and shows a photo-accumulation response. In contrast, white cell (chlorella-free *P. bursaria*) does not accumulate in a light-irradiated area, suggesting that symbiotic chlorella cells control the behavior of their host. The photo-accumulation response occurs as a result of multiple photo-responses, including photophobic response and orthokinesis. But it is not clear how these photo-responses (photophobic responses and orthokinesis) are involved in the photo-accumulation response of *P. bursaria*. Therefore, to elucidate the mechanism of light sensing and control of host behavior, we observed and analyzed light wavelength-dependence of photophobic responses (both step-up and step-down responses) and orthokinesis (swimming speed) in *P. bursaria* and white cell. When we examined the photophobic responses of swimming cells, we found that there was no difference between those of *P. bursaria* and white cell in green- and blue-light-irradiated areas, which are the effective wavelengths for the photo-accumulation response. We also examined the effect of green and blue light on the swimming speed of *P. bursaria* and white cell and found that the swimming speed of *P. bursaria* was significantly reduced by green- and blue-light irradiations, but that of white cell was not. These results suggest that the orthokinesis, in which the symbiotic chlorella cells decrease the swimming speed of the host cell under green or blue light condition, is largely responsible for the photo-accumulation response of *P. bursaria*.

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R1-06

Expression profile and immuno-localization of Skeleton Binding Protein 1 (SBP1) during gametocyte stage in *Plasmodium falciparum*

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Malaria is a mosquito-transmitted infectious disease caused by unicellular protozoan parasites belonging to the genus *Plasmodium*. Proliferation of the parasite in human erythrocytes underlies clinical manifestations of malaria. The intraerythrocytic stage is divided into ""asexual stage"" and ""gametocyte stage"". Gametocytes are sexual precursor cells of the malaria parasite that mediate the transmission from humans to *Anopheles* mosquitoes. During gametocyte development, the parasite undergoes five distinct morphological stages. In malaria patient, gametocyte stage V and immature asexual ring stage appear in the peripheral blood. The phenomenon due to modification of the infected erythrocyte surface by protozoan proteins. Our study focuses on modification of adhesive properties in *P. falciparum* gametocyte infected erythrocytes.

Maurer's clefts, parasite-derived membrane structures in the erythrocyte cytoplasm, are thought to function as a later sorting compartment. Skeleton Binding Protein 1 (SBP1), well-documented Maurer's cleft protein is reported to be expressed during asexual stage. This study therefore aimed at elucidating the expression profile and subcellular localization of SBP1 during gametocyte stage in *P. falciparum*. To characterize SBP1, we generated antibody using recombinant SBP1 protein which expressed using the wheat germ cell-free protein synthesis system. By IFA using anti-SBP1 antibodies signal was detected in the cytoplasm of erythrocytes parasitized with gametocyte stage I to V. To determine the precise localization of SBP1, we performed immuno-electron microscopy. Gold particles indicated SBP1 accumulation in Maurer's clefts in the cytoplasm of gametocyte infected erythrocytes. 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.

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R1-07

Comparative transcriptomics in a centric diatom *Pleurosira laevis* under two salinity conditions, with reference to stress response and morphological plasticity

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Pleurosira laevis is an euryhaline diatom distributed around the world. Compère (1982) described several forms and varieties of *P. laevis* based on their morphology, including *P. laevis* f. *laevis* and *P. laevis* f. *polymorpha*, distinguished by their flat valve face or dome-shaped valve face with protruding ocelli, respectively. In this study, we established 4 strains of *P. laevis* (3 of *P. laevis* f. *laevis* isolated from fresh or brackish waters, and 1 strain of *P. laevis* f. *polymorpha* isolated from a coastal area). Manipulating the salinity in culture showed that these *P. laevis* f. *laevis* and *P. laevis* f. *polymorpha* strains formed both flat (*laevis* form) and dome-shaped (*polymorpha* form) valves depending on the salinity. The morphological changes took place on the salinity boundary between 2‰ and 7‰ in all the strains, with transitional shapes between 3 and 6 ‰. This valve shape plasticity required only 5 ‰ salinity difference to induce the dynamic morphological change. We then performed a comparative transcriptome analysis using the strain grown under salinity 2‰ and 7‰, expecting to identify genetic factors responsible for determination of each valve shape as well as the ones related to salinity responses. As a result, 2,843 differentially expressed genes were detected between the two salinity conditions. The functional categories of the differentially expressed genes included transposons, osmolyte synthesis, membrane transport and cytoskeleton elements.

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R1-08

Acanthamoeba was induced differential gene expression by ingestion of Legionella pneumophila and Escherichia coli

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Acanthamoeba spp. feeds on bacteria, fungi, and algae to obtain nutrients from the environment. However, several pathogens can survive and multiply in Acanthamoeba. Mechanisms necessary for the survival and proliferation of microorganisms in Acanthamoeba remain unclear. The object of this study was to identify effective factors for survival of microorganisms in Acanthamoeba. Differentially expressed genes (DEGs) in A. castellanii infected by Legionella pneumophila or Escherichia coli were identified based on mRNA sequencing. A total of 2,342 and 1,878 DEGs were identified in Acanthamoeba with L. pneumophila and E. coli, respectively. Among these DEGs, 502 were up-regulated and 116 were down- regulated in Acanthamoeba infected by L. pneumophila compared to those in Acanthamoeba feed on E. coli. Gene ontology analysis showed that the genes encoded small GTPase-mediated signal transduction proteins in the biological process domain, intracellular proteins in the cellular component domain, and ATP binding proteins in the molecular function domain were up-regulated while integral components of membrane proteins in the cellular component domain were down-regulated in Acanthamoeba infected by Legionella compared to those in Acanthamoeba feed on E. coli. During endosymbiosis with Legionella, Acanthamoeba showed various changes in the expression of genes supposed to be involved in phagosomal maturation. Acanthamoeba infected by Legionella also showed high expression levels of aminotransferase, methyltransferase, and cysteine proteinase but low expression levels of RNA pseudouridine synthase superfamily protein and 2OG-Fe(II) oxygenase superfamily. These results provide directions for further research to understand the survival strategy of L. pneumophila in A. castellanii.

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R1-09

The HP1-like protein Hpl8p regulates vegetative growth and DNA elimination in *Tetrahymena*

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R2-01

Community response of protistan plankton and their mediated carbon flow to eddies

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The effect of oceanic mesoscale cyclonic eddies in an eastern boundary upwelling system on protistan community structures was examined using V9 18S rRNA gene amplicons. Therefore, we collected samples along a distance gradient from the centre of a cyclonic eddy towards the eddy periphery. At each transect station we sampled three depths (deep chlorophyll maximum DCM, end of photic zone EPZ and oxygen minimum zone OMZ). Additional stations outside the eddy were sampled as reference. We also conducted grazing experiments in samples of the DCM to determine the carbon transfer from bacteria to phagotrophic protists.
Alpha diversity of protistan communities was lowest in the DCM and increased with depth for all sampling stations. The protist community structure also changed along the horizontal transect and the vertical depth gradients. Dinoflagellates were the most abundant (in terms of sequence amplicons) in all three depths. In the DCM Stramenopiles were the second most abundant taxa which are superseded by Rhizaria in the EPZ and OMZ. Bacterial uptake rates of the protistan communities were six times higher within the eddy compared to the reference site. The top-down control of protists on bacteria accounted for a bacterial turnover rate of ca. 20% of the bacterial standing stock (BSS) within the eddy.
Our results suggest a high importance of ocean eddies for protistan community structures and carbon transfer within the microbial loop. In subsequent steps, this carbon is available for higher trophic levels, pinpointing the relevance of eddies for the ocean carbon pump and to sustain higher trophic levels, including fish.

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R2-02

Ciliates as models for the response to environmental changes: gene expression studies

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Ciliates, such as *Tetrahymena* and *Euplotes*, are eukaryotic microorganisms directly exposed to the aquatic environment and well characterized by omics technologies and genetic manipulations.
We use these organisms as models for studying the single cell response to environmental changes in temperature, salinity, and to several pollutants. The approach is both morphological and molecular, looking at changes in ciliates phenotype (e.g., cilia organization, structure and motility) and gene expression.
By differential expression and enrichment analysis (RNA-seq), we identified biological processes (phagocytosis, transport pathways, response to oxidative stress, glutathione peroxidase activity, proteolysis, and nitrogen metabolism process) and marker genes affected by environmental stresses in *T. thermophila* exposed to commercially available and stabilized silver nanoparticles (AgNPs) (Piersanti et al. Environ Pollut, 2021), that are increasingly diffused in industrial applications and well known as biocides. Performing the toxicity tests of AgNPs in *E. crassus*, we discovered that this marine ciliate is more sensitive by one order of magnitude than the freshwater *Tetrahymena* (EC50 at 24 h is 3,6 mg/L and 38 mg/L, respectively), suggesting that the marine environment could be more endangered by such a pollutant.
Currently, we are investigating in *E. crassus* the genes involved in the cellular response to these pollutants, like genes involved in the response to oxidative stress and in the cell cycle progression.

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R2-03

Group-specific phytoplankton carbon fixation in the south-west Pacific Ocean

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Small phytoplankton are significant contributors to plankton biomass and primary productivity, but their cell-specific rates of carbon fixation vary greatly among and within groups depending on the season and location. Yet, only 6 studies have directly measured the group-specific rates of carbon fixation of different small phytoplankton groups. Hence more measurements across diverse environmental gradients are needed to characterize group-specific variability and to improve current productivity estimates. We investigated the carbon fixation rates and community composition of three phytoplankton groups: the picocyanobacterial *Synechococcus*, picoeukaryotes (cell diameter from 0.2 to 3-µm) and nanoeukaryotes (cell diameter from 3 to 20-µm). The study was conducted in the south-west Pacific region across subtropical and subantarctic water masses that flank the subtropical front east of New Zealand. Picophytoplankton (consisting of cyanobacteria and picoeukaryotes) dominated phytoplankton biomass compared to nanophytoplankton in both subtropical and subantarctic water masses. In this study, we present measurements of group-specific rate estimates derived from flow cytometry sorting of ^{14}C incubated cells and explore its variability in contrasting water masses.. We also present data from DNA metabarcoding on sorted phytoplankton cells to characterise communities that are associated with higher or lower carbon fixation rates. Nanoeukaryotes had the highest group-specific rate of carbon fixation (measured in mgC/cell/day), followed by picoeukaryotes and lastly *Synechococcus*. The group-specific carbon fixation rate of smaller picophytoplankton changed across the environmental gradient, while the rate of larger nanophytoplankton remained similar. Our results suggest photosynthetic picoeukaryotes are significant contributors to primary productivity in subtropical and subantarctic oceanic waters despite their low cell abundance, which is consistent with previous studies.

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R2-04

Community structure variation and horizontal transfer of symbiotic protists of *Reticulitermes* termites revealed by partial SSU rRNA gene sequences

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Termites harbor symbiotic protist communities in their guts composed of species in two anaerobic taxa, Parabasalia and Oxymonada. These protists play a major role in wood degradation of host termites. In general the species composition of the symbiont community is host species-specific, reflecting vertical protist succession between host generations.

Seven species of *Reticulitermes* termites are known in Japan and five of them are distributed in the Ryukyu Archipelago. Their protist species composition have been regarded to show strong host species-specificity. Recently, however, we found a specific symbiont species of *R. speratus* from *R. amamianus* colonies in the Tokara Islands, the distribution boundary area of the two host species. This suggested the past host hybridization and horizontal transfer of symbiotic protist.

In this study, to reveal the variation of protist community structures and to verify the possibility of the past horizontal transfer, we collected a total of 70 host termite colonies from 21 Islands in the Japan Archipelago. We carried out a community composition analysis on these colonies by partial SSU rRNA gene sequencing using the Illumina MiSeq.

On average ca. 40,000 protist sequences were obtained from a host colony. 35.9% of the total reads were assigned as parabasalids, and the rest were as oxymonads. The community similarity pattern revealed by a principal coordinate analysis (PCoA) using Jaccard index largely corresponded to host species. However, protist compositions of the colonies in Okinoerabu Is., of *R. amamianus* were more similar to those of *R. okinawanus* than other conspecific colonies. From the colonies of three host species on the islands at the edge of distribution, we detected protist sequences which were shared by the colonies of different host species, but not by the conspecific colonies of other areas. These results indicate horizontal symbiont transfer should occur between neighboring host termite species more frequently than previously thought.

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R2-05

Mutually exclusive co-existence of the kleptoplastidic ciliate *Mesodinium rubrum* and its plastid donor cryptomonad *Teleaulax amphioxeia*

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KNU MR-MAL01, the first temperate strain of *Mesodinium rubrum*[1)] (*MR*), was tested for their growth pattern under different concentration of *Teleaulax amphioxeia* (*TA*) as plastid donor as well as ingested prey[2)]. Three sets of duplicate cultivation experiment each with 5-7 TA:MR ratios (0-1000) were set up with the fixed *MR* concentrations of 500, 1000, and 1400 cells ml^{-1}, respectively. From all the 54 combination culture bottles triplicate subsamples were collected daily for the microscopic observation and counting. In each sets, different critical concentration of *TA* (CCta) could be identified as the boundary between increased and decreased *MR* growth when compared with control *MR* bottles. Below CCta *MR* grows far better than control group by the promoting function of the ingested *TA* while *MR* growth is reduced in a *CR*-concentration dependent manner at inhibitory *TA* concentrations greater than CCta. This results highlight the importance of quantitative bispecific interaction for *MR* bloom formation at the point of the absolute *MR* concentration in the initial stage as well as of the simple TA:MR ratio. The duration of *MR* blooms and the maximum population level in each blooming case might be closely associated with not only the initial *MR* concentration of a season but also with the timing of CCta is met. Further understanding on the mutually exclusive co-existence of the kleptoplastidic ciliate *MR* and *TA* is pre-requisite for *MR* bloom control.
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R2-06

Protistan kleptoplastid chain of TPG clade origin and a cryptomonad species *Teleaulax amphioxeia*

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Uniqueness of cryptomonad plastids among phototrophic protists has long been known since the first report on the nucleomorph[1,2)] which enables the inherent, unique and incompletely remnant autonomy of the host plastid in cryptophytes[3)]. Among the TPG clade crypotomonads *Teleaulax amphioxeia* (*TA*) is the keystone plastid donor species for the kleptoplastid chain through *Mesodinium rubrum* (*MR*) and then *Dinophysis* spp. further to other dinoflagellate engulfment predators on *Dinophysis*.

In this presentation the whole scope of the protistan kleptoplastid chain of the TPG clade origin is envisioned as a still 'too complex to grip on' phenomenal entity. The aftermath of the cell organelles retention (CMCs and nuclei including nucleomorph in *TA*) by *MR* needs to be far better explored on the functional contribution of the sequestered organelles for *MR*'s ecological integrity. Third hand kleptoplastid retained in *Dinophysis* cells by feeding *MR*[3)] may be functioning through molecular genetically communication with host nucleus inside the labyrinthine structure of cellular biochemistry[4)]. Evolutionary timeline of *TA*, *MR*, *Dinophysis* species as wells as the history of kleptoplastidy of *MR* and *Dinophysis*[5)] may be resolved further deeply before science could touch the secret of the marine protist world.

Predators using *Dinophysis* spp. in marine ecosystem are another kind of organism for further understanding[6,7,8)]. The parasitic protist in the *Parvilucifera* infect *Dinophysis* spp. as well as the dinoflagellate predators of *Dinophysis* such as *Fragilidium duplocampanaeforme*[7)]. Thus, the protistan kleptoplastid chain of the TPG clade origin and the associated food web dynamics is wide open to the frontier marine ecosystem researchers.

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R2-07

Diversity of unclassified Rhizaria in the Indian Ocean hydrothermal vents

Je Bak An[1], Hyeon Been Lee[1], Dong Hyuk Jeong[1], Jung Min Choi[2], Young Ok Kim[2], Dong Sung Kim[2], Jong Soo Park[1]

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The submarine hydrothermal vent is widely but sparsely distributed on the seafloor deeper than 1500 m on Earth. Approximately 95% of the hydrothermal vent biota has been unknown taxa. Thus, the deep-sea hydrothermal eukaryote community remains poorly understood. Here, we investigated the benthic eukaryotic community near the Indian Ocean hydrothermal vents known as the Onnuri Vent Field (OVF, station: MC1906, 2019 m in depth) and Invent-B (station: MC1914, 4299 m in depth) using Illumina massive sequencing targeting the V4 and V9 regions of 18S rDNA. Two samples were collected from sediments at >2000 m in depth using a multiple corer, which appeared to be a reliable method capable of taking uncontaminated cores. In total, 505 operational taxonomic units (OTUs) and 680 OTUs were detected using the V4 and V9 primer sets, respectively, at two stations MC1906 and MC1914. Our data showed that Opistokonta, Alveolata, and Rhizaria groups dominated the vent eukaryote community. In particular, the Rhizaria including Foraminifera and Radiolaria, which comprises many of the amoeboid forms with fine pseudopodia, displayed the highest number of OTUs (66 OTUs from MC1906 and 72 OTUs from MC1914) and became a high-abundance group among eukaryotes (ranked second: 34% from MC1906 and 27% from MC1914) in the V9 dataset. Remarkably, at least 8 unclassified Rhizaria clades were newly discovered in the Rhizaria of the Indian Ocean hydrothermal vents (unclassified Rhizaria clade I to VIII). Our finding provides a hint that diverse unclassified Rhizaria are predominant groups around the hydrothermal vents and may play a critical role in one of the most unusual habitats.

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R3-01

An historical and contemporary review of the heterokont algae tree of life

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Heterokont algae are one of the largest and most diverse groups of eukaryotes, including unicellular and complex multicellular lineages; photosynthetic and nonphotosynthetic lineages; freshwater and marine lineages; microscopic and macroscopic lineages. Generally, they are characterized by an immature flagellum that bears tripartite tubular hairs and a mature flagellum lacking hairs. Despite a long and rich taxonomy tradition, the heterokont's classification was difficult and still remains to this day. The development of molecular phylogenies allowed overcoming the limitations of morphological and ultrastructural characters (e.g. one cannot compare naked amoeboid synchromophyte, silica-walled diatoms, or the cell wall of brown seaweeds). However, the phylogenetic classification of the heterokont algae still remains partly unresolved. We present here the result of recent multigene phylogenies based on either a large number of taxa or a large number of genes. These phylogenies allowed resolving a number of class level relationships within the heterokonts. However, they also generate conflicts with ancient literature that need to be addressed.
Following an historical review of the important developments in the study of heterokont algae, we will use various recent examples to illustrate how to address and resolve these conflicts. Finally, we discuss how our proposed phylogeny can serve as a framework to study the evolution of the heterokont algae and further developments.

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R3-02

Plastome phylogenomics elucidates evolutionary relationships and hidden conflicts in brown algae

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Since the advent of DNA sequencing techniques, phylogenomics approaches have contributed to understanding evolutionary relationships among various lineages of eukaryotes. Molecular phylogenetics approaches have also tremendously changed thoughts on evolutionary relationships among brown algae (Phaeophyceae), which was previously based on morphological (dis)similarities. However, the past phylogenetic studies made use of only several markers, which led to a poorly-resolved crown clade (BACR clade). This study successfully tackled this stochastic error (an error due to lack of sequence data) using full plastome data of 58 brown algal taxa. Also, the ancestral plastome structure of the SSDO and BACR clade was revealed. BACR clade is composed of 5 different clades (clade I-V), each of which was sequentially diverged from the rest of the BACR species and formed synapomorphic plastome structure. Moreover, we investigated conflicts between plastome (reference) phylogeny and gene phylogenies with gene concordance test and found substantial conflicts within several branches. Here, we suggest the sources of the conflicts with making a close examination on the two intra-order conflictual cases: Rearrangement of plastome structure (the Chordales case) and different inheritance mode (the Scytosiphonaceae case). The findings reject one-locus hypothesis of the plastid genome and suggest that researchers take note of biological sources of conflicts when dealing with highly-conflicted plastid gene trees.

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R3-03

Utility of hypervariable region V2 in SSU rRNA gene as phylogenetic signature of family Colepidae (Ciliophora: Prostomatea: Prorodontida)

Ratih Kusuma Wardani[1], Mann Kyoon Shin[1]
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The small subunit (SSU) rRNA gene sequence has been used extensively for phylogeny studies in several groups of ciliates. However, recent debate has arisen about the application and stability of SSU rRNA gene due to its copy number and polymorphism. Meanwhile, its secondary structure has been widely applied to infer the evolution and phylogeny of several taxa (ciliates, algae, metazoans). Family Colepidae Ehrenberg, 1838, consists of ten genera, and several major morphological characters (e.g., the number of armor tiers, the presence or absence of armor spines, the type of tier plates, and the number of adoral organelles) separate these genera. In previous studies, some genera within the family Colepidae show non-monophyletic relationships with other colepid genera. The problem remains unclear because of limited taxa and molecular data. The secondary structure of SSU rRNA can explain relationship between problematic taxa and support the phylogeny based on morphology and molecular data. In this study, we analyze the secondary structure of SSU rRNA to infer the relationship within colepid clade and add newly sequenced data of *Pinacocoleps similis* to increase taxon sampling. The helix E9 (part of V2 region), a part of secondary structure, distinguishes the relationship between *Coleps* and *Levicoleps*, and the helix E23_9 (part of V2 region) differentiates two genera *Coleps* and *Pinacocoleps*, which corroborate the relationships of these two genera based on the morphological character of armor spines by presence or absence. Furthermore, the structure of helix E43 (part of V2 region) discriminates the members of Colepidae into genera and it supports morphological criteria for the genus-level classification.

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R3-04

Exploration of protistan diversity from hydrothermal vent on the Central Indian Ridge, Indian Ocean

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The diverse protists play a critical role in controlling the flux of energy and transfer of materials in marine ecosystems including extreme environments. Deep-sea hydrothermal vents are one of the most unusual and highly dynamic environments, making it difficult to collect samples. To date, almost nothing is known about the diversity of protists inhabiting deep-sea hydrothermal vents based on the combinations of light microscopy and molecular sequencing. To investigate the diversity of the protistan community around the hydrothermal Onnuri Vent Field (OVF), the diverse samples around the OVF were collected by multiple corers, and TV grab on board R/V ISABU from June to July 2019. The sediment samples from the seafloor at a depth of 2020 m were analysed by Illumina high-throughput sequencing using the two V4 and V9 primer sets. Also, the ciliated protozoa were identified by light microscopic observations and molecular phylogeny of 18S rDNA sequences. Based on next-generation sequencing, the most abundant sequence reads in both the V4 and V9 regions of 18S rDNA amplicons were the supergroup Opisthokonta (V4: 93%, V9: 62%), and subsequently followed by the supergroups Rhizaria (V4: 2.5%, V9: 30.7%) and Alveolata (V4: 3.5%, V9: 1.0%). However, despite the relatively low sequence read, the supergroup Alveolata including ciliates displayed the highest OTUs (Operational Taxonomic Units) in the V4 and V9. A total of seven species were discovered by microscopical observation of the taxon Ciliophora, which was composed of each one species in Sessilid, Trachelocercid and Discocephalid, two *Euplotes* spp., *Pleuronema* sp., and *Dysteria* sp. Although the *Dysteria* sp. is considered as a species closed to *D. semiluaris*, its morphological characteristics of cell sized, shape and ciliary arrangements as well as 18S rDNA sequence differs from those of *D. semiluris*. Therefore, the *Dysteria* sp. is regarded as a candidate of new species. For species identification of other ciliate species, morphological and molecular data are needed through further investigations.

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R3-05

Redescription of three species of *Metopus* Ciliates (Ciliophora, Armophorida, Metopidae) from South Korea

Quoc Dung Nguyen[1], Nanda Dwi Kristanti[1], Novia Cahyani[1], Sahr Uzma[1], Mann Kyoon Shin[1]
[1]: Department of Biological Science, University of Ulsan, Republic of Korea

Metopid ciliates are known as cosmopolitan and inhabit hypoxic environments. They can be found in terrestrial, freshwater, and marine habitat worldwide. Although the genus *Metopus* is one of the most species-rich genera in metopid group, there are few descriptions of this genus using modern methods, particularly in South Korea. In this study, we redescribe three species of the genus *Metopus*, *M. contortus* (Quennerstedt, 1867), *M. es* (Müller,1776), and *M. vestitus* Kahl, 1927, collected from Korea, using live observation, protargol staining, and molecular phylogeny. *M. contortus* is characterized by a body shape with a wider equatorial part than anterior and posterior ends; body size *in vivo* 110–170 × 39–58 μm; somatic kinety comprises 34–45 rows, each kinety composed of "tri-kinetids"; preoral dome is broadly convex and extended over a half of body. *Metopus es* differs from the other species by elongate elliptical and slightly sigmoid body shape; preoral dome lightly convex, overhanging left margin. Korean population of *M. es* has body size *in vivo* 70–120 × 20–35 μm; one ellipsoidal macronucleus located in the anterior half of the body; one globular micronucleus located in the middle of the macronuclear depression; symbionts detected around macronucleus and cytoplasm; somatic kinety comprises 15–18 rows, each kinety composed of "di-kinetids". *M. vestitus* can be distinguished from other species by its cone-shaped body with conspicuous tail region; cell size *in vivo* 100–149 × 11–29 μm; accumulation of intracytoplasmic structure in the anterior end with the length 7–16 μm; and densely packed of bacteria on the cell surface. The molecular phylogeny based on small subunit ribosomal RNA gene sequences corroborates the morphological identification of these species and their phylogenies match with the previous studies.

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R3-06

A contribution to redescription of two poorly known *Sonderia* species of ciliates (Ciliophora; Plagiopylea; Plagiopylida; Sonderiidae) collected from Korea

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To investigate the Korean ciliate diversity, we collected two species of sonderiid ciliates from a saltmarsh in Mokpo, Korea. We identified them as *Sonderia* cf. *macrochilus* Kahl, 1930, and *S. vorax* Kahl, 1928 based on the observations of live and silver stainings. *Sonderia* cf. *macrochilus* has egg-shaped body with size *in vivo* 133–171 x 66–97 μm, body (length/width) ratio 1.7–2.3:1; oral cavity with narrow oral opening, right end dented downward about 18–22 μm in length, extended to left margin; the depth of oral cavity about 1/3–2/5 of body length; oral ciliatures consisted of pre-buccal on upper oral-lip with 26–36 rows and post-buccal on lower lip with 30–40 rows; somatic kineties consisted of 25–34 rows on ventral and 30–41 on dorsal surfaces; contractile vacuole located near posterior end without collecting canal; needle-shaped extrusomes (trichocysts) scattered in whole cytoplasm. Korean population of *Sonderia* cf. *macrochilus* is very close to German population of *S. macrochilus* in terms of body ratio and oral lip pattern but different regarding the depth of oral cavity (1/3–2/5 vs. >1/2), somatic kineties rows on the ventral surface (25–34 vs. about 18, based on drawing); trichocyst (present vs. absent) and contractile vacuole (present vs. absent). *Sonderia vorax* has ellipsoid body with size *in vivo* 82–114 x 43–65 μm (body ratio 1.6–2.1:1); oral cavity with wide oral opening, crescent-shaped, perpendicularly to body axis, the depth of oral cavity about 1/2 of body length; oral ciliatures consisted of pre-buccal on upper oral-lip with 19–27 rows and post-buccal on lower lip with 19–26 rows; somatic kineties consisted of 23–28 rows on ventral and 24–31 on dorsal surfaces; contractile vacuole located near posterior end without collecting canal; needle-shaped extrusomes scattered in whole cytoplasm. Korean population of *S. vorax* has minor differences compared with Italian and German populations in terms of depth of buccal cavity (1/3–2/3 vs. 1/3 vs. 1/2), direction of swimming rotation (both ways of clockwise vs. anti-clockwise vs. no data).

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R3-07

Pararosarium dinoexitiosum gen. et sp. nov. (Perkinsozoa, Alveolata), a new parasitoid infecting marine dinoflagellates with characteristic beaded sporocytes

Boo Seong Jeon[1], Myung Gil Park[1]
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Phylum Perkinsozoa is known as an exclusively parasitic group within alveolates, and is widely distributed in various aquatic environments from marine to freshwater environments. Nonetheless, Perkinsozoa is still full of numerous environmental rDNA sequences without taxonomically-defined, with their morphology, life cycle, the identity of the host, and physiological characteristics remaining unknown. During intensive sampling along the west coast of Korea in October and November 2017, a new parasitoid, which shares several characteristics with the extant families Perkinsidae and Parviluciferaceae, was discovered and three strains of the new parasitoid were successfully established in cultures. The new parasitoid shared many morphological and developmental characteristics with other Perkinsozoan parasites. Furthermore, through palintomic extracellular sporogenesis, it produced characteristic interconnected sporocytes resembling a string of beads. Phylogenetic analyses based on the small subunit and large subunit ribosomal DNA sequences revealed that the new parasitoid was distantly related to the family Parviluciferaceae, which is parasitoid group of dinoflagellates, and was more closely related to the families Perkinsidae and Xcellidae. Morphological, ultrastructural, and molecular data on the new parasitoid raised the need to erect a new family, i.e., Pararosariidae, within the phylum Perkinsozoa with *Pararosarium dinoexitiosum* gen. et sp. nov. as the type species. The isolation and establishment in culture of the new parasitoid outside the family Parviluciferaceae in the present study would contribute to the better understanding of the diversity of Perkinsozoan parasites and provide useful material for comparisons to other parasite species in the further study.

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R3-08

Characterizations of heterotrophic nanoflagellates isolated from coastal areas in Korea

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Heterotrophic nanoflagellates (HNFs, 2–20 μm in size) are substantially capable of controlling bacterial abundance in aquatic environments, and microbial taxonomists have studied ecologically important and abundant HNFs for a long time. Although many HNFs have been reported in Korea, our knowledge about the biodiversity of autochthonous HNFs remains poor in the natural ecosystems of Korea based on morphology and 18S rDNA sequencing. Here, previously reported five HNFs from non-Korean habitats were isolated from Korean coastal seawater or intertidal sediments for the first time. Light microscopic observations and 18S rDNA phylogenetic trees revealed that the five isolated species were *Cafeteria burkhardae* strain PH003, *Cafeteria graefeae* strain UL001, *Aplanochytriumminuta* (formerly *Labyrinthuloides minuta*) strain PH004, *Neobodo curvifilus* strain KM017 (formerly *Procryptobia sorokini*), and *Ancyromonas micra* (formerly *Planomonas micra*) strain IG005. Being morphologically and phylogenetically indistinct from its closest species, all isolates from Korea were therefore regarded as identical species detected in other countries. Thus, this result indicates an expansion of known habitats that range from those of the five isolates in natural ecosystems on Earth. Furthermore, the global distribution pattern of the five HNFs will be addressed through the sequence analysis for the hypervariable V4 region of the 18S rDNA from the *Malaspina*-2010 expedition surveyed in 120 stations at surface (3 m in depth)

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R3-09

Undescribed Cavosteliida amoeba isolated from *Auricularia* sp.

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The class Variosea is an amoebozoan group, which name is derived from “various” morphology, and its diversity has been recognized by the recent molecular phylogenetic study (Kang et al. 2016). Some variosean lineages have the protosteloid sporangium in their life cycle. The protosteloid sporangium is characterized by the simple structure, comprised of only 1–4 (typically one) spores and a non-cellular stalk. The protosteloid sporangium is obtained in various amoebozoan linages, but most protosteloid linages belong to the Variosea (Shadwick et al. 2009, Kang et al. 2016). However, the true biodiversity of variosean amoebozoans is not elucidated.
We isolated a new amoeba strain YIP-63 from the fruiting body of *Auricularia* sp. The trophic stage of YIP-63 was small and branched amoeba with filose pseudopodia, and sometimes formed lobose pseudopodia. In the lifecycle, YIP-63 made a tiny protosteloid sporangium, which had a non-deciduous spore (ave. 5.9 μm in diam.) and short stalk (ave. 2.3 μm in length). Flagellate stage and plurinucleate plasmodial stage were not observed.
Phylogenetic analysis based on SSU rRNA gene with amoebozoan dataset suggested the YIP-63 was placed sister to the Cavoseliida with a moderate bootstrap support (62%). The results of phylogenetic analyses with smaller datasets (Variosean dataset and Cavosteliida dataset) showed the YIP-63 was placed in the Cavosteliida and formed a clade with the genus *Tychosporium*, but the bootstrap supports were low.
The cavosteliida is comprised of three genera *Cavostelium*, *Schizoplasmodiopsis* and *Tychosporium*. All these genera have the prototsteloid sporangium stage with non-deciduous spore, and these genera were distinguished by the morphology of trophic amoeba and sporangium, and the presence or absence of flagellate and plasmodial stages. The strain YIP-63 shares non-deciduous spore of sporangium with cavosteliid members and branched amoeba producing filose pseudopodia with the genus *Schizoplasmodiopsis*. However, the amoeba of the YIP-63 is much smaller than any species of *Schizoplasmodiopsis* and the tininess of sporangium and the lifecycle without both plasmodial and flagellate stages are different from the all genera of Cavosteliida. Therefore, we conclude that the YIP-63 represents the undescribed species of the Cavosteliida and should be classified in a new genus. The detailed phylogenetic analysis based on the large dataset is necessary to clarify the taxonomic position of the strain.

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R3-10

Occurrences of *Cymbella distalebiseriata-liyangensis* species complex (Bacillariophyceae, Cymbellales, Cymbellaceae) from Japan

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Recently we discovered peculiar diatoms belonging to the genus *Cymbella* from three different rivers in Western Japan. They inhabited on a tortoise carapace in the Amano River (Shiga, Honshu), on a cobble in the torrent of the Chikusa River (Hyogo, Honshu), and on filamentous algae in the suspended water of the Onga River (Fukuoka, Kyushu). They are characterized by having valves with biseriate striae near the apices and uniseriate striae around the centre. The population from the Amano River is identified as *Cymbella distalebiseriata* B. Liu & D. M. Williams in Liu et al. (2018), while that from the Chikusa River is more suitable to identify as *Cymbella liyangensis* W.Zhang, I. Jüttner & E. J. Cox in Zhang et al. (2018). Those from the Onga River are similar to *C. distalebiseriata* in the valve shape, but morphometrically more similar to *C. liyangensis*. Because these two species are very similar in valve outline and stria density except for the difference of areola density, we regarded them as *Cymbella distalebiseriata-liyangensis* species complex. Both species were originally described from Yangtze River basin, China, and to our knowledge, these species have not reported from other countries or regions. We would assume that they have recently invaded Japan, but further studies are necessary to confirm if it is true.

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Young Protistologists' Association

Young Protistologists' Association

We have a special program for young researchers and students working on protistology to get to know each other and deepen their research interactions. This program is open to anyone in the world, not just people from Asian countries. The requirement to participate is that you must be under the age of 40 and register to participate. I have sent you the URL of the SpatialChat for the event (If you don't have the URL, please contact acop_young"a"protistology.jp; Please change "a" to @). At the start time (18:40 on November 19), please access the URL. For more information on the usage of the SpatialChat, please refer to the usage section on page 86 of this file.

The schedule for the event is as follows: First, I will explain the event, and then all participants will introduce themselves and their research in about 2 minutes using the materials already submitted. After that, we would like you to talk freely with anyone you want. Don't forget to bring your drinks and snacks. The session is scheduled to end at 20:00. We sincerely believe that this project will be a way to make good friends and an opportunity for future international collaborations among the participants, which will lead to a significant development of protistology beyond national borders. Have fun with this event!

If you have any other questions, please send an email to acop_young"a"protistology.jp. (Please change "a" to @)

All members of the Executive Committee of iACOP event for young researchers

Appendix

How to join Zoom and SpatialChat

Oral presentations will be held via Zoom, and poster presentations will be held via SpatialChat.

Zoom

The URL information for connecting to Zoom will be emailed to all participants a few days before the meeting.

SpatialChat

SpatialChat is optimized for Google Chrome and FireFox. It will also work with other web browsers, but you may experience delays, loss of audio, and may not be able to connect to view the shared. This platform is also best used on computers and laptops; Chromebooks, tablets, and smartphones can also run SpatialChat, but you won't be able to see the shared content and will likely experience connection issues. Holding multiple connections, which occurs when there are a large number of participants, can place a heavy load on the user's computer. To avoid compromising the user's convenience, reserve computer resources in advance.

It is very easy to participate in a poster session using SpatialChat. For detailed instructions, please watch a YouTube video (https://www.youtube.com/watch?v=gfgW1Ks66dA).

Presenters of poster sessions are requested to prepare poster image data in jpg or png format. Posters should be pinned in the designated SpatialChat locations by the presenter after Nov. 13. There will be a rehearsal time for presenters to freely participate as follows. Staff members will be available to help during this time.

Rehearsal 1: November 13, 2021, 18:00-19:50
Rehearsal 2: November 18, 2021, 14:00-15:50

Two channels of SpatialChat are used in ACOP-IV.

Channel 1: https://spatial.chat/s/acop4 (for poster sessions)
Channel 2: https://spatial.chat/s/acop4sc (for welcome get-together party and discussion/break room)

List of participants **(Date of issue: Nov. 21st. 2021)**

Name	Affiliation	Presentation
Argentina		
Gabriela, Kuppers	CONICET	
Australia		
Chan, Cheong Xin	The University of Queensland	Symposium 6
Shah, Sarah	The University of Queensland	
Austria		
Tian, Miao	Universität Wien	Symposium 2
Bangladesh		
Islam, Shafiq MD	Bangladesh ANSAR and VDP	
Brazil		
SILVA, MARCELO FRANCIS	UEMASUL	
Cambodia		
Sothary, Kim	Preah Sisowath High School	Symposium 1
China		
Chen, Guogui	Xiamen University	
Feng, Yao-Yu	South China Agricultural University	
Gao, Shan	Ocean University of China	Symposium 2
Lai, De-Hua	Sun Yat-Sen University	Plenary lecture
Li, Yuan	Ocean University of China	
liang, yubei	sun yat-sen university	
Liu, Yifan	Ocean University of China	
Long, Shaojun	China Agricultural University	Symposium 2
Lun, Zhao-Rong	Sun Yat-Sen University	
Luo, Jiayi	Sun Yat-sen University	
Miao, Wei	Chinese Academy of Sciences	Symposium 4
Mo, Yuanyuan	Chinese Academy of Sciences	
Pan, Bo	Ocean University of China	BPA
Sheng, Yalan	South China Normal University	BPA
Song, Liu Jie	Sun Yat-Sen University	
Tu, Jiawei	Chinese Academy of Sciences	Symposium 4
Wang, Ju-feng	Sun Yat-Sen University	
Wei, Fan	Institute of Evolution & Marine Biodiversity, Ocean University of China	
Wen, Jian-Fan	Chinese Academy of Sciences	
Xiong, Jie	Institute of hydrobiology, Chinese academy of sciences	BPA
Yang, Jiong	Sun Yat-Sen University	
Zhang, Peng	Sun Yat-Sen University	
Zhao, Xiaolu	Ocean University of China	Symposium 2
Zhou, Junyu	Sun Yat-Sen University	
Czech Republic		
Corre, Pia	Charles University	
Füssy, Zoltán	Charles University in Prague	
Hehenberger, Elisabeth	Biology Centre CAS, Czech Republic	
France		
Kawaguchi, Takayuki	Institut Jacques Monod	
Germany		
Gupta, Nishith	Humboldt University, Berlin	Symposium 2
Katzenmeier, Sven Nicolai	Technical University Kaiserslautern	Poster
Singh, Minakshi	Max Planck Institute for Developmental Biology	
Stoeck, Thorsten	Technische Universität Kaiserslautern, Ecology Group, Kaiserslautern - Germany	Symposium 4
India		
Bhadange, Mayuri Vasudeora	CSIR-National Chemical Laboratory, Pune	
BHULLAR, SIMRAN	Department of Genetics, University of Delhi, New Delhi, INDIA	
Dey, Aditi	Birla Institute of Technology and Science-Pilani, Hyderabad Campus	
Kumari, Rolly	Birla Institute of Technology and Science-Pilani, Hyderabad Campus	
Mund, Shivashis	Birla Institute of Technology and Sciences	
Mustafa, Mohammad	Birla Institute of Technology and Science-Pilani, Hyderabad Campus	
Peddiraju, Swaroop N V S J	Birla Institute of Technology and Science-Pilani, Hyderabad Campus	
Poosala, Ramya Sri	Birla Institute of Technology and Science-Pilani, Hyderabad Campus	
Srivastav, Ratnesh Kumar	Birla Institute of Technology and Science-Pilani, Hyderabad Campus	
Italy		
Buonanno, Federico	University of Macerata	Symposium 5
Miceli, Cristina	University of Camerino	Symposium 4
Modeo, Letizia	University of Pisa	
Piersanti, Angela	University of Camerino	Poster
Pucciarelli, Sandra	Università of Camerino	Symposium 4
Japan		
Akematsu, Takahiko	Nihon University	
Arikawa, Mikihiko	Kochi University	
Echigoya, Syun	Hokkaido University	BPA
Fujii, Toshikatsu	Kochi University	
Fujishima, Masahiro	Yamaguchi Unibversity	
Fukuda, Yasuhiro	Tohoku University	
Hamada, Mayuko	Okayama University	Symposium 5
Harada, Yuuya	Kochi University	
Harumoto, Terue	Nara Women's University	
Hasegawa, Yuya	Kochi university	

Hayakawa, Masashi Mark	Kobe University	BPA
Hikida, Hiroyuki	Kyoto University	
Hirakawa, Yoshihisa	University of Tsukuba	
Hirano, Yui		
Hirono, Masafumi	Hosei University	
Honda, Daisuke	Konan University	
Hori, Manabu	Yamaguchi University	
Horiuchi, Ryoka	Nara Woman University	
Hoshina, Ryo	Nagahama Institute of Bio-Science and Technology	
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Iida, hitoshi	CHIBA INSTITUTE OF SCIENCE	
Iida, Hitoshi	Chiba Institute of Science	
Imazato, Misaki	Nara Women's University	
Inagaki, Yuji	Center for Computational Sciences, University of Tsukuba	
Iriko, Hideyuki	Kobe University	Symposium 5
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Kobayashi, Yuki	Yamaguchi University	
Kodama, Yuuki	Shimane University/Institute of Agricultural and Life Sciences, Academic Assembly	
Komatsu, Kaho	Kochi University	
Kutomi, Osamu	University of Yamanashi	
Maeda, Marika	Nara Women's University	
Maeda, Kazuki	University of Tokyo	
Marumo, Akisato	University of Tokyo	
Marumo, Akisato	University of Tokyo	
Maruyama, Tadashi	Kitasato University	
Matsuda, Atsushi	National Institute of Information and Communications Technology	
Matsumoto, Genta	Hokkaido University	
Miura, Shota	Murorann Institute of Technology	
Miwa, Isoji	Ibaraki University	
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Sogame, Yoichiro	NIT, Fukushima College	
Sonobe, Seiji	Hyogo Prefectural University	
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Suzawa, Tamie	Institute of River Biology Ltd.	
Takagi, Yumi	Nara Women's University	
Takeshita , Yoko	Ochanomizu University	Symposium 1
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cho, kyoung won	Pukyong National University	
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Jeon, Gayeong	Pukyong National University	
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jo, jinseong	Chonnam national university	
Jung, Jae-Ho	Gangneung-Wonju National University	
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Kim, Miran	Honam Natinoal Institute of Biological Resources	
Kim, Sung Min	Chonnam National University	
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Tikhonenkov, Denis V.	Papanin Institute for Biology of Inland Waters, Russian Academy of Sciences	Symposium 3
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Lopes dos Santos, Adriana	Asian School of Environment - ASE	
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