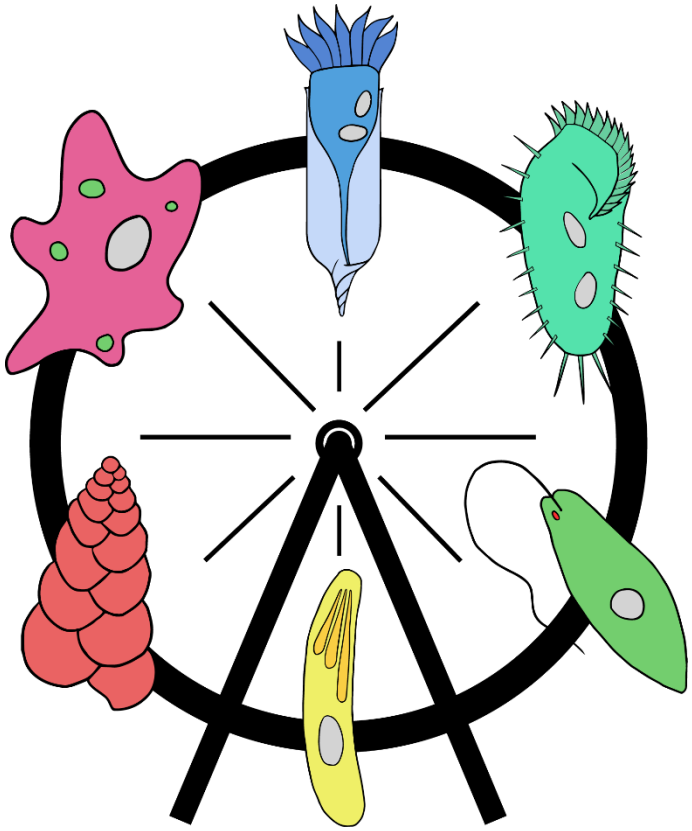

ECOP-ISOP joint meeting

"The Century of Protists"

9-14 July 2023

Vienna, Austria

Abstract booklet

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Vienna University of Technology

IX European Congress of Protistology & Annual Congress of the International Society of Protistologists

"The Century of Protists"

Dear Colleagues and Friends,

it is a great pleasure to welcome you to the **IX European Congress of Protistology (ECOP)** to be held as a joint meeting with the annual Congress of the **International Society of Protistologists (ISOP)**. The meeting is organized by the University of Vienna together in scientific cooperation with the **German Society for Protozoology (DGP)** in Vienna, Austria, **9-14 July 2023**.

The Congress of the European Societies is the most important event organized by FEPS, every four years.

Welcome in **Vienna**!

With kindest regards,
Julia Walochnik
Secretary General
Federation of European Protistological Societies

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- Vyacheslav Yurchenko

Contents

Program Overview

	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday
TIME	**9.7.2023**	**10.7.2023**	**11.7.2023**	**12.7.2023**	**13.7.2023**	**14.7.2023**
		8:30-9:00 WELCOME (Wiedermann; Griebler; Keeling; Arndt; Walochnik)				
9:00-10:30 **C1**		Chair: Rückert Alexandra Worden: **Marine Ecology & Global Change** Stefan Geisen: **Soil protists**	Chair: Simpson Patrick Keeling: **Phylogeny & Evolution** Joel Dacks: **Evolutionary Cell Biology**	**Holz-Connor Awards** Chair: Edgcomb **ISOP past president lectures:** Gaytha Langlois (2019-2021) Matthew Brown (2021-2022)	Chair: Brown Sonja Rückert: **Pathogenic Protists** Una Ryan: **Zoonotic Protists**	**GRELL AWARD LECTURES** Chairs: Arndt/ Walochnik Franziska Drews Clément Duckert Chair: Walochnik Matthias Horn: **Endosymbionts**
		coffee	**coffee**	**coffee**	**coffee**	**coffee**
11:00-12:30		**C1**: **Molecular evolution - genomics/ transcriptomics** Burki **C2**: **Soil protists** Mitchell **A**: **Extreme environments and climate change** Aberle/ Wickham **B**: **Taxonomy, systematics and barcoding**: Vďačný	**C1**: **Environmental genomics and bioinformatics** Cordier **C2**: **Symposium** Mayén-Estrada/ Song/Warren **Advances in taxonomy and phylogeny of sessilid peritrichs:** **A**: **Mixotrophy**: Johnson/ Moorthi **B**: **Pathogenic protists** Yurchenko	**C1**: **Symposium** Jeremy Wideman **Inferring protist cell biology using spatial proteomics** **C2**: **Ciliates as model organisms** Potekhin/ Simon **A**: **Biodiversity and biogeography**: Ptacnik **B**: **Protists associated with crops in land plants and aquaculture** Neuhauser	**C1**: **Ciliates as model organisms** Potekhin/ Simon **C2**: **Anaerobic protists** Cepicka **A**: **Cell biology and ultrastructure** Radek **B**: **Physiology and evolution of phototrophic protists** De Vries/Hess	**C1** **HUTNER TALKS** Chair: Keeling Enrique Lara (2019) Vladimir Hampl (2020) Fabien Burki (2021)
		lunch break	**lunch break**	**lunch break**	**lunch break**	**farewell**
13:30-15:00		**C1**: **Molecular evolution - genomics/ transcriptomics** Burki **C2**: **Soil protists** Mitchell **A**: **Extreme environments and climate change** Aberle/ Wickham **B**: **Taxonomy, systematics and barcoding**: Vďačný	**C1**: **Environmental genomics and bioinformatics** Cordier **C2**: **Symposium** Husník/ Lukeš **Protist symbioses** **A**: **Mixotrophy**: Johnson/ Moorthi **B**: **Pathogenic protists** Yurchenko	Hall 13:30-15:00 **Poster Session II** **N-Z** and coffee	**C1**: **Wilhelm Foissner-Symposium** Agatha **C2**: **Endosymbionts** Horn **A**: **Citizen science and science communication** Sonntag	13:00 Departure for National Park
		Hall 15:30-17:00 **Poster Session I** **A-M** and coffee	**coffee** **Springer/ Nature booth** Meet the Editors of *Parasitology Research*	**15:00** **Excursions** • **Bus for waste water plant (courtyard "Hof 10")** • **3rd man tour (start: 15:30 at Karlsplatz)** • **Natural History Museum** • **Josephinum**	**coffee**	
15:30-17:30	16:00-20:00 Registration		**Bioinformatics & genomes** Dunthorn **Symposium** Husník/ Lukeš **Protist symbioses** **Evolution** Simpson **Active compounds and cell death** Tsaousis		**NGTax Next Generation Taxonomy** Petroni/ Serra **Algae and cyanobacteria as endosymbionts** Pröschold/Darienko **Comparative genomics** Panek **Ecology and protist communities** Arndt	
17:30-18:00		**C1** **Elsevier Young Publishers' Workshop** **C2** **Carbon offsetting Workshop** Dorell/Dacks	**17:30** **Group FOTO**		**C1** Presentation of the **European Reference Genome Atlas** **C2** **Jury meeting**	
	18:00 Welcome (Hall)	**18:00** ISOP membership meeting (C2)	**18:00** DGP society meeting (C1) **20:00** Young protistologists' pub (Charlie P's Irish Pub)	**19:00** FEPS general assembly (C1)	**19:00** **Gala Dinner** **(Vienna Town Hall)**	

Program Details

Monday, 10th of July 2023

Room C1

TIME	TITLE	SPEAKER	SESSION	CHAIR
09:00-09:45	Marine ecology and global change	Worden, Alexandra	Plenary	Sonja Rückert
09:45-10:30	Soil protists: The new frontier in and beyond soil ecology	Geisen, Stefan		
11:00-11:15	Looking for genes withholding the secrets of uncultivable protist life cycles	Rizos, Iris	Molecular evolution: genomics/transcriptomics	Fabien Burki
11:15-11:30	Losing photosynthesis and the adaptation to heterotrophy	Nisbet, Ellen		
11:30-11:45	Microbial predators form a new supergroup of eukaryotes	Tikhonenkov, Denis		
11:45-12:00	Newly discovered deep lineage of eukaryotes with unusual morphology, ultrastructure, and mitochondrial genome	Valt, Marek		
12:00-12:15	Phylogenomics of Cercozoa: a single-cell transcriptome approach	Lax, Gordon		
12:15-12:30	Comparative genomics of the vampyrellid amoebae (Vampyrellida, Rhizaria)	Teixeira, Pereira Bassiaridis, Justin		
13:30-13:45	Phylogenomics show parasitism evolved twice in the marine alveolates (MALVs)	Holt, Corey		
13:45-14:00	Free-living relatives of highly abundant unicellular marine parasites elucidate plastid loss	Hehenberger, Elisabeth		
14:00-14:15	Single cell transcriptomics of heterotrophic taxa reveals new insights in dinoflagellate character evolution	Cooney, Elizabeth		
14:15-14:30	Spicing up the menu: Novel diversity of free-living bacterivorous colpodellids, relatives of Apicomplexa	Kolisko, Martin		
14:30-14:45	The hidden genomic diversity of orphan branches of the eukaryotic tree of life.	Blaz, Jazmin		
14:45-15:00	Integrating omics with 3D imaging to understand how endosymbionts become organelles	Husnik, Filip		

Room C2

TIME	TITLE	SPEAKER	SESSION	CHAIR
11:00-11:15	A triple metabarcoding strategy for discriminating active, dormant, and dead microbial eukaryotes in environmental samples	Gong, Jun	Soil protists	Edward Mitchell
11:15-11:30	Biotic interactions explain seasonal dynamics of the alpine soil microbiome	Fiore-Donno, Anna Maria		
11:30-11:45	Phylotranscriptomics support a temporal constraints model of development for the dictyostelid social amoebae.	Tice, Alexander		
11:45-12:00	Insights into protist activity, spatio-seasonal dynamics and multi-trophic linkages in a Canadian hypertidal mudflat	Kalu, Eke		
12:00-12:15	Island isolation and elevation as drivers of soil protist endemicity	Mitchell, Edward		
12:15-12:30	Long-read sequencing and soil eukaryote diversity	Stokke, Embla		
13:30-13:45	Measuring protist population growth in intact soil environments using quantitative stable isotope probing (qSIP)	Mau, Rebecca		
13:45-14:00	Protists as ideal models to study soil biodiversity-ecosystem functioning	Berlinches de Gea, Alejandro		
14:00-14:15	Deciphering the evolution of the early-diverging clades of heterotrophic flagellates Apusomonadida and Ancyromonadida	Torruella, Guifré		
14:15-14:30	Exploring eukaryote lateral gene transfer and its mechanisms in the model system *Acanthamoeba castellanii*	Colp, Morgan		
14:30-14:45	Decoding the doo-doo dilemma: The impact of bacteria on aggregative multicellularity in dung-inhabiting amoebae	Henderson, Tristan		
14:45-15:00	Chlamydial symbionts of amoeba impact top-down control in soil	Schwarzhans, Angelika		

Room A

TIME	TITLE	SPEAKER	SESSION	CHAIR
11:00-11:15	After years with partial mixing, complete water turnover boosts an intense phytoplankton bloom - how do ciliates react?	Schalch-Schuler, Martina	Extreme environments and climate change	Nicole Aberle-Malzahn/ Stephen Wickham
11:15-11:30	Ciliates in altered marine environments - plasticity, resilience & opportunism	Langlois, Gaytha		
11:30-11:45	Diversity and diurnal changes of microeukaryotes in mudflat biofilms: A molecular perspective	He, Cui		
11:45-12:00	Environmental bifurcation of two cyst forming acantharian clades in Arctic waters	Lovejoy, Connie		
12:00-12:15	Independent colonizations of athalassohaline water bodies by freshwater lobose testate amoeba (Arcellinida, Amoebozoa).	Useros, Fernando		
12:15-12:30	Insights into the dark matter of desert protists highlight unique community structures of cercozoans and amoebae across the driest desert on Earth, the Atacama Desert.	Acosta, Eduardo		
13:30-13:45	Relevance of climate, geography and habitat for the distribution of protists - lessons learned from a continental lake plankton survey	Boenigk, Jens		
13:45-14:00	The functioning of deep-sea microbial food webs influenced by sedimentation of organic debris	Dünn, Manon		
14:00-14:15	Assessing the impact of past environmental changes on marine protist biodiversity using sedimentary ancient DNA	Weiner, Agnes K.M.		
14:15-14:30	Quaternary Arctic sea ice reconstructions from ancient sedimentary DNA: updates from the AGENSI project	Cordier, Tristan		
14:30-14:45	Light/dark modulation of thiol oxidation in *Paulinella micropora* using redox proteomics	Lhee, Duckhyun		
14:45-15:00	Deep molecular characterization of microorganisms´ diversity and community composition in the tree canopies using a metatranscriptomics approach	Freudenthal, Jule		

Room B

TIME	TITLE	SPEAKER	SESSION	CHAIR
11:00-11:15	2D approach to reconstruct the evolutionary history of clevelandellids (Ciliophora, Armophorea) inhabiting the hindgut of the Panesthiinae cockroaches	Pecina, Lukáš	Taxonomy, systematics and barcoding	Peter Vďačný
11:15-11:30	A holistic approach to inventory the diversity of mobilid ciliates (Protista: Ciliophora: Peritrichia)	Zhang, Tengyue		
11:30-11:45	All we know about diversity of a neglected ciliate subfamily Clevelandellidae	Kotyk, Michael		
11:45-12:00	Characterisation and cultivation of new lineages of colponemids, a critical assemblage for inferring alveolate evolution	Simpson, Alastair		
12:00-12:15	Cleaving *Closterium*: A new feeding strategy found in the leptophryid amoebae (Vampyrellida, Rhizaria)	Suthaus, Andreas		
12:15-12:30	Effects of stressors on growth and competition between different cryptic taxa affiliated with ochromonadales (chrysophyceae)	Boden, Lisa		
13:30-13:45	First molecular evidence of hybridization in endosymbiotic ciliates (Protista, Ciliophora)	Obert, Tomáš		
13:45-14:00	Plasmodium and beyond - haemosporidian parasites of a Malagasy bird population	Musa, Sandrine		
14:00-14:15	Progress in taxonomy and phylogeny of the pleurostomatid ciliates (Ciliophora, Litostomatea, Pleurostomatida) in China	Pan, Hongbo		
14:15-14:30	The simple holocarpic oomycetes: Phylogeny and diversity	Buaya, Anthony		
14:30-14:45	UniEuk project update and future of the initiative	Berney, Cédric		
14:45-15:00	Unraveling *Paramecium* diversity by integrative taxonomy	Potekhin, Alexey		

Poster Session I

TIME	TITLE	SPEAKER
15:30-17:00	Genomes of diverse non-model Trypanosomatidae	Albanaz, Amanda
	Putative MutS2 homologs in algae: more goods in shopping bag?	Berdieva, Mariia
	Protist:bacteria metabolic associations might be pervasive in the Breviatae	Boisard, Julie
	Paramecium species in an acid rain-recovering lake	Bright, Lydia
	Morphological redescription of three *Sonderia* species (Ciliophora; Plagiopylea; Sonderiidae) based on Korean populations	Cahyani, Novia
	Phylogenomics and genomic metabolic features of gastrointestinal symbiotic ciliates of herbivorous mammals (Ciliophora, Trichostomatia)	Cedrola, Franciane
	A new symbiont ciliate of freshwater bivalvia, *Conchophthirus* n. sp. (Ciliophora: Scuticociliatia) from South Korea	Chae, Kyu-Seok
	New contributions to the taxonomy and phylogeny of the genus *Condylostoma* (Alveolata, Ciliophora, Heterotrichea)	Chi, Yong
	Bunch formation of *A. castellanii* infected with Marseilleviridae virus	Chihara, Akane
	Mophology, morphogenesis, and molecular phylogeny of a new species in the genus *Aspidisca* (Protozoa, Ciliophora) from South Korea	Choi, Ji Hye
	Metabarcoding analysis to detect protozoa and helminths in wild animals in South Korea.	Choi, Jun Ho
	Freshwater protist diversity analysis using long nanopore 18S rDNA amplicons reveals hidden diversity of 'Excavates'	Chwalińska, Małgorzata
	Unveiling the role of microtubule organizing centers (MTOCs) evolution in protists' diversity	Cirino, Luca
	Proteomics of the zoospore-to-vegetative cell transition in the thraustochytrid *Aurantiochytrium limacinum* (Labyrinthulomycota) reveals putative constituents of the bothrosome and ectoplasmic network	Collier, Jackie
	Diversity survey of endosymbionts associated with Polycystinea (Radiolaria) from the Sargasso Sea	Coots, Nicole
	Rumen ciliates (Ciliophora, Trichostomatia) in Brazilian domestic cattle feed diets with crescent urea levels	Costa Bordim, Suyane
	Polycycla (Poljansky, 1951) mobilines from South African holothurian hosts	de Jager, Gerhard
	Unrolling of R-bodies isolated from the *Paramecium* endosymbiont *Caedimonas varicaedens*	Dörr, Lennart
	New Arcellinida metabarcoding protocol	El Khouri Vidarte, Nura
	Evidence for a recent horizontal gene transfer from a close relative of *Paramecium* killer bacteria to *Blepharisma*	Emmerich, Christiane
	Anti-amebic activity and mechanism of action of synthetic pyrazolines	Espinosa, Avelina
	Identification of key enzymes for starch synthesis in chromerids and Apicomplexa	Fermont, Léa
	Ecology of Vampyrellida: broad insights from meta-analyses of molecular environmental surveys	Fiore-Donno, Anna Maria
	Bridging biology and physics – *Paramecium bursaria* as a model ambassador	Flemming, Felicitas Elisabeth

15:30-17:00	Unraveling cryptic speciation within *Ceratiomyxa fruticulosa*	Fry, Nicholas
	Description of new *Lambornella* (Tetrahymenidae, Oligohymenophorea, Ciliophora) and *Lambornella*-like isolates from Mexico	Gogoleva, Natalia
	Diversity of protist communities in the extreme environments revealed by metabarcoding	Gogoleva, Natalia
	A well-established symbiosis between *Dictyostelium giganteum* and a chlamydial symbiont under the influence of recurring multicellularity	Helmlinger, Lukas
	Standing above the rest: The unique behavior of new Thecamoebids	Henderson, Tristan
	A new group of endosymbiotic Legionellales of Euglenophyceae identified via metagenomic analyses	Hollender, Metody
	How did you get here? Protozoan diversity from freshwater volcanic crater lakes in the South Pacific	Irwin, Nicholas
	Impact of taxon sampling on the internal relationship of Archaeplastida by nuclear gene-based phylogenomic analyses	Isogai, Ryu
	Protistan plankton communities and the influence of a branch of Kuroshio in the northeastern East China Sea during the late spring	Kim, Yunhee
	Taxonomy of new non-photosynthetic species of the genus *Poterioochromonas* (Chrysophyceae) based on morphological and molecular evidence	Jeong, Minseok
	Morphology and molecular phylogeny of two planktonic hypotrichous ciliates (Ciliophora, Hypotrichia) from China	Jiang, Jiamei
	Giardia intestinalis – unique model for understanding the post – translational protein transport pathway into the endoplasmic reticulum membrane	Johánková, Alžběta
	Structure and composition of cyst envelopes of vampyrellid amoebae (Vampyrellida, Rhizaria) with special emphasis on chitinous substances	Kamp, Helen
	Metabarcoding of bacteria, protozoa, and helminths in the gut of *Apodemus agrarius*	Kang, Dongjun
	Pirsonia catenata sp. nov. (Pirsoniales, Pirsonea), a parasitic nanoflagellate infecting the marine centric diatom *Coscinodiscus radiatus* from coastal waters of Korea	Kim, Hyewon
	Detection of parasites and blood-meal hosts of tsetse flies from Tanzania using metagenomics	Kim, Myungjun
	Changes in marine bacterial community in response to parasite-induced dissolved organic matter from the harmful dinoflagellate *Akashiwo sanguinea*	Kim, Sunju
	A biological indication of a tintinnid species, *Tintinnidium primitivum*, of the Yellow Sea Bottom Cold Water	Kim, Young Ok
	Exploring the mitochondrial genetic code diversity	Klapuchová, Eliška
	More than a weed: Utilizing the diversity of Vannellid amoebae to uncover the developmental and evolutionary secrets of protostelid amoebae	Kleitz-Singleton, Felicity
	Two new species candidates of anaerobic ciliates genus *Tropidoatractus* (Ciliophora, Armophorea, Metopida, Tropidoatractidae) based on morphology and molecular phylogeny	Kristanti, Nanda Dwi
	Divergent ERMES complex is conserved in *Trichomonas vaginalis*	Kučerová, Jitka
	Predation by *Acartia* on the heterotrophic dinoflagellates *Gyrodinium* spp.	Lee, Moo Joon

15:30-17:00	Comparative mitochondrial genomics of Synurales (Chrysophyceae)	Lee, Nayoung
	NOX2-derived ROS-dependent calpain activation is involved in human hepatoma cells (HepG2) death induced by *Entamoeba histolytica*	Lee, Young Ah
	Trichomonas vaginalis-secreted lipid mediator LTB4 induces chemokine IL-8 production via dynamin-mediated endocytosis of LTB4 receptor BLT1 and phosphorylation of NF-kB	Lee, Young Ah
	Preliminary morphological and molecular analyses on free-living ciliates from South Africa	Lenti, Andrea
	Deciphering deep phylogenetic relationships among eukaryotic supergroups with improved gene and taxon sampling	Leroy, Romain
	Comparative transcriptome and antioxidant biomarker response reveal molecular mechanisms to cope with zinc ion exposure in the unicellular eukaryote *Paramecium*	Li, Lifang
	Distribution patterns of ciliate diversity in the South China Sea	Liu, Weiwei
	Evolution of a fibre-forming citrate synthase	Lometto, Stefano
	Oxygenic or anoxygenic? *Spirostomum teres* feeding preferences	Macek, Miroslav
	Gregarines from tenebrionid beetles of the Atacama Desert	Mach, Niclas
	An overview of the amoebae genera diversity in Mexico	Mayén-Estrada, Rosaura
	Epibiotic ciliate communities from a crayfish cultivated in artificial ponds in southern Mexico	Mayén-Estrada, Rosaura
	Developing gregarine apicomplexans as aquatic symbiosis model systems	McKinley, Kevin

Tuesday, 11th of July 2023

Room C1

TIME	TITLE	SPEAKER	SESSION	CHAIR
09:00-09:45	Horizontal gene transfer in the early evolution of protists: How eating changed our genes	Keeling, Patrick	Plenary	Alastair Simpson
09:45-10:30	Diversity of membrane-trafficking organelles in protists: new insights from evolutionary cell biology	Dacks, Joel B.		
11:00-11:15	Prevalence, succession, and activity of protistan grazers in particle-associated communities	Gleich, Samantha	Environmental genomics and bioinformatics	Tristan Cordier
11:15-11:30	Functional diversity of microbial eukaryotes in a meromictic lake: coupling between metatranscriptomics and a trait-based approach	Monjot, Arthur		
11:30-11:45	Microeukaryotic predators shape the wastewater microbiome	Heck, Nils		
11:45-12:00	Cultivation remains an indispensable tool for biodiversity discovery in heterotrophic protists	Eglit, Yana		
12:00-12:15	Digital PCR as a new cutting-edge tool for measuring ciliate abundance	Gross, Megan		
12:15-12:30	Chimera formation and detection in long-read amplicon sequencing data	Krabberød, Anders K.		
13:30-13:45	Global patterns and rates of habitat transitions across the eukaryotic tree of life	Jamy, Mahwash		
13:45-14:00	Global population structure of a unicellular marine predator lineage	Logares, Ramiro		
14:00-14:15	The eco-evolution of microbial eukaryotes: insights from molecular environmental surveys	Mendez Sandin, Miguel		
14:15-14:30	Freshwater plastid genomes through the lenses of metagenomics	Karlicki, Michał		
14:30-14:45	On the relationship between protist metabarcoding and protist metagenome-assembled genomes	Zavadska, Daryna		
14:45-15:00	Dissecting marine picoeukaryotic genomes from single cells and metagenomes	Massana, Ramon		
15:30-15:45	A long-read sequencing approach to metabarcoding protists from a diverse set of environments	Jones, Robert	Bioinformatics and genomics	Micah Dunthorn
15:45-16:00	Assessing the potential of Nanopore long-read sequencing for metabarcoding: A personalized molecular protocol for profiling protist communities in diverse habitats	Blandenier, Quentin		
16:00-16:15	There and back again, the genomics of free-living Diplomonads	Wisniewska, Monika		
16:15-16:30	Dollo parsimony overestimates ancestral gene content reconstructions	Galvez Morante, Alex		

16:30-16:45	High quality genome assemblies of six protists provide insights into giant virus and host defence mechanisms	Willemsen, Anouk		
16:45-17:00	A first glance at the transcriptome of *Crepidoodinium cyprinodontum* provides key insights into the evolution of fish ectoparasites among dinoflagellates	Maciszewski, Kacper		
17:00-17:15	Evolutionary studies of programmed genome rearrangements in ciliates, focusing on *Euplotes* and *Paramecium*	Gao, Feng		
17:15-17:30	The importance of model realism in addressing eukaryogenesis-related phylogenetic problems	Roger, Andrew		

Room C2

TIME	TITLE	SPEAKER	SESSION	CHAIR
11.00-11.15	The biology of peritrichs, including some historical perspectives	Warren, Alan	Symposium: Advances in the biology and systematics of sessilid peritrichs and other ciliates	Rosaura Mayén-Estrada
11:15-11:40	From phylogenomics to functional genomics of peritrichs	Jiang, Chuanqi		
11:40-12:05	New morphological and molecular data on peritrichs from Brazil provides new insights into the evolutionary relationships among epistylids	Dias, Roberto Júnio Pedroso		
12:05-12:30	Biodiversity of freshwater ciliates in the Lake Weishan Wetland, China	Song, Weibo		
13:30-14:00	Chlamydial endosymbionts of protists evolved through unexpected gene gain	Dharamshi, Jennah	Symposium: Protist symbioses	Filip Husník
14:00-14:15	The "bacterial" endosymbionts of *Symbiomonas scintillans* are actually prasinoviruses of the NCLDV phylum	Cho, Anna		
14:15-14:45	Methanogenic archaeal symbionts of anaerobic ciliates are host- and habitat-specific	Méndez-Sánchez, Daniel		
14:45-15:00	A unique symbiosome in an anaerobic single-celled eukaryote	Jerlström Hultqvist, Jon		
15:30-16:00	Transient and permanent residents: examples of euglenozoan-bacterial endosymbioses	Tashyreva, Daria		Julius Lukeš
16:00-16:15	Abortive infection and defensive symbiosis – how protists defend themselves against giant viruses	Arthofer, Patrick		
16:15-16:30	Infection of marine diatoms by *Pirsonia diadema*: a new model system to elucidate host-parasite interactions	Mathur, Varsha		
16:30-16:45	Anaerobic scuticociliates: A cosmopolitan lineage of anaerobic ciliates hosting diverse prokaryotic symbionts	Poláková, Kateřina		
16:45-17:00	Unexpectedly diverse protist community of *Reticulitermes tibialis*: Implications for symbiont inheritance and coevolution	Gile, Gillian		
17:00-17:05	Long-read sequencing sheds light on the origins of endogenous virophage and Polinton-like elements in the halophilic stramenopile *Halocafeteria seosinensis*	Gallot-Lavallée, Lucie		Filip Husník/ Julius Lukeš
17:05-17:10	Parasites of parasites: the diversity of metchnikovellids in marine gregarine apicomplexans	Park, Eunji		
17:10-17:15	An intriguing case of *Ciliophrys*: lack of plastid and presence of bacterial endosymbiont	Barcyte, Dovile		
17:15-17:20	Single-cell transcriptomics of *Hatena arenicola* and its symbiont	Okamoto, Noriko		
17:20-17:25	In search of Achilles' heel in ciliates	Sabaneyeva, Elena		

Room A

TIME	TITLE	SPEAKER	SESSION	CHAIR
11:00-11:15	The role of phagotrophy as an important iron acquisition strategy of marine dinoflagellates	Jang, Se Hyeon	Mixotrophy	Stefanie Moorthi/ Matthew Johnson
11:15-11:30	Physiology of ecdysis in dinoflagellates – presumably overlooked cyst type and directions of nutrient fluxes in the ocean	Matantseva, Olga		
11:30-11:45	To be or not to be a mixotroph? How niche modeling can help to tackle dinoflagellate trophic strategies in marine ecosystems	Bittner, Lucie		
11:45-12:00	The predicted plastid proteome of *Polytoma uvella* (Chlamydophyceae): contrasting differences between the metabolic roles of the nonphotosynthetic plastids of free-living and parasitic/pathogenic chlorophytes	Reyes-Prieto, Adrian		
12:00-12:15	Physiological and transcriptomic responses of mixotrophic *Ochromonas* to light regimes and prey availability	Zhang, Lu		
12:15-12:30	Never-ending story – intriguing trophic levels in pelagic microbial food webs	Piwosz, Kasia		
13:30-13:45	Revealing the kleptoplastidic symbiosis in the marine centrohelid *Meringosphaera*	Walraven, Anne		
13:45-14:00	Loss of metabolic autonomy in the photosynthetic ciliate *Mesodinum rubrum*	Johnson, Matthew		
14:00-14:15	Competition between mixotrophic and heterotrophic ciliates under dynamic light conditions and prey supply	Moorthi, Stefanie		
14:15-14:30	A new deep-branching lineage of predatory flagellates confirms the relationship between Pirsoniales and oomycete parasites (Stramenopiles)	Prokina, Kristina		
14:30-14:45	Creating a freshwater dinoflagellate transcriptome database	Mtawali, Mahara		
14:45-15:00	Phylogenomics reveals multiple independent lifestyle transitions within early-branching fungi	Thomé, Pauline C.		
15:30-15:45	Data mining Arthropoda genomes reveals diversity and host spectrum of Microsporidian parasites	Edwards, Sam	Evolution	Alastair Simpson
15:45-16:00	Unravelling the evolution of organellar genomes and metabolism of *Leontynka* lineage (Chlamydomonadales)	Corre, Pia		
16:00-16:15	Canonically circular mitochondrial genomes spread among Euglenids	Hałakuc, Paweł		
16:15-16:30	Simulating the origins of multicellularity by artificial selection on the filasterean *Capsaspora owczarzaki*	Bercedo-Saborido, Gonzalo		
16:30-16:45	The phylogenomic position of coral infecting corallicolid apicomplexan shows multiple independent losses of chlorophyll biosynthesis in obligate intracellular parasites	Jacko-Reynolds, Victoria		

16:45-17:00	The origin and evolution of symbiontid euglenozoans using single-cell transcriptomics	Maciejowski, William		
17:00-17:15	Evolution of splicing in *Pseudoloma neurophilia*: exploring the most reduced spliceosome	Whelan, Thomas		
17:15-17:30	Maturases and group II introns in the mitochondrial genomes of the deepest jakobid branch	Galindo, Luis Javier		

Room B

TIME	TITLE	SPEAKER	SESSION	CHAIR
11:00-11:15	A novel parasitoid (Oomycota) infecting the marine dinoflagellates	Jeon, Boo Seong	Pathogenic protists	Vyacheslav Yurchenko
11:15-11:30	Apical annuli are specialised sites for post-invasion secretion in *Toxoplasma gondii*	Waller, Ross		
11:30-11:45	*Babesia microti* and *Babesia vogeli* in *Rhipicephalus turanicus* ticks (Ixodida: Ixodidae) from Israel	Mumcuoglu, Kosta Y.		
11:45-12:00	Genetic diversity of Japanese *Toxoplasma* population based on genome-wide SNP analysis	Nagamune, Kisaburo		
12:00-12:15	Genome evolution of the apicomplexan parasite *Cryptosporidium parvum*	Castelli, Michele		
12:15-12:30	Isolation and molecular identification of *Vermamoeba vermiformis* strains from environmental samples in Castile and León, Spain	Pérez-Pérez, Patricia		
13:30-13:45	Microsporidia and protist parasites: an important future threat to the insect rearing industry	Bessette, Edouard		
13:45-14:00	Nephridiophagids (Chytridiomycota) reduce the fitness of their host insects	Strassert, Jürgen F. H.		
14:00-14:15	*Palythoa* aff. *clavata* as a source of bioactive compounds against the “brain eating” amoeba	Arberas-Jiménez, Iñigo		
14:15-14:30	Establishing a multi-omics approach to study *Cryptosporidium parvum* in calves	Tsaousis, Anastasios		
14:30-14:45	Phylogenomic analysis of adeleorina apicomplexans	Na, Ina		
14:45-15:00	The ancestral shape of the access proton path of mitochondrial ATP synthases revealed by the split subunit-a in *Trypanosoma brucei*	Gahura, Ondřej		
15:30-15:45	Antikinetoplastid activity of sesquiterpenes isolated from the zoanthid *Palythoa* aff. *clavata*	Bethencourt-Estrella, Carlos J.	Active compounds and cell death	Anastasios Tsaousis
15:45-16:00	Chlorhexidine caused imbalance oxidative state in *Acanthamoeba polyphaga*	Sifaoui, Ines		
16:00-16:15	Nitroxoline as an amoebicidal agent against *Acanthamoeba*: in vitro activity and detection of programmed cell death in *Acanthamoeba culbertsoni*	Rodríguez-Expósito, Rubén L.		
16:15-16:30	Repurposing of nitroxoline as an anti-*Naegleria* compound	Chao-Pellicer, Javier		
16:30-16:45	Holozoan protists shed light on the evolution of regulated cell death pathways at the onset of animal multicellularity	Leger, Michelle		
16:45-17:00	Exploring the efficacy of metabarcoding and non-target screening for detecting treated wastewater	Sieber, Guido		

Wednesday, 12th of July 2023

Room C1

TIME	TITLE	SPEAKER	SESSION	CHAIR
09:00-09:45	ISOP Past-President's Address 2019-2020 - Footprints in the sand	Langlois, Gaytha	Plenary	Virginia Edgcomb
09:45-10:30	ISOP Past-President's Address 2021-2022 - Curiosities of amoeboid protists: from genomics to development	Brown, Matthew		
11:00-11:05	INTRO	Wideman, Jeremy	Symposium: Inferring protist cell biology using spatial proteomics	Jeremy Wideman
11:05-11:17	Spatial proteomics of the free living anaerobic protist *Paratrimastix pyriformis*	Peña-Diaz, Priscila		
11:17-11:29	Spatial proteomics: the dark side of the method	Jirsova, Dagmar		
11:29-11:41	Spatial proteomics reveals the presence of complex subcellular compartimentalization in the Alveolate parasite *Perkinsus marinus*	Salas-Leiva, Dayana		
11:41-11:53	Comparative spatial proteomics of euglenozoans	Hammond, Michael		
11:53-12:05	Spatial proteomics of *Naegleria gruberi* with the focus on the evolution of the eukaryotic cell	Dolezal, Pavel		
12:05-12:30	PANEL DISCUSSION			

Room C2

TIME	TITLE	SPEAKER	SESSION	CHAIR
11:00-11:15	Morphological diversity and molecular phylogeny of five *Paramecium bursaria* (Alveolata, Ciliophora, Oligohymenophorea) syngens and the identification of their green algal endosymbiont	Pröschold, Thomas	Ciliates as model organisms	Alexey Potekhin/ Martin Simon
11:15-11:25	Intracellular bioaccumulation of the rare earth element Gadolinium in the ciliate *Tetrahymena pyriformis* resulting in biogenic particle formation and excretion	Kohl, Jana		
11:25-11:35	Spatial distribution and self-organized pattern formation in single-species systems – experiments with ciliates	Werner, Johannes		
11:35-11:45	*Paramecium bursaria* - a perfect model organism for so-called active Brownian particles and statistical physics?	Härtel, Andreas		
11:45-12:00	Fatty acid desaturases expression in *Euplotes focardii* as evolutionary adaptation to the Antarctic environment may expose this ciliate to higher risk under organic pollutants contamination	Piersanti, Angela		
12:00-12:15	Population genomic insights into syntrophic symbiosis between marine anaerobic ciliates and intracellular methanogens	Rotterova, Johana		
12:15-12:30	Protein-protein homo- and hetero-oligomerization phenomena explain autocrine (growth-promoting) and heterologous (mating-inducing) pheromone-cell interactions in *Euplotes*	Vallesi, Adriana		

Room A

TIME	TITLE	SPEAKER	SESSION	CHAIR
11:00-11:15	Characterization of a eukaryotrophic flagellate representing a novel major lineage within Stramenopila	Weston, Elizabeth	Biodiversity	Robert Ptacnik
11:15-11:30	On the life history, occurrence and microhabitat of a rare ciliate: *Apocarchesium arndti* Norf et Foissner, 2010 (Oligohymenophorea, Peritrichia)	Becz, Álmos		
11:30-11:45	RS, a new fully defined medium for cultivating diverse protists	Sigona, Cristiana		
11:45-12:00	Using long-read amplicon sequences to evaluate the diversity of understudied eukaryotic microbes	Rajter, Lubomir		
12:00-12:15	Apicomplexans symbionts of the coral reef	del Campo, Javier		
12:15-12:30	The photic-aphotic divide is a strong ecological and evolutionary force determining the distribution of marine ciliates (Alveolata, Ciliophora)	Santoferrara, Luciana		

Room B

TIME	TITLE	SPEAKER	SESSION	CHAIR
11:00-11:15	Phenotypic analysis of *Plasmodiophora brassicae* infection in various *Arabidopsis arenosa* genotypes: discovering similarities and dissimilarities in the parasite-host relationship	Neuhauser, Sigrid	Protists associated with crops in land plants and aquaculture	Sigrid Neuhauser
11:15-11:30	Genomic and transcriptomic advances in understanding the tripartite interactions between host plants, viruses and the protist vectors Polymyxa	Genard, Margaux		
11:30-11:45	Local endoreduplication of the host is a conserved process during Phytomyxea-host interaction	Hittorf, Michaela		
11:45-12:00	Phagocytosis underpins the biotrophic lifestyle of intracellular parasites in the class Phytomyxea (Rhizaria)	Garvetto, Andrea		
12:00-12:15	Seagrasses as model organisms to understand the biodiversity and evolution of phytomyxid parasites in natural settings	Kolátková, Viktorie		
12:15-12:30	What determines the assembly of protist microbiomes in the rhizosphere of plants?	Bonkowski, Michael		

Poster Session II

TIME	TITLE	SPEAKER
13:30-15:00	*Entamoeba histolytica*: Investigation of the lipopeptidophosphoglycan (LPPG) surface antigen and the monoclonal antibody EH5	Nagode, Anna
	Graphical tool for manual editing and annotation of sequence sampling in phylogenetic datasets	Nenarokov, Serafim
	Does *Blastocystis* shape the gut microbiota? A case study in a group of mothers and children volunteers from the Zanzibar Archipelago	Nguele, Aristide Toussaint
	Reinvestigation of two poorly known metopid ciliates: *Metopus major* and *M. pellitus* by Kahl, 1932 from South Korea (Ciliophora, Armophorea)	Nguyen, Quoc Dung
	Search for the new group-specific V9 hypervariable region metabarcoding primers	Novák, Jiří
	The metabarcoding of bacteria and protozoa of domestic pigeons in Seoul, Korea	Oh, Singeun
	Endosymbiotic relationship between the heterotrophic ciliate *Paramecium bursaria* and the green algae *Chlorella variabilis* under starvation	Okada, Kaoru
	Two new species of the eupelagonemids	Okamoto, Noriko
	Genetic manipulation of *Micromonas* - New tools for a marine model alga	Paap, Jöran
	Characterisation of a novel pan-microalgal chloroplast ATP-binding protein	Penot-Raquin, Mathias
	Towards deciphering the molecular mechanisms of RNA 3'-end modification in secondary plastids of euglenophytes	Pergner, Jiri
	Evidence and distribution of various Chlorovirus (Phycodnaviridae) strains in Lake Mondsee (Austria) infecting endosymbiotic green algae	Petlovana, Viktoriya
	Phyloplast: A plastid database with novel signal sequence prediction tool and a suite of programs for phylogenetic analyses	Pietluch, Filip
	Protist of the year 2023: Colony-forming ciliate *Ophrydium versatile* (Peritrichia)	Pröschold, Thomas
	Apofrontonia jejuensis n. sp. (Ciliophora, Oligohymenophorea), a new marine ciliate from Jeju Island, South Korea	Quintela-Alonso, Pablo
	Sessile ciliates colonizing the hindgut of higher termites	Radek, Renate
	The activity of PHMB and other guanidino containing compounds against *Acanthamoeba* and other ocular pathogens	Ratnayake, Dharanga
	Nitric oxide signaling controls collective contractions in a colonial choanoflagellate	Reyes-Rivera, Josean
	In vivo evaluation of bioenergetic parameters in heat-stressed *Cassiopea*	Royen, Edmee
	Specimens of common Baltic ciliates fixed with acid Lugol's solution - photomicrographs	Rychert, Krzysztof
	A dead end for both partners: hyperinfection with Ca. *Gortzia yakutika* in *Paramecium nephridiatum*	Sabaneyeva, Elena
	The first arctic rhizochromuline: morphology, ultrastructure, and position in the evolutionary tree of Rhizochromulinales (Ochrophyta, Dictyochophyceae)	Safonov, Pavel
	Composition of telomeric proteins in Blastochritidia nonstop	Saura, Andreu

	Does salinity matter? - Ciliates in the southern Baltic	Scherwass, Anja
	Genome of a Ca. *Megaira* endosymbiont from the cercozoan amoeba *Rhogostoma pseudocylindrica*	Seah, Brandon
	Effects of warming on the protist communities in the Southwestern Sea of Korea during the late spring	Seo, Hye Jin
	Absence of spliceosomal introns in the mixotrophic ciliate *Mesodinium rubrum* - how did they disappear?	Shaikhutdinov, Nurislam
	Identification and analysis of cilia-associated gene families in *Euplotes amieti* (Ciliophora, Hypotrichida）	Shen, Liheng
	Morphological reconstruction during cell regeneration in the ciliate *Spirostomum ambiguum*	Shimada, Maho
	Characteristics of a novel encystment-inducing pheromone released from the ciliated protozoan *Colpoda cucullus*	Shimada, Yuto
	Endemics ciliates of phytotelma exhibit high macroevolutionary rates	Silva Costa, Fabiola
	Ancient Protein Resurrection of the Arf1/6 progenitor: “Jurassic Park-ing” a 2 billion year old protein	Sivia, Mandeep
	Intracellular pigments and morphological changes as a stress response in a mesophilic *Ancylonema* species (Zygnematophyceae)	Slominski, Emilia
	Single-cell transcriptomics: establishing splitseq method for protist communities	Smacchia, Valentina
	Research and development of a novel biological water quality index based on Arcellinida using metabarcoding techniques	Soler-Zamora, Carmen
	New evidence of consistency between phylogeny and morphology for two large taxa in ciliated protists, the subclasses Oligotrichia (Protista, Ciliophora)	Song, Wen
	Integrative approach on key freshwater ciliates of the genus *Urotricha* (Alveolata, Ciliophora, Prostomatida)	Sonntag, Bettina
	A Deeper Investigation in Microsporidian Diversity	South, Lilith
	Genomic characterization of eight peritrich ciliates (Peritrichia: Sessilida), with new phylogenomic insights	Souza, Pedro
	It takes some guts: A termite-protist co-speciation analysis using long read amplicon sequences	Swichtenberg, Kali
	Long-read metagenomics of microbial communities in boreal forest soils	Thoen, Ella
	Interstitial environment of the Danube: home for an assemblage of rare filose testate amoebae	Török, Júlia Katalin
	Blastocystis under One Health - COST Action	Tsaousis, Anastasios
	Bioimaging in *Tetrahymena* by glucose analogs labeled at the C-1 or C-2 position with a fluorescent dansylamino group	Ueno, Hironori
	Mutual benefits from the symbiotic coexistence between bipolar *Euplotes* cells and *Parafrancisella* bacteria	Vallesi, Adriana
	Unexpected survival of various groups of benthic protists at anaerobic conditions	Wagenhofer, Julian
	Combining morphological and molecular data to explain the history and evolutionary pattern of problematic taxa in the genus *Frontonia*	Wardani, Ratih Kusuma
	Spatial and vertical distribution of pelagic nanoflagellate and nanoamoeba genotypes in relation to benthic and pelagic gene libraries of the Atlantic Ocean	Weiß, Antonia
	A new phylogenomic dataset for pinpointing the root of the eukaryote tree	Williamson, Kelsey

	Morphological plasticity and species determination: a case study from *Pseudokeronopsis erythrina* Chen et al. 2011 (Ciliophora, Hypotrichia, Urostylida)	Xu, Wenxin
	Morphology and molecular phylogeny of the semiterrestrial ciliate *Euplotes baugilensis* n. sp. (Protozoa, Ciliophora)	Yeo, Jeong Hyeon
	Morphological characteristics and phylogenetic position of *Eududoscquella* species infecting dinoflagellates	Yoo, Jiae

Thursday, 13th of July 2023

Room C1

TIME	TITLE	SPEAKER	SESSION	CHAIR
09:00-09:45	Pathogenic Protists	Rückert, Sonja	Plenary	Matthew Brown
09:45-10:30	Zoonotic Protists	Ryan, Una		
11:00-11:20	Semi-conservative transmission of DNA N6-adenine methylation in a unicellular eukaryote	Gao, Shan	Ciliates as model organisms	Alexey Potekhin/ Martin Simon
11:20-11:35	Functional division of two distinct methyltransferase complexes for eukaryotic DNA N6-adenine methylation	Wang, Yuanyuan		
11:35-11:50	Amplification of exogenous dsRNA trigger by RNA dependent RNA polymerases	Pirritano, Marcello		
11:50-12:10	Gametocyte-specific factor 1 (GTSF1) is required for genome rearrangement of *Paramecium tetraurelia*	Wang, Chundi		
12:10-12:30	Evidence for ciliates without extensive DNA elimination: the karyorelict *Loxodes magnus*	Seah, Brandon		
13:30-14:00	Wilhelm Foissner (1948—2020) --- Words of memory and admiration	Aspöck, Horst	Wilhelm Foissner Symposium	Sabine Agatha
14:00-14:15	Wilhelm Foissner (1948–2020), an outstanding taxonomist, and the future of a nomenclatural system of Ciliophora	Aescht, Erna		
14:15-14:30	Multiple lines of evidence allow a moderate revision of the oligotrichid genus *Strombidium* (Alveolata, Ciliophora, Spirotricha)	Agatha, Sabine		
14:30-14:45	The number of free-living ciliate species	Dunthorn, Micah		
14:45-15:00	"Blue Book" in new edition: Microscopic analysis of activated sludge assessing operational parameters in biological wastewater treatment and the quality of the inflowing municipal wastewater	Ettl, Marina		
15:30-15:45	A new litostomatean (Ciliophora) species from Tuscany, described accordingly to the standards of Next Generation Taxonomy	Allievi, Alessandro	NGTax: Next Generation Taxonomy	Giulio Petroni/ Valentina Serra
15:45-16:00	A pilot study of the transcriptomic response triggered by the bacterial infection in *Paramecium*	Potekhin, Alexey		
16:00-16:15	Contributions to the mobilid genus *Leiotrocha* Fabre-Domergue, 1888	de Jager, Gerhard		
16:15-16:30	Evolutionary origins and diversification of the association with eukaryotes and the intracellular condition among the Rickettsiales	Castelli, Michele		
16:30-16:45	*Holospora*-like bacteria "Candidatus *Gortzia yakutica*" and *Preeria caryophila*: new data on ultrastructure, biogeography and host specificity of the symbionts	Fokin, Sergey		

16:45-17:00	Integrative taxonomic data exploring ciliate diversity and community structure in Rift Valley and marine aquatic ecosystems of Kenya and Lake Mondsee in Austria	Owuor, Maxwell		
17:00-17:15	The multigene family of surface antigens in different *Paramecium* species	Simon, Martin		
17:15-17:30	Thinking outside the nucleus. A mitogenomic approach to ciliate phylogeny	Gammuto, Leandro		

Room C2

TIME	TITLE	SPEAKER	SESSION	CHAIR
11:00-11:15	ATP generation in *Paradiplonema papillatum*: a planktonic protist without the need for oxygen	Sveráková, Ingrid	Anaerobic protists	Ivan Čepička
11:15-11:30	Characterisation of the SUF FeS cluster machinery in the amitochondriate eukaryote *Monocercomonoides exilis*	Peña-Diaz, Priscila		
11:30-11:45	Evolution of Endosomal retrograde trafficking machinery in the Parabasalia and its free-living sister lineage	Shinde, Abhishek Prakash		
11:45-12:00	MRO-logy of Archamoebae species: Comparative analysis of mitochondrion-related organelles in anaerobic amoebozoans	Pašuthová, Kristína		
12:00-12:15	Novel Anaeramoebae strains exhibit an unexpected variety of symbioses with prokaryotic organisms	Pavlátová, Magdaléna		
12:15-12:30	Rediscovery of remarkably rare anaerobic tentaculiferous ciliate genera *Legendrea* and *Dactylochlamys* (Ciliophora: Litostomatea)	Pomahač, Ondřej		
13:30-13:45	Genomics of 'accessory' endosymbionts of the ciliate *Euplotes*: evolution of functional and ecological traits	Giannotti, Daniele	Endosymbionts and endosymbiosis	Matthias Horn
13:45-14:00	Methanogenic symbioses in Psalteriomonadidae	Čepička, Ivan		
14:00-14:15	Genomic insights into the evolution, diversity and biology of the eustigmatophyte-specific endosymbiont Candidatus *Phycorickettsia*	Eliáš, Marek		
14:15-14:30	Astonishing diversity of RNA viruses in *Leptomonas pyrrhocoris*, a trypanosomatid parasitizing firebugs	Kostygov, Alexei		
14:30-14:45	Endosymbionts in amoebae – A community approach	Török, Júlia Katalin		
15:30-15:45	A non-kareniacean dinoflagellate with a haptophyte-derived plastid indicates multiple tertiary endosymbioses	Takahashi, Kazuya	Algae and cyanobacteria as endosymbionts	Tatyana Darienko/ Thomas Pröschold
15:45-16:00	Elucidating early stages of plastid endosymbiosis with *Rapaza viridis* (Euglenophyta) kleptoplasty	Karnkowska, Anna		
16:00-16:15	Horizontal gene transfer contributes mechanistically to a facultative photosymbiosis in *Paramecium bursaria*	Irwin, Nicholas		
16:15-16:30	*Paramecia* in the spotlight – consequences of exposure to photosynthetically active radiation	Flemming, Felicitas Elisabeth		
16:30-16:45	Repeated haptophyte endosymbioses in the dinoflagellate family Kareniaceae	Inagaki, Yuji		
16:45-17:00	Single-cell transcriptomics reveal differential responses of mixed protist symbiont communities and host cells in corals during heat-stress	Bonacolta, Anthony		

17:00-17:15	The hidden world of green algal endophytes: diversity, relationship with host, and impact on aquaculture crops	Bjorbækmo, Marit F. M.		
17:15-17:30	Unexpected diversity of zoochlorellae revealed by multigene approach	Pröschold, Thomas		

Room A

TIME	TITLE	SPEAKER	SESSION	CHAIR
11:00-11:15	Parallel elaboration of contractile vacuole-associated membrane trafficking machinery in *Reclinomonas americana*	More, Kiran J.	Cell biology and ultrastructure	Renate Radek
11:15-11:30	Living well with evolution of beating heart: Contractile Vacuole in Ciliates	Kaur, Harpreet		
11:30-11:45	Preliminary studies of striated fibres and assemblins in Preaxostylans	Fang, Yi-Kai		
11:45-12:00	Chitin and chitin-related factors in microbial protoplast feeders point to new roles of understudied biopolymers in protists	Moye, Jannika		
12:00-12:15	Identification of key enzymes for starch synthesis in chromerids and Apicomplexa	Fermont, Léa		
12:15-12:30	Ethylene signaling in *Cyanophora paradoxa*: Revealing hormone usage in a glaucophyte alga	Burns, John		
13:30-14:00	Education and dissemination by eukaryotic microorganisms	Buonanno, Federico	Citizen science and science communication	Bettina Sonntag
14:00-14:30	Adventures in the microworld	Becz, Álmos		
15:30-15:45	Mitochondrial RNA editing functioning with diverse arsenal in a heterolobosean *Paravahlkampfia ustiana*	Mirzoyan, Seda	Comparative genomics	Tomáš Pánek
15:45-16:00	Mitochondrial RNA editing in ascetosporean amoebae	Yabuki, Akinori		
16:00-16:15	Nonconventional introns of euglenids as elements shaping the structure of genes and sequences of encoded proteins	Jagielska, Maria		
16:15-16:30	Non-host genome integration of the Nucleocytoviricota virus into the genome of eustigmatophyte alga *Characiopsis acuta*	Richtář, Michal		
16:30-16:45	Has the DNA polymerase of the proto-mitochondrion been retained in Discoba, Malawimonadidae, and Ancyromonadida? A novel mitochondrion-localized DNA polymerase with the phylogenetic affinity to the alpha-proteobacterial PolI	Harada, Ryo		
16:45-17:00	Lateral gene transfer into the “BRC”, a deep-branching group related to fornicate metamonads	Williams, Shelby K.		
17:00-17:15	RNA editing in non-model trypanosomatids	Yurchenko, Vyacheslav		
17:15-17:30	Herpes-like endogenous viral elements and subtelomeric ribosomal RNA genes in a thraustochytrid (Labyrinthulomycota) genome	Collier, Jackie		

Room B

TIME	TITLE	SPEAKER	SESSION	CHAIR
11:00-11:15	Saccoderm desmids are the key to understanding the evolution of zygnematophytes and their adaptations to terrestrial conditions	Busch, Anna	Physiology and evolution of phototrophic protists	Jan De Vries/ Sebastian Hess
11:15-11:30	Proteomics reveals the evolution of chlorarachniophyte pyrenoids	Hirakawa, Yoshihisa		
11:30-11:45	In vivo evaluation of bioenergetic parameters in heat-stressed *Cassiopea*	Royen, Edmee		
11:45-12:00	Microbiome in the mucus of *Ostreopis ovata* (Dinoflagellate) Mediterranean isolate	Angelici, Maria Cristina		
12:00-12:15	The evolution of characteristic AGP-glycosylation during the plant terrestrialization process	Pfeifer, Lukas		
12:15-12:30	Zoosporogenesis of chromerids and different fates of zoospores	Oborník, Miroslav		
15:30-15:45	Observing protistan communities using Leray-XT COI primers	Ewers, Isabelle	Ecology and protistan communities	Hartmut Arndt
15:45-16:00	Comparative analysis of Illumina and Nanopore amplicon sequences of eukaryotic communities from sediment samples	Bludau, Dana		
16:00-16:15	The dilemma of underestimating freshwater biodiversity: morphological versus molecular approaches	Schoenle, Alexandra		
16:15-16:30	Protist community ecology across an eddy dipole in the North Pacific Subtropical Gyre	Beatty, Jennifer		
16:30-16:45	Regulation of colony formation in a novel *Ventrifissura* species (Cercozoa)	Skamnelou, Margarita		
16:45-17:00	Free-living trichomonas: There and back again?	Kubánková, Aneta		
17:00-17:15	Environmentally-informed phenotyping of the pan-secondary red chloroplast proteome	Dorrell, Richard		
17:15-17:30	Mortality rates of planktonic ciliates	Weisse, Thomas		

Friday 14th of July 2023

Room C1

TIME	TITLE	SPEAKER	SESSION	CHAIR
09:00-09:20	A highly condensed genome without heterochromatin: orchestration of gene expression and epigenomics in *Paramecium tetraurelia*	Drews, Franziska	Grell Talks	Hartmut Arndt/ Julia Walochnik
09:20-09:40	Taxonomy for the actual end users based on morphology, molecules and nomenclature - examples from euglyphid and arcellinid testate amoebae	Duckert, Clement		
09:45-10:30	Amoebae as intracellular arena for bacterial symbionts and giant viruses	Horn, Matthias	Plenary	Julia Walochnik
11:00-11:30	Protist diversity patterns at the species level: The case of testate amoebae	Lara, Enrique	Hutner Talks	Patrick Keeling
11:30-12:00	Preaxostyla: A group that has succeeded in eliminating mitochondria	Hampl, Vladimír		
12:00-12:30	Eukaryotic Tree of Life	Burki, Fabien		

Abstracts:
Invited Talks
(in alphabetical order)

ISOP Past-President's Address 2021-2022 - Curiosities of amoeboid protists: from genomics to development

Brown, Matthew[1]

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Diversity of membrane-trafficking organelles in protists: new insights from evolutionary cell biology

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Beneath the easily recognized cartoon schematic of a eukaryotic cell lies a wondrous diversity of organellar form and function. Compartments shift in morphology: extend and contract in capacity. Two vignettes of organellar evolution and diversity will be presented, those of Golgi bodies and of peroxisomes. In both cases protist genomics and molecular cell biology have been applied in tandem, uncovering surprising new forms of the organelle. Organisms once considered not to possess Golgi bodies have been progressively demonstrated to encode the gene complement of Golgi-associated genes, in several cases with localization of those factors revealing un-stacked morphologies. Comparative genomics across protist diversity and of lineages with different Golgi morphologies is most consistent with a newly emerging hypothesis on the underlying genetic basis for Golgi stacking. By contrast comparative genomics of membrane-trafficking and peroxisome biogenesis has identified lineages where unusual peroxisomes may exist, including the most reduced, but putatively functional, peroxisome ever reported. Protist genomics, in a give and take with molecular cell biology, has and will continue to provide a powerful approach to exploring the diversity of eukaryotic organelles, with more surprises likely to come.

Soil protists: The new frontier in and beyond soil ecology

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We all agree on the importance of protists, which aligns with the theme of this ISOP-ECOP meeting. Yet, our perception is still lacking among soil scientists and the public. In contrast, the value of soil as the basis of One Health (health of e.g. soil, plant, animal, human) is increasingly appreciated. Here, I emphasize the immense value of a broader recognition of soil protists that spreads far beyond novel scientific insights, but might even change the way we appreciate the environment and treat soils. Starting with an overview on the diversity of soil protists, I will show their likely impact on soil functioning with a major focus on shaping microbiome functioning and, thereby, soil and plant health. My vision is that – as a community – we can expand beyond our protist bubble and have an increasing influence on (soil) science and beyond, including the public and (soil) management. Only thereby we can manage to actually stand up to make it a The Century of Protists.

Amoebae as intracellular arena for bacterial symbionts and giant viruses

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Protists often harbor bacterial endosymbionts, whose roles and modes of interaction with the host are often unclear. In this context, I will present chlamydial symbionts in amoeba as an exemplary model for investigating the diversity, biology, and evolution of such symbiotic associations. Chlamydial symbionts are intracellular bacteria that are widely distributed in various environments and eukaryotic hosts. They diverged from major human pathogens hundreds of millions of years ago, yet employ similar infection mechanisms and strategies. Their adaptation to microbial eukaryotic hosts involved evolutionary periods characterized by substantial genome expansion and the modulation of the canonical chlamydial developmental cycle. As symbionts of amoeba, chlamydiae can exhibit varying degrees of parasitism, ranging from highly virulent to neutral in terms of host fitness. The true benefits of these relationships become apparent only when the amoeba host encounters other microbial parasites. Through their bacterial symbionts, amoeba populations acquire immunity and successfully survive otherwise lytic infections caused by the amoeba pathogen Legionella pneumophila and nucleocytoplasmic large DNA viruses (known as giant viruses). Additionally, the influence of chlamydial symbionts on the fitness of the amoeba host affects the role of amoeba as predators, indirectly influencing the composition and functioning of microbial communities. Thus, bacterial symbionts play a pivotal role in the biology, ecology, and evolution of amoebae, with significant consequences on both partners and their environment.

Horizontal gene transfer in the early evolution of protists: How eating changed our genes

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Horizontal gene transfer (HGT) is the non-sexual movement of genetic information between different species. Horizontal transfer has been shown to have played such a pronounced role in the evolution of bacterial and archaea genomes, but it's role in eukaryotes has never been as clear. Eukaryotic genomes certainly bear evidence of HGT, in many cases with clear functional and ecological impacts, but compared with Bacteria and Archaea the scale of HGT is both reduced and more uneven across the tree of eukaryotes. This seemingly contrasts with arguments that phagocytosis, the uniquely eukaryotic act of cytoskeleton-mediated engulfment of other cells, should greatly enhance the opportunity for HGT, especially when those cells are sometimes retained as long-term endosymbionts. However, the origin of phagocytosis would have sent waves of changes throughout the evolution eukaryotes, and selection on the first phagotrophic predators (protists) would be affected by the fact that the cells around them were no longer competitors; they were food. The enhanced reliance on cellular structures and behaviours that this form of feeding required would also alter the kinds of variation that would be favoured by selection. In Bacteria and Archaea, HGT provides an abundant source of relevant variation because selection can easily favour highly modular metabolic genes. In phagotrophic protists, by contrast, selection would more likely favour developmental pathways that led to advantageous behavioural variants, and these would result from non-modular mutations to genetic regulatory networks. Looking to the most extreme cases, when engulfed cells are retained as endosymbionts, it not apparent that any increased opportunities afforded by the prolonged intracellular retention of foreign cells translate into high levels of HGT. There is no evidence for a major influx of genes from long-term endosymbionts to their host genomes in general, and even in genetically-integrated endosymbiont/organelle systems with protein-targeting, the first wave of genes for targeting proteins now appear to be acquired from sources other than the organelle itself, and most if not all the genes subsequently acquired from the organelle lineage are re-tarted to the organelle and do not function in the host. All this suggests that "opportunity" is not a significant factor modulating the frequency of HGT. Eukaryotic genomes are probably exposed to foreign genes at a very high frequency, but seldom have any use for them because the selective pressures usually do not tend to operate on that kind of variation.

ISOP Past-President's Address 2019-2020 - Footprints in the sand

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Progress in protistology provides a tapestry of ideas, methodologies, technological changes and creativity. This presentation will provide a retrospective view of protistan ecology and track the supply chain of ideas and innovations that have brought us to our present understanding of the ecological implications of ciliates in their respective habitats, as different trends have unfolded in the last 50 years. The challenge of embracing change while valuing past contributions and integrating existing data reservoirs into research design and interpretation is an unfolding but rewarding journey. Studies of selected ciliates will be utilized to illustrate these interwoven themes.

Pathogenic Protists

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Zoonotic Protists

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Marine ecology and global change

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Abstracts:
Oral Presentations
(in alphabetical order)

Insights into the dark matter of desert protists highlight unique community structures of cercozoans and amoebae across the driest desert on Earth, the Atacama Desert

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Protist life is ubiquitous and distributed along ecosystems playing a paramount role in environmental processes part of Earth's biogeochemical cycles since primal ages. Fast evolution and the ability to produce cysts allow these microbes to thrive every imaginable niche, even in the presence of the extreme range of factors including temperature, pressure, pH, salinity, solar radiation, etc. Here, we present the results of the environmental sequencing of protists in microhabitats comprising endangered and endemic cacti, terricolous lichens and microbial mats across the Atacama Desert and the Andes. We highlight the uniqueness of the explored microhabitats, hosting community structures, including the so-called dark matter of life as we aimed to register the unculturable microbes through metabarcoding of the V9 region of the 18S rRNA gene. Our results point to the presence of potentially interacting protists in network modules highlighting cercozoans, different flagellates and ciliates in soils and stramenopiles and amoebae from diverse clades in microbial mats of five high-altitude lagoons. Furthermore, we compare the sequence variants obtained by massive sequencing to phylotypes isolated in culture to assess the sequence variability between the culturable and unculturable protist diversity. Our results indicate the microbial mats as complex communities limited by biochemical gradients. The astonishing amount of unclassified sequence variants in microbial mats remark the necessity of studying underexplored habitats hosting protist species in aquatic systems affected progressively by anthropogenic activities and climate change as the Andean lagoons. Our results provide new insights into the biogeography, ecology patterns at microscale and function of the protist life in remote and understudied microhabitats part of the oldest desert on the planet.

Wilhelm Foissner (1948–2020), an outstanding taxonomist, and the future of a nomenclatural system of Ciliophora

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Foissner as a giant monographer considered both nomenclature (naming taxa) and taxonomy (defining and classifying taxa) as inseparable. This view has not been shared by many during the history of systematics and contemporary ciliatologists often show a striking disinterest for both fields. Since the authoritative works of Kahl (1930-1935), which were heavily affected by retroactively introduced rules of the 'first edition' of the International Code of Zoological Nomenclature in 1961, ciliatologists face a tremendous increase in numbers of names for taxa. Inofficial and/or arbitrary rules of nomenclature were (and still are) followed up to the works of Corliss, who has been a member of the International Commission on Zoological Nomenclature. Corliss found an incredible number of nomenclatural errors in the literature and therefore corrected the authorship and dates of many names and replaced junior homonyms. Likewise did Foissner starting from his first new species described in 1969. Although his main interest was collecting and observing specimens in all their morphological and ultrastructural aspects, Foissner became increasingly forced to deal with nomenclatural problems under three successive editions of the Code (last 1999) and thus provided synonymies that are not merely mentioned but also carefully discussed. He contributed to solve practical and theoretical difficulties, e.g. regarding the neotypification of protists, problematic designations of type species and established long-lasting standards for ciliate systematics. However the nomenclatural system, viz. assemblage of correct scientific names in a taxonominal hierarchy, of ciliate taxa is hampered by (1) oversimplified judgements between valid or invalid disregarding (un)available works, names and nomenclatural acts, (2) misunderstandings concerning types (must not be typical), spellings (i.e. mistaken for names) and application of terms (e.g. basionym and type family are outside the Code), (3) problems in interpreting Code and the "Amendment" of five articles to expand methods of publication in 2012 result in authorship- and dating-vagueness of a name (4) lack of pertinent directive anywhere in the Code concerning naming suprafamilial taxa. Ciliate classification, phylogeny and ecology are far from being understood because 90% of the species diversity is likely undescribed and sequences tell us nothing on the appearance of the organisms.

Multiple lines of evidence allow a moderate revision of the oligotrichid genus *Strombidium* (Alveolata, Ciliophora, Spirotricha)

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Wilhelm Foissner usually based his recent taxonomic studies and revisions on multiple lines of evidence, such as on data from light and electron microscopy as well as genetic analyses. We follow his approach in our moderate revision of the oligotrichid genus Strombidium Claparède & Lachmann, 1859, which mainly inhabits the marine pelagial. In molecular phylogenies, this speciose genus is not monophyletic, and cladistic analyses considering morphological and ultrastructural features indicate that it is exclusively characterised by plesiomorphies. Accordingly, new morphological and/or ultrastructural apomorphies are required for a reliable split of the genus, e.g., the extrusome arrangement and ultrastructure. Typically, the attachment sites of oligotrichid extrusomes form a stripe anteriorly to the girdle kinety. In Strombidium biarmatum Agatha, Strüder-Kypke, Beran & Lynn, 2005, however, light microscopy revealed additional extrusomes inserting between the collar membranelles and in the buccal lip. We investigated this special feature by transmission electron microscopy in specimens collected in the Baltic Sea. The results demonstrated two types of extrusomes differing not only in their position but also in size and shape. A similar situation had been described in two congeners which form a statistically fully supported cluster with S. biarmatum in 18S rRNA gene trees. Accordingly, both morphologic and genetic data corroborate the establishment of a distinct genus within the family Strombidiidae Fauré-Fremiet, 1970. By comparing the available ultrastructural data on oligotrichid extrusomes, differences of potentially taxonomic significance emerged. Based on a broader ultrastructural knowledge, these distinguishing features might be used for further splits of the non-monophyletic genus Strombidium and the family Strombidiidae in the future.

This study was financially supported by the FWF Projects P 28790-B29 and I3268-B29.

A new litostomatean (Ciliophora) species from Tuscany, described according to the standards of Next Generation Taxonomy

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The ciliate Lacrymaria was one of the first genera of ciliates ever described (1824), and during its taxonomic history was repeatedly merged and separated again from genus Phialina under different morphological criteria. It is rarely cultivated due to being a highly specialized predator of other ciliates. Here we describe a new Lacrymaria species, Lacrymaria venatrix sp. nov., recovered from freshwater in a Tuscan wetland, with the combination of methods prescribed by the new Next Generation Taxonomy standard. It shows a combination of morphological features that does not exactly correspond to any currently established species of either genus; in addition, 18S rDNA-based molecular phylogeny places it on a separate branch outside previously sequenced species, as well as suggesting that neither genus Lacrymaria nor Phialina as currently defined is monophyletic. It might therefore be appropriate to combine the two genera into one once again. We also provide data on the ultrastructure and mitogenomics of Lacrymaria venatrix sp. nov.

Microbiome in the mucus of *Ostreopis ovata* (Dinoflagellate) Mediterranean isolate

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Ostreopsis ovata is a harmful Dinoflagellate causing blooms all over the world included the Mediterranean Sea. This protist is a benthic species turning in a planktonic species in the course of the bloom: free to move in the water column and colonizing new sites. Its most notable features are to produce a mucous matrix during the benthic state and to produce ovatoxin, a chemical variant of palytoxin (PLTx). When O. ovata is in vitro cultured, it become disaggregated with free protists in the medium and re-aggregation in the mucosal structure is reached after few days. The matrix where this protist grows is characterized by presence of bacteria and other dinoflagellates species, producing palytoxin too. To understand the relationships between the eukaryotic and prokaryotic microbes living inside the mucus, relatively the protist trophism and toxin production, it's intriguing and interesting as O. ovata caused several toxic bloom in the Mediterranean sea, especially between 1970 and 2005 year. We cultivated a D849 strain of Ostreopsis ovata, kindly provided by the SZN of Naples. In vitro culture was standardized in 750 ml flask provided of light\dark period is 14\10 h, with a luminous intensity about 3000 Lux. In order to look for some associated microbial clades we choose primer specific for bacterial genes. Particularly we used primer for 16s RNA specific for coli-like bacteria and for PYR gene of Vibrio sp. bacteria. Qualitative PCR showed the presence not only of coli-like but also vibrio-like bacteria and a glycoprotein matrix in the mucus. Vibrio is a bacteria Genus living everywhere in the Ocean and some species have been found also in the Mediterranean Sea. These bacteria produce PLTx and are frequently associated to Coelenterates of Palythoa genus. Axenic cultures allowed us to study different Ostreopsis behaviors regarding to the mucous production and the microbe growth rate when the protist lifestyle is strictly autotrophic. SEM let us to see bacteria distribution in the mucous matrix and on isolates cells of Ostreopsis. Studies on the bacterial association with O. ovata and the toxin production mechanism both in non-axenic and in axenic cultures are in progress

Palythoa aff. *clavata* as a source of bioactive compounds against the "brain eating" amoeba

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Naegleria fowleri is a free-living amoeba belonging protozoa whose life cycle consists of three different stages: trophozoites, flagellated form and cysts. This parasite, also known as "brain eating amoeba", is the causative agent of the primary amoebic meningoencephalitis (PAM), a fulminant disease with a rapid progression that affects the central nervous system. Since the report of the first case in 1965, more than 440 have been described worldwide. The lack of standardized diagnostic techniques and effective treatments have provoked the lethality rate of the PAM to be higher than 97%. Therefore, the search of new anti-amoebic compounds remains as a key factor in the battle against the PAM. On the other hand, the zoanthid Palythoa aff. clavata has shown to be a source of active compounds against different protozoa such as Acanthamoeba spp., Leishmania spp. and Trypanosoma cruzi. In this work, the anti-Naegleria activity of twelve sesquiterpene lactones isolated from the zoanthid Palythoa aff. clavata was evaluated. The in vitro amoebicidal activity and cytotoxicity of the isolated compounds was evaluated using an alamarBlue® based fluorometric assay. These experiments were performed with the Naegleria fowleri ATCC 30808 strain and the ATCC J774A.1 murine macrophages cell line respectively. Moreover, the mechanism of action of the most active molecules was determined. For this purpose, different kits that outstand the presence of some of the cellular events that are characteristic of the programmed cell death process were employed. Results show that Anhydroartemorin and 1(10)Z,4E,14-acetoxy-costunolide were the most active molecules against Naegleria fowleri with inhibitory concentration 50 values of 23.02 ± 1.26 µM and 28.34 ± 6.27 µM, respectively. Moreover, both compounds induced the occurrence of metabolic events compatible with the programmed cell death. Hence, the Anhydroartemorin and 1(10)Z,4E,14-acetoxy-costunolide can be considered as good candidates for the development of novel therapeutic options against the primary amoebic meningoencephalitis.

Keywords: Naegleria fowleri, Palythoa; chemotherapy; meningoencephalitis; programmed cell death

Acknowledgments: CIBERINFEC (CB21/13/00100), Cabildo Insular de Tenerife 2023-2028, Ministerio de Sanidad, Consumo y Bienestar Social, ACIISI y Fundación La Caixa – Caja Canarias (2022CLISA26)

Abortive infection and defensive symbiosis – how protists defend themselves against giant viruses

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The discovery of giant viruses in amoeba changed our perception of the viral world and revealed a previously unseen diversity and complexity of viruses of microbial eukaryotes. With genome and particle sizes comparable to bacteria and many cellular features, giant viruses sparked a controversy about their evolutionary origin. Frequent host switches, competition with other viruses, and bacterial symbionts were proposed as drivers of giant virus evolution. While virophages have been shown to facilitate protist host survival during giant virus infection, very little is known about other antiviral defense mechanisms. Here, we report on two novel defense strategies observed with two different protists hosts and two different giant viruses. First, we studied the role of bacterial symbionts of free-living amoebae in establishing giant virus infections. To investigate these interactions in a system that would be relevant in nature, we isolated a giant virus (Marseilleviridae) and potential Acanthamoeba hosts infected with a bacterial symbiont, identified as Parachlamydia acanthamoebae, from the same environmental sample. Systematic co-infection experiments showed that the bacterial symbiont represses the replication of the sympatric giant virus as well as other giant viruses (Mimiviridae) in both environmental isolates as well as Acanthamoeba lab strains. Second, we studied a new giant virus isolate (Mimiviridae) infecting members of the amoeboflagellate genus Naegleria. We describe how infection of Naegleria may lead to abortive infection, in which viral replication and dissemination are blocked by premature host cell death, ensuring the survival of the amoeba host population. Together, we show that amoebae employ diverse and, as yet, underexplored strategies to cope with omnipresent giant viruses.

An intriguing case of *Ciliophrys*: lack of plastid and presence of bacterial endosymbiont

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Within the ochrophyte class Dictyochophyceae, two prominent colorless members are found, classified as the genera Pteridomonas and Ciliophrys. Based on previous molecular phylogenetic investigations, Pteridomonas and Ciliophrys are each specifically related to different photosynthetic dictyochophytes (pedinellids and Rhizochromulina, respectively), establishing them as two separate instances of photosynthesis loss. TEM investigation has revealed that Pteridomonas contains a residual plastid organelle, and the genome of the plastid has also been sequenced. However, the status of the plastid in Ciliophrys remains unresolved. To finally clarify this matter, we isolated from the Baltic Sea a new Ciliophrys strain and performed Illumina and Nanopore sequencing of its DNA. In addition, we also generated the transcriptome assembly of the studied organism. The 18S rDNA phylogeny confirmed our morphological identification of the organism as a Ciliophrys representative and revealed a substantial cryptic diversity within the genus. Furthermore, the assembled mitochondrial genomes of Ciliophrys sp. Baltic and Rhizochromulina marina showed similar characteristics, confirming their close relationship as sister taxa within the Dictyochophyceae. In contrast, no candidate for a plastid genome could be found in the assembly of total DNA from the Ciliophrys sp. Baltic culture. Furthermore, the organism seems to lack hallmark plastid proteins, as established by searches of the Ciliophrys sp. Baltic transcriptome assembly. These results suggest that Ciliophrys sp. Baltic lacks both a plastid genome and the organelle itself. Unexpectedly, the genome assembly from Ciliophrys sp. Baltic contained scaffolds corresponding to a bacterium of the order Rickettsiales, which is known to predominately consist of obligate intracellular endosymbionts. With the help of long sequencing reads, we were able to assemble the complete genome of the novel bacterium and demonstrate its inclusion within the broader Candidatus Megaira clade. We are investigating the endosymbiont genome sequence to illuminate the nature of its relationship to the host. The initial findings suggest that it is not necessarily a pure parasite, owing to its possession of a complete heme biosynthesis pathway that is apparently missing Ciliophrys sp. Baltic, suggesting the host may rely on the provision of heme by the endosymbiont.

Protist community ecology across an eddy dipole in the North Pacific Subtropical Gyre

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Oceanic eddies are common mesoscale features that can extend >100 km and maintain cohesiveness for months, potentially impacting biogeochemical cycles in the water column. We used direct microscopy and metabarcoding of sediment trap material, and imagery and metabarcoding of the planktonic community to investigate standing stocks of protists suspended in the water column and on sinking particles, at stations across an anti-cyclonic to cyclonic eddy-dipole near Station ALOHA in the North Pacific Subtropical Gyre during July 2017. The water column was sampled at 4 depths at each station from the surface to 500m and particle interceptor traps (PITs) were deployed at 150m. The greatest difference in protistan assemblage composition was observed between sample type with water column rDNA, water column rRNA, trap rDNA and trap microscopy revealing very different taxonomic compositions and dynamics of assemblages across the eddy-dipole. Water column metabarcoding revealed protistan assemblages dominated by Alveolates representing a mean of 63% of all water column sequences. Within planktonic assemblages, those assessed by rDNA were strongly affected by depth but less so for the assemblages assessed by rRNA. The protistan assemblages within sediment traps assessed with metabarcoding were dominated by Rhizarian protists representing a mean of 79% of total trap sequences. Microscopy observed communities of trap material showed an increased relative abundance of ciliates in the anticyclonic eddy center and an increase in diatoms in the cyclonic eddy center.

Adventures in the microworld

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Fortunately, there are more and more short videos available on social media and video-sharing sites on the subject of protistology. These can play a significant role in generating scientific interest. However, there are still very few nature films available on the subject of protistology. I had the opportunity to participate in the creation of such a nature film. This natural history documentary(Adventures in the Microworld) of the Naturefilm.hu Society presents the story of a protistological sampling expedition in the Carpathian basin. During the expedition, we were using a moving laboratory which was a small caravan. I was the main character in the film, and my task was also to film scenes about protists through a microscope. I would like to report on the shooting and reception of this nature film. With my presentation, I would like to encourage other protistologists to undertake similar projects.

On the life history, occurrence and microhabitat of a rare ciliate: *Apocarchesium arndti* Norf et Foissner, 2010 (Oligohymenophorea, Peritrichia)

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Due to the limited biogeographical distribution and the conspicuous appearance of the Apocarchesium species, they are considered to be important flagship species in ciliate biogeography. The genus Apocarchesium was first described in Japan and soon another species was described in Germany. An unexpected finding of an Apocarchesium arndti population in September 2019 proved to be the first record from the Carpathian Basin of this globally rare genus and species. We provide the first detailed description of the colony development of A. arndti. A novel zooid type has been identified from the Apocarchesium colony. We distinguished it from microzooids based on morphological features. The term, structural zooid, is proposed for this unique type of zooid. We provide a descriptive characterization of the ecological environment: community and microhabitat of A. arndti in the Middle Danube. Our finding supports the theory of moderate endemicity.

Simulating the origins of multicellularity by artificial selection on the filasterean *Capsaspora owczarzaki*

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The origin of multicellularity is one of the major evolutionary transitions in the history of life. However, little is known of the factors that triggered the evolutionary transition to macroscopic multicellularity at the onset of animals. To better understand this, we focused on the origins and evolution of mechanisms that regulate multicellular body size, and how do they correlate with the origins of spatial cell differentiation. To address these questions, we need to reconstruct how the last unicellular ancestor was. This can be achieved by studying the extant closest unicellular relatives of animals, from which we focus our work on the Filasterean protist Capsaspora owczarzaki. Additionally, to address the absence of macroscopic size metazoans until the Cambrian explosion, we recreated the atmospheric conditions from 500 Mya by duplicating the experiment under hypoxic conditions to test the role of oxygen in size regulation as an environmental constraint at the onset of multicellularity. We started a long term evolution experiment in which we have artificially selected for bigger C. owczarzaki aggregates by sedimentation, over more than 80 cycles of selection or 500 generations in 12 different populations, in both normoxic and hypoxic conditions (2% O_2, or 10%PAL). During these cycles we were able to observe and measure changes in shape and size of the aggregates e.g. differences in the degree of compaction in some evolved populations, leading to corresponding differences in sedimentation rates. Furthermore, we use genomic sequencing and differential gene expression profiling to examine genetic and transcriptomic changes that took place in different populations over the course of the experiment. This allows the identification of key mutated genes that are related to body size regulation. With our genetic approach we also sought to identify possible spatial cell differentiation evidence by performing single-cell sequencing in the largest evolved aggregates, looking for heterogenic gene expression patterns. This work promises to deepen our understanding on the origins of macroscopic multicellularity and the processes that accompany the evolution in size, it also settles the ground to future experimental evolution projects on C. owczarzaki and other aggregative protist and cell differentiation in other groups (e.g. nucleariids, acrasids, labyrinthulomycetes, cercozoans.)

Protists as ideal models to study soil biodiversity-ecosystem functioning

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Biodiversity and ecosystem functioning (BEF) are often positively related. This BEF relationship has been mainly shown for plants where a higher diversity of plants is related to higher plant biomass among other ecosystem functions. However, this BEF relationship is poorly studied for one of the most diverse ecosystems in the world (soils), hosting potentially millions of species, including protists. Even more striking is the lack of knowledge on the effect of (interactive) global change drivers (GCDs) on this sBEF relationship, as for aboveground studies it has been shown that higher biodiversity might reduce the negative impacts of (interactive) GCDs on ecosystem functioning. Here I show the results obtained in 2 greenhouse experiments that investigated the soil BEF (sBEF) relationship with a focus on predatory protists under both biotic and abiotic GCDs. I tried to uncover sBEF by manipulating the species richness from 0 to 30 protist species in non-treated controls, under abiotic stress (fertilization or drought), biotic stress (nematodes dominated by root-feeders), and a combination of biotic and abiotic. Then we tested plant biomass (tomato and Cannabis sativa) and biochemical soil parameters related to nutrient cycling (such as soil organic carbon (SOC) or Microbial Biomass Carbon and Nitrogen) as ecosystem functions. Increasing biodiversity showed a wide range of impacts on plant biomass and nutrient cycling, not only positively as mentioned in the literature (here under nematode infection or fertilization) but also negatively as shown under drought. Two stressors combined led to additive patterns canceling out the negative drought effects and positive nematode effects. I conclude that protists are great models to study sBEF relationships in soils, which we show to not generally follow those of plants as ranging from positive to negative, necessitating fundamental changes in claims on (s)BEF. These results are a starting point to uncover the sBEF relationship under ongoing and future climate change and that protists should be part of new endeavours.

UniEuk project update and future of the initiative

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The UniEuk project is the ISOP-supported, community-based and expert-driven international initiative to build an online universal taxonomic framework for eukaryotes (focusing primarily on protists) linked to sequence data in the International Nucleotide Sequence Database Collaboration (hosted by DDBJ, EMBL-EBI/ENA, and NCBI). The project started in 2016 and was designed to become the community hub of choice to centralize, safeguard and promote our current global knowledge on eukaryotic diversity and evolution, integrating morphology and ecology with key molecular information. It is based on three complementary modules and accompanying public resources. EukRef is the standardized, open-source bioinformatics pipeline that allows taxonomic curation of publicly available 18S rDNA sequences, generating homogeneous sets of aligned sequences and phylogenetic trees. EukMap is the user-friendly representation of the taxonomic framework in the form of a publicly navigable tree, where each node/taxon is associated with standardized features (name, contextual data, links to pictures and literature, etc.), and allowing registered users to submit proposals of changes that can be validated by experts within the research community. EukBank is a public repository of high-throughput metabarcoding datasets that facilitates monitoring of eukaryotic diversity across biomes, and identification of ecologically relevant new lineages. Linked to the project are also two reference databases of 18S rDNA sequences (EukRibo) and genome-scale predicted proteins (EukProt). As the project reaches the end of its second round of funding, and directly following a final workshop linking EukRef curation and a EukMap tutorial, we will report on the successful deployment of all modules and the challenges we faced during the project's implementation, and discuss the future of the initiative and community involvement moving forward.

Microsporidia and protist parasites: An important future threat to the insect rearing industry

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Little is known about what future risk Microsporidia and protists pose to insects reared for food and feed. However, past experiences with intensive invertebrate rearing indicate that these parasites whilst often benign, in particular microsporidia, can rapidly sweep through populations causing extensive damage. To ascertain whether communities of microsporidia and protists, with an emphasis on gregarines, are present in various reared insect species; we are applying a PCR and sequencing Nanopore based investigation, as well as a bioinformatic mining approach of insect omic' data. The PCRs include two approaches, the first one using broad anti-metazoan primers to target the 18S gene of microeukaryotes and the second one group specific primers of diverse Microsporidia and protists. The use of adapted PCR and sequencing high throughput methods, coupled to data mining aims to provide insights into these parasites' prevalence and host spectrum as well as novel genomic data for overlooked protists. So far, the sequencing of amplicons has shown that Gregarines were representing the major part of detected protists. Eventually, to assess the potential effects of gregarines on different life traits of cricket hosts, bioassays with Acheta domesticus are undertaken.

Keywords: rDNA, Nanopore, micro-eukaryotes, parasites, reared insects, bioassays

Antikinetoplastid activity of sesquiterpenes isolated from the zoanthid *Palythoa* aff. *clavata*

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Leishmaniasis and Chagas disease are neglected tropical diseases that affect millions of people worldwide, causing problems in developing countries. Globalization and the rise in migration has meant that this pathology is having an increasing impact in non-endemic countries. Available treatments for these pathologies are not fully effective and can have serious side effects, so there is a need to find new effective molecules, and safe for patients. The search for active compounds of natural origin has made possible to obtain new and novel molecules with therapeutic activity against these parasites, such as the molecules obtained from the zoanthid coral Palythoa aff. clavata, which has reported activity against other protozoa such as Naegleria fowleri or Acanthamoeba spp. In this work the trypanocidal and leishmanicidal activity of 13 sesquiterpenes obtained from Palythoa aff. clavata were studied, using a colorimetric assay based on the alamarBlue reagent. At the same time the cytotoxicity against murine macrophages were done. In addition, the mechanisms of action of the most active compounds was determined to determine the type of death that these molecules produce in the parasites. Results shown that these sesquiterpenes have activity against Leishmania amazonensis, Leishmania donovani and Trypanosoma cruzi. The sesquiterpene lactones anhydroartemorin (2), cis,trans-costunolide-14-acetate (3) and 4-hydroxyarbusculin A (11) were the most selective against the kinetoplastid species studied. These molecules induce the mechanisms involved in an apoptotic-like death or programmed cell death (PCD) in the kinetoplastids.

Keywords: Trypanosoma cruzi, Leishmania spp., Palythoa aff. clavata, PCD.

Acknowledgements: Proyect PI18/01380 ISCIII; CB21/13/00100 (CIBERINFEC); Cabildo de Tenerife (2023-2028), MEDI y FDCAN; 21/0587 MICINN, AEI and ERDF; Fundación La Caixa-CajaCanarias 2022CLISA26. ACIISI 85% co-funded by the FSE (TESIS2020010057 C.J.B.E.; R.L.R.E.; D.S.N.H.; I.A.J.; P.P.P.).

To be or not to be a mixotroph? How niche modeling can help to tackle dinoflagellate trophic strategies in marine ecosystems

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Dinoflagellates are ubiquitous unicellular eukaryotes that frequently dominate marine planktonic communities. They fill diverse ecological roles (primary producers, consumers, decomposers) and show a wide metabolic and physiological diversity. Most lineages are capable of mixotrophy, i.e., realizing both phototrophy and phagotrophy within the same cell. Nutritional mode depends on multiple factors, which can be intrinsic (e.g., evolutionary history of the lineage and how the trophic mode(s) are acquired) and extrinsic (e.g., light, nutrient and/or prey availability). By focusing on abiotic factors, this study aims to build the current biogeography of dinoflagellate species and their associated trophic types. Using metabarcoding data and multivariate statistics, the determinants of community structure are highlighted and then the distributions of species and their traits are projected at a global scale with species distribution models (SDMs). Nutrient availability, light and temperature gradients discriminate communities efficiently and distinguish coastal, eutrophic, polar environments from oligotrophic, open- ocean, tropical waters. The coexistence of the 3 trophic types represents more than half of the global oceanic surface, and phototrophs, which are less ubiquitous, do not occur alone. Conversely, heterotrophs can exist alone in more than 20% of oceanic waters, and coexist with mixotrophs to a lesser extent. These results must be considered regarding to the trophic assignment of dinoflagellates. Indeed, a higher precision in the trophic assignment and a larger species sampling could contribute to a better understanding of the determinants of these distributions, useful for future predictions and potentially biogeochemical modeling.

The hidden world of green algal endophytes: diversity, relationship with host, and impact on aquaculture crops

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Not all green algae are free living, some have evolved an endophytic lifestyle, and live inside algal hosts. Endophytism is a common phenomenon in several evolutionary lineages of green algae and occurs in a wide diversity of hosts in both aquatic and terrestrial habitats. Here, based on a literature review of green algal endophytes of seaweed hosts, we have compiled a database that facilitates comparisons of the diversity of these endophytes and their hosts. The majority of hosts belong to the red algae (Rhodophyta), an ancient lineage that originated over a billion years ago, at approximately the same time as green algae (Chlorophyta). Most of the endophytic green algae in seaweed hosts belong to the Ulvophyceae, which is one of the core chlorophyte lineages. The origin of an endophytic lifestyle in some ulvophyceans is unknown. However, the Ulvophyceae, went through a rapid radiation in the late Neoproterozoic and early Paleozoic Era, which coincides with Snowball Earth conditions and the Cambrian explosion (respectively). Earlier studies have hypothesised that the evolution of multicellularity and macroscopic growth in ulvophyceans may have been triggered by the introduction of novel grazing pressures by animals in the late Cambrian and Ordovician periods. We hypothesise that the evolution of endophytism in some ulvophyceans may also have been triggered by the same factors: as a survival strategy during glaciation periods and/or to escape grazing. Further, we address how current green algal endophytes might be affected by global warming and how this might impact on aquaculture crops.

Assessing the potential of Nanopore long-read sequencing for metabarcoding: A personalized molecular protocol for profiling protist communities in diverse habitats

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Metabarcoding is a widely used strategy for surveying biodiversity in multiple scientific domains, including agriculture and medical research. Typically, the v4 short region of the ribosomal small subunit 1 (18S) gene is sequenced using Illumina technology to assess eukaryotic diversity. However, this approach has been criticized for its bias in recovering certain protist clades, such as Amoebozoa, due to the heterogeneity of the v4 fragment size and variability of its priming regions. With the emergence of Oxford Nanopore Technologies, amplicons can now be sequenced without size limitations, potentially revealing a more accurate picture of microbial communities. However, obtaining and analyzing longer fragments, along with lower sequencing depth and higher sequencing errors, presents challenges that must be addressed to obtain meaningful and reproducible data. In this project, we describe a molecular protocol for sequencing the full 18S gene with the latest nanopore kit and flow cell on the MinION platform, starting from raw environmental samples. We assess the performance of our method for both quantitative and qualitative data using a mock community and 24 samples with diverse compositions (e.g., soils, freshwater, marine water, bark, and dead leaves), and evaluate its potential for capturing relevant communities. Our goal is to provide an honest assessment of the strengths, weaknesses, costs, and challenges of long-read sequencing with Nanopore, and to guide colleagues in their research.

The hidden genomic diversity of orphan branches of the eukaryotic tree of life

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Ancyromonads and mantamonads are clades of free-living heterotrophic flagellates that deeply diverge from all extant eukaryotic supergroups, therefore representing key lineages for our understanding of the origin and early evolution of eukaryotes. Despite their evolutionary relevance, their biology and diversity are still poorly explored. In this work, we isolated, cultured, and described several new species within the Ancyromonadida and Mantamonas lineages. The assembly of the first high-quality reference genomes for these organisms using short- and long-read sequencing allowed us to investigate their phylogenetic placement and the macroevolutionary patterns shaping their gene content. Our phylogenomic analyses confirmed the early divergence of ancyromonads within the eukaryotic tree of life and the monophyly of the genus Mantamonas within the CRuMs supergroup. Albeit their morphological similarities, the genomes of these organisms are diverse in size (from 25 to 39 Mb), number of genes (from 10K to 16K) and repeat content (from 3 to 29%). The ancestral gene content reconstruction over 141,634 gene families distributed across eukaryotes revealed a significant gene gain in the ancestor of ancyromonads. Most of these genes have unknown functions but also encompass many related to signal transduction mechanisms and cell motility. Furthermore, ancyromonad genomes have retained ancestral gene families since their divergence from the last eukaryotic common ancestor (LECA) that have been lost in other members of the Amorphea, allowing us to identify new LECA genes previously thought to be only present in Diaphoretickes. We characterized the gene expression patterns of Ancyromonas sigmoides under shifting environmental conditions, which will allow us to shed light on the roles of the uncharacterized fraction of the gene content and improve our knowledge of the biology of these enigmatic organisms. By proxy, this might also inform us on the putative ancestral function of these genes in the LECA.

Comparative analysis of Illumina and Nanopore amplicon sequences of eukaryotic communities from sediment samples

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For the assessment of ecosystem functions advanced understanding of environmental diversity is vital. Metabarcoding enables precise classification of distinct species through DNA sequencing. In recent years, high-throughput sequencing techniques evolved from short-read Illumina sequencing to long-read Nanopore sequencing. Despite advantages of Nanopore sequencing like cost reduction and sequencing of full-length genes, error rates are still higher than those of high accuracy Illumina sequencing. Also, suitable tools and workflows for Nanopore read processing are rare. Since existing comparative analyses deal almost exclusively with prokaryotes, it is unclear to what extent both techniques are comparable regarding eukaryotic marker genes. Therefore, the short 18S V9 gene region, extracted from mesocosm sediment samples was sequenced with both platforms. In addition, full-length 18S sequences were obtained with Nanopore sequencing. Initial processing of raw-reads was accomplished with the open-source pipeline Natrix. Moreover, the pipeline was adapted for additional Nanopore-specific read processing by implementing tools for read orientation, trimming, error correction and adjusted OTU clustering. Obtained data were analyzed for variations in sequencing depth, quality scores, pipeline effectiveness and community compositions. Observed differences in quality scores with lower values for Nanopore reads were associated with lower accuracy. However, regions of higher errors rates were mostly sequence specific and applicable to both sequencing approaches. Despite variations in sequencing depth, the number of clustered OTUs was similar and correlation was strong for Illumina and both Nanopore approaches processed with the adapted pipeline. Though the similar number of classified taxa, significant differences between community compositions were found, explainable by a high number of presumably rare species. Since the unadjusted processing pipeline failed for Nanopore full-length 18S reads and number of OTUs and classified taxa for Nanopore 18S V9 reads processed with the unmodified pipeline differed greatly from the other approaches, Natrix v1 was shown to be unsuitable for Nanopore reads. However, the adjustments made the pipeline an optimal tool for processing Nanopore raw data. In conclusion, Nanopore short 18S V9 and long 18S reads were found to be comparable to short Illumina 18S V9 reads with perhaps even better resolution of community composition with respect to rare species.

Effects of stressors on growth and competition between different cryptic taxa affiliated with Ochromonadales (Chrysophyceae)

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Molecular studies revealed a high cryptic diversity among morphologically similar flagellates such as colourless non-scaled chrysophytes. This high cryptic diversity evolved through parallel evolution in different linages. However, its ecological significance remains unclear. In this study, we investigated the effects of heat waves and salinization on growth and competition between cryptic taxa affiliated with the clades C1, C2 and C3 within Ochromonadales (in particular Spumella spp., Pedospumella spp. and Poteriospumella spp.). We designed the FISH probes O1C531, O2C613 and O3C723 targeting the C1-, C2-, and C3-clade, respectively, to identify and quantify each taxa when grown together in mixed cultures. Increased salinity significantly decreased growth rates for all three taxa. An increase in temperature, however, stimulate particularly the growth of Poteripspumella lacustris (member of C3-clade). P. laucstris showed a high competitive strength, which resulted in a shift of community composition in mixed cultures. Our results suggest that morphological similarities may mask a turn-over of cryptic species differently adapted to abiotic factors. Since predator-prey interactions largely correlate with morphology, such a turn-over of cryptic species may stabilize microbial food webs facing environmental change.

Relevance of climate, geography and habitat for the distribution of protists - lessons learned from a continental lake plankton survey

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Is climate change in particularly temperature a factor driving distribution pattern of microbial eukaryotes? If so, past cold-adapted taxa and communities may be conserved at least partially in azonal cool islands such as high mountain ranges. Alternative strategies preventing or buffering local extinctions upon warming could be high dispersal capabilities with respect to either geographic distance or habitat type or both. High geographic dispersal capabilities should be reflected by a missing or negligible biogeography of taxa while a high capability to invade potential retreat habitats should be reflected by a high taxon overlap between these habitats. We investigated these aspects based on the molecular diversity of lake plankton on a European scale. Our analyses demonstrate that protists have a restricted geographical distribution even though biogeographic pattern do not mirror pattern known from macro-organisms. In particular, many OTUs were found exclusively in high mountain lakes and several putatively endemic OTUS occurred in mountain regions. High proportions of region-specific alpine OTUs indicate an increased occurrence of distinct lineages within each mountain range and thus, suggested either separated glacial refugia or post-glacial diversification within mountain ranges. However, a few alpine specialists were shared between mountain ranges suggesting a post-glacial recolonization from a common lowland pool. Regarding the ability to invade different habitats we show that the overlap of organisms co-occurring in freshwater and soil habitats is surprisingly low. Even though closely related taxa occur in both habitats distinct OTUs were mostly habitat–specific and most OTUs occur exclusively in either soil or freshwater. The distribution pattern of the few co-occurring lineages indicates that their presence in both habitat types is probably based on a stochastic drift of particularly abundant but habitat-specific taxa rather than on established populations in both types of habitats.

Single-cell transcriptomics reveal differential responses of mixed protist symbiont communities and host cells in corals during heat-stress

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Marine heatwaves caused by climate change leads to the stress-induced breakdown of coral-algal symbiosis referred to as "bleaching", threatening the continued survival of coral reef ecosystems worldwide. Since bleaching is at its core a cellular-response to heat-stress and since corals host a vast diversity of protist symbionts including many different dinoflagellates and apicomplexans, we developed a single-cell RNA sequencing (scRNA-seq) strategy to characterize the bleaching phenomenon from the microbial ecology perspective. We exposed replicate fragments of a colony of the endangered Caribbean coral Orbicella faveolata containing co-dominating dinoflagellate symbionts (Durusdinium and Breviolum; Family Symbiodiniaceae) to thermal stress and generated thousands of Symbiodiniaceae and coral cellular transcriptomes at four stages of bleaching. This allowed us to study, for the first time, the contemporaneous gene expression of individual microeukaryotic symbionts and coral cells under stress. In addition to identifying coral cell-specific responses to heat-stress, we found that Durusdinium symbionts, unlike Breviolum, showed significantly increased expression of antioxidants, allowing them to survive weeks of bleaching conditions. In contrast, we found not one but two different clusters of Breviolum, based on their transcriptomics profile, which decreased nitrate and ammonium transporter gene expression to different degrees in response to heat-stress. The Breviolum cluster which showed the least amount of decrease appeared to be more resilient than the other. It also exhibited higher expression of peridinin-chlorophyll a binding proteins and chloroplast envelope transporters compared to the less thermally tolerant Breviolum. This experiment has been repeated with the branching Caribbean stony coral, Acropora palmata, which is dominated by different Symbiodiniaceae dinoflagellates from O. faveolata as well as with the Mediterranean red gorgonian, Paramuricea clavata, which does not bleach and is dominated by Corallicolids and Syndiniales to compare and contrast the microbial gene expression between different anthozoans harboring different microeukaryotic symbionts under heat-stress conditions. These results highlight the utility of scRNA-seq in studying microbial symbioses and deepen our understanding of marine host microbial ecology at the cellular level.

What determines the assembly of protist microbiomes in the rhizosphere of plants?

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It is by now well proven that plant species select for distinct subsets of microbiota from bulk soil – their individual rhizosphere microbiomes. Also Cercozoa (Rhizaria) show plant species-specific rhizosphere microbiomes. In maize, root growth advances several centimeters each day, with the locations, quality and quantity of rhizodeposition changing. So where and when do the microbiomes assemble on the growing roots? Protists are assumed to be the most important predators of rhizosphere bacteria. Does predation then shape bacterial community composition - or is the assembly of protist communities rather driven by bacteria? What are the consequences for plant performance? If plants orchestrate microbiome composition, where are the potential key-genes located on the root axis, and which genes might control the composition of bacterial and cercozoan microbiomes? I will provide answers to these questions in my presentation.

The simple holocarpic oomycetes: Phylogeny and diversity

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Oomyceta are fungal-like heterotrophic organisms traditionally studied by mycologists, even though they belong to the kingdom Straminipila. The phylum is best known for its plant pathogens and freshwater saprobes in managed and natural ecosystems. However, little is known about the biology and evolution of the simple holocarpic forms, which mostly are obligate endobiotic parasites that develop their thallus entirely into reproductive structures. Even though they are widespread in aquatic and terrestrial environments, the biology and ecology of these bizarre organisms remains obscure. Most studies on these oomycetes have been reported decades ago, and only a handful have been rediscovered and investigated for their molecular phylogeny over the past few years. Here, we showcase the taxonomy, phylogeny and diversity of the simple holocarpic oomycetes encountered in our on-going studies. These encompass species that are obligate endoparasites of diatoms (Coscinodiscus, Pseudo-nitzschia, Rhizosolenia, Pleurosigma, Gyrosigma, Licmophora, Synedra, Acnanthes, Nitzschia, Pinnularia, Ulnaria, Minidiscus, Craticula, Cymbella, Fragillaria, Navicula, Melosira, Striatella, Acnanthes, Hantzschia), aquatic oomycetes (Saprolegnia, Achlya, Pythium, Aphanomyces), algae (Ceramium, Capsosiphon, Polysiphonia, Chondrus, Palmaria, Spirogyra, Ancylistes, Oedogonium, Zygnema, Pylaiella, Lyngbya), freshwater invertebrates (Ostrocods, Rotifera), and plants (Pinus). We isolated them from various aquatic (marine, brackishwater, freshwater) and terrestrial habitats in Germany (e.g. North Sea, Black Forest, various lakes and rivers), Norway (Oslo Fjord), Iceland (e.g. Heiðharvatn, Skagaströnd, Einbúalækur, Breiðdalsvegur, Astjörn, Blávík, various lakes and rivers), Denmark (Kolding, Copenhagen, Esbjerg, Borkhavn), and Mexico (Zempoala). A total of 49 newly described species and combinations, 3 new families, 3 new genera, and 4 new orders were introduced or reinstated. In addition, 13 rediscovered species and some recent insights into their diversity will be reported.

Education and dissemination by eukaryotic microorganisms

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Microscopic life forms are everywhere on Earth, and eukaryotic microbes (mostly protists) are no exception. Indeed, they are very common in all fresh- and salt water, as well as in moist soils, and a significant number of species can be found as parasites or symbionts of several organisms. Despite their abundance, their presence in some important ecological activities, and their relevance to human and environmental health, protists are often ignored both by the educational community and the lay public. Nevertheless, in our experience, protists represent unique models to teach fundamental topics of biology, ecology, systematic and evolution, especially to undergraduate students, as they perform all life functions within the small space of a single cell. In addition, protists may help counter popular misconceptions about microbes as “inferior” and less evolved organisms. For these reasons, since several years, we have been offering theoretical and practical experiences on protists for first and secondary grade schools. In particular, we have recently been collaborating with some museums and one zoological park to install permanent "exhibitions” for the observation of some species of protists (especially ciliates) as well as educational trails. These trails will be organized with an appropriate language for the non-scientific community and may serve to show the relevance of protists in various fields, such as evolution, scientific research, and public health applications.

Ethylene signaling in *Cyanophora paradoxa*: Revealing hormone usage in a glaucophyte alga

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Glaucophytes, an enigmatic group of freshwater algae, occupy a pivotal position within the Archaeplastida, providing insights into the early evolutionary history of plastids and their host cells. These algae possess unique plastids, known as cyanelles, that retain certain ancestral features, enabling a better understanding of the plastid transition from cyanobacteria. We investigated the role of ethylene signaling, a potent hormone used by land plants to coordinate stress responses, in the glaucophyte alga Cyanophora paradoxa. We demonstrate that C. paradoxa converts 1-aminocyclopropane-1-carboxylic acid (ACC), the ethylene precursor in land plants, into gaseous ethylene and produces ethylene natively in response to abiotic stress. Reactive oxygen species accumulation occurs following abiotic stress and ACC treatment, leading to growth inhibition in C. paradoxa. Our results also indicate a possible interaction between a second hormone, abscisic acid, and ACC/ethylene signaling in this alga, with evidence of crosstalk between the two signaling pathways. Furthermore, we reveal that ACC treatment induces the upregulation of senescence-associated proteases in C. paradoxa, consistent with the observation of growth inhibition. This is the first report of hormone usage in a glaucophyte alga, extending our understanding of hormone-mediated stress response coordination to the common ancestor of the Chloroplastida and glaucophytes. While the genes responsible for the conversion of ACC to ethylene in glaucophytes have not yet been identified, we note that C. paradoxa has homologs of pyridoxal-5'-phosphate dependent enzymes, the active domain in plant ACC synthases, and 2-oxoglutarate and Fe (II)-dependent dioxygenase domain enzymes, the active domain of ACC oxidases, which are candidates for ethylene synthesis activity in glaucophytes. By unraveling hormone signaling pathways in glaucophytes, we pave the way for a more comprehensive understanding of the evolution and diversification of hormone-mediated communication across the tree of life, ultimately enhancing our knowledge of the adaptive strategies employed by diverse lineages.

Saccoderm desmids are the key to understanding the evolution of zygnematophytes and their adaptations to terrestrial conditions

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The Zygnematophyceae are the closest algal relatives of embryophytes and well-suited objects to study the evolution of land plants. Because of their interesting phylogenetic position, the zygnematophytes gained much attention in the recent years. The latest phylogenomic studies helped to resolve the internal branching pattern of major zygnematophyte clades, but much of the known diversity remains uncaptured. It appears that sampling is still biased towards the beautiful placoderm desmids and filamentous species. However, there are unicellular zygnematophytes with a rather simple cell morphology (saccoderm desmids), whose diversity is still poorly represented in the current research. Interestingly, many representatives of the saccoderm desmids live on land and display various strategies to cope with terrestrial stressors (e.g. high light, poor nutrient supply). This makes saccoderm desmids well-suited for studying zygnematophyte adaptations to a life on land. We isolated saccoderm desmids from aquatic and terrestrial habitats and established axenic cultures to study the morphology and molecular phylogeny of the strains. Some traditional genera turned out to be highly polyphyletic; the genus Mesotaenium for example comprises more than ten separate lineages. We also explore the cellular adaptations, that help these algae thrive in high light and nutrient poor habitats with laboratory experiments, and finally generated transcriptome data of relevant saccoderm desmids. These data are used to infer an updated multigene phylogeny of the Zygnematophyceae and to gain insights into the molecular basis of terrestrial adaptations.

Evolutionary origins and diversification of the association with eukaryotes and the intracellular condition among the Rickettsiales

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The Rickettsiales are an alphaproteobacterial order encompassing multiple host-associated representatives, traditionally studied as vector-borne pathogens and reproductive manipulators in arthropods. Actually, Rickettsiales overall display a much broader host range, prevalently including protists, such as ciliates, amoebas, chlorophytes, euglenids, and cercozoans. These bacteria engage in intricate and largely undisclosed interactions with their hosts. The majority of host-associated Rickettsiales reside and multiply inside host cells. At least one exception is documented, namely a representative of an under-investigated sublineage living extracellularly attached to the surface of its host Paramecium. In any case, Rickettsiales are generally host-dependent but not host-confined, being able to horizontally transfer even among very distantly related hosts. From an evolutionary perspective, available data indicate a probable ancient association with aquatic protists. Nevertheless, key aspects on the origin and evolution of host association and intracellularity are not well understood, in particular it is not yet clear whether these are ancestral or convergently evolved features, and which mechanisms underlie such processes in different Rickettsiales. In order to address such major open questions, we assembled an unprecedentedly large and phylogenetically-balanced genomic dataset, by targeted sequencing of novel protist-associated organisms, as well as accurately selecting published genomic and metagenomic assemblies. Our detailed investigations clearly indicate that obligate host-association and intracellularity evolved “late” among Rickettsiales, independently in different sublineages. We infer a scenario involving multiple independent series of horizontal acquisitions of membrane transporters, leading to the progressive and convergent loss of the biosynthetic capabilities for nucleotides, amino acids and other metabolites. Such evolutionary trajectories were comparable but not fully equivalent in each sublineage, producing distinct conditions of host-dependence. Our reconstructions suggest that each clade has experienced a different pattern of evolution of the ancestral set of interaction apparatuses, including acquisition and/or development of specialised effectors involved in the lineage-specific mechanisms of host cell adhesion/invasion and intracellular (or extracellular) interactions. Obtained results may also offer novel perspectives on the evolution of interactions with eukaryotes among other lineages showing an ancient and diversified evolutionary history of interactions with protists and other eukaryotes, such as Legionellales, Chlamydiae, and Holosporales.

Genome evolution of the apicomplexan parasite *Cryptosporidium parvum*

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Cryptosporidium spp. (Apicomplexa) are globally distributed parasites causing severe gastrointestinal disease in humans and animals. In humans, the zoonotic species Cryptosporidium parvum is highly prevalent in high-income countries, while C. hominis is the major pathogen in low-income countries. The epidemiology of the infection is complex, and the reason(s) for the predominance of specific variants are unknown. Very young children and neonatal ruminants are particularly at risk of severe infections, and no effective drugs or vaccines are currently available. Here, we performed the largest comparative genomics study of C. parvum to date (140 total isolates) by generating novel whole genome sequence (WGS) data from human and ruminant isolates collected across Europe (n=85), and including available WGS of isolates from European and extra-European countries (USA, China, Egypt). By Single Nucleotide Polymorphism (SNP)-based analyses, we identified three highly supported populations within C. parvum. Population 1 comprises the majority of European isolates and all those from the USA, Population 2 is formed by the remaining European isolates, and Population 3 by isolates from China and Egypt. Interestingly, isolates from USA are monophyletic, and their sister group is made by isolates from the United Kingdom. As expected, isolates from known outbreaks presented high genetic similarity (<100 SNPs distance). Further groups of isolates without known epidemiological links presented comparable genetic distances (<100 SNPs). Collectively, the majority of such highly related isolates belonged to Population 1. Focusing on the evolutionary history of C. parvum in Europe, we provide evidence that Population 2 may be ancestral, while Population 1 likely originated more recently, expanded in Europe and in the USA, and caused multiple outbreaks. We investigated patterns of recombination, gene presence/absence, and selective pressures, thus identifying few candidates that may be responsible of putative advantageous traits in Population 1. In conclusion, our results illustrate a complex history of C. parvum genomic evolution in Europe and North America, and indicate that genome data from currently under-represented geographical areas are needed to better understand the evolutionary and epidemiological history of this parasite.

Methanogenic symbioses in Psalteriomonadidae

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Psalteriomonadidae is a group of free-living anaerobic protists belonging to Heterolobosea, which inhabit various, mainly freshwater, sediments. Psalteriomonadids are ancestrally amoeboflagellates, but some species have lost the flagellate stage while some others have lost the amoeba stage. Three species of Psalteriomonas, the type genus of Psalteriomonadidae, are known to form syntrophic symbioses with methanogenic Archaea, which were thought to belong to the genus Methanobacterium (Methanobacteria: Methanobacteriales). The symbiotic methanogens presumably use hydrogen and possibly other metabolites of the host's mitochondrion-related organelles (hydrogenosomes) for methane production, a potent greenhouse gas. Although the symbioses of anaerobic protists and methanogens are quite common in anoxic environments, this phenomenon was almost exclusively studied in ciliates and Archamoebae. To extend the knowledge about the identity and diversity of methanogenic symbionts of psalteriomonadids, we Sanger-sequenced the partial 16S rRNA gene of the symbionts of ca. 20 strains of long-term cultivated Psalteriomonas spp., including several undescribed species, and verified the obtained data by Illumina amplicon sequencing. Unlike in the previous works, the symbionts were identified exclusively as members of the genus Methanoregula (Methanomicrobia: Methanomicrobiales). Sanger and Illumina data consistently show a single dominant methanogen in each Psalteriomonas strain. Phylogenetic analysis shows remarkable host specificity of the symbionts.

Repurposing of nitroxoline as an anti-*Naegleria* compound

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Primary amoebic meningoencephalitis (PAM) is a central nervous system affecting disease caused by the opportunistic protozoa Naegleria fowleri. The infection generally occurs in healthy children and young adults with a history of swimming and other recreational activities. The trophozoite stage of this amoeba penetrates the nasal cavity and migrates through the olfactory nerves to the brain causing infection and subsequent death a few weeks after the onset of the first symptoms. Although the disease has a case fatality rate higher than 97%, currently available therapies are not at all effective and it is associated with a wide range of serious side effects. Therefore, the search for molecules with anti-Naegleria activity and low toxicity remains an urgent issue in the fight against the PAM. For this reason, the aim of the present study was to assess the potential of nitroxoline for the treatment of another Free-living amoeba (FLA) beside Balamuthia mandrillaris: Naegleria fowleri. In addition, the presence of different metabolic events that are characteristic of the programmed cell death were evaluated in Naegleria fowleri trophozoites. To evaluate the selectivity against Naegleria fowleri, activity assays were performed on two strains of the pathogen (ATCC® 30808™ and ATCC® 30215™) and, in turn, cytotoxicity assays against a murine macrophage cell line. Once the high selectivity of nitroxoline against Naegleria fowleri was demonstrated, nitroxoline was used in order to demonstrate the induction of programmed cell death (PCD) process. These metabolic events studied were DNA condensation, plasma membrane damage, reduction of mitochondrial membrane potential and ATP levels, generation of ROS or presence of autophagosomes. As a result, nitroxoline shows a high anti-Naegleria activity against the trophozoite and cyst stage. Moreover, the type of cell death process that produces the antibiotic was also determined, showing a programmed cell death induction in treated cells. For this reason, quinoline nitroxoline could be useful as a potent amoebicidal compound for the treatment of the primary amoebic meningoencephalitis.

The “bacterial” endosymbionts of *Symbiomonas scintillans* are actually prasinoviruses of the NCLDV phylum

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Symbiomonas scintillans is a small (~2 µm) heterotrophic bikosian stramenopile (Guillou et al., 1999). The genus was named based on the presence of endobacteria within the host, however, the identity or the role of these endobacteria were not explored. We generated whole genome amplified (WGA) sequencing data of S. scintillans strain RCC257 yet, were unable to detect any bacterial sequences from known endosymbiotic lineages. Instead, scaffolds with up to 200x coverage were identified as prasinoviruses – specifically Ostreococcus lucimarinus and Bathycoccus prasino viruses (OlVs and BpVs). Using the published BpV2 genome as a reference-guide, we recovered a 190kbp draft genome. When a OlV genome was used as a reference-guide, only a partial draft genome was recovered. All hallmark prasinovirus genes were detected, and phylogenomics placed the two viruses as sister to the BpV2 (Moreau et al., 2010) and OlV-4. Similar BpV-like and partial OlV-like draft viral genomes were obtained from another S. scintillans strain, RCC24. Using transmission electron microscopy, we identified a ~200 nm virus-like particle (VLP) from strain RCC24, corroborating with the morphology and the size of the first EM image of S. scintillans from 1999. Overall, our data suggests the possibility of dormant BpV-like and OlV-like viruses, rather than endobacteria as previously reported.

Herpes-like endogenous viral elements and subtelomeric ribosomal RNA genes in a thraustochytrid (Labyrinthulomycota) genome

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We used short-read and long-read sequencing to produce a telomere-to-telomere genome assembly for the heterotrophic stramenopile (thraustochytrid) protist Aurantiochytrium limacinum MYA-1381. Its ~62 Mbp nuclear genome comprises 26 linear chromosomes ranging from ~1 to ~4 Mbp which are not overly rich in repetitive sequence and end with telomeric repeats of TTAGG. Strikingly, the subtelomeric regions of all chromosomes have a shared novel configuration: rDNAs are located at consistent distances from and orientations relative to the telomeric repeats, and are interspersed with long repeated sequence elements denoted as LOng REpeated - TElomere And Rdna Spacers (LORE-TEARS). These repeats may play a role in chromosome end maintenance. In addition to the 26 contigs representing linear chromosomes, a ~300 Kbp contig (CE1) predicted to be circular, and lacking telomeres, rDNAs, and LORE-TEARS, is present at a high copy number and transcribed at levels comparable to the linear chromosomes. We suggest that CE1 represents an endogenized viral genome that might be maintained by a plasmid-like mechanism and/or might represent the sort of latent viral element responsible for previous reports of 'virogenic' thraustochytrid strains that produce viral particles only under specific growth conditions. Additionally, a related but distinct 269 Kbp virus-like element was found to reside between two complete sets of rRNA and LORE-TEAR sequences on one end of chromosome 15, indicating recent recombination. A previously reported thraustochytrid dsDNA virus (SmDNAV) belongs to the relatively well-known nucleocytoplasmic large DNA viruses (NCLDVs). However, Blast, HMM, and phylogenetic analyses of proteins characteristic of different groups of viruses suggest that both of these novel A. limacinum genomic elements belong instead to another group of large dsDNA viruses, the recently recognized Mirusviricota. Our data reveal novel chromosome organization as well as new types of endogenous viral elements originating from herpes-like viruses and existing as either 'stand-alone' or integrated elements in A. limacinum.

Exploring eukaryote lateral gene transfer and its mechanisms in the model system *Acanthamoeba castellanii*

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Lateral gene transfer (LGT) is well known as a driver of prokaryote evolution, especially as a major contributor to the spread of bacterial virulence factors and antibiotic resistance. Its importance for the evolution of eukaryotes is less certain; genome sequence data seem to provide ample evidence for LGT, but there is little experimental evidence to suggest its mechanisms or frequency. Acanthamoeba castellanii is being developed as a model organism in which we will attempt to gain a foothold on the cell and molecular biology related to acquiring foreign DNA that can inform similar investigations across eukaryote diversity. Having recently produced a high-quality, chromosome-level reference genome sequence for A. castellanii, we now seek to capitalize on this resource to explore some aspects of eukaryote LGT as can be observed in this model system. This investigation attempts to address two main questions: what can we learn about the genome biology of A. castellanii when it encounters foreign DNA, and how much LGT has A. castellanii undergone during its evolutionary history including from which donors and for which functions? Artificial transformation has been used to expose the A. castellanii nuclear genome to exogenous DNA, and clonal isolates have been generated from the resulting transgenic cultures. These transformants have been probed with long- and short-read sequencing as well as molecular biological methods to search for patterns that may elucidate mechanisms for incorporating or expressing the foreign genes. To explore the evolutionary history of LGT in A. castellanii, a phylogenetic approach was used. Homologs were retrieved for all predicted proteins in the A. castellanii str. Neff and str. C3 genomes, and single protein trees were analyzed for indications of lateral gene transfer. Putative laterally transferred genes were further investigated for their inferred donor taxa and predicted functions to form a more holistic understanding of the role of LGT in the evolution of A. castellanii.

Single cell transcriptomics of heterotrophic taxa reveals new insights in dinoflagellate character evolution

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Dinoflagellates are diverse and ecologically important protists characterized by many unique features that set them apart from other eukaryotes. These features include but are not limited to massive genomes organized using bacterially-derived histone-like proteins (HLPs) and dinoflagellate viral nucleoproteins (DVNP) rather than histones, and a complex history of photobiology with many independent losses of photosynthesis, numerous cases of serial secondary and tertiary new plastid gains, and the presence of horizontally acquired bacterial rhodopsins. Elucidating how this all evolved depends on knowing the phylogenetic relationships between dinoflagellate lineages. However, although half of known dinoflagellate species are heterotrophic, existing molecular data is strongly biased toward photosynthetic taxa due to their prevalence in culture collections. Here, we collected single cells from the Salish Sea and sequenced them using single cell transcriptomics to expand molecular data for dinoflagellates to include many more heterotrophic taxa. Using these data, we performed the most comprehensive search to date for proteins involved in chromatin packaging, plastid function, and photoactivity across dinoflagellates. These searches reveal that 1) two known types of HLP were horizontally acquired around the same time (rather than sequentially as previously thought); 2) multiple rhodopsins are present across the dinoflagellates, acquired multiple times from different donors; and 3) the warnowiids are the only heterotrophs that retain a partial photosystem assemblage, although some photosynthesis-related electron transport genes are widely retained in heterotrophs, likely as part of the iron-sulfur cluster pathway that persists in non-photosynthetic plastids.

Quaternary Arctic sea ice reconstructions from ancient sedimentary DNA: Updates from the AGENSI project

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Sea ice is a crucial component for the functioning of Arctic ecosystems and has a crucial role in the regulation of climate system. Our knowledge of its historical variation is limited to few decades of satellite records, which considerably hampers our understanding on how past climate has influenced sea ice extent in the Arctic. Projections indicate that the Arctic may be sea ice free during summer by 2050, and so understanding the implications of such a change on the Arctic Ocean ecosystem is paramount. Here, we present work conducted within the AGENSI project (www.agensi.eu), where we aim to build a reference DNA metabarcodes database of modern environmental samples, i.e. surface sediments, sea water and sea ice samples, along gradients of sea ice cover in the Arctic. This DNA metabarcodes database is compared to modern oceanographic parameters, and their relationships are used to build and calibrate eukaryotic DNA-based sea ice proxies. These proxies will then be applied to Late Quaternary marine sedimentary records, where DNA metabarcoding data can be used to document past biodiversity and be used to reconstruct the past sea ice variability.

Unravelling the evolution of organellar genomes and metabolism of *Leontynka* lineage (Chlamydomonadales)

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Genus Leontynka is a non-photosynthetic lineage of Chlamydomonadales isolated from freshwater hypoxic sediments and comprised of two species, L. elongata and L. pallida. Interestingly, the latter possesses the largest ptDNA among non-photosynthetic algae due to repeats expanded in both, coding and non-coding regions. To better understand mechanism responsible for repeat proliferation, we re-sequenced organellar genomes of L. pallida after four years of continuous cultivation and we newly obtained organellar genomes of L. elongata using combination of Illumina and Oxford Nanopore technology. We estimated number of generations between the two L. pallida organellar genome sequencing, annotated organellar genomes and analyzed expansion of repeats. We also investigated variations in cell structure of Leontynka in a greater detail, studied its mitochondria, and performed comparative transcriptomics to predict energetic metabolism to understand how Leontynka generate ATP in an oxygen-low environment. Using molecular phylogeny, we have also traced origin of key enzymes.

Contributions to the mobilid genus *Leiotrocha* Fabre-Domergue, 1888

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Representatives of the genus Leiotrocha are all ectozoic mobiline commensals of marine invertebrate hosts. Hosted by molluscs, brachiopods, polychaetes and echinoids, very little is known about host specificity or taxonomic status. As members of the family Urceolariidae, many leiotrochan species have erroneously been classified within the monotypic genus Urceolaria, the latter from the epidermis of the freshwater triclad planarians. The present study examined Leiotrocha species from the ctenidia of two polyplacophorans (Acanthochitona garnotti; Dinoplax gigas), a sabellid polychaete (Pseudobrachiomma longa), all from the southern coast of South Africa, and two patellogastropods (Patella caerulea; P. rustica) from the Italian west coast. As part of the Next Generation Taxonomy platform, it was possible to utilise various specialised microscopic and genomic techniques to obtain a deeper understanding of this neglected group. Morphometric analysis by means of compound microscopy revealed that minimal differences are observed between individual leiotrochans within a population per host, but significant morphological and ecological differences between the mobilines found on various South African hosts. Scanning electron and confocal microscopy further illustrated differences in pellicle texture, the presence of vestigial aboral cilia on the adhesive disc, pronounced marginal cilia and an abundance of endosymbionts. Phylogenetic analysis revealed that the genus Leiotrocha indeed forms a sister clade to Urceolaria. Data illustrated that the South African chiton and polychaete populations were extremely host specific, while Leiotrocha patellae, collected from limpets in Italy, were ubiquitous in Mediterranean patellids. This study also proposes a method for a morphological and morphometric description to discriminate between species.

Apicomplexans symbionts of the coral reef

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Coral reefs are biodiversity hotspots and providers of crucial ecosystem services to coastal communities. However, it is only recently that we have begun to unveil the diversity and role of the myriad of protists that are part of the coral reef ecosystem. Among them, one of the most recent discoveries has been the widespread presence of a coccidian symbiont in corals, the corallicolids. However, corals are not the only hosts for apicomplexans in the reef. Haemohormidium -like apicomplexans are blood parasites reported from tropical reef fishes such as damselfishes (Pomacentridae) or blennies (Blenniidae). After sequencing the genomic DNA from the blood of an infected red lip blenny (Ophioblennius macclurei), we have been able to obtain the mitochondrial and the plastidic genome from this Haemohormidium-like parasite. Using these genomes to build phylogenies we have been able to confirm that, as suggested by SSU data, these Haemohormidium-like apicomplexans are coccidians instead of piroplasmids, as previously thought, and actually are sister to the corallicolids. These results significantly aid our understanding of the diversity of coral reef associated apicomplexans.

Chlamydial endosymbionts of protists evolved through unexpected gene gain

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Studying the ancient origins of endosymbionts is vital for unravelling the processes that mediate host-symbiont evolution. One highly successful group of obligate endosymbiotic bacteria is the phylum Chlamydiae, which has a one-billion-year-old evolutionary history with eukaryotic hosts. Chlamydiae are notorious as animal pathogens, but are also hosted by diverse protists. Despite a conserved lifestyle, these protist-infecting chlamydiae have larger genome sizes and greater metabolic versatility than other phylum members. Our view on chlamydial biology was recently widened through the cultivation-independent discovery of novel lineages, such as anaerobic chlamydiae. To interpret how this expanded chlamydial diversity influences our understanding of endosymbiont evolution, we reconstructed Chlamydiae evolutionary history using gene-tree aware ancestral state reconstruction. The Chlamydiae ancestor was reconstructed with key genes indicative of endosymbiosis, providing further evidence of an ancient capability to infect eukaryotic hosts. In addition, the Chlamydiae ancestor was reconstructed as a likely facultative anaerobe. Unexpectedly for strict endosymbionts, this revealed that major shifts in chlamydial aerobiosis and energy metabolism were driven by extensive horizontal gene transfer, potentially through co-infecting symbionts. In particular, we found that the protist-infecting chlamydiae only later gained characteristic metabolic and aerobic genes, such as electron transport chain complexes that allow energy generation using a proton motive force. We had expected the chlamydial ancestor to resemble these protist-infecting chlamydiae given that endosymbionts are thought to primarily evolve through metabolic streamlining. However, we found that protist-infecting chlamydiae have instead increased in metabolic versatility since their divergence from the Chlamydiae ancestor. Together, these findings highlight unexpected metabolic transitions in Chlamydiae and provide new insights into endosymbiont evolutionary trajectories in protist hosts.

New morphological and molecular data on peritrichs from Brazil provides new insights into the evolutionary relationships among epistylids

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The peritrichs are classified into two assemblages: the free-living Sessilida and the parasitic Mobilida. Although a comprehensive recent review is not available, these ciliates comprise at least 1000 described species. Recent molecular studies have shown that most of the order Sessilida families are not monophyletic. In this work, we studied the morphology and 18S-rDNA phylogeny from marine (plankton), freshwater (streams and bromeliads) and terrestrial (lichens) Brazilian ecosystems. The morphological study of forty species of peritrich ciliates was carried out using in vivo, protargol-stained and SEM observations. This study also presents the results of a phylogenomic investigation containing eight new genomic sequences from peritrich ciliates. To shed more light into the evolutionary relationships within peritrichs, 18S-rDNA sequences of thirty sessilids species and genomic sequences were used to construct phylogenetic trees. Our results show that (1) the alpha-taxonomic information on peritrichs is expected to improve the knowledge about geographic distribution and diversity of these ciliates in the neotropics; (2) we obtained eight peritrich new genomes with 2,827 ~ 79,758 contigs and N50 length of 1,644 ~ 56,289; (3) genome size (29.4 ~ 88.2 Mb; completeness_BUSCO_Alveolata_odb10 45.6 ~ 74.9) and GC content (25.45 ~ 45.59 %) were similar to those observed in other peritrich genomes available in public repositories; (4) first genomic records for the Lagenophryidae and Opisthonectidae families and the first genomic record for the genus Platycola; (5) many morphological characters used to define some genera and families that compose the subclass Peritrichia do not reflect evolutionary divergence which imply that the diagnoses should be improved; and (6) Epistylididae needs to be reorganized.

Keywords: Peritrichia, Neotropical area, taxonomy, evolution, 18S-rDNA, phylogenomic.

Spatial proteomics of *Naegleria gruberi* with the focus on the evolution of the eukaryotic cell

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Protists are excellent models for studying the evolution of mitochondria and other cellular compartments. Their organelles retained many bacterial traits that have been lost in the most studied experimental models. For instance, bacteria have evolved several sophisticated protein secretion nanomachines such as type II secretion system (T2SS) to deliver toxins, hydrolytic enzymes, and effector proteins into their environments. Recent findings have demonstrated that several components of the T2SS are localised in mitochondria of some eukaryotic lineages, including the heterolobosean amoeba Naegleria gruberi. We have employed the DC-LOPIT approach to study the overall cellular architecture of N. gruberi with the focus on the mitochondrial T2SS-derived system, the interaction of mitochondria with other cellular compartments and the discovery of novel cellular compartments that could be unique to Heterolobosea.

Environmentally-informed phenotyping of the pan-secondary red chloroplast proteome

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Eukaryotic algae have evolved many times through the endosymbiotic acquisition of chloroplasts, which are supported by both chloroplast- and nuclear-encoded proteomes. The most abundant algal groups in the world ocean, such as diatoms, haptophytes and pelagophytes, predominantly possess chloroplasts acquired through the multiple secondary and higher endosymbioses of eukaryotic red algae. Beyond this simple evolutionary synapomorphy, the specific molecular actors that allow algae with secondary red chloroplasts to adapt to different environmental conditions across the contemporary ocean remain poorly understood. Here, I will outline recent progress from my group using combined phylogenomic, meta-genomic and functional phenotyping approaches to identify novel nucleus-encoded and chloroplast-targeted proteins, many of which are of non red-origin, that underpin the striking success of the pan-secondary red chloroplast. These include a complete chloroplast glycolytic pathway that likely evolved in the common ancestor of diatoms and pelagophytes, and may have subsequently been shared endosymbiotically with haptophytes, which is predominantly expressed in high-latitude stations sampled within the Tara Oceans Expedition. CRISPR knockout lines of chloroplast glycolytic pathway in the model diatom Phaeodactylum show growth retardation and dysregulated primary chloroplast metabolism in response to both continuous illumination and low temperature. We suggest that this pathway functions as a primary adaptive modulator of diatom chloroplast metabolic poise at high latitudes; emphasise the value of environmental data for not only validating, but predicting functional phenotypes of model laboratory species. I will finally briefly outline two further projects underway within my group, relating the similar functional characterisation of a novel chloroplast-to-mitochondria metabolite transporter, and a highly divergent homologue of a chloroplast ATP synthase subunit, both shared across and restricted to the secondary red chloroplast tree of life.

A highly condensed genome without heterochromatin: orchestration of gene expression and epigenomics in *Paramecium tetraurelia*

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The vegetative macronucleus of Paramecium tetraurelia shows some extraordinary features such as high polyploidy and the absence of heterochromatin along with a high coding density due to short intergenic regions and tiny introns. Due to these distinctive characteristics, accurate transcriptional initiation, elongation, and termination must be carried out in a way that is fundamentally unique. The positioning of nucleosomes, which serve as chromatin's primary subunit and are a crucial component of the intricate regulation of gene expression, has a significant impact on the accessibility of chromatin to the transcription machinery. Still little is known about the distribution of nucleosomes over the gene body in correlation to gene regulation in Paramecium. To understand how differential gene expression is accomplished in the MAC epigenome, the presented study catalogized the distribution of nucleosomes, histone modifications and the Polymerase II complex. We profiled the histone marks H3K4me3, H3K9ac, H3K27me3 by ChIP-seq and nucleosome positioning by Mnase-seq aside with the distribution of the PolII complex from one biological sample of fixed cell material. Our study revealed some unique features of the epigenome encountering textbook knowledge on gene expression regulation: silent genes are lowly occupied by nucleosomes while recruitment of nucleosomes into the ORF drives gene activation. H3K27me3, a mark associated with gene silencing and heterochromatin in mammalian systems, can be detected by mass spectrometry together with the other investigated marks. Signals from ChIP-seq argue against a classic repressive function of H3K27me3 but infer a dynamic function in combination with H3K4me3 patterns in genes with high expression plasticity. In addition to the particular epigenome features, Paramecium possess a highly divergent PolII complex, with the largest subunit Rpb1 missing the heptameric repeats in the C-terminal domain, a site well studied for regulation of transcription by phosphorylation in other model organisms. Our ChIP-seq data reveals a high occupancy of PolII along highly expressed ORFs, thus transcriptional elongation appears to be quite different from that of other species. Together the presented studies provide the first description of Paramecium macronuclear epigenome and PolII function exhibiting a variety of intriguing characteristics and variations from canonical transcription dynamics.

Taxonomy for the actual end users based on morphology, molecules and nomenclature – examples from euglyphid and arcellinid testate amoebae

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The use of protists as bioindicators implies a sound taxonomic framework. However, the diversity and the taxonomy of these organisms is still far from being understood, as most species have not been described. Protists were mainly characterized with light microscopy, but the absence of characteristic morphological traits - and the fact that the phenotypical plasticity of protists was considered as being extremely high - has led naturalists to underestimate their diversity for a long time. It is only recently with the appearance of staining protocols, electron microscopy and molecular biology that we start to assess the true diversity of these organisms. With these new tools, it appears that many described species were in fact morphospecies complexes including more than one biological species that, sometimes, could be completely unrelated. There is then a need for taxonomists to describe this hidden diversity and to update old descriptions to build a sound taxonomy to better use protists as scientific tools. Furthermore, due to the general difficulty to identify them (most species could likely not be correctly identified even if their taxonomy was solved), protists are rarely identified to the species level in routine work and are instead treated into broad morphotypes. In the case of testate amoebae, given the lack of an agreed upon classification this approach suffers from several shortcomings leading to confusion, consistency issues and low comparability between studies because how morphotypes and their names should be formed are not well defined. Nonetheless, the usage of broad morphotypes reveals the inadequacy of the current taxonomy framework and the need for a user-friendly method to sort the morphological variation of the organisms encountered on the field.

The functioning of deep-sea microbial food webs influenced by sedimentation of organic debris

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Due to the absence of light, deep-sea ecosystems strongly depend on the production and sedimentation of organic material from surface waters. As this carbon flux showed to decrease with depths, the deep sea is assumed to be an oligotrophic environment with relatively stable conditions. However, in recent years, this paradigm of a food-poor environment was drawn into question due to high records of sedimented macroalgae (e.g. Sargassum) which were found to represent an important source of carbon sequestered to deep-sea ecosystems. We analyzed the influence of this irregular and large-scale sedimentation events on the microbial food web by conducting a long-term in-situ experiment located at a deep-sea area in 1000 m depth in the Atlantic Ocean close to Madeira Island. With the help of the manned submarine LULA1000 and the Rebikoff-Niggeler Foundation, we deposited different types of organic materials (macroalgae, cyanobacteria and fish) on the deep-sea floor and investigated the response of the microbial food web after different time intervals of 4 days up to 14 months. Using a combination of metabarcoding techniques, abundance estimations and cultivation techniques, we analysed the taxonomic composition, structure and abundance of the benthic protist communities in the different experimental treatments.

The number of free-living ciliate species

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Wilhelm Foissner was right, or nearly-right, about a lot of things. And even when he was wrong, his ideas were at least easily testable. One example of where Foissner was right, was his view that the number of free-living ciliate species is a lot higher than commonly thought. He came to his high species numbers based on his deep understanding of the morphological diversity within the ciliates (including minutely observing characteristics of both cells and cysts), and on his extrapolations from his novel observations when he explored environments usually ignored by ciliatologists. In line with Foissner's views, using available metabarcoding data from EukBank (part of the international UniEuk initiative), 19,000 OTUs were inferred from world-wide samples that were sequenced for the V4 region of SSU-rRNA. This number of free-living ciliate OTUs will likely become much higher as more and more environments are sequenced, especially from terrestrial communities.

Data mining Arthropoda genomes reveals diversity and host spectrum of Microsporidian parasites

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Jaroslav Weiser once said “my experience in distribution of microsporidia of invertebrate hosts indicates that there may be a microsporidian in every living invertebrate”. In order to get an idea of the extent of the vast microsporidian diversity, we mined WGS assemblies in Genbank for misassembled microsporidian contigs using 99 conserved microsporidian proteins. Tblastn of ~3.200 WGS identified 182 Arthropod WGS assemblies positive for microsporidia. We retrieved positive contigs from WGS assemblies from the subphyla Chelicerata (Arachnids, mites, etc…), Crustacea and Hexapoda (insects and Collembola). Using phylogenetic analyses, we aim to understand microsporidian host spectrum. Furthermore, host origin allows us to infer microsporidian co-evolutionary relationships to host using factors such as taxonomical and ecological traits.

Cultivation remains an indispensable tool for biodiversity discovery in heterotrophic protists

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The diversity of microbial eukaryotes has long been examined through cultivation and identification by light microscopy. Our understanding of microbial diversity was greatly expanded and accelerated by the introduction of cultivation-independent environmental molecular sequencing, particularly among groups previously resistant to growth in captivity. Nevertheless, not only are cultivation approaches still in use, but were behind most of the deepest-branching novel lineages discovered in the previous decade (Burki et al. 2020 Tr. Ecol. Evol.). We obtained three dozen sequences from a culturing program that targeted phylogenetic novelty amongst benthic free-living heterotrophic protists across several eukaryotic supergroups. Using a combination of BLAST and evolutionary placement analysis tools, we searched public short read environmental sequence (SSU rDNA V4 and V9) datasets for plausible sequence matches. Perhaps surprisingly, we found environmental matches for only half of our novel sequences. This prompted us to investigate examples of other understudied (and often overlooked) heterotrophic flagellates, particularly free-living anaerobes and eukaryotrophs, and how they behave in environmental sequence analyses.

To compare and discuss cultivation-dependent vs. environmental approaches, we highlight three categories:

1. sequences for which abundant matches were identified in environmental sequence data;

2. sequences from organisms frequently observed in enrichments but with few and/or sporadic matches found in environmental sequence data;

3. organisms observed multiple times in samples and enrichments but missing environmental sequence matches altogether.

Additionally, we present a possible pipeline for searching for specific unannotated query sequences in public environmental databases. In summary, 'low-throughput' cultivation-based approaches not only provide additional opportunities for downstream analyses, but also comprise a so-far-irreplaceable tool for discovering and characterising biodiversity. We believe that a combination of environmental sequencing and cultivation-based approaches can be particularly powerful.

Genomic insights into the evolution, diversity and biology of the eustigmatophyte-specific endosymbiont Candidatus *Phycorickettsia*

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Previously we discovered a novel endosymbiotic bacterium, Candidatus Phycorickettsia (Rickettsiaceae), which was found to inhabit several members of the algal class Eustigmatophyceae (Ochrophyta). Sequencing and analysis of a single Phycorickettsia representative revealed several interesting features of the bacterium, most notably the presence of an uncharacterized six-gene cluster, denoted the ebo operon, hypothesized to mediate the synthesis of a prenylated cyclitol derivative that is presumably useful to the algal host. However, a more detailed picture of the occurrence, diversity, and endosymbiont-host functional associations is lacking for Phycorickettsia. By surveying newly generated genome data from >30 eustigmatophytes we confirmed or uncovered Phycorickettsia in six additional strains from both major eustigmatophyte subgroups (Eustigmatales and Goniochloridales) and obtained draft genome assemblies of the endosymbionts. By confirming the lack of Phycorickettsia-derived sequences in public genomic and transcriptomic resources from non-eustigmatophyte eukaryotes, we cemented the view of Phycorickettsia as a eustigmatophyte-specific endosymbiont. At the same time, exploration of available environmental 16S rRNA gene sequence data revealed that Phycorickettsia is a highly diverse bacterial radiation commonly occurring throughout the globe. The phylogenetic relationships of the seven Phycorickettsia strains inferred from the complete or draft genome sequences did not match the host phylogeny, consistent with Phycorickettsia being horizontally disseminated among eustigmatophytes. Significantly, the ebo operon was found in all seven Phycorickettsia strains sequenced and its expression was detected in the endosymbiont stage, attesting to its physiological significance. Notable is also the acquisition from a Legionellales-related donor of the DNA repair enzyme (6-4) photolyase by a particular Phycorickettsia subclade, which presumably compensates for the Phycorickettsia-specific loss of the RecFOR pathway. The rarity of this enzyme in Rickettsiales is explained by the fact that it requires as a light-absorbing chromophore the riboflavin biosynthesis intermediate 6,7-dimethyl-8-ribityllumazine, which can be provided by the algal host of Phycorickettsia but not by the heterotrophic hosts typical for other rickettsialean bacteria. Finally, we found out that eustigmatophyte nuclear genomes contain Phycorickettsia-derived DNA segments, indicating that DNA transfer from the endosymbiont into the host nuclear or plastid genome is not limited to the previously described transfers of the acpP gene or the ebo operon.

“Blue Book” in new edition: Microscopic analysis of activated sludge assessing operational parameters in biological wastewater treatment and the quality of the inflowing municipal wastewater

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Globally, the activated sludge process is the backbone of biological wastewater treatment. Activated sludge consists of a diverse microorganism community. Each biocoenosis develops based on the framing environmental factors, thus indicating special operational characteristics of the treatment process as well as the quality of the inflowing wastewater. Based on publications of the “Bayerisches Wasserwirtschaftsamt” in Germany in the 1980s, in 1999 "Das mikroskopische Bild bei der biologischen Abwasserreinigung" - known as the "Blue Book" - was published and became the standardized tool to determine the microorganisms. Typical protozoa, some metazoa and characteristic filamentous bacteria have been defined as indicators to assess the plants’ operational conditions by microscopic analysis. This biological monitoring supplements the analyses of the chemical-physical parameters which are a part of the regular self-monitoring. Conclusions allow to confirm stable plant operation or uncovers operating faults, with reference to suitable optimization measures. Along with changing treatment requirements as nitrogen (nitrification/de-nitrification) and phosphorus (biological P-elimination, “bio-P”) removal and resulting changes in plant design and operation, the microbial activated sludge communities changed, too. As a consequence, compared to the version published in 1999 new protozoa taxa were detected, that had to be included in the new evaluation scheme. Accordingly, the “Blue Book” was fundamentally revised, based on data and empiric insights gathered by microscopic analyses of activated sludge samples in European countries during the last decades. The new edition of the “Blue Book” has 276 pages and provides an updated identification key, detailed profiles for the indicator organisms and standardized instructions to interpret the results. Above all, the recording of the microscopic results in a newly developed excel tool accelerates the evaluation and enables an easier and quicker analysis process for everyday laboratory work. The printed version of the new “Blue Book”, the excel tool and the evaluation forms as additional working materials are available on the website of the "Bayerisches Landesamt für Umwelt” (ISBN 978-3-936385-98-4). With these updates, the “Blue Book” continues to serve as a standard work for quality assurance and plausibility check of many measurement results within the scope of self-monitoring of the municipal wastewater treatment plants.

Observing protistan communities using Leray-XT COI primers

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Metabarcoding allows you to see some taxa, but it can also make you blind to other taxa. This ability to observe taxa depends on choosing the right primer pair for amplification, and on having the right reference sequences for taxonomic assignments. Here, I evaluate the efficacy of the Leray-XT primer pair, which amplifies the cytochrome c oxidase subunit I gene for observing marine protistan communities. I found that while some taxa have available reference sequences for the COI gene deposited in the NCBI reference database, such as Rhodophyta, Ochrophyta, and Oomycota, other taxa have no available COI reference sequences, such as Apusozoa, Picozoa, and Radiolaria. Therefore, even if the Leray-XT primer pair amplified these taxa, we would not be able to identify their sequences as belonging to them. I further found that while the Leray-XT primer pair can potentially amplify some commonly abundant marine protistan taxa using in silico amplifications, such as the Bacillariophyta, Dinoflagellata, and Haptophyta, it cannot amplify other taxa such as the Ciliophora. Given these findings, the Leray-XT COI primer pair is suitable for the observation of some marine protistan taxa, but the primer pair will blind you to some other taxa due to reference database issues and primer biases. If you are however interested in the taxa that the Leray-XT primer pair allows you to observe, then it is suitable for analysing ecological questions of those taxa.

Preliminary studies of striated fibres and assemblins in preaxostylans

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The flagellar apparatus, comprising the basal bodies and the microtubular and fibrous structures directly associated with them, is the core of the cytoskeleton in most protists. The similar attachment, positioning, and paths through the cell of the non-microtubular structures suggest the possibility of homology in different lineages. The system I fibres of green algae and the microribbons of the gut parasite Giardia are composed respectively of striated fibre assemblin (SFA) and giardins, which are known to be homologous non-microtubular components. Recent work has greatly extended the number and diversity of SFA in both taxonomic diversity and paralogy, however, SFAs have only been characterized in a few species. We had previously found putative SFAs in the anaerobic preaxostylans Paratrimastix pyriformis and oxymonad Monocercomonoides exilis. To investigate their possible roles in the cell we constructed hemagglutinin (HA) tagged proteins of putative SFAs in M. exilis and P. pyriformis for antibody generation, and two of the raised antibodies against P. pyriformis proteins exhibited sufficient targeting specificities to SFAs. The preliminary results from IFA and expansion microscopy confirmed these two P. pyriformis SFAs are closely associated with the entire length of the right, left and singlet microtubular roots attached to the posterior basal body. The results suggest that the SFA are basic components of the cytoskeleton of excavates.

Identification of key enzymes for starch synthesis in chromerids and Apicomplexa

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Starch and glycogen are the two most common forms of storage polysaccharides in living organisms and define two states of the same type of polysaccharide. Despite being both composed of α-1,4-linked and α-1,6-branched glucose chains, they have different physicochemical characteristics due to the different distribution of branching points. Starch is crystalline and allow to store huge quantities of glucose, but is only found in eukaryotes derived from plastid endosymbiosis and is linked to the appearance of Archaeplastida, and has for now always been linked to the presence of specific enzymes. However, starch accumulation also occurs in some Alveolata, such as Dinoflagellates, Chromerids, or Apicomplexan parasites, which arose from other endosymbiosis involving not a cyanobacteria but a eukaryotic alga. Until now, functional analysis was mainly focused on Chloroplastida and led to the identification of key enzymes, like the isoamylase required for starch crystallization, or some specific starch synthase for initiation. Comparative analysis performed in silico of metabolisms in different eukaryotic lineages reveals that many eukaryotes and especially Chromerids and Apicomplexa do not contain sequences encoding those types of enzymes. Moreover, it is still unclear if the initiation of starch synthesis involves a specific starch synthase as in plants. To identify the enzymatic mechanism in those organisms, a collection of 95 Chromera velia UV mutants deficient in starch synthesis was generated. Targeted sequencing highlighted mutants containing distinct alleles altered in a different gene acting on carbohydrates. Among these, an allelic series of an atypical protein-encoding gene, found only in starch accumulating Chromerids and Apicomplexan, shows drastic deficiencies in starch or soluble polysaccharides production in mutants impacted in this enzyme, suggesting a potential defect in the initiation of storage polysaccharide synthesis. Further structural characterization of soluble polysaccharides accumulated by these mutants reveals they adopt an atypical structure. Finally, while its function remains unknown, preliminary works to define the enzymatic and physiologic activities of the identified protein suggest its potential role during the very first steps of polysaccharides synthesis, and in C. velia viability under certain conditions.

Biotic interactions explain seasonal dynamics of the alpine soil microbiome

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The soil alpine microbiome is dependent on season and elevation, yet there is limited understanding of how complex communities are differentially shaped by abiotic and biotic factors. Here we investigated the spring-to-summer dynamics of soil microbiomes in alpine grasslands, focussing on soil food web interactions. To this end, we conducted a survey along altitudinal transects in three mountains in the Alps, in spring at snowmelt and in the following summer, recorded vegetation and topographic, climatic and edaphic parameters for 158 soil samples. By using metatranscriptomics, we simultaneously assessed prokaryotic and eukaryotic communities, further classified by nutrition guilds. Our results show: (i) that biotic interactions could explain more variation of the microbial communities than topographic and edaphic variables, more for consumers than for preys, and this effect was stronger in summer than in spring; (ii) a seasonal dynamic in biotic interactions: the consumers' pressure on preys increases from spring to summer, resulting in a higher diversity and evenness of preys. In alpine grasslands, consumers effectively contribute to maintain the diverse soil bacterial and fungal community essential for ecosystem functioning.

Paramecia in the spotlight – consequences of exposure to photosynthetically active radiation

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Paramecium bursaria lives in close mutualistic symbiotic association with photosynthetically active microalgae, mostly Chlorella variabilis or Micractinium conductrix. Within its genus, it exhibits a unique behavioral trait, so-called photoaccumulative behavior, i.e., cells accumulating in photosynthesis favoring areas. A refined protocol to qualitatively but also quantitatively analyse photobehavioral responses as a consequence of exposure to photosynthetically active radiation was used to assess differences in behavioral responses of P. bursaria (i) infected with different intracellular algae, (ii) naturally symbiont-free cells, and (iii) cells experimentally deprived of their algal endosymbionts. Previous studies reported contradictory observations for the latter, i.e., aposymbiotic cells either still exhibiting such behavior or not. Different hypotheses on how photoaccumulation is actually mediated were proposed. Our data provide a unifying explanation by considering the aposymbiotic cultivation duration, the time elapsed between the cell's confirmed aposymbiotic status and the respective experimental assessment. Moreover, under laboratory conditions aposymbiotic P. bursaria successfully re-establish symbiosis with their algal endosymbionts independent of the algae's identity. We exposed aposymbiotic P. bursaria strains for which complete loss of photoaccumulation was experimentally confirmed to their original and other photobionts, respectively. Six of these successfully re- and cross-infected strains were subjected to photoaccumulation assays to test whether re-establishment of symbiosis leads to re-induced photoaccumulative behavior. Our results indicate an overall positive response to light accompanied by subsequent accumulation. Based on our data we interpret photoaccumulative behavior as a complex, adaptive strategy of P. bursaria to its symbiotic lifestyle. Currently, we are investigating interactions between the cyanobacterium Synechocystis ssp. and P. bursaria.

Holospora-like bacteria "Candidatus *Gortzia yakutica*" and *Preeria caryophila*: New data on ultrastructure, biogeography and host specificity of the symbionts

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Two already known representatives of Holospora-like bacteria – "Candidatus Gortzia yakutica" from Paramecium putrinum and Preeria caryophila from P. aurelia complex were found in new hosts: P. nephridiatum and P. polycaryum, respectively. Bacteria were investigated using morphological and molecular methods. For "Ca. Gortzia yakutica", the first details of the electron microscopic structure in the main and new hosts are provided; for Pr. caryophila, the ultrastructural description of bacteria is supplemented by several features unknown earlier. The new combinations of Holospora like bacteria with ciliates are discussed from biogeographical and ecological points of view. Host specificity of symbionts as a general paradigm is assessed as well.

Deep molecular characterization of microorganisms´ diversity and community composition in the tree canopies using a metatranscriptomics approach

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More than 3 trillion trees exist worldwide whose canopies form numerous diverse and dynamic habitats. Previous metabarcoding approaches revealed highly abundant and specialized community compositions of heterotrophic protists. However, metabarcoding approaches are limited in detecting the whole microbial diversity due to the lack of suitable barcoding primers. To investigate the entire microbial community composition and diversity in tree canopies, we sampled the bark of three different tree species - Quercus robur, Tilia cordata, and Acer pseudoplatanus - of the Leipzig floodplain forest with the Leipzig Canopy Crane facility. Using shotgun metatranscriptomic (RNA) sequencing data we were able to assess the mainly active microbial community composed of 645 prokaryotes, 114 algae, 558 fungi, 154 heterotrophic protists, and 16 microscopic metazoa genera respectively. We found tree-species dependent differences between alpha and beta diversity in the canopy, and putative trophic interactions within. Among our findings, we report a high relative abundance of myxomycetes and the importance of algae for the food web composition.

The ancestral shape of the access proton path of mitochondrial ATP synthases revealed by the split subunit-a in *Trypanosoma brucei*

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F1Fo-ATP synthases are rotary molecular machines that convert the proton-motive force across energy-transducing membranes of bacteria and endosymbiotic organelles to the chemical energy of ATP. Passage of protons across membranes through the ATP synthases spins their rotors and drives production of ATP. While the principle of torque generation by proton transfer is known, the mechanisms and routes of proton access and release and their evolution are not fully understood. Here, we explore structures and sequences of the lumenal half-channel forming elements across species and show that the N-terminal region of subunits-a form a short a-helix shared by all alphaproteobacterial and vast majority of mitochondrial ATP synthases. In Trypanosoma brucei and other Euglenozoa, this helix is a part of a different polypeptide chain encoded by a nuclear gene that is a product of fragmentation of the mitochondrial gene for subunit-a. We document that the helix defines the proton entry site and contributes to the shaping of the inner part of the half-channel. The position of the helix blocks one of two proton routes known in Escherichia coli and other non-alphaproteobacterial bacteria, resulting in the single proton path in mitochondrial and alphaproteobacterial ATP synthase. Thus, the proton entry site and the shape of the access half-channel predate eukaryotes and originated in the lineage from which mitochondria evolved by endosymbiosis. On the contrary, the proton transfer mechanism involving a chain of ordered water molecules is most likely common to all ATP synthases. Our analyses further revealed that the entire subunit-a gene has been transferred to the nuclear genome only three times in the evolution, namely in the ancestors of the present-day myzozoans, ctenophores and Chlamydomonadales. We argue that the transfer was facilitated by the loss of the first transmembrane helix, a possible barrier to the insertion of the protein into the inner mitochondrial membrane from the intermembrane space.

Maturases and group II introns in the mitochondrial genomes of the deepest jakobid branch

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Ophirinina is a recently described suborder of jakobid protists (Excavata) with only one described species to date, Ophirina amphinema. Despite the acquisition and analysis of massive transcriptomic and mitogenomic sequence data from O. amphinema, its phylogenetic position among excavates remained inconclusive, branching either as sister group to all Jakobida or to all Discoba. From a morphological perspective, it has several typical jakobid features but also unusual traits for this group, including the morphology of mitochondrial cristae (sac-shaped to flattened-curved cristae) and the presence of two flagellar vanes. In this study, we have isolated, morphologically characterized, and sequenced genome and transcriptome data of two new Ophirinina species: Ophirina chinija sp. nov. and Agogonia voluta gen. et sp. nov. Ophirina chinija differs from O. amphinema in having rounded cell ends, subapically emerging flagella and a posterior cell protrusion. The much more distantly related A. voluta has several unique ultrastructural characteristics, including sac-shaped mitochondrial cristae and a complex 'B' fibre. Phylogenomic analyses with a large conserved-marker dataset supported the monophyly of Ophirina and Agogonia within the Ophirinina and, more importantly, resolved the conflicting position of ophirinids as the sister clade to all other jakobids. The characterization of the mitochondrial genomes showed that Agogonia differs from all known gene-rich jakobid mitogenomes by the presence of two group II introns and their corresponding maturase protein genes. A phylogenetic analysis of the diversity of known maturases confirmed that the Agogonia proteins are highly divergent from each other and define distant families among the prokaryotic and eukaryotic maturases. This opens the intriguing possibility that, compared to other jakobids, Ophirinina may have retained additional mitochondrial elements that may help to understand the early diversification of eukaryotes and the evolution of mitochondria.

Long-read sequencing sheds light on the origins of endogenous virophage and Polinton-like elements in the halophilic stramenopile *Halocafeteria seosinensis*

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Virophages are small icosahedral dsDNA viruses that require a coinfecting giant virus in a eukaryotic host to replicate. Polintons are found in diverse eukaryotic genomes and are evolutionarily connected to virophages. Although initially thought to be endogenous transposable elements, the discovery of capsid genes in Polinton genomes suggest that Polintons have a virus-like lifestyle. Virophages integrated into the nuclear genomes of single-celled eukaryotes are also known. The exact evolutionary relationship between virophages, Polintons and the so-called 'Polinton-like viruses' (PLVs) recently discovered in metagenomic data is unclear. The latter are related entities mainly characterized from metagenomic data although two PLVs have been isolated with their single-celled algal host, and a few are found in protist genomes. Having identified virophage- and PLV-like sequences in short-read genomic data of the heterotrophic stramenopile Halocafeteria seosinensis, we used long-read Oxford Nanopore technology to produce a highly contiguous nuclear genomic assembly for this extremophilic protist. Analysis of our long-read assembly (62 contigs, 39 Mbp) revealed the presence of dozens of integrated elements, some of which belong to the virophage clade while others are most closely related to PLVs. Each of these are only distantly related to previously characterized elements. Furthermore, the diversity inside each clade suggests that H. seosinensis has taken up virophages and PLVs on several occasions. The virophages and PLVs are themselves colonized by several families of transposable elements. Our analysis of the H. seosinensis nuclear genome paves the way for future experimental work on the complex interplay between viruses, virophages, polinton-like viruses, transposable elements, and the protists they inhabit in nature.

Dollo parsimony overestimates ancestral gene content reconstructions

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Ancestral reconstruction is a technique that can be used to understand the history of gain and loss of gene families over evolutionary time scales, and to produce hypotheses on how these gains and losses may have influenced the evolutionary trajectories of extant organisms. Ancestral reconstructions can be based on different phylogenetic inference methods, such as maximum likelihood or Bayesian inference, but many current studies are based on Dollo parsimony. We hypothesize that Dollo parsimony is not appropriate for ancestral gene content reconstruction inferences based on sequence homology, as Dollo Parsimony assumes that a complex character can only be gained once. This premise does not accurately model molecular sequence evolution, in which homology can result from sequence convergence or lateral gene transfer, and could lead to distortions in phylogenetic studies. Therefore, the aim of this study is to test Dollo parsimony's suitability and compare its inferences with a maximum likelihood approach, which allows a gene family to be gained more than once within a tree. In order to test our hypothesis, we first compared the performance of the two methods on a simulated dataset of 5,000 genes across a spectrum of evolutionary rates, and then we reconstructed protein domain evolution on a phylogeny representing known eukaryotic diversity. We observed numerous ancestral gene content overestimations produced by Dollo parsimony, which led us to the conclusion that, confirming our hypothesis, Dollo parsimony is not an appropriate method for ancestral reconstruction studies based on sequence homology.

Thinking outside the nucleus: A mitogenomic approach to ciliate phylogeny

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In recent years, the usage of mitochondrial genes, or even entire mitochondrial genomes, to investigate phylogenetic relationships between organisms had seen a bloom in popularity. These kinds of analyses had been used, for example, to successfully depict cryptic species in protist, annelids and arthropods, or to discriminate between subspecies of Apis mellifera. In this regard, ciliates possess very peculiar mitochondrial genomes, being among the first to be identified as linear, showing several split rRNA genes and protein genes and being relatively long (~ 40 Kbp) in respect of other eukaryotes. Nevertheless, the low amount of available mitochondrial genomes of ciliated organisms in online databases, as well as the absence of representatives of many classes of this phylum, didn't allow, up to now, to perform extensive and in-depth analyses using mitochondrial genomes. Here we present a first attempt to perform a phylogeny of phylum Ciliophora, using the whole mitochondrial genome. All the available mitochondrial genomes in online databases were collected. Moreover, genomic project involving ciliates were inspected, and when possible related reads were downloaded and analyzed in order to reconstruct mitochondrial genomes previously neglected or overlooked. For at least two representatives of each missing class in online databases, the whole DNA material was extracted, and the mitochondrial genome was sequenced and bioinformatically assembled. A selection of 17 protein coding genes was then used to perform the phylogenetic analysis, among almost 100 representatives of the phylum. Lastly some considerations about pros and cons about using mitochondrial genomes for phylogenetic analyses will be presented, as well as some consideration about synteny and genome content in ciliate mitochondria.

Evolutionary studies of programmed genome rearrangements in ciliates, focusing on *Euplotes* and *Paramecium*

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Genome evolution in ciliates has to be interpreted in light of their dual genomes, the presence of both germline and somatic genomes within each cell. Programmed genome rearrangements occur extensively by removing massive amounts of repetitive, or selfish DNA elements found in the silent germline genome during development of the somatic genome. The processes and mechanisms of genome rearrangements are quite diverse, both within a given ciliate species as well as among different ciliates. Studies on ciliate genome rearrangements are focusing on only a handful species. To expand our knowledge of the diversity of genome structures and the evolution of complex genome rearrangements, we focus on the highly differentiated euplotids, Euplotes vannus. We revealed the genome rearrangement patterns of E. vannus, including the sequence characteristics of chromosomal breakage sites (CBS), macronucleus destined sequences (MDS), internal eliminated sequences (IES) and pointers. About 4% and 10% of MAC chromosomes are scrambled or alternatively processed, respectively. We also explored its molecular mechanism of genome rearrangements by sequencing and analyzing small RNA, long noncoding RNA, mRNA and proteins in different stages of its life cycle. We found that 30nt sRNAs and long double-strand RNAs specifically derive from the maternal somatic nucleus during early conjugation and accumulate prior to the formation of zygotic nucleus. The precursor of these noncoding RNA is bidirectionally transcribed using the conserved CBS-related motif at sub-telomeric region as the promoters. The 30nt sRNAs can recognize and protect MDSs in the developing new MAC. Microinjection of synthetic 30nt sRNAs corresponding to IESs leads to their retention in the sexual progeny, which can achieve the purpose of gene knockout in Euplotes. Differential gene expression analysis revealed some potentially important and species-specific genes involved in genome rearrangement, and multiple homologous genes played different roles at different stages. We also explored and compared genome rearrangement processes in Paramecium caudatum, a species closely related to the P. aurelia complex but with a gigantic germline genome. This work highlights genome rearrangement complexities within lineages and expands our understanding of the dynamics of eukaryotic genomes.

Semi-conservative transmission of DNA N6-adenine methylation in a unicellular eukaryote

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While DNA N6-adenine methylation (6mA) is best known in prokaryotes, its presence in eukaryotes has generated great interest recently. Biochemical and genetic evidence supports that AMT1, a MT-A70 family methyltransferase (MTase), is crucial for 6mA deposition in unicellular eukaryotes. Nonetheless, 6mA transmission mechanism remains to be elucidated. Here we provide definitive evidence for semi-conservative transmission of 6mA, showcased in the unicellular eukaryote Tetrahymena thermophila. In wildtype (WT) cells, 6mA occurs at the self-complementary ApT dinucleotide, mostly in full methylation (full-6mApT); hemi-methylation (hemi-6mApT) is transiently present on the parental strand of newly replicated DNA. In ΔAMT1 cells, hemi-to-full conversion fails and 6mA predominantly occurs as hemi-6mApT. In vitro methylation of both Tetrahymena oligos and human chromatin by reconstituted AMT1 complex recapitulates preferential targeting of hemi-6mApT sites, supporting AMT1's intrinsic and autonomous role in maintenance methylation. We conclude that 6mA is transmitted by a semi-conservative mechanism: full-6mApT is split by DNA replication into hemi-6mApT, which is restored to full-6mApT by AMT1-dependent maintenance methylation. Our study reveals a molecular pathway for 6mA transmission with striking similarity to 5-methyl cytosine (5mC) transmission at the CpG dinucleotide and establishes 6mA as a bona fide eukaryotic epigenetic mark.

Phagocytosis underpins the biotrophic lifestyle of intracellular parasites in the class Phytomyxea (Rhizaria)

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Phytomyxea are intracellular biotrophic parasites infecting plants and stramenopiles and they include agriculturally impactful pathogens such as Plasmodiophora brassicae. Recent molecular investigations assigned them to the clade Rhizaria, mainly composed of free-living amoebae and amoeboflagellates where phagotrophy is a widespread mode of nutrition. Phagocytosis is a complex multigene trait specific to eukaryotes, well documented in free-living unicellular eukaryotes and specialised cellular types of animals. Studies on phagocytosis in intracellular biotrophic parasites are scant, since the direct consumption of host organelles and cellular components is seemingly at odds with the biotrophic requirement of keeping the invaded cell alive. Here we provide evidence that phagotrophy is part of the nutritional strategy of phytomyxea, using morphological and genetic data (including a novel transcriptome of the brown algae parasite Maullinia ectocarpii). We document intracellular phagocytosis in P. brassicae and M. ectocarpii by transmission electron microscopy and fluorescent in situ hybridization. Our investigations confirm the presence of molecular signatures of phagocytosis in Phytomyxea and hint at a small specialised subset of genes used for intracellular phagocytosis. Microscopic evidence confirms the existence of intracellular phagocytosis, which in Phytomyxea targets primarily host organelles. Phagocytosis seems to coexist with the manipulation of host physiology typical of “traditional” biotrophic interactions. Our findings resolve long debated questions on the feeding behaviour of Phytomyxea, suggesting an unrecognised role for phagocytosis in biotrophic interactions.

Genomic and transcriptomic advances in understanding the tripartite interactions between host plants, viruses and the protist vectors *Polymyxa*

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Polymyxa spp. are root obligate endoparasite protists classified among the Plasmodiophorids. These protists are studied in agronomy as they transmit a diversity of viruses (29 viruses reported so far) to numerous host plants (monocotyledons and dicotyledons) in both temperate and tropical areas. Our research aims to assess how the host plants, the protists Polymyxa and the viruses they vector interact, mainly focusing on two pathosystem models for which genomic data are available : (1) the Polymyxa betae – Beet necrotic yellow vein virus (BNYVV) on sugar beet and (2) the Polymyxa graminis – Rice stripe necrosis virus (RSNV) on rice. Many questions remain so far regarding the nature and the specificity of host-Polymyxa and Polymyxa-virus interactions. This work aims to present the results of recent genomic (Decroës et al., 2022a) and transcriptomic (Decroës et al., 2022b) data of the vector Polymyxa. The study of the distinct effects of Polymyxa alone or associated with a virus on its host opens new opportunities to understand how Polymyxa manage to by-pass host defenses and paves the way for identifying effectors involved in host defenses manipulation. It also gives insight on how does the virus impact Polymyxa and its interactions with its host and the specificity of Polymyxa-virus interactions.

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Genomics of 'accessory' endosymbionts of the ciliate *Euplotes*: Evolution of functional and ecological traits

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Protists frequently engage in symbiotic associations with prokaryotes. Particularly, ciliates of the genus Euplotes host a diverse array of endosymbionts, most of which are not essential to the host survival, yet little to nothing is known about their role in the relationship. Among these, members of the family 'Candidatus (Ca.) Midichloriaceae' (Rickettsiales) have been detected multiple times in these ciliates, but are also known to colonize a wide range of hosts and environments. Nevertheless, a coherent picture of the diversity and evolutionary trends in this group has been long overdue. Another endosymbiont of Euplotes, 'Candidatus Nebulobacter yamunensis' (Thiotrichales), shares 100% sequence identity over the 16S rRNA gene with a free-living strain. Such genetic similarity between two organisms with such contrasting ecologies provides a unique opportunity to investigate what genomic changes may be involved in the transition to endosymbiosis. We added up to five bacterial genomes to reconstruct the phylogeny and functional traits of Euplotes' endosymbionts. All available genomes of 'Ca. Midichloriaceae' were analyzed, and their traits were mapped on phylogenetic trees to uncover potential evolutionary patterns. The traits varied considerably even between closely related taxa, potentially indicating frequent evolutionary transitions. Relevant traits such as metabolic pathways, flagella, and secretion systems have deteriorated multiple times independently in different hosts, supporting the idea of bacteria independently evolving 'professionally symbiotic' features, irrespective of their host identity. Moreover, our results show that members of 'Ca. Midichloriaceae' mainly colonize aquatic organisms, an important clue to reconstruct the evolution of this clade. The genome of 'Ca. Nebulobacter''s symbiotic strain was compared to that of the free-living strain, while cultivation was attempted on both strains in parallel. While the cultivation experiments confirmed their distinct ecological strategies, the symbiont's genome remarkably displayed no typical signature of obligate endosymbiosis. Instead, it shared extreme similarities with that of the free-living strain. This suggests that 'Ca. Nebulobacter' might have transitioned to an obligate intracellular lifestyle extremely recently, and not enough time has passed for the typical signatures of symbiosis to appear in its genome, while other overlooked and perhaps unpredictable changes might be involved in the transition.

Unexpectedly diverse protist community of *Reticulitermes tibialis*: Implications for symbiont inheritance and coevolution

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Wood-feeding termites have a nutritional symbiosis with gut-dwelling flagellate protists (Metamonada). This symbiosis is mutually obligate and vertically inherited, resulting in co-diversification of hosts and protists. Each termite species harbors a characteristic set of protist species, with closely related hosts typically harboring closely related symbionts. The richness of these symbiont communities varies widely across host lineages, from as few as 1 species in Termitogeton to likely more than 20 in Hodotermopsis. Molecular approaches tend to reveal higher diversity than morphological approaches, but most termite symbionts have yet to be characterized by molecular methods. The symbionts of Reticulitermes hosts mainly belong to Trichonymphida, Spirotrichonymphida, and Pyrsonymphidae (Oxymonadida). The number of protist morphospecies described from Reticulitermes ranges from 8 in R. hageni to 14 in R. speratus. Here we have characterized the symbiont diversity of Reticulitermes tibialis, the arid land subterranean termite, collected at multiple locations in Arizona. Using light microscopy and single cell PCR of the 18S gene, we found 29 species-level symbiont lineages, the highest symbiont richness of any termite species investigated to date. Our phylogenetic analyses indicate at least 10 major clades that include symbionts from both North American and Asian Reticulitermes species, suggesting that they were present in the ancestor of all extant Reticulitermes. It seems likely that symbiont richness in other Reticulitermes species is currently underestimated. A few of the R. tibialis symbionts we identified were previously described from other North American Reticulitermes species, including R. hesperus, R. flavipes, and R. okanaganensis, all of which can be found within the geographical range of R. tibialis. These shared species may be due to symbiont transfer or delayed diversification in the symbionts relative to their hosts. To distinguish between these possibilities, further characterization of other Reticulitermes species symbionts is needed. This work was carried out as a course-based undergraduate research experience at Arizona State University.

Prevalence, succession, and activity of protistan grazers in particle-associated communities

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Heterotrophic and mixotrophic protists affect the cycling of organic carbon in the ocean by transferring organic carbon to higher trophic levels, contributing to or taking from the dissolved organic matter pool, and remineralizing organic carbon and nutrient elements. While grazing by free-living heterotrophic and mixotrophic protists is known to play a significant role in marine carbon cycling dynamics, less is known about the relative importance of protistan heterotrophy in particle-associated microbial communities. Three 'decomposition' incubation studies were carried out to (1) compare the diversity and metabolism of particle-associated and free-living protists and to (2) identify how the composition and metabolism of particle-associated protistan assemblages change over time as particulate organic matter is degraded. Eukaryotic metatranscriptomes were obtained from water column (i.e., free-living) and sediment trap (i.e., particle-associated) microbial communities at 150 m depth in the North Pacific Subtropical Gyre to identify differences in the composition and metabolism of these protistan assemblages. A fraction of the particle material was incubated and metatranscriptomes were obtained after 3 days and 6 days of incubation to investigate how the protistan assemblages changed over time as particulate organic matter was presumably degraded. The results of this analysis revealed that transcripts associated with photosynthesis were enriched in the free-living communities, while transcripts that may be associated with heterotrophic processes were generally enriched in the particle-associated samples. Additionally, dinoflagellate, haptophyte, and pelagophyte transcripts were more abundant in the free-living samples, while excavate, choanoflagellate, and amoebozoan transcripts were more abundant in the particle-associated samples. There was substantial variability in the composition and metabolism of the particle-associated protistan assemblages over time; however, the relative abundances of bicoeceans and labyrinthulomycetes (Stramenopiles) increased between day 0 and day 3 in all of the incubation experiments and were expressing genes that may be related to the breakdown of organic matter. Together, these observations revealed stark taxonomic and metabolic differences in free-living and particle-associated oceanic, protist assemblages and provided insight on the roles that specific particle-associated protists play in the breakdown, transformation, and remineralization of organic carbon.

A triple metabarcoding strategy for discriminating active, dormant, and dead microbial eukaryotes in environmental samples

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Microbial communities have been widely characterized using metabarcoding of environmental DNA, which is derived from living (including dormant and active forms) and dead cells. Despite advances in discriminating living and dead cells, methodologies for further separation of dormant from active members are lacking. Propidiummonoazide (PMA)is a photoreactive dye that can selectively cross-link to the DNA of broken cells. By taking advantage of PMA, we propose an approach combined DNA, RNA and PMA-DNA based Illumina sequencing to discriminate dormant phylotypes from active and dead forms in nature. We applied this approach to characterize the diversity and community structure of microeukaryotes (protists and fungi) in intertidal sediments that underwent a wetting-drying-rewetting incubation cycle. We found that1) considerable proportions of OTU richness detected in bulk DNA pools were attributed to the encysted and dead microeukaryotes, indicating that the “active” microeukaryotic richness is largely overestimated; 2) the assemblage structure of active fraction discriminated by our approach was comparable to those based solely on metabarcoding of 18S rRNA transcripts; 3) the environmental drivers behind the dynamics of these fractions varied. Taken together, by using the triple metabarcoding strategy, our study demonstrates that cysts are a non-negligible and dynamic part of microeukaryotes in coastal sediments. Hence, this methodology should enable a better understanding of the persistence and resilience of microeukaryotic diversity, seasonal and spatial distributions, and function.

Digital PCR as a new cutting-edge tool for measuring ciliate abundance

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Observing the abundances of ciliates is pivotal to understanding their ecological importance in different ecosystems. Although numerous morphological and molecular methods have been developed to estimate microbial abundances, we still lack a standardized technique for accurate and precise estimations of ciliate abundances. The novel digital PCR (dPCR) technology has recently been successfully used to analyze copy number variation among different groups of organisms. Here, we show that dPCR is not only applicable for the estimation of copy number variations in ciliates, but can also be used to quantify their relative abundances. Single-cell analysis of rDNA copy number variation within the model ciliate Paramecium tetraurelia showed that dPCR is a precise and easy to handle tool to quantify intraspecific copy number variations of ciliates. Despite these variations, we found that digital PCR was able to successfully detect increasing cell numbers of mock communities. Our results demonstrate that digital PCR can be applied to future environmental studies that aim to quantify abundances of individual ciliate species.

Canonically circular mitochondrial genomes spread among euglenids

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The peculiar organisation of mitochondrial DNA is one of the defining features of Euglenozoa. Mitochondrial genomes spread among thousands of circular molecules are well known in Kinetoplastids and Diplonemids, with a few reports of fragmented linear mitochondrial genomes in Euglenids. While some indications of circular mitogenomes in early branching Euglenozoans were known before, here we present several independent instances among Euglenids. Circular mitogenomes in Colacium, Eutreptiella and Peranema vary in size (20-122kbp) and contain 0-5 group I introns. Despite different sizes, they always encode the same set of eight mitochondrial proteins. Still, the most common mitogenome structure we observe among 30 diversly sampled Euglenid species is short linear fragments with one protein gene per fragment. In some genomes, we recovered longer contigs, indicating a variable level of fragmentation. Gene content is consistent across all sampled taxa, with just one or two proteins missing in some of them. Group I introns are spread irregularly, but when present, they are always found in only six positions in cob, cox1 and nad4 genes. In some species, introns encode LADLIDADG endonucleases, known to be involved in group I introns spread. The presence of circular mitogenomes in distantly related taxa challenges the loss of circular mitogenomes in the Euglenozoan common ancestor. We hypothesize that the complex picture we observe across Euglenids can be explained by the presence of both linear and circular chromosomes in Euglenid mitochondria. We are currently investigating this hypothesis using cell-fractioning and long-read sequencing of mitochondrial DNA.

Comparative spatial proteomics of euglenozoans

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The now-defunct supergroup of Excavata is presently recognised as a variety of distantly related protists, spread across the Eukaryotic domain. However, the shared possession of certain ultrastructural features suggests that such distantly-related members may in fact most closely resemble the form of the Last Eukaryotic Common Ancestor among extant organisms, of which, research into such protists holds the promise to elucidate evolutionary insight into common features beyond morphology. Accordingly, we initiated a spatial proteomics investigation into the less resolved clades of Euglenozoa, choosing free-living members of Euglena longa (representing euglenids) and Paradiplonema papillatum (representing diplonemids). Using LOPIT-DC, we generate spatial datasets consisting of over 5,000 high-quality proteins per organism, and define a variety of organelle and structural compartments using the comparatively well-investigated proteome datasets of the kinetoplast sister clade. Our work defines common protein constituents shared between these organisms, which in turn provides proteomic insights into a group of protists regarded as one of the most basal amongst Eukaryotes.

Preaxostyla: A group that has succeeded in eliminating mitochondria

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Approximately 160 species of the genus Preaxostyla represent a minor group of protists with no significant impact on natural processes and no known application potential. Yet, a closer understanding of them has proved surprisingly fruitful for the debates about some evolutionary trends and events. My lecture will pay tribute to this eukaryotic lineage. Preaxostyla includes species of Trimastix and Paratrimastix with a beautifully developed excavated cytoskeleton, which represent suitable models to study its protein composition. Other species, classified in oxymonads, have lost mitochondria, an otherwise ubiquitous structure of extraordinary importance for the evolution of eukaryotes. We now understand the circumstances of this event and its surprisingly small consequences. The lack of mitochondria has not resulted in a visible simplification or decrease in their sizes and, in some cases, quite the opposite. Admittedly, most amitochondriate species live as gut endobionts, which also affects their physiology and morphology, and so the lesson from the evolutionary experiment performed by oxymonads does not provide a clear and simple conclusion. The absence of mitochondria in this group is secondary, and such status cannot be easily compared to the situation in a putative amitochondriate intermediate during eukaryogenesis, which is disputed mainly on the energetic ground. Still, I will discuss how these inspiring arguments withstand the existence of secondary amitochondriate oxymonads. Finally, the existence of amitochondriates provides a model system to tackle some questions on eukaryogenesis experimentally and will show the results of one experiment revealing some circumstances of the origin of protein targeting into the emerging mitochondria.

Has the DNA polymerase of the proto-mitochondrion been retained in Discoba, Malawimonadidae, and Ancyromonadida? A novel mitochondrion-localized DNA polymerase with the phylogenetic affinity to the alpha-proteobacterial PolI

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The replication of mitochondrial (mt) genomes requires DNA polymerases that cooperate with other proteins encoded by the nuclear genomes. So far, evolutionarily distinct types of DNA polymerase have been reported to be localized in mitochondria. DNA polymerase gamma (Polγ) has been well studied as the mt-localized DNA polymerase in human and yeast. Trypanosomatids are known to possess multiple DNA polymerases (PolIA, B, C, and D) for their mitochondrial DNA replication. Another type of mt-localized DNA polymerase (plant and protist organellar DNA polymerase or POP) has been found in phylogenetically diverse protists. All of these DNA polymerases bear a sequence similarity to bacterial DNA polymerase I (PolI). In this study, we surveyed PolI-like DNA polymerases in diverse eukaryotes to depict the diversity and evolution of mt-localized DNA polymerases. We detected none of the above-mentioned mt-localized DNA polymerases in the species belonging to Malawimonadidae, Ancyromonadida, and Discoba (except for Euglenozoa). Instead, these species appeared to share a unique type of PolI-like polymerase. The novel PolI-like polymerases likely bear typical mitochondrial-targeting signals (MTS) at the N-termini, and the N-terminal amino acid sequences functioned as the MTS in yeast cells. Thus, we conclude that the novel PolI-like polymerases are localized in the mitochondria of Malawimonadidae, Ancyromonadida, and Discoba, all of which are the candidates for early branches of the tree of eukaryotes. Furthermore, the novel PolI-like polymerases showed a strong phylogenetic affinity to the PolI sequences of alpha-proteobacteria. Combined, we propose the mt-localized PolI-like polymerase occurred very early in eukaryotic evolution or is the direct descendant of the PolI of the alpha-proteobacterium that gave rise to the mitochondrion (i.e., proto-mitochondrion).

Paramecium bursaria - a perfect model organism for so-called active Brownian particles and statistical physics?

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It is well known that Paramecium bursaria can live in facultative, mutualistic symbiosis with Chlorella-like algae ("green" paramecia). It was shown that green paramecia accumulate in the light, a behavior that is influenced but not caused by the symbiont. Apart from biological questions, we are particularly interested in the physical properties of such behavior and in proving Paramecium bursaria to be a good model organism for so-called active Brownian particles. In recent years "active matter" gained an enormous interest from the community of statistical physicists. Flocks of birds, schools of fish, microbial biofilms, sperm, and even cars and people – they all can be modeled as "particles" with a self-propulsion mechanism. Physicists are interested in, for instance, the function of the propulsion mechanism, changes between individual and collective motion, and the influence of external stimuli like light, walls, food, or concentration on the behavior. Such knowledge allows to tune and guide the motion in order to develop functional materials that hinders biofilm growth or to drive or even separate particles, according to certain properties. A famous example is given by designed colloidal particles that show a "motility induced phase separation", where dense, cluster-like formations of particles form when the system is illuminated. Turning off illumination, the clusters immediately "melt" and one homogeneous "phase" remains where all particles distribute equally. I will present a broad overview over the interests physicists have in active matter. In particular, I will focus on similarities between the random-like motion of run-and-tumble organisms and diffusion of passive particles that obey Brownian motion. I will briefly explain the underlying stochastic processes and, in which sense, active matter particles can be modeled using methods from equilibrium statistical physics. Importantly, I will demonstrate why green paramecia are, indeed, an excellent model organism and how this can be exploited to tackle the question whether random walkers in a maze show sub-, normal, or super-diffusive motion. Finally, I will discuss how our findings and questions might be transferred to other organisms and species and I look forward to have stimulating discussions that may open new perspectives and directions of joint research.

Diversity and diurnal changes of microeukaryotes in mudflat biofilms: A molecular perspective

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Benthic biofilms contain diverse prokaryotic and eukaryotic microorganisms that are dominated by diatoms, which migrate diurnally and vertically in the surface sediments. However, not much is known about the diversity of other microeukaryotic groups in benthic biofilms. In this study, we investigated the diversity, community structure, and diurnal variations of microeukaryotes (protists and fungi) in benthic biofilms on an intertidal mudflat in the Pearl River Estuary in both summer and winter. We analyzed the metabarcoding data of 18S rDNA and rRNA transcripts (cDNA) in an integrated manner by focusing on the active operational taxonomic units (OTUs), which were identified in both rDNA and rRNA datasets of a given sample. Our results showed that the Raphid pennate diatoms were the most dominant groups, comprising approximately 97% and 80% of reads in the microeukaryotic communities in summer and winter, respectively. Other taxa, e,g., Dinoflagellata, Cercozoa, Fungi, and Chrompodellids were relatively minor. Statistical analyses indicated that there were significant seasonal and diurnal differences in diatom rRNA abundance, community structure, and diversity of microeukaryotes. Diatom rRNA abundance was lower during the day than at night in the winter. In the summer, diatom rRNA abundance in the surface layer (top 0-2 mm) decreased with increasing sunshine in the morning and reach the lowest level at noon, indicating downward migration. The diurnal variation in the community structure of microeukaryotes was mainly driven by nitrate in the winter but phosphate in the summer. Our study also demonstrated that the analysis of either rDNA or rRNA dataset alone led to great overestimations of the richness of active microeukaryotes, because of the existence of abundant extracellular rDNA and variants of rRNA transcripts in the sediments.

Microeukaryotic predators shape the wastewater microbiome

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Prokaryotes are the main actors in wastewater treatment and the physicochemical parameters that shape their community composition are extensively studied. In contrast to prokaryotes, wastewater microeukaryotes are poorly investigated and it is unknown whether the predation pressure they exert on prokaryotes significantly shapes the wastewater microbiome. Here we show that the microeukaryotic community composition does shape the prokaryotic community. Using metatranscriptomics data of a bioreactor that was sampled over 14 months at weekly intervals, we were able to investigate the wastewater microbiome in its entirety, including the often neglected microeukaryotes. We show that, while prokaryotes are not affected by a seasonal change in water temperature, they are affected by a seasonal, water temperature-induced change in the microeukaryotic community. These findings indicate that predation, selectively exerted by microeukaryotic predators, is a major shaping factor for the prokaryotic community in wastewater. Our study emphasizes the need for closer investigation of the wastewater microbiome in its entirety to fully develop an understanding of wastewater treatment.

Free-living relatives of highly abundant unicellular marine parasites elucidate plastid loss

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Although photosynthesis has been lost many times, complete loss of the plastid organelle is considered to be very rare, since plastids are integrated into host metabolism in many ways. But understanding plastid loss is critical to understanding the evolution of photosynthesis and endosymbiosis more broadly. Plastid loss is a central force underlying the distribution of eukaryotic photosynthesis, and also key to understanding the impact of endosymbiosis on the host genome. Endosymbiosis is also widely held to facilitate a massive influx of new genes into the host genome, which may continue to function in the organelle, or may take on functions in other subcellular compartments. This leads to the prediction that after plastid loss the nuclear genome might retain numerous functional genes originating from the former plastid, and by extension the presence or absence of such a genetic “footprint” has been used to argue for or against cryptic, ancient endosymbioses. But our current understanding of the fate of such genes and plastid loss in general is insufficient to make such claims. Currently the only two relatively clear cases of plastid loss are in highly reduced parasites, neither of which have closely-related free-living relatives with plastids to compare. Accordingly, we lack even the fundamental expectations to distinguish a genome where a plastid once existed and was lost from one where a plastid never existed. Here we describe a novel lineage, the eleftherids, and use phylogenomics to show that they are the closest sister group to the Marine Alveolates (MALVs), an abundant, diverse, and widespread group of parasites thought to play a major role in marine food webs. Moreover, MALVs are one of the two lineages thought to have lost plastids. Eleftherids are free-living predators and, although not photosynthetic, retain a metabolically active plastid, which allows for a more detailed model for how the MALV plastid was lost leaving virtually no genomic trace. By challenging the notion that plastid origins are expected to make a pronounced and lasting mark in the host genome, the eleftherids and MALVs also raise important questions about the process and impact of endosymbiotic organelle origins more broadly.

Decoding the doo-doo dilemma: The impact of bacteria on aggregative multicellularity in dung-inhabiting amoebae

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Aggregative multicellularity has independently evolved in eukaryotes at least seven times, leading to intriguing questions about its triggers and convergent evolution. Copromyxa protea, a dung-dwelling amoeba, forms macroscopic tree-like sorocarps through aggregative behavior, yet the factors initiating this behavior remain elusive. Our experimental evidence reveals that bacterial communities from herbivore dung play a crucial role in this process for C. protea. Our research demonstrates that the bacterial trigger exists within animal dung and the sorocarps constructed by C. protea cells. Furthermore, reintroducing a dung-maintained bacterial community restores aggregative behavior in strains previously believed to have lost this capability. We observed that the bacterial community of multicellular strains on dung is more diverse than those that do not aggregate, suggesting a direct influence of bacterial communities on aggregative behavior. Employing a testable and manipulatable system, our experimental methods allow for a detailed exploration of how environmental context and bacteria can impact the evolution of aggregative behaviors in protists. Notably, this pattern of behavior may also be observed in dung-inhabiting sorocarpic members of three supergroups (A rhizarian, stramenopile, and amoebozoan). Our findings underscore the significance of examining bacterial roles in the evolution of aggregative behaviors in protists, and pave the way for further investigation into the mechanisms underlying this behavior in these diverse and fascinating organisms.

Proteomics reveals the evolution of chlorarachniophyte pyrenoids

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Pyrenoids are electron-dense structures found in photosynthetic plastids of various eukaryotic algae and hornworts. It is believed that Rubisco proteins and CO2 are concentrated in a compartment to facilitate CO2 fixation. Recent extensive studies on the model green alga Chlamydomonas reinhardtii have revealed the pyrenoid composition and function. However, the molecular mechanisms of pyrenoids remain unclear in many other algae. In this study, we investigated pyrenoid proteomics in the chlorarachniophyte alga Amorphochlora amoebiformis, which possesses complex plastids derived from the secondary endosymbiosis of a green alga. We identified pyrenoid-related proteins by mass spectrometry, and further localization experiments demonstrated that several proteins were specifically targeted to pyrenoids. Although many of them were functionally unknown, we found an intrinsically disordered protein as a candidate of Rubisco linker. Unexpectedly, the composition of pyrenoid proteins, other than Rubisco, was found to be significantly different between C. reinhardtii and A. amoebiformis. It seems that chlorarachniophyte pyrenoids have been independently evolved rather than endosymbiotically derived from a green alga.

Local endoreduplication of the host is a conserved process during Phytomyxea-host interaction

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Endoreduplication is a modified cell cycle in which cells duplicate their DNA without subsequent mitosis. This process is common in plants and can also be found in other organisms like algae and animals. Biotrophic plant pathogens have been shown to induce endoreduplication in their host to gain space and/or nutrients. Phytomyxea (divided into the Plasmodiophorida, the Phagomyxida, and the Marinomyxa clade) are obligate biotrophic parasites of plants, diatoms, brown algae, and oomycetes. Here, we tested if phytomyxids induce local endoreduplication in two distant hosts (plants and brown algae). By combining fluorescent in situ hybridisation (FISH) coupled with nuclear area measurements and flow cytometry, we confirmed that endoreduplication is induced by Plasmodiophora brassicae (Plasmodiophorida) in infected plants and demonstrate this process in combination with Maullinia ectocarpii and Maullinia braseltonii (Phagomyxida) in brown algae. We identified molecular signatures of endoreduplication in RNA-seq datasets of P. brassicae-infected Brassica oleraceae and M. ectocarpii-infected Ectocarpus siliculosus. Cell cycle switch proteins (CCS52A1 and B in plants and CCS52 in algae) as well as the protein kinase WEE1 (in plants) were identified as genes potentially important for the phytomyxean-induced switch from the mitotic cell cycle to the endocycle. Their expression pattern changed in infected plants and brown algae accordingly. In this study we expand the knowledge on Phytomyxea-host interactions by showing that induced endoreduplication in the host is a conserved feature in phytomyxid infections. The induction of this cellular mechanism by phytomyxid parasites in phylogenetically distant hosts further points at a fundamental importance of endoreduplication in these biotrophic interactions.

Phylogenomics show parasitism evolved twice in the marine alveolates (MALVs)

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The Marine Alveolates (MALVs) are a largely uncharacterised collection of dinoflagellates predominantly represented by SSU rRNA gene sequences from host-associated and environmental microbiome surveys. Also referred to as the Syndiniales, the MALVs were originally thought to form a monophyletic group sister to the core dinoflagellates (i.e. parasitism evolved once in the group), although some analyses suggested they are paraphyletic (i.e. parasitism may have evolved multiple times). Resolving this question has been restricted by the absence of genome-wide data from one of the two major MALV sub-groups. With the isolation of a new MALV I-related parasite from the dinoflagellate Polykrikos sp., we use single-cell transcriptomics and phylogenomics to show that the Syndiniales are actually polyphyletic: MALV II/IV and MALV I arose independently from two distinct, free-living ancestors. While MALV II and IV form a sister group to the newly discovered, eleftherids, MALV I is related to Oxyrrhis marina. Despite sharing morphological characteristics with other Eudubosquella-like dinoflagellate parasites, rRNA analysis shows a clear distinction between the Polykrikos-infecting species and other lineages within the genus. We therefore create a new genus, Deorella, to distinguish this lineage from ciliate-infecting Eudubosquella spp. and suggest MALV I be renamed the Ichthyodiniales to formalise their distinction from the remaining Syndiniales (MALV-II and IV).

Integrating omics with 3D imaging to understand how endosymbionts become organelles

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Mitochondrial and plastid origins were the two most important symbioses in the evolution of life. That these cellular organelles started as symbiotic bacteria is now supported by overwhelming evidence. However, after more than 1.5 billion years of tight host-symbiont integration, present-day mitochondria and plastids no longer allow us to fully study the endosymbiont-organelle transition. Younger (<400 Mya) bacterial endosymbionts are thus emerging as powerful model systems for studying the origin and evolution of endosymbiotic organelles. These novel systems, however, come with their own experimental challenges since we often lack high-resolution methods for distinguishing organism-specific signals in highly nested and interdependent systems. Introducing innovative three-dimensional approaches to such models is thus crucial for answering basic questions about how endosymbionts become organelles. Here, I will present our results on symbioses in protist hosts where we integrated single-cell genomics/transcriptomics with 3D imaging (FIB-SEM, SBF-SEM, and confocal imaging) and subcellular proteomics. Creatively combining and optimizing these methods allows us to answer basic questions about the structure, function, and evolution of highly integrated symbiotic systems. Overall, our results suggest that organellogenesis can follow a winding path with species-specific outcomes.

Repeated haptophyte endosymbioses in the dinoflagellate family Kareniaceae

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The majority of photosynthetic dinoflagellates possess plastids containing peridinin. Intriguingly, several types of “non-canonical” plastids, which are evolutionarily distinct from peridinin plastids, were established in a number of dinoflagellate lineages. One type of non-canonical plastids found in dinoflagellates has been derived from a haptophyte endosymbiont, and such “haptophyte-derived” plastids have been found exclusively in members of the family Kareniaceae. Despite uncertainty about when precisely the haptophyte-derived plastid first emerged, the haptophyte endosymbiosis is thought to have happened early in the evolution of Kareniaceae. We here report evidence of a recent haptophyte endosymbiosis occurring after the divergence of members of the genus Karlodinium, one of the genera comprising Kareniaceae. We completely sequenced the plastid genome of a member of Karlodinium and noticed that the Karlodinium plastid genome determined in the current study possesses >99% nucleotide identity to the plastid genome of the free-living haptophyte Emiliania huxleyi. Thus, we conclude that haptophyte endosymbiosis occurred at least twice—once in the early evolution of and the other after the divergence of Kareniaceae, and the latter event gave rise to the plastid of the Karlodinium species studied here. We are currently searching for the nuclear genes encoding plastid proteins in the dinoflagellate of our interest to explore the evolutionary origins of the proteins working in the new plastid and examine whether endosymbiont gene transfer from the second haptophyte endosymbiont took place in this system.

Horizontal gene transfer contributes mechanistically to a facultative photosymbiosis in *Paramecium bursaria*

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Horizontal gene transfer (HGT) provides a mechanism for rapid genic and phenotypic innovation. In contrast to vertical inheritance, where genetic material is passed from parent to offspring, HGT involves the movement of genes between disparate organisms. These gene transfers frequently occur within symbiotic contexts and are known to have played an important role in the evolution of endosymbiotic organelles. However, when, and how horizontally acquired genes contribute to organellogenesis remains unclear. Here, we surveyed the genomes of five strains of Paramecium bursaria, a ciliate that forms a facultative photosymbiosis with the green alga, Chlorella. We identify numerous gene transfers of various ages which were derived from two disparate sources, bacteria, and large double stranded DNA viruses. Functional annotation of these genes revealed that these sources have provided P. bursaria with distinct gene sets including metabolic enzymes and glycosylhydrolases from bacteria and autophagy and endosomal regulators from viruses. Using RNA-interference we tested the function of some of these genes and demonstrate their requirement for endosymbiont control. These data provide new insights into the molecular toolkits available through HGT which can contribute to organellogenesis and raise interesting questions about the prevalence and origins of endosymbiosis in ciliates and the plastid evolution in eukaryotes more broadly.

The phylogenomic position of coral infecting corallicolid apicomplexan shows multiple independent losses of chlorophyll biosynthesis in obligate intracellular parasites

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Apicomplexans evolved from a free-living photosynthetic ancestor, still reflected in a few photosynthetic relatives like Chromera and Vitrella, as well as in the retention of a cryptic plastid in most apicomplexans. The transition from a free-living phototroph to an obligate intracellular parasite completely transformed the genomes and functions of the plastid, resulting in an apicoplast that plays a role in only four biosynthetic pathways. Recently, however, a new intriguing remanent of their photosynthetic ancestry was identified in the apicoplast of corallicolid apicomplexans, whose plastid genomes still encode genes for the chlorophyll biosynthesis pathway. How we interpret this relict pathway is not clear though, because the phylogenetic positions of the nuclear and plastid SSU rRNA gene of corallicolids are contradictory: plastid phylogeny places them distantly related to all other the apicomplexans, but nuclear rRNA phylogeny places them sister to the coccidia. Here, we describe the first genomic survey of corallicolid apicoplexans, and using this data to perform a phylogenomic analysis concludes corallicolids are sister to protococcidia. These first multiprotein phylogenetic analyses of corallicolids supports their nested position within the apicomplexan crown group, although in a different position suggested by rRNA alone. Moreover, we also identify nucleus-encoded genes for plastid targeted proteins representing the rest of the chlorophyll biosynthesis pathway in corallicolids and their sister Eleutheroschizon. Altogether this shows that chlorophyll biosynthesis was retained throughout much of apicomplexan evolution, but repeatedly lost in the ancestors of most extant lineages.

Nonconventional introns of euglenids as elements shaping the structure of genes and sequences of encoded proteins

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Euglenids genomes remain poorly understood, mainly due to their large sizes, complexity and repetitiveness. So far only a draft genome of Euglena gracilis, with a few genes annotated has been published. One of the most remarkable features of euglenids genomes is the presence of distinct type of introns, not observed in other organisms – so called nonconventional introns. This type introns differ from spliceosomal ones, common in nuclear genes of eukaryotes including euglenids. They have non-canonical borders, lack polypyrimidine tract and form a stable RNA secondary structure bringing together intron ends, as well as the ends of adjacent exons. The length of nonconventional introns ranges from tens of bases to several kilobases, and their RNA secondary structure is rather weakly preserved. It was revealed that these atypical introns can be inserted at new positions (mobile genetic elements), and are rapidly removed from transcripts (in circular form) but later than conventional ones, most likely post-transcriptionally, by unknown mechanism of removal (most likely spliceosome-independent). Meticulous analyses of genomic and transcriptomic sequences from three species (Euglena gracilis, E. hiemalis, E. longa) have shown other peculiarities related to nonconventional introns, which were subsequently tested and confirmed experimentally. One of them is the occurrence of additional introns within existing ones – such structures are called twintrons. Moreover, it has been shown that nonconventional introns can be alternatively excised. This phenomenon relies on recognising alternative borders of introns in pre-mRNA, which result in different mRNA, coding for different proteins. It seems that nonconventional introns could be evolutionary hotspots, the presence of which may favour the creation of new proteins. Such discovery shows not only the influence of nonconventional introns on repertoire of proteins in contemporary euglenids, but suggests also that spliceosomal introns could play similar role in early eukaryotes before the precise spliceosome machinery evolved.

Global patterns and rates of habitat transitions across the eukaryotic tree of life

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The colonization of new habitats has played a fundamental role in the evolution of life. One of the most difficult habitat boundaries to cross is the salt barrier—the boundary between marine and non-marine (including freshwater and soil) habitats—but the role of this barrier in shaping eukaryotic diversity remains unclear. Traditional views suggest that marine/non-marine transitions are rare and ancient events, but the recent discovery of several closely related marine and non-marine lineages casts doubt on this dominating view. Here, we investigate habitat transitions across the tree of eukaryotes using a unique set of taxon-rich phylogenies inferred from a combination of long-read and short-read metabarcoding data spanning the ribosomal DNA operon. Our results indicate that marine and non-marine microbial communities are generally phylogenetically distinct, but transitions have occurred in both directions in most major eukaryotic lineages, with hundreds of transition events detected globally. Some groups in particular have experienced high rates of transitions, most notably fungi, diatoms, and chrysophytes. At the deepest phylogenetic levels, our analyses suggest that the two largest known eukaryotic assemblages, TSAR and Amorphea, arose in different habitats. Overall, our findings indicate that the salt barrier has played an important role in eukaryotic evolution, and demonstrate that using metabarcoding data in a phylogenetic framework is a powerful approach for addressing ecological and evolutionary questions.

The role of phagotrophy as an important iron acquisition strategy of marine dinoflagellates

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Although well known for toxin production of dinoflagellates in coastal and estuarine waters, they are often present and occasionally dominate the protistan communities within offshore regions where iron concentrations are variable and frequently growth limiting. Despite their seemingly higher iron requirements compared to other phytoplankton groups, many marine dinoflagellates may benefit from their diversity of trophic modes, including autotrophy, heterotrophy and mixotrophy, with respect to iron acquisition. Through a combination of laboratory and field experiments, we investigated the iron requirements and acquisition strategies in oceanic and coastal dinoflagellates exhibiting various trophic modes. The mixotrophic dinoflagellate Karlodinium spp. isolated from iron-limited waters of the Northeast Pacific Ocean exhibits increased relative growth rates under low iron conditions compared to a coastal counterpart. Yet the cellular iron contents between isolates are not significantly different so that reductions in iron requirements cannot be used to explain the observed growth discrepancies. The heterotrophic dinoflagellate Gyrodinium dominans displays reduced growth and prey ingestion rates when grown in medium absent of dissolved iron and provided with iron-limited prey, suggesting this dinoflagellate emulates the iron status of it's food. Lastly, during a simulated upwelling experiment using subsurface natural plankton communities in the California Upwelling Zone, heterotrophic dinoflagellates were found to dominate the assemblages over time following the addition of a strong iron chelator which inhibits dissolved inorganic iron uptake. Overall, our findings support the role of phagotrophy as an important iron acquisition strategy that may provide a competitive advantage to mixotrophic plankton under certain scenarios.

A novel parasitoid (Oomycota) infecting the marine dinoflagellates

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Oomycetes are known as pathogens of plants, insects, crustaceans, vertebrate animals, fish, and various microorganisms. Among the oomycetes that infect microorganisms, many studies have conducted on the morphology, life cycle, and phylogeny of oomycete parasites that infect diatoms, whereas those on oomycete parasites of marine dinoflagellates remain poorly known. During intensive sampling along the east and the west coast of Korea in May and October 2019, a new species of oomycete was discovered and two strains of the new parasitoid were successfully established in cultures. The new parasitoid penetrated the dinoflagellate host cell and developed to form a sporangium, very similar to the Perkinsea parasitoids that infect dinoflagellates. The most distinctive feature of the new parasitoid was a central large vacuole with a formation of several germ tubes. The molecular phylogenetic trees based on a small subunit (SSU) ribosomal DNA (rDNA) and cytochrome c oxidase subunit 2 (cox2) revealed that the new parasitoid was in a distinct branch unrelated to other described species and had a sister relationship with the genus Miracula, which is a parasitic group infecting the diatom. Cross-infection experiments showed that among the examined microorganisms including Dinophyceae, Raphidophyceae, Cryptophyceae, Litostomatea, Euglenoidea, and Bacillariophyceae, only six genera belonging to Dinophycea were infected by the new parasitoid.

A unique symbiosome in an anaerobic single-celled eukaryote

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Symbiotic relationships drive evolutionary change and are important sources of novelty. Here we demonstrate a highly structured syntrophic symbiosis between species of the anaerobic protist Anaeramoeba (Anaeramoebae, Metamonada) and bacterial ectosymbionts. We dissected this symbiosis with long-read metagenomics, transcriptomics of host and symbiont cells coupled with fluorescent in situ hybridization (FISH), and microscopy. Genome sequencing, phylogenomic analyses and FISH show that the symbionts belong to the Desulfobacteraceae and were acquired independently in two different Anaeramoeba species. We show that ectosymbionts likely reside deep within cell surface invaginations in a symbiosomal membrane network that is tightly associated with cytoplasmic hydrogenosomes. Metabolic reconstructions based on the genomes and transcriptomes of the symbionts suggest a highly evolved syntrophic interaction. Host hydrogenosomes likely produce hydrogen, acetate, and propionate that are consumed by the symbionts dissimilatory sulfate reduction, Wood-Ljungdahl and methylmalonyl pathways, respectively. Because the host genome sequences encode several vitamin B12-dependent enzymes but appear to lack the ability to biosynthesize this vitamin, we hypothesize that the symbionts supply their hosts with B12. We detected numerous lateral gene transfers from diverse bacteria to Anaeramoeba, including genes involved in oxygen defense and anaerobic metabolism. Gene families encoding membrane-trafficking components that regulate the phagosomal maturation machinery are notably expanded in Anaeramoeba spp. and may be involved in organizing and/or stabilizing the symbiosomal membrane system. Overall, the Anaeramoebae have evolved a dynamic symbiosome comprised of a vacuolar system that facilitates positioning and maintenance of sulfate-reducing bacterial ectosymbionts.

From phylogenomics to functional genomics of peritrichs

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In 2019, we launched the Protist 10,000 Genomes Project (P10K, https://ngdc.cncb.ac.cn/p10k/). As a part of this project, extensive isolation of ciliates from freshwater samples in China was performed. By establishing a large-scale fast genome/transcriptome assembling-decontamination-annotation pipeline, over 350 genomes and transcriptomes by sequencing from cultured cells, wild specimens, and single cells of peritrich ciliates. The omic data obtained were used to conduct a comprehensive phylogenomic analysis to address the phylogenetic relationship of different peritrichs, which is one of the challenging questions in ciliate taxonomy. Using the high-quality genomes, comparative genomic analysis was performed between the peritrichs with and without stalk, colonial and solitary, and a number of genes that constitute the stalk were identified, and verified by Mass Spectrometry with low protein input. Antibodies of several key proteins, including the stalk “shell” proteins and spasmoneme proteins, were generated and imaged using high-resolution microscopy, and interesting patterns of these proteins were revealed. In general, our study provides new insight into the fast contraction and colonial style of peritrichs.

Loss of metabolic autonomy in the photosynthetic ciliate *Mesodinum rubrum*

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The Mesodinium rubrum species complex is widespread in coastal marine ecosystems where it is a year-round member of the plankton and in some regions forms red tides. The ciliate acquires its phototrophic potential by stealing organelles from Teleaulax-like cryptophytes and by exploiting their entire metabolism. This process is made possible by the ciliate stealing the nucleus of its prey, i.e. the kleptokaryon, which is non-replicating but remains transcriptionally active and facilitates control and division of sequestered plastids and mitochondria. Using multi-omics approaches we show that M. rubrum depends on its acquired metabolism, not just for photosynthesis, but biosynthesis of a broad array of molecules, including amino acids, fatty acids, purines, and pyrimidines. This synthesis is not just metabolic surplus, but has apparently replaced many of the ciliate's own basic anabolic pathways for essential metabolites. Analysis of multiple M. rubrum variants show similar trends, while the more mixotrophic M. chamaeleon and heterotrophic M. pulex retain complete anabolic gene pathways. Our results suggest that adaptation to a phototrophic lifestyle in M. rubrum-like ciliates has profoundly reshaped their genome, and reveal that basic cell metabolism has become obligately dependent upon a foreign nucleus. This precarious relationship reveals striking similarities to known protist parasites and illuminates an evolutionary mechanism by which foreign organelles and their metabolism can become integrated with their host to form an obligate relationship.

A long-read sequencing approach to metabarcoding protists from a diverse set of environments

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The 18S rRNA gene is a commonly used marker for studying eukaryotic diversity in various environments. Metabarcoding using short-read sequencing of hypervariable regions as the V4 and V9 regions of the 18S has revolutionized analyses of microbial diversity. Oxford Nanopore long-read sequencing technology offers longer reads, making it a promising approach for full-length eukaryotic 18S metabarcoding. In this study, we developed a pipeline for full-length eukaryotic 18S metabarcoding using this new sequencing technology. We extracted DNA from various environmental samples and amplified the 18S rRNA gene using universal primers. The resulting amplicons were sequenced using the Oxford Nanopore platform and analyzed using our customized pipeline. The pipeline included quality control, read filtering, taxonomic classification, and statistical analyses. Our pipeline demonstrated high accuracy and resolution in identifying diverse eukaryotic classifications, including rare and novel taxa. The full-length reads strengthened our confidence of the classifications. In conclusion, our study demonstrates the effectiveness of using Oxford Nanopore long-read sequencing technology in full-length eukaryotic 18S metabarcoding studies. The developed pipeline can provide a more accurate and comprehensive understanding of eukaryotic diversity in various ecosystems, enabling better monitoring and managing these important communities.

Insights into protist activity, spatio-seasonal dynamics and multi-trophic linkages in a Canadian hypertidal mudflat

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Protists constitute much of the phylogenetic and functional diversity among eukaryotes and play essential roles in nutrient and energy cycling. Yet, they remain relatively understudied in marine sedimentary ecosystems like the intertidal mudflats in the hypertidal Bay of Fundy (Atlantic Canada). The harsh conditions of the intertidal zone and high tidal energy in the Bay of Fundy provide an ideal system for gaining insights into benthic protist communities. As such, we used 18S rDNA metabarcoding, metagenomics and metatranscriptomics to gain specific insights into (1) major food web players, (2) spatial and seasonal diversity patterns, (3) ribosomally active diversity, (4) trophic linkages with ecosystem-engineering animal co-residents, and (5)potential drivers of heterogeneous spatial distribution among protist communities in a Bay of Fundy mudflat. We found that protist community diversity and dynamics were dominated by eukaryvorous and bacterivorous cercozoans (thecate, benthic gliding forms), cosmopolitan typically-planktonic diatoms, a toxigenic bloom-forming dinoflagellate, omnivorous ciliates, diverse gregarine parasites and uncultured syndinean parasitoids. Although metabarcoding detected relatively abundant diatom and dinoflagellate assemblages in hypoxic subsurface sediments, meta-omic examination revealed that most, but not all, of these lineages were ribosomally inactive and unlikely to play active roles in ecosystem functioning. A field experiment on multi-trophic interactions found that although spatial factors, such as patchiness and depth distribution, contributed most to protist community dynamics, trophic factors also played a role, albeit limited. Our experiment revealed that among the top predators in the mudflat, mud snail presence significantly changed protist community composition while migratory shorebird presence did not. The effect of experimental nutrient addition (commercial fertilizer) was inconsistent, and conditional when present. Our findings highlight the combined marine and terrestrial influences on protist communities inhabiting intertidal sediments and suggest that synergistic interactions of both local and regional processes, notably benthic-pelagic coupling, may drive microbial dynamics in high-energy coastal systems. Our results also highlight the limited yet complex role of multi-trophic interactions in shaping protist community dynamics. Our study is the first of its kind in the Bay of Fundy and contributes to the broader understanding of processes structuring microbial communities in diverse environments.

Freshwater plastid genomes through the lenses of metagenomics

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Microbial eukaryotes (protists) are important contributors to freshwater ecosystems. However, due to the insufficient sequencing efforts of freshwater environments, protistan diversity and functions remain understudied in those environments. Metagenomics changed our perception of the diversity of prokaryotes, and it is still pretty challenging for research of the protist's communities. We believe that plastid genomes, which are compact and occur in high copy numbers, can be a promising target in metagenomic-based analyses of photosynthetic protists. Here, we present the results of the study of phototrophic microbial eukaryotes based on their plastid genomes recovered from the metagenomic dataset of 1096 samples. Samples were obtained from ten-year-long time series from four lakes characterised by different trophic statuses. To tackle those tremendous data, we developed a pipeline that minimises computational resources, including screening for eukaryotic contigs by tiara and alignment-based validation of putative plastid-like contigs and manual binning in Anvi'o. Using the established protocol, we have detected and reconstructed environmental plastid genomes (ptMags) from eight co-assemblies. We recovered more than 200 plastid genomes representing major groups of photosynthetic protists belonging to Chlorophyta, Stramenopila, Haptophyta, Cryptophyta, Alveolata, and Euglenophyta. ptMags were further used for i) phylogenomics utilising 61 plastid-encoded genes and ii) abundance over a sampling time range – to assess phytoplankton dynamics related to changing environmental conditions. In summary, in this project, we shed light on the genetic diversity and ecology of freshwater protistan phototrophs over ten years. These results clearly show that plastid genomes could be successfully recovered from metagenomic data and serve as valuable data for further studies of microbial eukaryotes ecology.

Elucidating early stages of plastid endosymbiosis with *Rapaza viridis* (Euglenophyta) kleptoplasty

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The phenomenon of endosymbiosis, in which one organism lives within another, led to major evolutionary transitions, such as the origin of mitochondria and plastids. However, despite its significance, we still do not understand the mechanism of endosymbiotic integration. Only by examining more recent endosymbioses in various phases of integration can we make progress in our understanding of the host-endosymbiont integration process. One such organism constitutes mixotrophic euglenid Rapaza viridis (Euglenophyceae) and its green-algal kleptoplasts. Here, we investigated R. viridis kleptoplasty using microscopy observations, biochemical analysis, genomics, and transcriptomics. Experiments indicated that the kleptoplasts of R. viridis are functionally active. We identified 276 sequences encoding putative plastid-targeting proteins and 35 sequences of presumed kleptoplast transporters in the transcriptome of R. viridis. These genes originated in a wide range of algae other than Tetraselmis sp., the source of kleptoplasts, suggesting a long history of repeated horizontal gene transfer events from different algal prey cells. Many of the kleptoplast proteins and the protein-targeting system in R. viridis were shared with members of the Euglenophyceae, providing evidence that the early evolutionary stages in the green alga-derived secondary plastids of euglenophytes also involved kleptoplasty. Rapaza viridis constitutes a unique example of a transient association with host-encoded proteins targeted to the temporary symbiont, and obtained data advance our understanding of the origin and evolution of plastids not only in Euglenophyceae, but also in eukaryotes as a whole.

Living well with evolution of beating heart: Contractile vacuole in ciliates

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The contractile vacuole responsible for osmoregulation in different supergroups across eukaryotic diversity, precisely in freshwater and soil-dwelling microbes. Ciliates, single-celled eukaryotes belong to group alveolates in the SAR clade. Diversity of ciliates at both morphological and molecular levels makes them a predominant model system for evolutionary studies of cellular organelles and associated membrane trafficking proteins. The contractile vacuole facilitates the survival of ciliates in varied living conditions which drives us to study and identify the conserved machinery related to the contractile vacuole activity and their evolution across the ciliate lineage. Tetrahymena thermophila and Paramecium tetraurelia from class Oligohymenophorea, Oxytricha granulifera from class Spirotrichea and Stentor coeruleus from class Heterotrichea were selected as representatives from different classes. A contractile vacuole specific clade had been identified in P. tetraurelia. Further, transcriptomics and comparative genomics approach will be implemented to predict the conserved core genes and class-specific genes related to contractile vacuoles in selected ciliate species. Differential expression of genes followed by their molecular phylogenetics will contribute to build a foundation in unfolding the evolution of osmoregulatory organelle.

Intracellular bioaccumulation of the rare earth element Gadolinium in the ciliate *Tetrahymena pyriformis* resulting in biogenic particle formation and excretion

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The ciliate Tetrahymena pyriformis is a model organism in many ecotoxicological studies. It is known that ciliates can tolerate high concentrations of toxic heavy metals and are often found even in polluted environments. The understanding of the cellular pathways of organisms that have established processes to deal with heavy metal pollution is a promising approach to find new bio-inspired ways of combating environmental pollution. Also, the recycling of strategic and rare elements is becoming more and more important in current times. In our study, we investigated the process of tolerance, cellular uptake and bioaccumulation of the rare earth element gadolinium (Gd) in the ciliate Tetrahymena pyriformis. Gd^{3+} ions are toxic because they can interfere with the Ca^{2+} signalling of cells. Gd is mainly used in complexed form as diagnostic tool in MRT contrast agents, that cannot be filtered out by wastewater treatment plants. The fate and speciation of anthropogenic Gd in freshwater environments is the subject of current research. Gd treatment of T. pyriformis results in the intracellular formation of uniformly sized biogenic Gd-containing particles. Particle formation occurs in a time frame of a few hours, thereafter the particles are excreted into the surrounding medium. This cellular process effectively removes dissolved Gd from the organic growth medium by 53% within 72 h. Light and electron microscopic observations reveal an intracellular detoxification pathway by which the cells take up toxic Gd^{3+} ions from the medium by endocytosis. The Gd is sequestered inside food vacuoles and further processed to Gd-containing particles. Over time, a reduction in size of the particles can be observed. Particles excreted through exocytosis are sufficiently stable and can afterwards be removed from the solution. The particles with a constant diameter of about 3 µm consist of the elements Gd, C, O, P, Na, Mg, K, and Ca, as shown by elemental analysis (EDX). The understanding of the cellular processes of bioaccumulation by ciliates broaden the view of metal ion accumulation and are of relevance to understand environmental elemental cycles.

Seagrasses as model organisms to understand the biodiversity and evolution of phytomyxid parasites in natural settings

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Phytomyxids (SAR: Rhizaria: Endomyxa: Phytomyxea) are a monophyletic group of obligately biotrophic intracellular parasites infecting various plants, algae and oomycetes, and dreaded pathogens of numerous crops of great economic significance. Despite their infamy in agriculture, the diversity and evolutionary history of phytomyxids – the majority of which parasitize hosts from non-artificial ecosystems – remain significantly understudied. Since the symptoms of naturally occurring phytomyxid infections are often inconspicuous, the greatest limitation to uncovering their diversity is finding infected host specimens. To shed new light on these protists, we recently initiated a systematic search for long-overlooked phytomyxid taxa associated with seagrasses, a paraphyletic group of flowering plants adapted to life in the marine environment. Our sampling surveys, supported by analyses of a global amplicon sequencing dataset, revealed that phytomyxids are highly specialized and ubiquitous residents of coastal ecosystems, in contrast to previous speculations that they are rare. In seagrasses of the genus Zostera – important foundation species in coastal areas and estuaries worldwide; primary phytomyxid infection stages were found in the roots of >99% of examined plants. Furthermore, the diversity of marine phytomyxids appears to be much larger than previously anticipated, with our preliminary findings suggesting that they parasitize at least 5/11 recognized seagrass genera. Molecular analyses of the 18S and 28S rRNA phylogenetic markers show that seagrass-associated phytomyxids form distinct clades within the group and indicate long coevolutionary host-parasite relationships resulting in high host-specificity. Given the low number of contemporary seagrass taxa (~60 species), their global distribution and ecological significance, we believe that research on phytomyxid infections in seagrass hosts can provide a deeper understanding of evolutionary and speciation patterns of Phytomyxea outside of agricultural settings.

Spicing up the menu: Novel diversity of free-living bacterivorous colpodellids, relatives of Apicomplexa

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Colpodellids are free-living marine, freshwater, and soil predatory microbial eukaryotes. All described species live aerobically and specifically feed on microbial eukaryotes. Colpodellids consume their prey through a unique process called myzocytosis, in which the predator attaches to and penetrates the prey's surface and cell membrane before 'sucking up' the cytoplasmic contents. Remarkably, the closest relatives of colpodellids are the free-living algae Chromera and Vitrella along with the Apicomplexa. Most apicomplexans are obligatory intracellular parasites that invade their host cells using a characteristic apical complex. Despite the dramatic difference in life history, colpodellids also have an apical complex that facilitates predation by myzocytosis, where attachment to the prey is followed by formation of a microtubule ring derived from the pseudoconoid that delimits the connection between the predator and prey. Overall, colpodellids together with Chromera, Vitrella and Apicomplexa represent an incredibly diverse group of organisms whose lifestyles range from primary production, through predation to parasitism. Moreover, they all share the apical complex that has been repurposed for different activities. We have recently discovered a colpodellid lineage containing isolates that break the paradigm inferred from all other known colpodellids, as they are bacterivorous and clearly capable of living both aerobically and anaerobically. Here, preliminary morphological and molecular characterizations for one of these novel Colpodellids are presented. We will show light and electron microscopy observations demonstrating feeding behaviour and apical-complex structures, as well as results of phylogenomic analysis and differential expression analyses of cells grown aerobically and anaerobically. These results add to the existing diversity of life histories within colpodellids, apicomplexans, and chromerids.

Astonishing diversity of RNA viruses in *Leptomonas pyrrhocoris*, a trypanosomatid parasitizing firebugs

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Parasitic flagellates of the family Trypanosomatidae, some of which are infect humans, domestic animals, and cultural plants can harbor RNA viruses. At least in human pathogens Leishmania spp. such viruses impact on the course of the disease. Leptomonas pyrrhocoris, a parasite of firebugs that is closely related to leishmaniae, has been recently shown to host viruses of groups not detected in other trypanosomatids. This along with the worldwide distribution and high prevalence of this parasite stimulated us to further investigate the diversity of RNA viruses in it. We surveyed 106 axenic cultures of L. pyrrhocoris (collected in 27 localities from 13 European countries) and detected viruses in 60% of them. The analysis of NGS data demonstrated the presence of seven viral species (up to five at once in a single isolate) belonging to four viral groups. In addition to the previously documented tombus-like virus and Ostravirus, there were four new species of the trypanosomatid-specific family Leishbuviridae, and a new species of Qinviridae, a family earlier documented only in metatranscriptomes from invertebrates. Our phylogenetic reconstructions point to interactions between L. pyrrhocoris strains resulting in reassortment of the highly prevalent tombus-like virus. Two out of the four new species of Leishbuviridae represent early branches in the evolution of this family and shed light on the process of the genomic reduction that started in the ancestor, apparently similar to the insect-parasitic Phenuiviridae, and culminated in the crown Leishbuviridae. The wide range of viruses in L. pyrrhocoris and the simultaneous presence of up to five of them in a single isolate are unprecedented among protists and suggest that this flagellate is unique. We argue that the wide distribution, high abundance and some habits of its host stipulate the profuseness of L. pyrrhocoris and its exposure to a broader spectrum of viruses compared to other trypanosomatids combined with a limited ability to transmit these viruses to its relatives.

All we know about diversity of a neglected ciliate subfamily Clevelandellidae

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Cockroaches (Blattodea) exhibit an immense variation in life-history and foraging strategies with xylophagy of termites being one of the most widely known. However, wood-eating has evolved multiple times independently also in non-termite Blattodea, mostly in the distantly-related cockroach family Blaberidae, the species-rich subfamily Panesthiinae being the most striking example. In contrast to termites, the hindgut of panesthiins is not inhabited by the giant and visually attractive, wood-digesting hypermastigotes from the phylum Parabasalia. Instead, they possess bizarre symbionts of their own – ciliates of the family Clevelandelliae. These protists exhibit a peculiar morphology. Their unusually long buccal cavity is situated at the posterior of the cell, which is frequently extended into a peristomal projection. Despite the family along with 8 included species being erected more than 80 years ago, only 12 species have been described since, 8 of them, however based only on molecular sequences and without morphological descriptions. Moreover, apart from three species found in the termite Capritermes incola and cockroach genera Salganea and Macropanesthia, the majority of the species was described from just a single cockroach host genus – Panesthia. In this study we report the diversity of Clevelandellidae from 12 host populations (which is so far the widest host spectrum assessed) from four host genera, including representatives of two as-yet-unexplored host genera: Ancaudellia and Miopanesthia. We characterized a majority of previously described species of Clevelandellidae by molecular and modern morphologic methods and describe six new species in genus Clevelandella. We also discuss a number of unusual and previously overlooked or misinterpreted morphologic features of the group.

Chimera formation and detection in long-read amplicon sequencing data

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High-throughput sequencing of environmental samples is a powerful tool for mapping and discovering protist diversity. Until recently, the field has been dominated by short-read sequencing techniques, which typically generate sequences up to 500 base pairs long. However, these short sequences have limited phylogenetic signals. Long-read approaches, which allow the sequencing of longer regions (10-15 kilobases) with stronger phylogenetic signals, are becoming more popular. However, long-read sequence data are often hampered by two types of errors: PCR/sequencing mutations and chimeric sequences. While point mutations can be corrected by denoising or clustering methods similar to shorter reads, there are currently no good tools specifically designed for detecting chimeras in long-read amplicons. Chimeric sequences occur when two or more different DNA templates are fused into one during PCR. It is reasonable to assume that chimeric sequences are more prevalent among long-read amplicons, as there are more regions where templates can hybridize. In this study, we assessed the degree to which chimeric sequences emerged during long-read PCR/sequencing by analyzing two mock communities and more than 100 environmental samples from soil. We used both general eukaryotic primers and primers specifically targeting a microscopic group of fungi called Archaeorhizomycetes. The amplicons ranged from 3000 to 4500 bp, covering most of the ribosomal operon, and were sequenced using PacBio Sequel II (HiFi-reads). We tested modifications of the current UCHIME-like chimera detection algorithms implemented in VSEARCH, including assuming more than two parental sequences and checking a higher number of sequence partitions, to see if they could recover more chimeric sequences. In the mock community containing two closely related fungal species, we observed a high number of chimeric sequences. In the other mock community and the less homogeneous natural samples, we observed fewer chimeras but were still able to detect a large portion of chimeric sequences. Our study concludes that chimeras are commonly formed during long-read metabarcoding and must be thoroughly checked for, preferably using improved chimera detection approaches under development.

Free-living trichomonas: There and back again?

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The phylum Parabasalia (Metamonada) is of interest primarily because it includes important parasites of humans and domestic animals and also wood-digesting symbionts of termites. Although some free-living parabasalians also exist, they have been largely neglected and most of their known diversity has been discovered only recently. Importantly, the free-living parabasalians form several independent evolutionary lineages suggesting that at least some of them may be secondarily free-living. However, there is a serious limitation of molecular data, since the phylogenetic analyses of free-living Parabasalia have been based exclusively on the SSU rRNA gene, which lacks a phylogenetic signal sufficient to resolve the phylogeny of Parabasalia. To this end, we have sequenced eight transcriptomes of various parabasalians with the focus on the free-living representatives. In our preliminary analyses, the recently discovered order Pimpavickida (Alexandriella bishopae and Pimpavicka limacoides) appear to be sister to the rest of Parabasalia, which suggests they may represent primarily free-living organisms, while other sequenced free-living parabasalians cluster within endobiotic taxa (e.g., Honigbergiellida, Trichomonadida) and are likely secondarily free-living.

Ciliates in altered marine environments - plasticity, resilience & opportunism

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Some ciliates in oiled environments show remarkable resilience to a complex array of stressors, particularly in habitats characterized by chronic releases of hydrocarbon residues, while other common species do not flourish or are absent from samples. Questions remain regarding the mechanisms that permit such resilience and adaptation. This presentation will provide a review of recent research regarding the plasticity associated with ciliate species that can thrive in contaminated environments and attempt to decipher whether there is a greater breadth of tolerance to varying conditions or special abilities to extrude contaminants or otherwise block the effects of toxic exposure. With climate change scenarios posing dramatic changes in shallow coastal waters, including warmer temperatures and possible acidification, understanding these mechanisms seems important in begin able to assess water quality and ecosystem health.

Protist diversity patterns at the species level: The case of testate amoebae

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Species are the basic units of diversity. Since the turn of the 21st Century, scientists agree that biodiversity is presently undergoing a deep crisis with massive numbers of species extinctions, with tremendous consequences on ecosystems. These conclusions have been based on macroscopic species, plants and animals, which are exceptions in a mostly microbial eukaryotic tree. However, it is still not clear how far the rules that explain how biodiversity is distributed on Earth can be extended to small organisms as well. To address this question, protist models are needed; here, I will present the testate amoebae (Arcellinida and Euglyphida). A first prerequisite to study protist diversity distribution is a sound taxonomic framework backed with molecular data, where both deep and recent nodes are resolved. The search for variable genetic markers with species-level resolution permits the development of specific metabarcoding protocols, which can then be applied to large sampling designs. The datasets produced can then be used to infer the evolutionary histories of the groups or the influence of environmental drivers on their diversity. Such approaches can also be applied to monitor environmental quality. Altogether, evidence accumulation suggests that the rules that drive biodiversity distribution tend to be the same for all eukaryotes, large or small

Phylogenomics of Cercozoa: A single-cell transcriptome approach

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The Cercozoa are a large group of Rhizaria, with a dizzying number of diverse morphologies, ranging from many substrate-associated flagellates and amoeboflagellates to planktonic forms such as Ebria and phaeodarians, the latter being large enigmatic amoebae with skeletal structures that are abundant in the deep pelagic ocean. What we know about the phylogeny and evolutionary history of Cercozoa is constricted by the data for most groups being limited to the SSU rDNA gene, the phylogeny of which lacks the resolution that a comprehensive multigene phylogenetic analysis would provide. To enable a multigene phylogenetics approach, we photodocumented and isolated more than 30 cercozoan single cells from marine benthic and pelagic environments, and generated single-cell transcriptomes with SmartSeq2 and placed these transcriptomes in a multigene framework of 240 marker genes. Most of our single-cell transcriptomes only recovered a portion of the marker genes, but it nevertheless enabled a well-resolved multigene phylogeny. Most of our cells fall within Cryomonadida, Thaumatomonadida, and Marimonadida, and we recover these as well-supported clades. Ebria branches among Thecofilosa, in agreement with previous SSU rDNA results. Some of our cells belong to previously unsampled branches, others to clades previously known from environmental reads only.

Holozoan protists shed light on the evolution of regulated cell death pathways at the onset of animal multicellularity

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Regulated cell death involves the destruction of cells in a deliberate and controlled manner in response to damage or infection, and has been reported in a variety of eukaryotes. A subset of regulated cell death processes known as programmed cell death occurs as a normal part of tissue homeostasis or embryonic development. Because programmed cell death is essential to the development and health of animals, understanding its emergence is a key part of understanding the evolution of animal multicellularity. The holozoan protists comprise several lineages related to animals, encompassing diverse morphologies, lifestyles and gene complements. The genomes of many of these organisms encode homologs of signaling proteins, adhesion proteins and transcription factors that were previously believed to be animal-specific and likely facilitated the emergence of multicellularity in the animal stem lineage. By studying these organisms, we trace the emergence of regulated cell death-related proteins during holozoan, and identify common interactors of regulated cell death-related proteins in holozoan protists and in animals with a view to understanding the functional and regulatory changes undergone in regulated cell death pathways as animals emerged.

Light/dark modulation of thiol oxidation in *Paulinella micropora* using redox proteomics

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Redox regulation is an important strategy for controlling metabolic pathways in response to changing environmental conditions. In chloroplasts, thiol oxidation is linked to the excitation of photosynthetic electron transport, allowing for light-responsive control of chloroplast functions. In this study, we investigated whether Paulinella micropora, which has photosynthetic organelles resulting from independent primary endosymbiosis of Archaeplastida plastids, possesses a redox control system regulated by light. We employed a quantitative proteomic approach using resin-assisted enrichment with isobaric tandem mass tag labeling to measure reversibly oxidized thiols. We quantified the redox dynamics of Cys-sites under light and dark conditions, including the percentage of oxidation (reversibly oxidized/total thiols) for Cys-sites. Our findings provide new insights into the evolution of photosynthetic organelles in Paulinella micropora.

Global population structure of a unicellular marine predator lineage

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Protists play critical roles in marine ecosystems and substantially impact the dynamics of marine food webs. Among these, MAST-4 constitutes a critical lineage of unicellular ocean predators. Understanding their genetic diversity, population structure, and adaptations to environmental factors is crucial for comprehending the role of protists in the ocean ecosystem. This study investigated the genomic diversity and population differentiation of four MAST-4 species across the global ocean using single-cell genomics and metagenomics data from the Tara Oceans expedition. By calling and analyzing Single Nucleotide Variants (SNVs), we identified distinct patterns of population-level genomic divergence in the four MAST-4 species. Some species exhibited strongly differentiated populations due to potential limitations in gene flow or adaptation to different environmental conditions. Temperature and salinity emerged as the primary factors structuring MAST-4 populations. We found that the number of SNVs in MAST-4 species was not associated with genome size. We calculated the dN/dS ratio for each MAST-4 gene to identify those that may represent the basis of differential adaptation between populations. We detected positively selected gene clusters in all MAST-4 species associated with genomic populations and oceanic basins, for example, in the Mediterranean population of MAST-4A or the North Atlantic samples of MAST-4E. The analysis of the functional role of the genes under positive selection provided insights into the metabolic functions that have been the focus of selection. In conclusion, our results expand our knowledge about the population genomics of a key unicellular predator in the ocean, MAST-4. This information contributes to a better understanding of marine protist populations and their adaptations. Our study highlights the importance of incorporating population genomics into the analysis of protists, with potential implications for a better comprehension of marine food webs and ecosystem functions.

Environmental bifurcation of two cyst forming acantharian clades in Arctic waters

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Acantharia, which are large protists with bio-mineralized skeletons of strontium sulfate (SrSO4), have received little attention as a component of pelagic food webs, though they are reported to contribute to planktonic biomass in global oceans. Their large cell-size and potential for mixotrophy in some clades has implications for food web dynamics, but to our knowledge, their diversity not been explored in the Arctic Ocean. To address this knowledge gap we used amplicon sequencing of the V4 variable region of the 18S rRNA gene to detect acantharian sequences in pelagic eukaryote communities in the Western Arctic in the late summer. Acantharian distribution patterns differed by region and water mass. In the Canada Basin, Clade B1 acantharians accounted for up to 81% of total microbial eukaryotic reads in the Pacific Winter Water mass, and were occasionally detected at high relative abundance in Pacific Summer Water, while in Nares Strait between Greenland and Ellesmere Island, Clade C3 could be as much as 35% of total reads in the deep Atlantic Water of southern Kane Basin and northern Baffin Bay, but was not abundant in upper Arctic water masses. Detection of acantharians in deeper samples from size-fractionated samples < 3 µm near the end of summer was consistent with reported acantharian life cycles, but future projections are difficult given ongoing changes to ice extent that currently menace the Arctic Ocean biosphere.

Protein-protein homo- and hetero-oligomerization phenomena explain autocrine (growth-promoting) and heterologous (mating-inducing) pheromone-cell interactions in *Euplotes*

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Families of cell type-specific, water-borne signaling protein pheromones regulate Euplotes growth and union in mating pairs by binding competitively target cells in autocrine or heterologous fashion. Membrane-bound pheromone isoforms function as pheromone receptors on the cell surface. They originate through an intron-splicing mechanism from the same macronuclear genes specifying the soluble forms, and this common genetic determination makes the pheromone and receptor's extracellular (ligand-binding) domain of each cell type structurally identical. In consideration of this identity, the pheromone/receptor interactions on the cell surface were inquired as they were mimicked by the interactions that associate pheromone molecules into crystals. These crystals were obtained from native preparations of the E. raikovi pheromones Er-1 and Er-13, that are distinctive of two interbreeding cell types and show largely overlapping molecular structures based on a three-helix fold. In spite of marked differences in the symmetry space group, the Er-1 and Er-13 crystals equally show to be formed by molecules that rigorously take mutually opposite orientations and use two faces (mostly provided by amino acid sidechains lying on helix-3) to associate and oligomerize into linear chains. Of the two faces, one shows to be substantially conserved between Er-1 and Er-13 molecules and this conservation ensures their effective association, albeit only to form heterodimers. While oligomerization of Er-1 and Er-13 molecules well accounts for observations that autocrine pheromone/receptor complexes cluster and undergo internalization in growing cells, heterodimerization between Er-1 and Er-13 molecules accounts for observations that heterologous pheromone/receptor complexes lack clustering and remain anchored on the cell surface to be likely used in mating unions.

The origin and evolution of symbiontid euglenozoans using single-cell transcriptomics

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The Euglenozoa consists of mostly predatory microbial eukaryotes that fall into four major subgroups: Euglenida, Kinetoplastida, Diplonemea and Symbiontida. Although the diversity and phylogenetic relationships of euglenids, kinetoplastids, and diplonemids are relatively well understood, our understanding of symbiontids remains poor, especially their phylogenetic position within the euglenozoan tree. Symbiontids are challenging to study because they occur in low-oxygen marine sediments and are rarely encountered. Only two species have been characterized at both molecular phylogenetic and ultrastructural levels, namely Calkinsia aureus and Bihospites bacati, and no cultures for members of this group have been established to date. A third species, Postgaardi mariagerensis, has only been characterized at the ultrastructural level using single-cell SEM and TEM. However, environmental DNA surveys of microbial diversity have shown that symbiontids are more diverse than just these three species, indicating that symbiontids play an understated role in low-oxygen ecosystems. The three species that have been reported so far are all enveloped by a dense layer of episymbiotic bacteria that are thought to be metabolically integrated with their symbiontid hosts (e.g., superficial layers of highly modified mitochondria). Major differences in the ultrastructure and episymbiont communities between the three known species suggests that uncharacterized symbiontids are reservoirs of novel cellular characteristics and interactions. Surprisingly, we were able to uncover cells of Bihospites and Calkinsia, a symbiontid previously isolated from deep sea sediments 500 m below the ocean surface, in an intertidal sample gathered from Quadra Island, British Columbia. Twelve cells of the known Calkinsia morphotype were individually isolated for single-cell transcriptomics along with nine cells displaying a similar but novel morphotype potentially representing a new species of Calkinsia. The transcriptomes will be used in multi-gene phylogenomic analyses to infer the position of symbiontids within the euglenozoan tree. Furthermore, we have identified a novel symbiontid in our sampling, displaying characteristics previously not defined within the group, such as a cytoproct-like opening at the posterior of the cell. These data are expected to provide insights into our overall understanding of ultrastructural evolution and symbiotic interactions within the group.

A first glance at the transcriptome of *Crepidoodinium cyprinodontum* provides key insights into the evolution of fish ectoparasites among dinoflagellates

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Dinoflagellates (Alveolata, TSAR) constitute one of the most physiologically and ecologically diverse groups of protists, including key primary producers in the global ocean, essential coral symbionts, but also a broad variety of parasites. Among the last of the aforementioned groups is an assemblage represented by three genera of fish ectoparasites, some of which are documented to cause massive fish kills, in particular in aquacultures. However, our current knowledge on this group includes mostly descriptions of their morphology and life cycles, revealing similarities on both accounts, but the available molecular data are limited to the recently published transcriptome of one marine species (Amyloodinium ocellatum) and the SSU rRNA sequence of one freshwater strain (Piscinoodinium sp.). To deepen our understanding of the evolution and lifestyle of these parasites, we obtained single-cell transcriptomic data from their least-studied representative - the ectoparasite Crepidoodinium cyprinodontum. Having reconstructed the SSU rDNA-based phylogeny of dinoflagellates, including the aforementioned parasitic taxa, we observed that each of the three genera is located in a different dinoflagellate family with moderate to high support. This observation suggests that despite the prevalence of parasitism among dinoflagellates in general, the fish-associated ectoparasitic lifestyle evolved at least three times independently and somewhat convergently. Furthermore, a preliminary reconstruction of the metabolic pathways in C. cyprinodontum has revealed an intact major part of plastid metabolism, including the photosynthetic apparatus. This observation holds particular importance, as not only the functions, but even the presence of plastid organelles in these lineages have thus far been uncertain. Future comparative analysis of C. cyprinodontum with the other fish ectoparasitic lineages, as well as their free-living relatives, will not only bring more important insights into the convoluted evolutionary paths of dinoflagellates, but will also help elucidate the persisting questions about the evolution of endosymbiotic organelles, parasite-host interactions, and physiological adaptations in microbial eukaryotes.

Dissecting marine picoeukaryotic genomes from single cells and metagenomes

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Retrieving genomic data from uncultured marine picoeukaryotes is still in its infancy, and so far the most profitable approach is the use of Single Cell Genomics. However, each single cell provides only a partial and random snapshot of the complete genome. One way to circumvent this limitation is combining the reads of several single cells from the same population, while another possibility would be to use metagenomic data from the same community. From a coastal sample in the Northwestern Mediterranean Sea, we produced a substantial collection of single-cell picoeukaryotic genomes and assessed the potential of simultaneously gathered metagenomic data to increase their completeness. Metagenomes were obtained from microbial biomass collected by filtration as well as by sorting phototrophic and heterotrophic picoeukaryotic cells by flow cytometry, which produced datasets dominated by eukaryotic genes. Here I will present the collection of novel and improved picoeukaryotic genomes generated by combining these different gene datasets. This new resource provides tools to improve our knowledge in biodiversity and microbial functions in the ocean and may contribute to a better understanding of genome evolution across the eukaryotic tree of life.

Physiology of ecdysis in dinoflagellates – presumably overlooked cyst type and directions of nutrient fluxes in the ocean

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Dinoflagellates represent a large eukaryotic lineage with many peculiar physiological and morphological features ranging from pronounced ability to mixotrophy to the organization and stressor-induced rearrangement of a cell wall. This cell-wall rearrangement, called ecdysis, was repeatedly observed in the laboratory and, in some cases, in natural systems, while its biological relevance remains unclear. It is thought to be linked to the production of various cyst types characteristic to dinoflagellates, but not much is known about the physiology of this process and a cell undergoing it. We studied stressor-induced ecdysis in the culture of Prorocentrum cordatum, a cosmopolitan armored dinoflagellate responsible for the occurrence of harmful algal blooms worldwide. We combined several techniques of cell biology, including stable isotope incubations followed by transmission electron microscopy (TEM), that allowed precise determination of the stage of cell covering rearrangement, and nanoscale secondary ion mass spectrometry (NanoSIMS) to characterize structural changes during ecdysis and acquisition of carbon and nitrogen by ecdysing cells. We demonstrated that ecdysing P. cordatum cells preserved their ability to take up bicarbonate and nitrate at unexpectedly high rates, even though they shed flagella, became immotile, and their transcriptomes revealed patterns typical to dormant stages of other eukaryotes, e.g. suppression of photosynthesis and changes in the expression of the abscisic acid metabolism genes involved in the control of seed germination in plants. At the same time, over 2000 annotated genes were upregulated, mostly reflecting the activation of cell signaling and cell covering maturation, i.e. genes involved in calcium and MAPK-dependent signaling, ionic and nutrient transport, cytoskeleton, cellulose and lipid metabolism. Cells of P. cordatum undergoing ecdysis lack cell wall structures typical to the well-known dinoflagellate cyst types, such as the pellicle, and are hard to distinguish from active vegetative cells at the level of light microscopy. We refer to them as to thecate cysts and assume they can be widely distributed in natural environment. Their ability to take up and accumulate nutrients while being relatively dormant can add complexity to our view of the nutrient fluxes, and be especially relevant during blooming events.

Infection of marine diatoms by *Pirsonia diadema*: a new model system to elucidate host-parasite interactions

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Parasitism has evolved multiple times independently across the eukaryotic tree of life, and studying host-parasite interactions in diverse eukaryotes is important to reconstruct how the transition from free-living to parasitic lifestyles takes place. A significant yet understudied group of parasites are the Pirsoniales, a lineage of stramenopiles that infect the bloom-forming diatom, Coscinodiscus. These parasites play a fundamental role in marine ecosystems by regulating diatom blooms yet the mechanisms and dynamics of this interaction are poorly understood. To study the Pirsonia-Coscinodiscus system we have collected isolates, established long-term cultures and have begun developing methodologies to study this interaction. Using PacBio long-read sequencing we have assembled high quality reference genomes for Pirsonia and Coscinodiscus in order to examine both parasite and host genome evolution. To complement this, we are conducting differential expression analyses to study gene expression changes of both parasite and host during infection. And finally, to probe the mechanism of the interaction we have developed a phenotyping assay based on high-throughput automated imaging that is capable of monitoring infection progression at a single cell level. I will be presenting preliminary findings from each of these directions.

Measuring protist population growth in intact soil environments using quantitative stable isotope probing (qSIP)

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Soil is a complex environment supporting thousands of species of bacteria, archaea, fungi, protists, and animals. The activity of these organisms drive global nutrient cycling; however, understanding population-level dynamics and linking them to ecosystem functioning remains a challenge. Quantitative stable isotope probing (qSIP) is a novel technique which allows for the quantification of population dynamics in situ, improving upon methods which require extraction and isolation of organisms from their native environment. By measuring the incorporation of an isotopic tracer into the DNA of growing organisms, taxon-specific population dynamics can be measured in complex systems where conditions such as habitat complexity, nutrient availability, and predator-prey dynamics remain intact. Here, we present relative growth rates of protists and metazoa in intact soil systems exposed to four different soil moisture contents (20%, 40%, 60%, 80% of field capacity), using H218O as a universal tracer. Surprisingly, amplicon sequencing of the V4 region of the 18S rRNA gene did not reveal significant differences in protist and metazoan richness nor the presence/absence composition between the water treatments. However, wetter soils did support faster growth of most taxa. For example, organisms from the family Leptophryidae (Cercozoa; Endomyxa; Vampyrellida) did not significantly differ in relative abundance between the moisture treatments, but increased soil water content stimulated their relative growth rates from 0.0057 day-1 in the lowest to 0.035 day-1 in the highest water treatment. Similarly, nematodes from the class Enoplea showed significant growth at 80% field capacity (0.01 day-1) though their relative abundance decreased with increasing water availability. As we try to better link terrestrial ecosystem functioning with organisms in the environment, methods such as qSIP will be vital in providing quantitative measurements of population dynamics in intact systems.

The eco-evolution of microbial eukaryotes: Insights from molecular environmental surveys

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Recent molecular dating analyses suggest that eukaryotes originated more than 2 billion years ago. Following this life-changing event, however, current evidence point to an initial slow diversification process, a period that is sometimes referred to as the “boring billion”. A critical question is thus, what evolutionary processes led eukaryotes to evolve into this incredible diversity that we observe today after the boring billion? In our project, we fit macro-evolutionary models into an ecological perspective to explore the diversification of eukaryotes through geological times at a broad phylogenetic scale. Uniquely, we integrated long-read environmental rDNA sequences along with phylogenetically curated references to access the majority of the known eukaryotic molecular diversity. We then combined this phylogeny of eukaryotic diversity, containing more than 70 000 OTUs, with 76 established fossil calibrations to date the eukaryotic tree of life and infer diversification patterns throughout the history of eukaryotes. While early eukaryotic diversification was initially slow, biotic interactions established the main groups that we observe today and set the basis for the later expansion. Archaeplastida was the most diverse supergroup during the Proterozoic possibly due to the establishment of an endosymbiosis with cyanobacteria. All major eukaryotic supergroups show abundant shifts in diversification rates over the Proterozoic indicating a high biological activity. Here we show that the “boring billion” was perhaps not so boring after all and that eukaryotes were diverse and abundant long before the second, and most pronounce, Great Oxidation Event.

Methanogenic archaeal symbionts of anaerobic ciliates are host- and habitat-specific

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Anaerobiosis has evolved multiple times in ciliates resulting in the transformation of their mitochondria to hydrogen-producing mitochondrion-related organelles and the acquisition of prokaryotic symbionts that uptake their metabolic end-products. Methanogenic archaea have been found in symbiotic interactions with anaerobic ciliates; however, little is known about their diversity. We studied the diversity of methanogenic archaeal symbionts of 54 strains of ciliates belonging to 33 species, mainly metopids, using Sanger and Illumina sequencing of the 16S rRNA gene. Autofluorescence, FISH, and TEM were also used to reveal the presence of methanogenic archaea and their distribution in the cytoplasm of the ciliates. The Sanger and Illumina amplicon sequencing demonstrated that these ciliates almost always harbor a single dominant methanogenic archaeon belonging to either genus Methanobacterium, Methanoregula, or Methanocorpusculum. There is a degree of host-symbiont specificity at the genus level, and ecologically, particular methanogen genera are restricted to ciliates from particular habitats, i.e., marine niches. Furthermore, co-cultivation experiments demonstrated that ciliates that harbor a particular methanogen do not exchange their symbionts over time when co-cultivated with another ciliate species that hosts a different methanogen.

Mitochondrial RNA editing functioning with diverse arsenal in a heterolobosean *Paravahlkampfia ustiana*

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To date, mitochondrial RNA editing events have only been documented in three Heterolobosea species: Naegleria gruberi and Acrasis kona perform C-to-U substitutions in mRNA, while heterolobosean sp. BB2 inserts guanosine residues in all types of mitochondrial RNA products (mRNAs, tRNAs, rRNAs). After assembling and annotating the mitochondrial genome of Paravahlkampfia ustiana, we discovered the existence of C-to-U substitutions in multiple mitochondrial mRNAs, as well as guanosine insertions in most mitochondrial mRNAs, in several tRNAs, and in both LSU and SSU rRNAs. In addition, a unique case of A-to-U substitution was identified in the T-loop of trnY(gua). Our survey of pentatricopeptide repeat (PPR) protein genes in the nuclear genome and transcriptome of P. ustiana discovered the presence of genes encoding DYW-type PPR proteins known for their cytidine deaminase function during C-to-U substitution events as well as their mRNA transcripts, which further reinforces the active status of the C-to-U substitution editing system. Interestingly, Paravahlkampfia ustiana, Naegleria gruberi, and Acrasis kona are the only members of Heterolobosea that possess these genes. However, putative PPR loci are present in diverse eukaryotic lineages, while genes encoding DYW-type proteins have only been identified in some organisms including Gefionella okellyi (Malawimonadida), Plasmodiophora brassicae (Rhizaria), and Physarum polycephalum (Amoebozoa).

Island isolation and elevation as drivers of soil protist endemicity

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MacArthur and Wilson's island biogeography theory, based on the observation of macroscopic plants and animals, predicts that species diversity declines and the rate of endemicity increases with decreasing island size and increasing distance to the nearest continent. This theory was latter applied to micro-organisms when the definition of island in ecology was extended to any type of isolated habitats (e.g., lakes, tree holes), but it has not yet been tested for soil protists over continental and oceanic islands. Here we present results of elevation gradients diversity analyses of soil protists in two continental mountain ranges and seven islands by soil eDNA high throughput sequencing. We hypothesized that the proportion of endemic taxa would increase i) with increasing distance to continents, following the classical island biogeography theory, and ii) but would be highest at low elevation (i.e., in forest vs. alpine habitats) due to the elevation gradient of wind-exposure controlling the dispersal potential of soil protists. The distance to the nearest continent appeared to be a strong driver of endemicity rate, which was significantly higher in the forest than in the alpine habitats. Most clades also displayed a significant linear increase in endemicity with distance to continent in forests, while only clades of larger protists such as Ciliophora and Imbricatea showed also this trend in the alpine habitats. These results support the idea of a higher dispersal ability and adaptability of smaller protists in alpine habitat, while most forest soils protists have restricted geographical distribution.

Functional diversity of microbial eukaryotes in a meromictic lake: coupling between metatranscriptomic and a trait-based approach

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The advent of high-throughput sequencing has led to the discovery of a considerable diversity of microbial eukaryotes in aquatic ecosystems, nevertheless the role of some of them (e.g. parasites or mixotrophs) remains poorly characterized especially considering freshwater ecosystems. Based on metabarcoding data obtained from a meromictic lake ecosystem (Pavin, France), we performed a trait-based approach to infer functional groups of microbial eukaryotes. Metatranscriptomic data were also analyzed to assess the metabolic potential of these groups across day/night cycle, size fraction, sampling depth and periods. Our analysis highlights a huge microbial eukaryotes diversity especially in the monimolimnion characterized by numerous saprotrophs expressing transcripts related to sulfur and nitrate metabolism as well as organic matter degradation. We also describe strong seasonal variations of microbial eukaryotes in the mixolimnion especially for parasites which dominate the microbial diversity (~20%) and mixotrophs. It appears that the stirring (occurring during spring and autumn) which benefits photosynthetic hosts communities also promotes parasitic fungi dissemination and over-expression of genes involved in zoospore phototaxis and stage transition in parasite cycle. Mixotrophic haptophytes over-expressing photosynthesis-, endocytosis- and phagosome-linked genes in November under nutrients limitations also suggest that phagotrophy may provide them an advantage over strictly autotrophic phytoplankton.

Competition between mixotrophic and heterotrophic ciliates under dynamic light conditions and prey supply

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Combining phototrophy and heterotrophy, two functionally different modes of nutrition, mixotrophic protists are able to use inorganic and organic carbon sources for biomass production. If limited by total cell resources, the investment in one nutritional mode would require the allocation of resources from the other. Based on this energetic trade-off, mixotrophs are often considered inferior to their purely heterotrophic and photoautotrophic counterparts concerning photosynthetic carbon fixation, inorganic nutrient acquisition and feeding. By supplementing or substituting photosynthetic energy supply under light or nutrient limitation, and heterotrophic energy supply during prey shortages, mixotrophic protists are thought to gain starvation resistance facilitating growth or survival under resource depletion. We investigated competitive interactions of heterotrophic and mixotrophic ciliates belonging to the genera Euplotes and Coleps in 48-day chemostat experiments. Same genus (C. het – C. mixo, E. het – E. mixo) and different genus (E. het – C. mixo, C. het – E. mixo) combinations were exposed to different resource regimes, providing algal prey (Cryptomonas) either continuously or in pulses under constant or fluctuating light, entailing periods of resource depletion in fluctuating environments, but overall providing the same amount of prey and light. Based on the above-mentioned assumptions, we expected heterotrophs in the chemostat system to become and remain dominant under constant prey supply due to their superior feeding traits, while starvation resistance should allow mixotrophs to coexist in low numbers. Pulsed prey supply should result in density oscillations of heterotrophs, whereas mixotrophic populations should remain stable and coexist during food shortages despite inferior feeding traits. While competitive dynamics of the same genus combination Coleps – Coleps were in line with the assumptions based on energetic trade-off and starvation resistance, competitive dynamics in the Euplotes – Euplotes combination were not. Here, the mixotroph dominated irrespective of light and prey supply. Different genus combinations were both Euplotes dominated. Our results do not support the assumption that mixotrophs are generally inferior to heterotrophs. Instead, competition between mixotrophic and heterotrophic ciliates in our experiment was strongly affected by functional trait differences of the species involved, which have the capacity to override possible energetic trade-off effects.

Parallel elaboration of contractile vacuole-associated membrane trafficking machinery in *Reclinomonas americana*

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The osmoregulatory contractile vacuole (CV) is one of the few ubiquitous eukaryotic organelles that has not been confidently inferred in the last eukaryotic common ancestor (LECA). For such organelles (e.g. flagella, Golgi, mitochondria), presence in the LECA has been inferred through a combination of morphological studies and comparative genomic analyses of characteristic genes. CVs, though near ubiquitous in freshwater protists, are generally absent from closely related marine or parasitic relatives. Additionally, CVs are poorly characterized from a molecular standpoint, in large part due to their absence from the typical cell biological models, and no exclusive molecular marker has been found in all organisms studied. The limited molecular data available from the protist CV models instead show a pattern of involvement of membrane trafficking proteins known from other organelles (e.g., Rab11, which is canonically involved in recycling endosome exocytosis). This combination of patchy distribution and lack of unique marker proteins, along with the substantial morphological diversity, makes the evolutionary history of CVs unclear—are they derived from a single organelle found in the LECA, or are they more recent cellular innovations? As part of a larger research program to examine CV evolution, I characterized the CV of Reclinomonas americana (Discoba) using microscopy, transcriptomics, and phylogenetics. Cultures of R. americana were exposed to low, control, and high osmolarities, which were then harvested for RNA for differential expression analysis. Transcripts of the membrane trafficking system that increased as osmolarity decreased were considered putatively CV-associated. Phylogenies of the associated proteins were then used as a proxy to investigate the evolutionary history of the organelle. Several common CV-associated proteins were detected as differentially expressed in R. americana; however, phylogenetics indicated that these were the result of lineage-specific expansions. In contrast to most common eukaryotic organelles studied from a comparative genomics perspective, we have not been able to recover a strong signal for a single origin of CVs in LECA. Rather, this work adds to a growing number of examples of parallel expansions in CV-associate membrane trafficking machinery.

Chitin and chitin-related factors in microbial protoplast feeders point to new roles of understudied biopolymers in protists

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Chitin, a polymer of N-acetylglucoseamine, is one of the most abundant polysaccharides in the environment. Together with its deacetylated form chitosan are these relatively resistant biopolymers best known for their structural and protective function in extracellular structures, e.g. in fungal cell walls, arthropod exoskeletons and protist cysts. Orciraptor agilis (Viridiraptoridae, Cercozoa) is a heterotrophic amoeboflagellate, which feeds on diverse freshwater green algae by perforation of the prey cell wall and subsequent phagocytosis of the protoplast. Comparative transcriptomic data of Orciraptor agilis revealed a large set of chitin-related proteins including a chitin synthase, putative chitin-binding modules (families CBM18 and CBM50), chitinases (families GH18 and GH19) and lytic polysaccharide monooxygenases (LPMOs). Interestingly, some chitin-binders and chitinases were clearly upregulated during the attack on the cells of zygnematophycean green algae, even though the latter are not known to contain chitin or related substances. With the application of fluorescent probes against chitin and chitosan, we demonstrate the presence of both biopolymers in Orciraptor agilis and provide evidence that this material is deposited on the algal prey cells during the feeding act. Our microscopic findings are supported by mass spectrometric analyses, which reveal a quite unusual composition of the potential chitin/chitosan co-polymer. We hypothesize that this co-polymer could act as an adaptor material that enables Orciraptor agilis to bind to the algal cell wall, due to the specific physico-chemical properties of the found biopolymer and by chitin-binding proteins. In our ongoing research, we aim to understand the interaction of chitin/chitosan co-polymers with algal cell walls and to characterize the putative chitin-binding proteins of Orciraptor.

Creating a freshwater dinoflagellate transcriptome database

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Dinoflagellates comprise a large and impactful portion of the aquatic microbial community due to their abundance, trophic niche and photosynthetic capabilities. Subsequent to the Marine Microbial Eukaryote Transcriptome Sequencing Project, many dinoflagellate transcriptomes were generated and available leading to a rapid expansion in the understanding of the genetic makeup and phylogeny of marine species. There is, however, a conspicuous absence of transcriptomic data for freshwater dinoflagellates, subtending a massive hole in the literature surrounding the diversity and role that dinoflagellates play in freshwater ecosystems. The evolutionary history of freshwater adaptations in dinoflagellates is also consequently obscured. Here, we use a combination of single cell isolation from locally collected environmental samples as well as RNA extractions from cultures from the Canadian Centre for the Culture of Microorganisms to generate transcriptomic data for several freshwater dinoflagellate species. Using these data, we generate a maximum likelihood phylogeny to resolve the placement of these taxa. This analysis confirms that the transition into fresh water has happened multiple times independently.

Babesia microti and *Babesia vogeli* in *Rhipicephalus turanicus* ticks (Ixodida: Ixodidae) from Israel

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Background: Rhipicephalus turanicus is a prevalent tick species in Israel and was found to be positive for bacterial and protozoan pathogens. The aim of this study was to identify Babesia pathogens found in questing ticks collected from all over the country.

Materials and Methods: Ticks were collected by flagging from 16 localities. Pools of ticks were first screened by conventional PCR for Babesia spp. using the primers BJ1 and BN2, and later by amplifying Babesia spp. target DNA region encoding the 18S rRNA region in protozoans.

Results: Overall, 124 adult specimens of R. turanicus ticks were collected by flagging and 43 pools were prepared. In one pool collected in northwestern part of the country (Hadera) Babesia microti and in another one collected in the Negev Desert area (Re'im) Babesia vogeli were detected.

Conclusions: To the best of our knowledge, B. microti is reported for the first time in Israel. Earlier, B. vogeli DNA was reported from questing R. turanicus ticks, and Babesia caballi DNA was found in R. turanicus collected from horses in the country

Plasmodium and beyond - haemosporidian parasites of a Malagasy bird population

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Haemosporida are important intracellular protozoan parasites. In addition to the genus Plasmodium, other genera also belong to this order: Haemoproteus and Leucocytozoon, among others. Whereas species of the order Plasmodium can be found in all vertebrate taxa, Haemoproteus and Leucocytozoon are specialized on birds. Basic knowledge of prevalence, diversity and impact of all avian haemosporidian parasites is still scarce. A long-term, large-scale molecular study of a bird population in Madagascar is providing new insights into the parasite population that exists there. An enormous number of unknown haemosporidian lineages were found, showing that Madagascar is not only a biodiversity hotspot for birds, but also for their parasites. Each haemosporidian parasite differs in its degree of host specialization and abundance. Understanding those parasite-host interactions is crucial to assess the impact of those parasites on the environment.

Phylogenomic analysis of adeleorina apicomplexans

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Our understanding of the major relationships in apicomplexan phylogeny has been revolutionized by transcriptomics and phylogenomics, including the establishment of new major lineages like Marosporida and Squirmida, and the realization that parasitism, plastid loss, and photosynthetic loss have evolved multiple times in the group overall. This work has now gone so far as to make genomic data available from all major subgroups of apicomplexans, except for one, the Adeleorina, a significant major group with expansive host range, but no generic data and conflicting results when present in single-gene phylogenetic analyses. To provide a baseline apicomplexan phylogeny containing all known major groups, including members of the Adeleorina, we obtained transcriptomes from two Adeleorina genera, Klossia and Legerella, and examined the phylogenomic relationship of Adeleorina to other major apicomplexan groups. Contrary to expectations, the adeleorinids branched completely outside of Coccidia, and instead emerged as sister to a clade composed of Coccidia, Haematozoa, Protococcidia, and Nephromycida, with Marosporida branching sister to this clade and Adeleorina. This position reveals clues about ancient common ancestors of these major apicomplexan lineages, and the evolution of a fully-intracellular life history.

Genetic diversity of Japanese *Toxoplasma* population based on genome-wide SNP analysis

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Toxoplasma gondii is a unicellular parasite that infects virtually all warm-blooded animals including human. Infections with T. gondii typically remains asymptomatic; however, it may lead to severe and disabling disease for children as a result of transplacental infection. The worldwide samples reported to date are genetically subdivided into 16 haplogroups (HGs), and some at least show differential pathogenicity to patients as well as mouse models. Unfortunately, fine-scale genotyping data from Asian countries other than China are still scarce, and thus the risk of severe symptoms when people exposed to T. gondii in everyday life in Japan is unclear. To uncover the fine-scale genetic structure of Japanese Toxoplasma population, we performed high-throughput sequencing of 12 Japanese isolates sampled from Hokkaido to Okinawa, one of which is a clinical isolate obtained from a patient of congenital toxoplasmosis. The result shows that the most isolates have genetic background unique to Japan; many isolates from Okinawa belong to a novel haplogroup, and other isolates are a similar but distinct subtype of HG2. There were only two isolates of HGs 2 and 3, which characteristics are well known to date. Our results suggest that the present knowledge accumulated for European and American isolates is not sufficient to deduce the pathology of Japanese isolates. The risk of severe symptoms for these isolates is incomparable to those in European and American isolates and remains to be elucidated. Further sampling from Japan and neighboring countries is required to understand the genetic diversity of Asian Toxoplasma population and their pathological characteristics.

Phenotypic analysis of *Plasmodiophora brassicae* infection in various *Arabidopsis arenosa* genotypes: Discovering similarities and dissimilarities in the parasite-host relationship

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Plasmodiophora brassicae is an obligate biotrophic parasite within the class Phytomyxea (Rhizaria). The pathogen causes clubroot disease in brassicas, resulting in reduced plant growth and gall formation in roots. Here we tested how eight different genotypes of Arabidopsis arenosa react to an infection with P. brassicae via assessing pathogen phenotype, above ground plant hormone levels and by calculating the disease index and disease severity index. Four different types of interactions between the pathogen and host were discovered after the A. arenosa genotypes were infected. All genotypes showed typical gall formation, whereas a variation in infection rate and intensity was found. Additionally, marked differences in the expression of above ground disease symptoms were observed between the genotypes. In this study, the phenotypic reaction of A. arenosa varies depending on the genotype supporting the hypothesis of a natural variation in the defense response within a species. The pathogen phenotypes of tested A. arenosa genotypes range from heavily infected roots and detectable shoot symptoms to a weaker clubroot development along with less symptomatic aerial parts. Three genotypes of A. arenosa showed no significant impact on the upper plant part although pronounced gall formation was induced by P. brassicae. No clear correlation of disease symptoms with the polidy of the hosts, the ecotype or the geographical origin could be identified. Genetic diversity can influence plant response to pathogens and A. arenosa could be the ideal choice for exploring the pathogen-host dynamics of P. brassicae in greater depth.

Losing photosynthesis and the adaptation to heterotrophy

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Across the eukaryotes, many organisms have lost the ability to carry out photosynthesis. This transition to heterotrophy requires a dramatic remodelling of biochemical cycles within the cell. Using the dinoflagellate species Symbiodinium microadriaticum, we have evolved in the laboratory a number of strains which are reliant on glucose and no longer reliant on photosynthesis. Symbiodinium has a fragmented chloroplast genome, with genes on individual plasmid-like minicircles. We have characterized a number of glucose-requiring Symbiodinium strains, and have shown that they have lost key minicircle-encoded genes, encoding proteins involved in photosynthesis. The photosynthetic ability of these strains is fundamentally changed, as shown by RNA-seq analysis, genome sequencing and in vivo analysis of photosynthesis. Photosystem II activity is impaired to various extent in these strains. We will discuss the implications of our findings, and the consequences of gene loss on the transition to heterotrophy.

First molecular evidence of hybridization in endosymbiotic ciliates (Protista, Ciliophora)

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Hybridization is an important evolutionary process that can fuel diversification via formation of hybrid species or can lead to fusion of previously separated lineages by forming highly diverse species complexes. We provide here the first molecular evidence of hybridization in wild populations of ciliates, a highly diverse group of free-living and symbiotic eukaryotic microbes. The impact of hybridization was studied on the model of Plagiotoma, an obligate endosymbiont of the digestive tube of earthworms, using various phylogenetic methods and multidimensional morphometrics. Phylogenetic analyses indicated that gene flow slowed down and eventually hampered the diversification of Lumbricus-dwelling plagiotomids, which collapsed into a single highly variable biological entity, the P. lumbrici complex. The distribution of the species boundaries was also suggested by conspicuously increased diversity in the nuclear rDNA cistron and the host structural specificity of the P. lumbrici complex was also weakened. On the other hand, the other detected species P. aporrectodeae showed no signs of introgression, no variability in the rDNA cistron, and very high host specificity. These contrasting eco-evolutionary patterns indicate that hybridization might decrease the alpha-diversity by dissolving species boundaries, weaken the structural host specificity by broadening ecological amplitudes, and increase the nuclear rDNA variability by overcoming concerted evolution within the P. lumbrici species complex.

Zoosporogenesis of chromerids and different fates of zoospores

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Chromerids, algae with a complex rhodophyte-derived plastids, are the closest known photosynthetic relatives of apicomplexan parasites. The two chromerids formally described to date, Chromera velia and Vitrella brassicaformis, differ significantly in their morphology, life cycles, and genomes. While Chromera lives in a simple asexual life cycle, Vitrella develops in a complex cycle that includes zoospore fusion. However, both algae produce zoospores that appear to have different fates. Chromera zoospores develop in small numbers in zoosporangia that resemble the cysts of the related colpodellids. The zoospores are rapidly motile, contain a single large chloroplasts and are repelled by light. Their production is controlled by the two endogenous factors, circadian rhythm and yet unspecified chemical signal, and by exogenous light. They probably serve to disperse Chromera in the environment and possibly infect coral larvae. We see rhythmicity of zoosporogenesis in C. velia even for two days under constant dark conditions. Light induction of zoosporogenesis is mediated exclusively by blue light in the chromerid. Consequently, we identified six cryptochrome-like genes, two genes possibly related to CCA/LHY, whereas we did not find a homolog of any animal, cyanobacterial, or fungal circadian clock gene. The second chromerid, V. brassicaformis, produces two types of zoospores, both in dozens per sporangium. The first type develops from the multinucleate mother cell by budding and already has flagella in the sporangium. The second type contains intracytoplasmic axonemes and expels flagella when the spores are released into the environment. Sporogenesis in Vitrella does not appear to be affected by light, and zoospores fuse during the sexual part of the cycle. Despite the lack of rhythmicity in zoosporogenesis in V. brassicaformis, we have identified five of six cryptochromes from C. velia here. Based on these results, we hypothesise a different fate of zoospores in chromerids. While the zoospores in C. velia serve to disseminate and infect the host, the zoospores in V. brassicaformis function as gametes.

Single-cell transcriptomics of *Hatena arenicola* and its symbiont

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Endosymbiotic plastid acquisition (EPA) drives the evolution of photosynthetic organisms. EPA happened multiple times and resulted in today's diverse plants and algae. Current cases of endosymbiosis between a heterotrophic protist and its photosynthetic partner give us insights into the cellular process during the past EPAs. The symbiotic relationship between Hatena arenicola and its partner Nephroselmis sp. is one such case. At each cell division, the symbiont is inherited only by one of the host daughter cells; meanwhile, the other daughter cell acquires the symbiont from the environment to re-establish the symbiosis from scratch. This symbiosis would give us an insight into the cellular process of the early stage of EPA. We collected H. arenicola cells with the symbiont that shows the various degree of integration to conduct single-cell transcriptomic analysis to investigate the changes in gene expression in the host and the symbiont.

Integrative taxonomic data exploring ciliate diversity and community structure in Rift Valley and marine aquatic ecosystems of Kenya and Lake Mondsee in Austria

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Ciliates are excellent indicator protists for water quality assessment. However, ciliate communities on submersed plants and stones from lake shorelines are relatively unknown. Therefore, we here integrated morphological and molecular datasets of several ciliate species to present an informative and reliable taxonomic approach on studying ciliate diversity and community structure in Rift Valley dam, river, lakes and marine ecosystems of Kenya and a freshwater lake in Austria. Live observation, morphometric measurements and protargol staining were used for revealing morphological details of the ciliates. Moreover, their molecular sequences (ribosomal operon, ITS) were investigated. The most abundant ciliates belonged to the Oligotrichida, the Hymenostomatia, the Haptorida, the Colpodea, and to the Peritrichia. According to this integrated approach, we were able to determine the water quality of several water bodies based on ciliates as indicators.

Progress in taxonomy and phylogeny of the pleurostomatid ciliates (Ciliophora, Litostomatea, Pleurostomatida) in China

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Pleurostomatid ciliates are predatory and widespread microbial eukaryotes in various aquatic habitats. This group is clearly outlined as a monophyletic lineage and can be easily recognized by its bilaterally compressed body, the anterior ventral margin located oral slit, and the bristle-like cilia of the left kineties. However, it is one of the most confusing groups in the ciliate class Litostomatea. The main reason is that this group is underexplored and most members have a similar body shape. In addition, the type of each genus is not sufficiently studied with integrative methods, which impedes the clarification of species affiliations. Previous investigations in China already reveal that the diversity of the Pleurostomatida is underestimated. Our recent studies discovered 12 new species, and phylogenetic analysis based on SSU rRNA gene sequences indicate part of them represent new lineages: 1) we established a new family, Paralitonotidae Zhang et al., 2022, for Paralitonotus Zhang et al., 2022, Pseudolitonotus Wu et al., 2021 and Apolitonotus Pan et al., 2020, with the diagnostic character that the right somatic kineties are distinctly shortened in the anterior portion; 2) Heterolitonotus rex gen. nov., sp. nov., also exhibits some special characters and is positioned in a peripheral branch in SSU rRNA gene trees of Pleurostomatida, indicating it is likely another new lineage; 3) Novilitonotus clampi gen. nov., comb. nov. (basionym Protolitonotus clampi Pan et al., 2020) is divided from Protolitonotus. After new amphileptid ciliates are sequenced, the ectocommensal genus Pseudoamphileptus is monophyletic, whereas the genus Amphileptus is still paraphyletic.

Parasites of parasites: the diversity of metchnikovellids in marine gregarine apicomplexans

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Microsporidians are a highly diverse group of obligate intracellular parasites that mainly infect metazoan hosts. Metchnikovellids are early branching microsporidia that are exclusively found in gregarine apicomplexans as hyperparasites (i.e., parasites of parasites). Currently, about 30 species are known from three genera, most of which were described before the early 1900s. While crucial for understanding the evolution of microsporidia, only a few small subunit ribosomal RNA (SSU) sequences are currently available, and genomes have been sequenced for only two genera, Metchnikovella and Amphiamblys. With extensive sampling efforts, we visually examined marine gregarines from diverse polychaete hosts for metchnikovellids along the complex coast of British Columbia, Canada. We obtained transcriptomes and genomes of 7 new metchnikovellid species using single-cell isolates of infected single gregarines. In addition, we detected 3 metchnikovellid species from previously obtained transcriptomic and genomic data of gregarines. In total, we obtained genetic data for 10 different metchnikovellid species, including two Amphiacantha species, from diverse gregarine genera. Molecular phylogenetic analysis of SSU rDNA sequences showed several major lineages of metchnikovellids, but could not resolve relationships among them. Therefore, we inferred a multigene phylogeny from transcriptomic and genomic data to resolve deeper relationships among metchnikovellids. We discuss the overlooked diversity of the group, patterns of host specificity, and the need for taxonomic revision.

MRO-logy of Archamoebae species: Comparative analysis of mitochondrion-related organelles in anaerobic amoebozoans

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Archamoebae is a group of amoeboid protists with free-living or endobiotic life strategies, all inhabiting anaerobic or microaerophilic environments. Instead of aerobic mitochondria, they house mitochondrion-related organelles (MRO) of various metabolic capacities. In this study, we compared predicted MRO proteomes of eight species (six genera) and proposed the scenario of their evolution. The common ancestor of Archamoebae likely possessed a reduced/divergent protein translocation machinery similar to that in extant species. On the other hand, the MRO metabolic capacity decreased lineage-specifically. The glycine cleavage system is widely conserved among Archamoebae, except in Entamoeba, probably owing to its role in catabolic function or one-carbon metabolism. Pyruvate metabolism was disposed of in Entamoebidae and Rhizomastixidae lineages, and the sulfate activation pathway was lost in three isolated cases - Rhizomastix libera, Mastigamoeba abducta, and Endolimax sp. A bacterial NIF-type of the Fe-S cluster assembly system was acquired through lateral gene transfer in the common ancestor of Archamoebae and duplicated in the common ancestor of Mastigamoebidae and Pelomyxidae, with one copy participating in Fe-S assembly within MRO. In Entamoebidae and Rhizomastixidae, dual localization of the system in the cytosol and the MRO may in principle allow Fe-S cluster assembly in both compartments. We could not find evidence for changes in the MRO metabolic functions in response to the transition to an endobiotic lifestyle, suggesting that environmental drivers do not strongly affect MRO reduction in this group.

Novel Anaeramoebae strains exhibit an unexpected variety of symbioses with prokaryotic organisms

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Anaeramoebae is a recently recognized phylum of anaerobic marine amoebae and amoeboflagellates belonging to the Metamonada supergroup. So far, six species of the genus Anaeramoeba have been described on the basis of the light-microscopic morphology and sequences of SSU rRNA gene. Here we present three new strains of Anaeramoeba representing two species. At least one is novel and has by far the smallest cell among Anaeramoebae. The representatives of the genus Anaeramoeba are known to live in a syntrophic relationship with prokaryotic symbionts. Recently was shown that the symbionts are sulfate-reducing Desulfobacter-related bacteria, which were acquired at least twice independently within the Anaeramoeba lineage. Although they were considered endosymbionts, they likely reside within deep cell membrane invaginations and communicate with the outer environment. Research of our new strains revealed two new types of symbiosis with prokaryotes, and it is showing a significant variability within the Anaeramoeba species and their symbionts.

2D approach to reconstruct the evolutionary history of clevelandellids (Ciliophora, Armophorea) inhabiting the hindgut of the Panesthiinae cockroaches

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The ciliate order Clevelandellida unites endosymbionts of the digestive tract of a variety of invertebrates and vertebrates. The primary and secondary structures of nuclear and hydrogenosomal rRNA molecules were employed to reconstruct the phylogenetic relationships and to estimate the divergence times of clevelandellids inhabiting the hindgut of the Panesthiinae cockroaches. The secondary structure information was incorporated in phylogenetic analyses using two different strategies: indirectly through 2D-guided alignments and directly through so-called pseudo-protein data. Nuclear and hydrogenosomal markers carried a consistent phylogenetic signal and robustly supported the monophyletic origin of the family Clevelandellidae as well as of its four genera. According to Bayesian relaxed molecular clock analyses, the last common ancestor (LCA) of the family Clevelandellidae very likely emerged during the Late Cretaceous in the Oriental region. Its descendants most likely expanded to Australia in concert with the Neogene colonization and radiation of their host Panesthiinae cockroaches. Taking into account the time-calibrated phylogenies and the fact that early branching members of the order Clevelandellida inhabit the digestive tract of amphibians, it is tempting to speculate that the LCA of the Clevelandellida evolved in ectothermic vertebrates. Amphibians could have brought clevelandellids to the land, where they may have been transmitted to the cockroach digestive tract upon feeding on amphibian feces.

Characterisation of the SUF FeS cluster machinery in the amitochondriate eukaryote *Monocercomonoides exilis*

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Monocercomonoides exilis is the first eukaryotic organism described as a complete amitochondriate, yet it shares common features with heterotrophic anaerobic/microaerophilic protists, some of which bear divergent mitochondrion-related organelles or MROs. It has been postulated that the retention of these organelles stems from their involvement in the assembly of essential cytosolic and nuclear FeS proteins, whose maturation requires the evolutionarily conserved mitochondrial ISC and cytosolic CIA machineries. The amitochondriate M. exilis lacks genes encoding the ISC machinery yet contains a bacteria-derived SUF system (MeSuf), composed of the cysteine desulphurase SufS fused to SufD and SufU, as well as the FeS scaffolding components MeSufB and MeSufC. Here, we show that expression of the M. exilis SUF genes, either individually or in tandem, can restore the maturation of the FeS protein IscR in the Escherichia coli double mutants of ▲sufS iscS and ▲sufB ▲iscUA. In vivo and in vitro studies indicate that purified MeSufB, MeSufC and MeSufDSU proteins interact suggesting that they act as a complex in the protist. MeSufBC can undergo conformational changes in the presence of ATP and assemble FeS clusters under anaerobic conditions in presence and absence of ATP in vitro. Altogether, these results indicate that the dynamically interacting MeSufDSUBC proteins may function as an FeS cluster assembly complex in M. exilis thereby being capable of replacing the organelle-enclosed ISC system of canonical eukaryotes.

Spatial proteomics of the free living anaerobic protist *Paratrimastix pyriformis*

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The loss of mitochondria in oxymonad protists has been associated with the redirection of the essential Fe-S cluster assembly to the cytosol. Yet as our knowledge of diverse free-living protists broadens, the list of functions of their mitochondrial-related organelles (MROs) expands. We revealed another such function in the closest oxymonad relative, Paratrimastix pyriformis, after we solved the proteome of its MRO with high accuracy using Localisation of Organelle Proteins by Isotope Tagging (LOPIT). The newly assigned enzymes connect to the glycine cleavage system (GCS) and produce folate derivatives with one-carbon units and formate. These are likely to be used by the cytosolic methionine cycle involved in S-adenosyl methionine recycling. The data provide consistency with the presence of the GCS in MROs of free-living species and its absence in most endobionts, which typically lose the methionine cycle and, in the case of oxymonads, the mitochondria.

Isolation and molecular identification of *Vermamoeba vermiformis* strains from environmental samples in Castilla y León, Spain

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Free-living amoebae (FLA) are ubiquitous protozoans that are widely distributed in the environment including water, soil, and air. Although the amoebae of the genus Acanthamoeba are still the most studied, other species, such as Vermamoeba vermiformis are the subject of increased interest. Worldwide, it has been isolated from natural freshwater reservoirs, tap water, swimming pools and hospital environments. On the other hand, numerous cases have been reported where this amoeba can be isolated from keratitis. Furthermore, V. vermiformis have been described in many occasions as the most common carriers of pathogens of high medical relevance such as Legionella pneumophila and Mycobacterium spp. In this study, the presence of this amoeba was evaluated in water and soil samples from the autonomous community of Castilla y León. The water samples were subjected to the membrane filtration technique, while soils were grown directly on plates of non-nutritious 2% agar (ANN). Both samples were incubated at room temperature and monitored daily for the presence of this free-living amoeba. Molecular characterization was carried out by amplifying the 18S rDNA gene and DNA sequencing, confirming that the isolated strains belonged to Vermamoeba vermiformis species. From the total 71 samples collected, Vermamoeba vermiformis was isolated from 9 water samples and 3 soil samples. To the best of our knowledge, this is the first report on the presence of FLA in environmental sources in Castilla y León. Moreover, the two strains isolated in this study were collected in recreational areas with close contact with humans and thus awareness should be raised.

The evolution of characteristic AGP-glycosylation during the plant terrestrialization process

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Charophyte green algae (CGA) are assigned to be the closest relatives of land plants and can therefore help to enlighten crucial processes in colonization of terrestrial habitats. As arabinogalactan proteins (AGPs) are considered common for all land plant cell walls, the question when these special glycoproteins evolved in plant kingdom is in the focus of our research. With an analysis of available genomic and transcriptomic data from several plant species within the green lineage, we were able to show that AGP protein backbones seem to have evolved prior to characteristic AGP glycosylation. Carbohydrate attachment seem to have occurred firstly within the group of CGA. Our investigation therefore focussed on a number of algae from the Charales order, as well as on Spirogyra pratensis (Spirogyrales). AGPs were isolated via the use of β-Glc-Yariv reagent and their composition and fine-structure analysed by GC-FID/MS and AGP antibodies. Interestingly, no AGPs precipitated and no hydroxyproline was detected in all investigated members of the Charales. Within the Spirogyrales, the absence of arabinose and the presence of rhamnose side-chains (= RGP), together with occurrence of an AGP-like galactan backbone in Spirogyra Yariv-precipitates lead to the concept of a conserved galactan backbone structure with more flexibility in the decorating sugars.

Fatty acid desaturases expression in *Euplotes focardii* as evolutionary adaptation to the Antarctic environment may expose this ciliate to higher risk under organic pollutants contamination

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Fatty acid desaturases (FADs) catalyse the production of polyunsaturated fatty acids by inserting double bonds into the fatty acyl chain of membrane phospholipids. The cell ability to acclimate to low temperatures is explained, at least in part, by the capacity to desaturate the fatty acids (FAs) for adjusting the fluidity of the membranes to the new cold environment. Several studies have shown that Antarctic algae and fishes upregulate FADs expression under low temperature (He et al. Biotech, 2019; Lu et al. Extremophiles, 2009; Palmerini et al. J Membr Biol, 2009). In ciliates, the incorporation of unsaturated FAs into the lipid bilayer increases its fluidity to allow different membrane functions, including regulation of ciliary motility. In Tetrahymena thermophila the importance of FADs in the regulation of cold adaptation processes has been demonstrated (Sanchez Granel et al. Biochimica et Biophysica Acta (BBA), 2019). The Antarctic Euplotes focardii is a validated model organism to study adaptation to the cold (Mozzicafreddo et al. Scientific Reports, 2021). Our interest in the E. focardii FADs emerged by an analysis of its response to plastic pollutants such as polystyrene nanoparticles (PS-NPs) and derivatives like Bisphenol A (BPA), that were assayed at concentrations comparable to those found in the environment, including the Antarctic seawater. E. focardii appeared much more sensitive to the pollutants with respect to E. crassus by both toxicity tests and microscopical observations, showing a higher level of cell damages, probably due to a higher penetration of pollutants at membrane level. We are currently investigating the expression profiles of four E. focardii FAD genes (annotated as three fatty acid desaturases and one polyunsaturated fatty acid delta-5-desaturase) and of their homologs in E. crassus at variable temperatures and concentrations of PS-NPs and BPA. We are also performing in silico structural comparisons of FADs identified by genomic data in Euplotes and other ciliates. Our working hypothesis is that changes in FADs' gene expression and structure protect E. focardii at low temperature by increasing the membrane fluidity, but this condition exposes the ciliate to a higher risk in the presence of new organic pollutants such as BPA.

Amplification of exogenous dsRNA trigger by RNA dependent RNA polymerases

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RNAinterference (RNAi) by exogenous RNA is a widespread mechanism among many different species and involves the specific down-regulation of genes in a homology-dependent manner. The exogenous trigger RNA is usually double stranded (dsRNA) and is believed to be processed by Dicer into primary small interfering RNAs (1°siRNAs). Those siRNAs can attack transcripts, causing their degradation and hence, phenotypical down-regulation of the target gene. In Paramecium tetraurelia, several of the key enzymes involved in this pathway have been identified. While the involvement of some enzymes in the biogenesis and function of the 1°siRNAs is not surprising, like Dicer1, the necessity of RNA-dependent-RNA-Polymerase (RdRP) activity remains unknown. Usually, the function of RdRPs is the synthesis of a complementary strand using a single-stranded RNA as a template. Due to the intitial trigger RNA already being double-stranded, this function of RdRPs in this context seems counterintuitive at first. In this work, we focus on the specific function of the involved RdRPs in the biogenesis of 1°siRNAs. For this, we produced a heteroduplex dsRNA with mismatches to the endogenous targets by in vitro transcription. In addition, we established the stable formation of Dextran-dsRNA nanoparticles for packaging of this dsRNA. To allow for efficient phagocytotic uptake, the particles were loaded onto bacteria. Our data suggest initial activity of RdRPs on the trigger dsRNA due to the presence of antisense siRNAs containing mismatches specific for the sense dsRNA-strand, accumulating antisense-directed siRNAs from both strands preferably. Additionally, we observe poly-uridylation of some smallRNA subspecies. This specific uridylation of some smallRNA subspecies might also play a role in the processing of the trigger dsRNA by RdRPs. Our results indicate that RdRPs process the initial trigger of exogenous RNA: this could be due to a mechanism of trigger amplification or may represent a mechanism that allows dissecting self and non-self RNAs preventing the immediate addition of exogenous RNA to the endogenous RNAi pool.

Never-ending story – intriguing trophic levels in pelagic microbial food webs

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The functional diversity of pelagic protistan predators is conceptually well recognized but the trophic role of ecologically relevant groups is little known. The current model of microbial food webs, proposed in the 1980s, assumes almost linear energy and carbon flow from bacteria via bacterivorous nanoflagellates (NF) to microzooplankton and higher trophic levels while growing experimental evidence showed that the NF feed also on other eukaryotes. Moreover, it is unknown to what extent the NF community is bottom-up or top-down controlled. Here, we conducted a size fractionation experiment on a native microbial community from the Baltic Sea and a freshwater reservoir to disentangle the role of NF in contrasting pelagic microbial food webs. Our results indicate strong top-down control in the Baltic Sea, mostly by ciliates in spring and ciliates and dinoflagellates in autumn. In freshwaters, prostomatid ciliates top-down controlled the NF's spring community compared to the prominent role of prostomatids and scuticociliates in autumn. Adding bacterial prey induced rapid growth of bacterivores (cryptomonads, Paraphysomonas in spring, cryptomonads, Spumella and kinetoplastids in autumn), followed by an increase in predatory and omnivory NF (MAST-2, Mataza and kathablepharids) in the Baltic Sea. In the reservoir, bacterial additions promoted the rapid growth of aplastidic cryptomonads and Cry-1 in spring and autumn, and of kinetoplastids and perkinsozoa in autumn. Predatory katablepharids and cercozoan, as well as bacterivorous or detritivorous ciliates, increased towards the later part of the experiment in both seasons. Our results enhance the understanding of microbial food webs both in marine and freshwater, allowing for fine-tuning of the novel model of pelagic processes in these environments.

Anaerobic scuticociliates: A cosmopolitan lineage of anaerobic ciliates hosting diverse prokaryotic symbionts

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Anaerobic ciliates are predominant bacterivores in oxygen-depleted environments, playing an indispensable role in microbial food webs. Importantly, most if not all anaerobic ciliates harbor prokaryotic symbionts and thus represent a particular habitat themselves. To study this phenomenon, we have chosen anaerobic scuticociliates (class Oligohymenophorea), a cosmopolitan ciliate lineage with largely underexplored diversity in anoxic habitats. Broad sampling of both marine and freshwater anoxic habitats together with the combination of both single-cell and cultivation-based approaches enabled us to study anaerobic scuticociliates and their prokaryotic symbionts in detail. Symbiotic associations between anaerobic scuticociliates and prokaryotes can be quite complex. Symbionts can be found as ecto- and/or endosymbionts and multiple prokaryotic lineages can be found in a single strain. In freshwater anoxic environments, methanogenic Archaea are the prevailing symbionts of scuticociliates. In marine anoxic habitats, sulfate-reducing deltaproteobacteria seem to be the common symbionts but the prokaryotes able to enter symbioses with marine scuticociliates are much more diverse. Here, we present a first study investigating multiple strains of anaerobic scuticociliates and using the combination of morphological methods, fluorescence in situ hybridization, electron and confocal microscopy, and microbiome sequencing to explore the ecologically important symbiotic interactions of both marine and freshwater representatives of this ciliate lineage.

Rediscovery of remarkably rare anaerobic tentaculiferous ciliate genera *Legendrea* and *Dactylochlamys* (Ciliophora: Litostomatea)

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Free-living anaerobic ciliates are of considerable interest from an ecological and an evolutionary standpoint. Extraordinary tentacle-bearing predatory lineages have evolved independently several times within the phylum Ciliophora, including two anaerobic litostomatean genera, Legendrea and Dactylochlamys. Both genera are considered extremely rare since their first descriptions more than 100 years ago (a once in a lifetime observation for most ciliatologists) with only a few published reports and only a few website images and videos. We found and studied all three valid species of the genus Legendrea and the only species of the genus Dactylochlamys. We offer the first morphological and enhanced phylogenetic characterization of these two poorly known groups of predatory ciliates. We provide the first phylogenetic analysis of the monotypic genus Dactylochlamys and the three valid species of Legendrea based on 18S-ITS-28S rRNA gene sequences. Prior to this study, neither group had been studied by silver impregnation methods. We identified methanogenic archaeal symbionts of both genera and a bacterial symbiont related to Syntrophaceae from Legendrea using Illumina sequencing, and fluorescence in situ hybridization to corroborate presence of the symbionts. We also obtained a unique video material including documentation, for the first time, of the hunting and feeding behavior of a Legendrea species.

A pilot study of the transcriptomic response triggered by the bacterial infection in *Paramecium*

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Preeria caryophila is the infectious bacterium with a complex lifecycle that belongs to the family Holosporaceae (Alphaproteobacteria). These bacteria colonize macronucleus of several Paramecium species, among them representatives of the P. aurelia complex. The associations may be very stable, though Preeria, apparently, parasitise on ciliates. The symbiotic system Paramecium-Preeria is advantageous to study molecular cross-talk and interactions of the partners during infection. Autogamy, a regular specific sexual process resulting in self-fertilization, makes P. aurelia strains completely homozygous, thus facilitating genetic dissection of the symbiotic system. We studied in detail the first days of Preeriacaryophila infection in two species of the P. aurelia complex. The first single bacteria appear in the host macronucleus within 1-1.5 h after entering the ciliate cell with food, but mostly they remain in the food vacuoles for 24-60 h, taking the phagosomes, where they happen to reside, out of cyclosis. Only after 72 h the infection starts to develop faithfully in the macronucleus. We prepared the time-scale series of total RNA samples for the P. biaurelia strain experimentally infected with Preeria caryophila. The transcriptome sequencing of the samples taken at the starting point, 2 h after adding homogenate containing infectious bacteria to the recipient cells, and 26 h after the start of the experiment, in parallel with the control set of samples, was performed. We documented certain changes in P. biaurelia transcriptomes at the 2 h post-infection point, and a strong transcriptomic response 26 h post-infection, the numerous up- and downregulated genes are currently under analysis. We also obtained the first evidence that small RNAs may be involved in Paramecium response to the bacterial infection. Numerous clusters of noncoding small RNAs were differentially expressed after 2-6 h from the start of infection, and majority of these clusters were activated upon experimental infection. Preferentially, small RNAs were produced from the coding sequences of the Paramecium genome. The functions of these coding regions remain mostly unknown.

Unraveling *Paramecium* diversity by integrative taxonomy

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Ciliates are a remarkable group of protists where speciation is actively ongoing, and numerous morphological, cryptic, and genetic species may exist in each genus. Paramecium, one of the model ciliates studied for almost 250 years, is distributed in fresh and brackish waters all over the planet except Antarctica. Many Paramecium species were described by morphological features in former times, later molecular phylogenetic studies confirmed most of them, simultaneously putting some under question. The representatives of some clades in the molecular phylogenies have never been checked morphologically, thus suggesting certain hidden diversity within the genus. However, up to recent it was believed that most of Paramecium diversity was already known, and some yet undescribed species might lurk in remote understudied regions of the world. Nevertheless, in the last decade three new Paramecium morphological species were discovered (two of them, P. buetschlii and P. lynni, were sampled in Europe), one neglected morphological species, P. chlorelligerum, was found in Europe and redescribed, and at least two cryptic species were distinguished (one of them, P. fokini, is also present in Europe). Our group took part in several of these studies. In parallel, with our sampling field expeditions we tried to evaluate Paramecium diversity in earlier understudied regions, such as Mexico and Thailand, where we found one new sibling species of the P. aurelia complex, and one cryptic species related to P. caudatum. In our work we follow the principles of integrative taxonomy, applying thorough morphological examinations on big representative sets of strains, performing multi-loci molecular phylogenetic analysis, and, when possible, testing reproductive barriers among the related groups of strains. In my talk I will provide an overview of the state-of-the-art in the systematics of Paramecium. Currently the genus includes 16morphological species, among them P. aurelia and P. bursaria are recognized as the species complexes, and also 2 cryptic species. The special focus of my presentation will be on the Cypriostomum subgenus of Paramecium, where we recently found two more new species (again in Europe) and clarified relations and status of the previously known species attributed there.

A new deep-branching lineage of predatory flagellates confirms the relationship between Pirsoniales and oomycete parasites (Stramenopiles)

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Stramenopiles define one of the largest and most diverse lineages among eukaryotes, including free-living heterotrophic flagellates, various photosynthetic groups (brown algae, xantophyceae and diatoms), and important parasites (oomycetes, Blastocystis). However, the phylogenetic relationships of major groups within stramenopiles are poorly resolved, due to both lack of massive sequence data, especially for heterotrophic representatives, and lack of data on yet undescribed diversity of stramenopiles, including many predicted by metagenomic studies (e.g., many MAST lineages). In this study, we have isolated a new divergent strain of predatory flagellates, Colp-PM2, which feeds on the unicellular red algae Erythrolobus coxiae. To resolve its phylogenetic position, we generated transcriptome data for this organism as well as two new strains of predatory flagellates belonging to the Pirsoniales (Stramenopiles, Bigyromonada). Maximum likelihood phylogenomic trees based on 303 conserved markers placed the new strain Colp-PM2 at the base of Pirsoniales, forming the sister group to the Oomycetes. Morphological observations show similarities of this strain with the typical oomycete zoospores, including swimming patterns and cell morphology and, notably, the ability to self-aggregate. The phylogenetic position and morphological features of this new organism suggest that it has kept several ancestral features of both oomycetes and Pirsoniales. Its characterization will help to understand the evolution of oomycetes from a free-living phagotrophic ancestor to a parasitic lifestyle.

Morphological diversity and molecular phylogeny of five *Paramecium bursaria* (Alveolata, Ciliophora, Oligohymenophorea) syngens and the identification of their green algal endosymbiont

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Paramecium bursaria is a mixotrophic ciliate species, which is common in stagnant and slow-flowing, nutrient-rich waters. It is usually found living in symbiosis with zoochlorellae (green algae) of the genera Chlorella or Micractinium. We investigated P. bursaria isolates from around the world, some of which have already been extensively studied in various laboratories, but whose morphological and genetic identity has not yet been completely clarified. Phylogenetic analyses of the SSU and ITS rDNA sequences revealed five highly supported lineages, which corresponded to the syngen and most likely to the biological species assignment. These syngens R1–R5 could also be distinguished by unique synapomorphies in the secondary structures of the SSU and the ITS. Considering these synapomorphies, we could clearly assign the existing GenBank entries of P. bursaria to specific syngens. In addition, we discovered synapomorphies at amino acids of the COI gene for the identification of the syngens. Using the metadata of these entries, most syngens showed a worldwide distribution, however, the syngens R1 and R5 were only found in Europe. From morphology, the syngens did not show any significant deviations. The investigated strains had either Chlorella variabilis, Chlorella vulgaris or Micractinium conductrix as endosymbionts. As consequences of our findings, we taxonomically revised the P. bursaria species complex and subdivided it into five new species, P. protobursaria, P. deuterobursaria, P. tritobursaria, P. tetratobursaria, and P. pentobursaria.

This study was funded by the Austrian Science Fund (FWF): project P28333-B25.

Unexpected diversity of zoochlorellae revealed by multigene approach

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Zoochlorellae or Chlorella-like are widely distributed in distant related mixotrophic ciliates, Heliozoa and other invertebrates such as freshwater sponges. In the literature, those endosymbiotic green algae were often described as uniform and therefore often only designated as Chlorella, Chlorella-like or zoochlorellae. Recent phylogenetic analyses have revealed that this group of green algae are highly diverse representing different genera and species. For example, even in different isolates of the model ciliate species Paramecium bursaria, three species of the Chlorellaceae have been discovered. Other ciliate species such as Coleps, Euplotes, and Cyrtolophosis have other Chlorellaceae as green algal endosymbionts. Most freshwater sponges have Choricystis parasitica as endosymbiont, which is not closely related to the Chlorellaceae. Only one strain isolated from Spongilla lacustris represented a new genus of the Chlorellaceae, Lewiniosphaera. All zoochlorellae were characterized using an integrative approach, which included multiple gene phylogenies and genetic synapomorphies, physiological and biochemical features and ecological parameters. In addition, most zoochlorellae could be infected by highly specific Chloroviruses (a group of double-stranded DNA viruses), if cultivated outside of their hosts. We established an easy diagnostic PCR and DNA Barcoding approaches for identification of the zoochlorellae at generic and species levels without isolation and cultivation. The internal transcribed spacer regions of both nuclear and chloroplast ribosomal operon have clear signature at species and population level.

This study is funded by the Austrian Science Fund (FWF): project P28333-B25.

Using long-read amplicon sequences to evaluate the diversity of understudied eukaryotic microbes

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As long-read sequencing with the Pacific Biosciences platform (PacBio) is gradually becoming more affordable, some protistologists have already applied this technique for environmental sequencing in the framework of ecological studies. Yet, most studies report only the presence and abundance of protist lineages above the family level, leaving the diversity on lower taxonomic levels superficially explored. This provides a potential for re-evaluating the publicly available long-read amplicon sequences to learn more about the diversity and evolution of particular protist lineages. Here, we show how to fully exploit such data with a new pipeline for analyzing Pac-Bio long-read amplicon sequences. The raw reads are first quality-checked, filtered, denoised, and finally clustered into OTUs. The OTUs are then taxonomically assigned by VSEARCH performing pairwise sequence comparisons and EPA-ng performing phylogenetic placements. Based on the resulting taxonomic assignment, the individual OTUs can be filtered out according to the taxon of interest and evaluated independently. We tested the pipeline by assessing the diversity of vampyrellid amoebae (Vampyrellida, Rhizaria) using rRNA gene sequences generated from PacBio Sequel 2. These sequences came from already published sequences (Jamy et al. 2022, 2022) and newly sequenced samples. Both taxonomic assignment methods—pairwise alignment and phylogenetic placement—assigned the same OTUs to the Vampyrellida. The phylogenetic placement assigned the OTUs to lower taxonomic levels than VSEARCH, showing that phylogenetic information increases the taxonomic assignment resolution. The new pipeline allows to study overlooked or rare taxa by long amplicon reads, which will continue to accumulate in public repositories in the future.

The predicted plastid proteome of *Polytoma uvella* (Chlamydophyceae): contrasting differences between the metabolic roles of the nonphotosynthetic plastids of free-living and parasitic/pathogenic chlorophytes

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The loss of photosynthesis (LoP) in eukaryotes is typically associated with a switch to parasitic/pathogenic lifestyle. Nevertheless, photosynthesis has also been lost frequently in free-living algae. The "core chlorophytes" (Chlorophyta) contain taxa with different trophic lifestyles that have lost the autotrophic capacity. Investigations of plastid genomics and the role of the organelle after the LoP have been focused on parasitic chlorophytes, such as Helicospuridium and Prototheca (Trebouxiophyceae). But during the last decade we have gain insights into the evolution and functions of colorless plastids in free-living members of the class Chlamydophyceae that have lost photosynthesis independently. Here, we present a comparative analysis of the plastid proteomes of the free-living colorless chlamyodomandalean chlorophytes Polytoma uvella and Polytomella parva, the trebouxiophyceans Prototheca wickerhamii (opportunistic parasite) and Helicosporidium sp. (pathogen), and diverse photosynthetic relatives. The P. uvella plastid proteome was predicted from protein sequence models (assembled transcripts; RNAseq) with two complementary criteria. First, we used sequence similarity to identify homologous proteins reported in experimental proteomic studies of Chlamydomonas rehinhardtii and Arabidopsis thaliana. Then, the sub-cellular localization of the proteins was predicted with diverse software tools. Plastid-localized pathways of P.uvella, and other algae, were modeled with the KEGG Automatic Annotation Server. Among the 48,148 P. uvella protein models, we predicted 1,675 to be plastid localized. Only 314 proteins were assigned to known plastid pathways involved in the metabolism/biosynthesis of amino acids, carbohydrates, lipids, nucleotides, and energetic metabolism. We identified convergent similarities in the plastid protein repertoires of parasitic and free-living chlorophytes (e.g., terpenoid and starch biosynthesis; purine and pyruvate metabolism). We did not detect P. uvella proteins involved in photosynthesis, but most enzymes required for carbon fixation, except RuBisCO, were identified. Our analysis revealed the heavy involvement of the P. uvella plastid in amino acid biosynthesis (17 amino acids), which is in stark contrast with the limited biosynthetic role of the plastids from parasite/pathogenic chlorophytes. Also, the P. uvella plastid possesses a richer set of enzymes participating in fatty acid biosynthesis and glycerolipid metabolism than other colorless algae. Differences between the metabolic roles of colorless plastids in free-living and parasitic/pathogenic chlorophytes will be discussed.

Non-host genome integration of the Nucleocytoviricota virus into the genome of eustigmatophyte alga *Characiopsis acuta*

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Recent analyses across the diversity of protist genomes unveiled a surprisingly broad spectrum of so-called endogenous viral elements (EVEs), traces of past encounters with dsDNA viruses left in the host genomes. One type of these elements called GEVEs (giant EVEs), created by integrated Nucleocytoviricota virus genomes, can reach up to more than 1 Mbp, create a significant portion of the host genome content and influence the host genome evolution. These enormous integrated elements are prominent mainly in algal genomes. Here, we present a peculiar virus integration into the genome of the eustigmatophyte Characiopsis acuta SAG 14.97. A whole-genome assembly from the alga yielded a ~1,765 Mbp long genomic scaffold with an internal segment of ~300 Kbp containing Nucleocytoviricota marker genes; the segment was flanked by regions containing typical eustigmatophyte nuclear genes, confirming it belongs to the nuclear genome of C. acuta. Strikingly, the coding regions in the putative GEVE were interrupted by in-framed UAG codons, with a strong tendency of occurring at positions corresponding to conserved serine residues. This was reminiscent of an altered genetic code recently suggested to be utilized by the green algal genus Scotinosphaera. Analysis of newly generated or reassembled genomic data from two Scotinosphaera species revealed the presence of putative virus-derived sequences sharing the UAG=Ser reassignments with the host algae and, crucially, specifically related to the GEVE in the eustigmatophyte C. acuta. Thus, our findings indicate a virus normally infecting Scotinosphaera has integrated into a genome of a eustigmatophyte that is extremely unlikely support the propagation of the virus owing to exhibiting the standard genetic code. In conclusion, our analyses unveiled the first and so far, the only known non-host Nucleocytoviricota virus integration and also documented the first Nucleocytoviricota virus with a non-canonical genetic code.

Looking for genes withholding the secrets of uncultivable protist life cycles

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Protist lineages represent the majority of eukaryotic life on earth. Apart from exhibiting a great variety of shapes, sizes and trophic modes, the study of protist model species suggests a diversity of life stages and life cycle structures. Yet, among the protistan life one can detect in the environment, the majority remains tedious to culture in vitro, hindering the comprehension of their biology and ecology. This is the case of many free-living heterotroph protists like Radiolaria, Rhizaria. Up to date, description of radiolarian life cycles is fragmentary and mainly based on knowledge acquired in the 80's-90's. Among the key aspects that remain enigmatic about Radiolaria is the transition from one generation to another. Accumulating observations on the field has led to the proposal of diverse reproductive strategies for the 4 extant radiolarian groups (Acantharia, Spumellaria, Nassellaria, Collodaria), including the formation of cysts, transition from solitary to colonial stages and the production of flagellated swarmers. Swarmers have been hypothesised to be linked to sexual reproduction, nevertheless, swarmer ploidy has never been formerly determined and their fusion is still undocumented. By combining in situ observations of various radiolarian life stages to single-cell transcriptomics, we investigate the presence of reference protist genes involved in sexual processes, like meiosis and gamete fusion. With an approach bypassing the limits of cultivability, we aim at providing functional genetic descriptions of radiolarian life stages. These results will contribute to resolve the long-speculated sexuality of radiolarians and provide a better insight into the ecological implications of each life stage while expanding the current knowledge around the intriguing life of free-living rhizarians.

Nitroxoline as an amoebicidal agent against *Acanthamoeba*: In vitro activity and detection of programmed cell death in *Acanthamoeba culbertsoni*

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The most commonly detected Free living amoebae (FLA) in the environment are the Acanthamoebae. Their life cycle shows a vegetative form (trophozoite) as a motile trophic stage, when the amoeba is in a humid, favorable and nutrition rich environment. These trophozoites form a resistant cyst as soon as they are exposed to adverse conditions. Acanthamoebae are highly resistant to disinfection measures or medical treatment. This high tenacity enables them to tolerate a wide range of environmental or mqn-made stress. The broad environmental distribution results in a high-level rate of contact with humans. The pathogenicity varies among the Acanthamoeba species or strains. As etiological agents of the so-called Acanthamoebiasis, they can trigger different specific disease symptoms in humans: granulomatous amebic encephalitis (GAE) or Acanthamoeba Keratits (AK). Nitroxoline, a hydroxychinoline derivate, has been used for many years to treat urinary tract infections. The drug is known for its benefits as antibacterial, amoebicidal, antifungal or antiviral. In this study the in vitro amoebicidal activity of Nitroxoline was tested against trophozoites and cysts of Acanthamoeba castellanii Neff, A. polyphaga, A. griffini, A. quina, A. culbertsoni and A. sp. L-10 (clinical strain). The Inhibition Concentration 50 (IC50) was determined using the methods based on the alamarBlue® colorimetric assay. A. griffini was the strain that demonstrated the lowest IC50 in trophozoites and cyst (0.68 ± 0.01 and 1.53 ± 0.17 µM, respectively). Also, the evaluation of Nitroxoline mechanism of action demonstrated that this compound induced a programmed cell death (PCD) in trophozoites of A. culbertsoni. Cells treated with Nitroxoline showed mitochondrial damage, chromatin condensation, formation of autophagy vacuoles and disorganization of actin and microtubules. In conclusion, Nitroxoline demonstrated amoebicidal activity and induced features compatible with apoptosis in the tested strains of Acanthamoeba. Therefore, this compound could represent a new horizon in the field of Acanthamoeba current therapies.

Keywords: Acanthamoeba, Nitroxoline, PCD, amoebicidal activity.

Acknowledgements: Proyect PI18/01380 ISCIII; CB21/13/00100 (CIBERINFEC); Cabildo de Tenerife (2023-2028), MEDI y FDCAN; 21/0587 MICINN, AEI and ERDF; Fundación La Caixa-CajaCanarias 2022CLISA26. ACIISI 85% co-funded by the FSE (TESIS2020010117 R.L.R.E.; C.J.B.E.; D.S.N.H.; I.A.J.; P.P.P.).

The importance of model realism in addressing eukaryogenesis-related phylogenetic problems

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Two high profile studies used branch length ratios from large sets of phylogenetic trees to determine the relative ages of mitochondrial, archaeal and duplicated in the evolution of eukaryotic cells. This approach can be straightforwardly justified if substitution rates are constant over the tree for a given protein. However, such strict molecular clock assumptions are not expected to hold on the billion-year timescale. I will discuss an alternative set of conditions under which comparisons of branch length distributions from multiple groups of phylogenies of proteins with different origins can be validly used to discern the order of their origins. Then I will show that functional divergence in proteins adapting to new cellular roles during the prokaryote-eukaryote transition has differentially affected edge length distributions of genes of different origins, complicating attempts to order the events that occurred during eukaryogenesis.

Population genomic insights into syntrophic symbiosis between marine anaerobic ciliates and intracellular methanogens

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Multi-domain symbioses between microbial eukaryotes and intracellular methanogenic archaea are crucial to our understanding of the origins and mechanisms of eukaryotic anaerobiosis. Nearly all anaerobic ciliates, ecologically important protists commonly found in diverse oxygen-depleted environments, host methanogenic endosymbionts, sometimes with additional bacterial partners, that facilitate their anaerobic metabolism. The methanogenic endosymbionts of anaerobic protists represent the only known intracellular archaea. Though it is known that vertical symbiont transmission occurs over the short-term via synchronous host-symbiont division, there is also evidence that symbiont-switching occurs. However, the factors influencing patterns of host-symbiont specificity and intraspecific variability remain mostly unknown. Here, we present the first intra-specific genomic analysis of both host and symbionts in such partnerships, providing key insights into the fidelity of eukaryotic-prokaryotic liaisons in anoxia. We assessed the symbiont-host co-diversification and genetic variation by analyzing symbiont genomes (Methanocorpusculum sp.) and host mitochondrial genomes from 78 holobiont cells from 12 populations of a marine anaerobic ciliate Metopus sp. (Armophorea, SAL), across various geographic scales. Symbiont comparative and population genomics enable us to further comprehend the complex nature of these multi-partner syntrophic symbioses, which are crucial to our perception of cell-cell interactions across domains of life.

In vivo evaluation of bioenergetic parameters in heat-stressed *Cassiopea*

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The symbiotic partnership between cnidarians and dinoflagellates from the family Symbiodiniaceae constitutes the basis of remarkably diverse ecosystems. This tight association between the cnidarian host and the intracellular photosymbionts displays a complex energetic metabolism, involving respiration from both partners and photosynthesis from the dinoflagellate symbionts. Despite the major importance of these two critical processes, their interplay and regulations remain poorly studied. Abiotic factors can unsettle the symbiotic balance, leading to the collapse of the partnership and threatening the survival of entire ecosystems. Among them, the rise in sea water temperature is getting more and more concerning as global warming takes place. To address this topic, our first approach consisted in subjecting Cassiopea, an established model organism for photosynthetic jellyfish, to a mild hyperthermic stress for 28 days. Despite an increase of 6°C of the water temperature, the stressed jellyfish kept on growing over the experiment, their symbiont density stayed constant and their photosynthetic capacity was barely impacted (Fv/Fm, rETR-PSII, rETR-PSI, oxygen exchanges). The measurement of the level of pigments in Symbiodinium cells hints at an adaptation of the photosynthetic apparatus to heat stress, with the slight increase of the amount of total pigments per cell and the transient rise in the deepoxidation of xanthophylls. Despite the minor impact of heat stress on the photosynthesis of symbiotic Cassiopea, an increased respiration along with a rise in bell pulsation rate were observed in the heat-stressed population. To dig more into the cellular scale and understand the factors that ensure photosynthetic stability, we are repeating this long-lasting heat stress experiment, collecting samples to carry out bottom-up proteomics analysis. The production of reactive oxygen species is also under investigation, along with the fatty acid composition of both symbiotic partners. All in all, our work aims at taking advantage of the biophysical and spectroscopic techniques in the context of the mutualistic partnership between cnidarians and dinoflagellates, as well as its disruption. Our results show the tolerance of Cassiopea andromeda jellyfish and their dinoflagellate symbionts to a long-lasting hyperthermic stress.

In search of Achilles' heel in ciliates

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Symbiotic associations, encompassing all kinds of relationships between the partners, commensalism, mutualism, and parasitism, are common in ciliates. Susceptibility to such interactions differ in various genera: Paramecium and Euplotes often get involved in symbioses, while others, like Tetrahymena and Stylonychia, seemingly avoid engagement in symbiotic systems. Moreover, closely related species or even strains of one and the same species can differ in their ability to be infected with endosymbiotic microorganisms. Thus, cryptic species of Paramecium aurelia complex demonstrate different susceptibility to infection with the microsporidium Globosporidium paramecii, while strains of P. caudatum are known to vary in their susceptibility to the endonucleobionts belonging to the genus Holospora. The reason for such differences remains unclear. Presumably, in each case the host has at least one weak spot in its life style that is an essential prerequisite for the infection. Since initial steps of infection are crucially dependent on phagocytosis of the host cell, one of the approaches to study susceptibility/resistance to infection in ciliates is to focus on phagocytic abilities of the potential host keeping in mind possible importance of lectin-glycoconjugate interactions in symbiotic partners' communication. Experimental infections of P. caudatum with H. obtusa and H. undulata in the presence of the WGA lectin specifically binding to terminal N-acetyl glucosamine or after treatments with N-acetyl glucosaminidase and neuraminidase demonstrated that the presence of terminal N-acetylglucosamines on the host cell surface receptors is a prerequisite for phagocytosis of both, infectious forms of Holospora and food bacteria in paramecia. The presence of terminal N-acetylneuraminic acid residues on the surface glycoconjugates in ciliates promotes the uptake of the infectious forms of H. undulata by the host. Eukaryotic endosymbionts, apparently, take advantage of the later steps of phagocytosis in the host, the occasional invader of P. bursaria, the yeast Rhodotorula mucilaginosa, using the same pathway as the host's common inhabitant green alga Chlorella. Assessment of the protein glycosylation profile and enzyme levels in vulnerable and resistant cell lines might contribute to understanding the causes of susceptibility to infection.

Supported by RSF grant #22-24-00335.

Spatial proteomics reveals the presence of complex subcellular compartimentalization in the alveolate parasite *Perkinsus marinus*

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One of the most intimate achievements of endosymbiosis is the integration of an endosymbiont's genome into its host genome while maintaining most of the symbiont's structural compartments and functions. Most plastid endosymbionts retain some genes on a relict genome (plastome), and the site of its maintenance, expression and interactions with nucleus-encoded plastid-tageted proteins define this space as a direct inherence from the endosymbiont. Perkinsus marinus (Alveolate) represents an opportunity to study the implications of transfer of genetic control to the host as it is suspected to contain a relic plastid, but the retention of such a cellular space has been difficult to verify. To investigate subcellular compartmentalization, in particular, what plastid-derived structures occur in this parasite we 1) sequenced the cell's genomes, and 2) applied global spatial proteomics (LOPIT) to these cells. Genome analyses shows no evidence of a plastome, but supports retention of plastid-derived genes in the host nucleus. LOPIT analysis resolves up to 19 subcellular protein niches, one of which is consistent with a plastid-like organelle capable of isoprenoid and Fe-S cluster synthesis. The plastid proteome lacks any plastome maintenance or expression machinery which corroborates the loss of the plastome. Surprisingly, it also lacks the cannonical plastid translocons necessary for protein import across multiple membranes. Expression of a reporter-tagged plastid protein reveals multiple small discrete compartments as the sites of these plastid proteomes. Our observations share similarities with experimentally induced plastome and plastid loss in Plasmodium falciparum that results in the formation of multiple single-membrane vesicles that can still perform plastid functions. Our observations in Perkinsus suggest similar derived compartments and raise the question, are they plastid compartment relics or in fact a novel type of organelle now contained in endomembrane vesicles: a putative Endosymbiont Legacy Organelle. This is a tantalizing hypothesis in which the subcellular compartment itself would no longer be of endosymbiont origin but a new host-synthesized compartment capable of carry out the functions that were acquired via endosymbiont gene transfer.

The photic-aphotic divide is a strong ecological and evolutionary force determining the distribution of marine ciliates (Alveolata, Ciliophora)

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The bulk of knowledge on marine ciliates is from shallow and/or sunlit waters. We studied ciliate diversity and distribution across epi- and mesopelagic oceanic waters, using DNA metabarcoding and phylogeny-based metrics. We analyzed sequences of the 18S rRNA gene (V4 region) from 369 samples collected at 12 depths (0-1,000 m) at the Bermuda Atlantic Time-series Study site of the Sargasso Sea monthly for 3 years. The comprehensive depth and temporal resolutions analyzed led to three main findings. First, there was a gradual but significant decrease in alpha-diversity from the surface to the deepest waters analyzed. Second, multivariate analyses of beta-diversity indicate that ciliate assemblages change significantly from photic to aphotic waters, with a switch from Oligotrichea to Oligohymenophorea prevalence. Third, phylogenetic placement of sequence variants and clade-level correlations show Oligotrichea, Litostomatea, Prostomatea, and Phyllopharyngea as anti-correlated with depth, while Oligohymenophorea (especially Apostomatia) have a direct relationship with depth. Two enigmatic environmental clades include either prevalent variants widely distributed in aphotic layers (the Oligohymenophorea OLIGO5) or subclades differentially distributed in photic versus aphotic waters (the Discotrichidae NASSO1). These results settle contradictory relationships between ciliate alpha-diversity and depth reported before, suggest functional changes in ciliate assemblages from photic to aphotic waters (with prevalence of algivory and mixotrophy versus omnivory and parasitism, respectively), and indicate that contemporary taxon distributions in the vertical profile have been strongly influenced by evolutionary processes. Integration of DNA sequences with organismal data (microscopy, functional experiments) and development of databases that link these sources of information remain as major tasks to better understand ciliate diversity, ecological roles, and evolution in the ocean.

After years with partial mixing, complete water turnover boosts an intense phytoplankton bloom - how do ciliates react?

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In recent years, climate-induced warming of surface water and increasing density differences to deep water led to a reduced mixing depth in Lake Zurich (maximum depth 135 m). This resulted in a reduced transport of nutrients from the near bottom to the euphotic surface zone. In consequence, nutrient depletion negatively affected the development of vernal phytoplankton blooms, as well as their main predators, i.e. ciliates. However, not only did the temperature of the surface water rise, but also the deep water became warmer and reached temperatures above 5 °C. In winter 2020/21, a cold winter caused a strong heat loss of the surface layer, which induced isothermal conditions in the lake. Together with strong winds, complete water turnover occurred in spring 2021, leading to a three-fold rise in orthophosphate concentrations in the euphotic zone. High frequency sampling was conducted during spring 2020 (partial mixis), 2021 (complete mixis) and 2022 (deep mixis) to study the effects of water turnover on the succession of organisms. Beside environmental parameters, abundances of bacteria, heterotrophic nanoflagellates (HNF), algae and rotifers were determined. A focus was set on abundances of ciliate species, applying the quantitative protargol staining technique. High orthophosphate concentrations in 2021 triggered an intense phytoplankton bloom with four times more diatoms and three times more cryptophytes compared to the previous year. Many ciliate species, especially Balanion planctonicum and tintinnids benefited from the increase in potential food sources, but not all like Histiobalantium bodamicum. One side effect of the complete water turnover was an increased oxygenation of the deep zone, resulting in a reduced remineralization and accumulation of orthophosphate. In consequence, despite a deep mixis in 2022, less nutrients got upwelled and primary production during spring was as low as in 2020 (partial mixis). Complete water turnover still occurs in Lake Zurich, whereby up-welling nutrients may boost intense phytoplankton blooms. Thus, effects of lake warming in deep stratifying lakes are not as unidirectional as previously presumed, with deep-water warming even facilitating complete mixing events anew.

The dilemma of underestimating freshwater biodiversity: Morphological versus molecular approaches

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Heterotrophic protists form a major link from bacteria to algae and higher trophic levels in marine and freshwater systems. While freshwaters cover around 0.8 % of the earth's surface, its habitats provide essential ecosystem services that are fundamental and supporting ~10 % of all described living species including human livelihoods. Freshwater systems are a hotspot of biodiversity and highly complex ecosystems. However, knowledge and description of its biodiversity across all trophic levels in freshwater habitats is still incomplete to resolve the complexity of interactions, especially beyond the micro scale. Here, we introduce a long term ecological research project (LTER) to investigate the Rhine-Eco-Evolutionary System (REES), where we aim to generate a holistic perspective on the eco-evolutionary dynamics of the River Rhine and associated water bodies. The project focuses on an area at the Lower Rhine in North Rhine-Westphalia (district Rees), which can be described as landscape of ecological succession including several gravel pit lakes, Rhine oxbows and abandoned meanders, as well as the main river channel. This wider system of standing and flowing freshwater bodies offers great opportunities to study dynamic fluctuations in the composition of biodiversity at all levels, from species diversity of communities to genomic diversity at the molecular level of individuals and populations. Along a selected trophic cascade (from protists to fish), representative species are ecologically and (population-)genomically assessed through time. We will present first results on protist diversity from the study area including two riprap river Rhine sections, one oxbow as well as two gravel-pit lakes in the flood plain. Diversity was assessed through morphotype richness and abundances, molecular approaches (metabarcoding and individual barcoding) as well as accounting for sediment composition. Using linear mixed-effect models, possible dependencies were evaluated within and between the different water bodies. We recovered 946 ASVs assigned to heterotrophic protists, while we only identified 84 protist morphotypes during live counting within all stations. We found high discrepancies between ASVs and morphotype richness in all size classes as well as available freshwater sequences within public databases showing the importance to integrate different measures to assess protist diversity for long term projects.

Chlamydial symbionts of amoeba impact top-down control in soil

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Protists in soil contribute to primary production and play key roles in decomposition pathways as major consumers of bacteria and other microbes. Acanthamoeba species are members of the Amoebozoa, a dominant group of soil protists, that have been used as model organisms, unravelling the significant effects of protist predation on soil microbial communities and ecosystem processes. These amoebae frequently harbour obligate intracellular bacterial symbionts such as members of the phylum Chlamydiota. We hypothesize that by modulating their hosts' fitness, chlamydiae have the potential to play an important, yet unstudied, role in shaping protist communities and thereby indirectly also microbial communities and ecosystem functioning. Here, we investigated the impact of the highly parasitic and attenuated chlamydial symbionts Parachlamydia acanthamoebae and Protochlamydia amoebophila and their Acanthamoeba castellanii host in a microcosm setup, mimicking a simplified soil ecosystem. Over a period of 35 days we tracked bacterial community, chlamydial and amoebal growth by digital PCR and monitored changes in the active bacterial soil community with 16S rRNA amplicon sequencing. Additionally, changes in microbial biomass, DNA turnover, respiration and nutrient mineralization were recorded, using established stable isotope probing techniques. We observed that the impact of chlamydial symbionts depends on their fitness-costs for the amoeba host. The presence of attenuated symbionts increased predation, with having significant effects on bacterial cell numbers, community composition, biomass turnover, respiration, and nitrogen mineralization. Opposing effects were observed when the highly parasitic strain was present. Taken together, this study reveals bacterial symbionts of protists as important yet overlooked contributors to soil ecosystem functioning

Evidence for ciliates without extensive DNA elimination: The karyorelict *Loxodes magnus*

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Unlike most eukaryotes, ciliates have two types of nuclei in each cell: the germline micronucleus (MIC), which is usually transcriptionally silent, and somatic macronucleus (MAC), where most gene expression takes place. In most ciliates, MACs develop from MIC precursors following gametic nuclear fusion. Thousands of interspersed DNA segments called internally eliminated sequences (IESs) from tens to thousands of bp in length are eliminated, and chromosomes also experience fragmentation, rearrangement, and amplification, although the extent varies between species. The MAC genome content is hence a subset of the MIC genome. During cell division, MACs replicate in parallel to MICs. Karyorelictea, however, are the exception; as their MACs do not divide, but must develop anew from MICs with each cell division. Here we report an additional exception in the karyorelict ciliate Loxodes magnus – an apparent lack of genome editing. We separated MIC and MAC by flow cytometry and confirmed purity by morphology and Western blotting against MAC-specific markers. Unexpectedly, sequence libraries prepared from purified nuclei showed only a small fraction of k-mers specific to the MIC libraries. Indels found by mapping MIC reads against MAC draft genomes appeared to represent allele variants rather than IESs. Both genomes were large for ciliates: after filtering out low-complexity repetitive sequences (up to 43% of total), assemblies were 450-470 Mbp (MAC) vs. 460-480 Mbp (MIC). Although we cannot definitely rule out editing in the form of rearrangements or elimination of low-complexity sequences, they hence appear to lack abundant interspersed IESs typical of most ciliates. Nonetheless, histone markers and nucleosome profiling suggest that the MACs are the site of active gene expression, like other ciliates. Thousands of retrotransposon-like sequences, encoding reverse transcriptase domains, were also present in both MAC and MIC genomes. We hypothesize that the loss or streamlining of genome editing avoids costly overheads during cell division in karyorelicts.

Evolution of endosomal retrograde trafficking machinery in the Parabasalia and its free-living sister lineage

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Endosomes are a nexus point for sorting of different transmembrane cargo; either for lysosomal degradation through ESCRT pathway or recycling to the Golgi complex or plasma membrane. Endosomal retrieval machinery in eukaryotes is governed by two anciently homologous machineries, namely the Retromer complex and Retriever complex. The Retromer complex was first discovered in S. cerevisiae, and until very recently was assumed to be the only endosomal retrograde trafficking machinery in eukaryotes. However, with the recent discovery of the Retriever complex in mammalian system, it was confirmed that Retromer-independent rescue of endomembrane proteins can occur. Using evolutionary bioinformatics tools such as comparative genomics, we identified an expansion of the Retromer complex components contrasting to the paralogue count of the Retriever complex in the parasitic Parabasalia and its free-living sister lineage of Anaeramoeba spp. Our novel identification of unexpanded gene paralogues of the Retriever complex specific components and of its accessory factors such as the WASH complex and the CCC complex (CCDC22-CCDC93-COMMDs) in this clade gave us a good contrast of both the machineries. Our findings hint towards the expansion of the specific retrograde trafficking machineries being dependent on the expansion of their specific cargo proteins as both the machineries are responsible for rescuing differential cargoes from lysosomal degradation. We also discovered partial loss of the membrane deformation complex in Anaeramoeba spp and its complete loss in parasitic parabasalia. Using phylogenetic analyses, we confirm that the independent evolution of Retromer and Retriever complex proteins was a result of ancient duplication events in LECA, however, the expansion of Retromer complex components was a result of lineage specific and species-specific duplication events in both the parabasalia and Anaeramoeba lineages. Our work focuses on the complete reconstruction of these evolutionarily conserved endosomal retrograde trafficking machineries in parabasalia and its newly identified sister lineage of Anaeramoeba.

Exploring the efficacy of metabarcoding and non-target screening for detecting treated wastewater

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Wastewater treatment processes are capable of eliminating many pollutants; however, not all are removed, leading to the introduction of organic compounds and microorganisms from treated wastewater into receiving waters. These microorganisms and organic compounds have the potential to adversely impact downstream ecosystem processes, but their presence is currently not being monitored. In this study, we aimed to investigate the effectiveness and sensitivity of non-target screening of chemical compounds as well as of 18S V9 and full-length 16S metabarcoding techniques in detecting treated wastewater in receiving waters. We investigated the effect of an introduction of 33% of treated wastewater versus a control in a triplicated large-scale mesocosm setup over a 10-day exposure period. Our study revealed that the discharge of treated wastewater significantly altered the chemical signature as well as the microeukaryotic and prokaryotic diversity of the receiving waters. Non-target screening, 18S V9, and full-length 16S metabarcoding were all able to detect these changes, with significant covariation of the detected pattern. Nevertheless, the sensitivity varied among these methods. While full-length 16S metabarcoding was most sensitive initially, non-target screening became more effective than 16S metabarcoding in the mid- to long-term. However, the sensitivity of V9 metabarcoding was most consistent throughout the investigated period.

Chlorhexidine caused imbalance oxidative state in *Acanthamoeba polyphaga*

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Acanthamoeba keratitis is a rare but vision-threatening parasitic infection. Affecting broadly the contact lens wearers, this infection is caused by an ubiquitous opportunistic protozoan pathogen Acanthamoeba sp. Current treatment protocol usually include polyhexamethylene biguanide (0.02%) and/or chlorhexidine (Chx) (0.02%). Although the use of chlorhexidine is widely reported, its action mode still undefined. The aim of the present work was to investigate the effect of chlorhexidine on the oxidative state of Acanthamoeba polyphaga. To assess the oxidative state of treated and untreated cells, the antioxidant capacity and the ROS level was measured. The antioxidant capacity was determined using different antiradical methods including ABTS, DPPH and FRAP, and by measuring the activity of a couple of antioxidant enzyme namely SOD, NADH-FRD and SDH. As for the ROS level, CellRox deep red was used to measure the general oxidative stress level and the Mitochondrial Superoxide Dye was used to detect the superoxide production. The chlorhexidine was able to induce oxidative imbalance in treated Acanthamoeba polyphaga by over expression of reactive oxygen species and/or inhibiting the antioxidant enzymes. In addition to enhancing the antiradical activity in response to oxidative stress, the present drug was able to reduce the activity of two antioxidant enzymes, superoxide dismutase (SOD) and reduced flavin adenine dinucleotide-fumarate reductase (NADH-FRD), to 30% and 40%, respectively. To cope with the overproduction of ROS we did observe an increase of the antiradical capacity of cell's lysate supernatant. The inhibition of both antioxidant enzymes :SOD and NADH-FRD by the Chx could have a major role in the cell oxidative unbalance.

Keywords : Oxidative stress; Antioxidant activity; Acanthamoeba polyphaga; Chlorhexidine

Funding: CIBERINFEC (CB21/13/00100), Cabildo Insular de Tenerife 2023-2028, Ministerio de Sanidad, Consumo y Bienestar Social, ACIISI y Fundación La Caixa – Caja Canarias (2022CLISA26)

RS, a new fully defined medium for cultivating diverse protists

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Protists occupy a key position in earth's ecosystem and form the backbone of the eukaryotic tree of life. Cultivating diverse protist species is a powerful method to characterize their cell biology and behavior. Nearly all of the most commonly used media recipes for protist cultivation are either specific to a group of species or a trophic mode (autotrophic, heterotrophic, etc.), or contain ingredients of unknown or variable composition such as natural seawater, peptone or yeast extract. Thus, we currently lack a fully defined medium suited for general protist growth, whose chemical composition can be precisely modified as needed for laboratory experiments. By combining elements of several frequently used protist and bacterial growth media with a natural pH buffering system, we have designed the RS medium, a generally usable fully defined medium designed to mimic conditions in the ocean, and which can be easily modified to include/exclude any component. It is characterized by a pH of ~7, a salinity of 3.3% and a Redfield ratio (C:N:P) similar to typical ocean conditions. Based on current media recipes for growing diverse eukaryotes and bacteria, we selected ingredients for the RS medium in multiple categories: high and low concentration salts, heavy metals, amino acids, vitamins and carbon sources. We have used RS medium to isolate new protist species from seawater samples off the Blanes coast (Spain), leading to a wide range of cultivated diversity and trophic modes. In order to test the utility of RS for currently cultivated species, experiments are ongoing to compare growth rates of diverse species in the Roscoff Culture Collection (RCC) in their habitual growth medium versus RS. We hope that RS medium can be of general use to the protist community, both to culture new species and to enable growth experiments that rely on specifically modifying one or more chemical components of the medium.

The multigene family of surface antigens in different *Paramecium* species

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Paramecium is a free-living ciliate that undergoes antigenic variation and still the functions of these variable surface antigen coats in this non-pathogenic ciliate remain elusive. Switching the surface antigens was described to follow environmental parameters and some reports also mention alteration of the surface antigen due to infection with symbiotic bacteria. Only a few surface antigen genes have been described, mainly in the two model species P. tetraurelia strain 51 and P. primaurelia strain 156. Given the lack of suitable sequence data to allow for phylogenetics and deeper sequence comparisons, we screened the genomes of six different Paramecium species for serotype genes and isolated 548 candidates. Our approach identified the subfamilies of the isogenes of individual serotypes that were mostly represented by intrachromosomal gene duplicates. These showed different duplication levels, and chromosome synteny suggested rather young duplication events after the emergence of the P. aurelia species complex, indicating a rapid evolution of surface antigen genes. We were able to identify the different subfamilies of the surface antigen genes with internal tandem repeats, which showed consensus motifs across species. The individual isogene families showed additional consensus motifs, indicating that the selection pressure holds individual amino acids constant in these repeats. This may be a hint of the receptor function of these antigens rather than a presentation of random epitopes, generating the variability of these surface molecules.

Characterisation and cultivation of new lineages of colponemids, a critical assemblage for inferring alveolate evolution

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There are several alveolate groups outside the well-studied ciliates, dinoflagellates, and apicomplexans that are crucial for understanding the evolution of this major taxon. One such assemblage is the "colponemids", which are eukaryotrophic biflagellates, usually with a ventral groove associated with the posterior flagellum. Previous phylogenetic studies show colponemids forming up to three distinct deep branches within alveolates (e.g. sister groups to Myzozoa or to all other alveolates). We have developed dieukaryotic (predator-prey) cultures of several colponemid isolates that include representatives of two previously uncharacterised new lineages, and described those as Neocolponema and Loeffela. Neocolponema saponarium is a swimming alkaliphile with a large groove and conspicuous vane on the posterior flagellum, which feeds on a kinetoplastid. Loeffela hirca is halophilic, has a subtle groove, a vestigial vane, usually moves along surfaces, and feeds on Pharyngomonas and Percolomonas. Prey capture in both new taxa is raptorial and involves a specialized structure that protrudes to the right of the proximal posterior flagellum and that houses the presumed extrusomes. These observations extend and clarify our knowledge of feeding in colponemids. The relationships amongst Myzozoa, ciliates, and the (now) five described colponemid clades are unresolved, signaling that colponemid diversity represents both a challenge and important resource for tracing deep alveolate evolution.

Regulation of colony formation in a novel *Ventrifissura* species (Cercozoa)

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Expanding our knowledge on protist diversity and biology is of utmost importance, as protists form the backbone of the eukaryotic tree of life. We describe the isolation, culturing and characterisation of a novel Ventrifissura species from the Western Mediterranean. Ventrifissura is a ventricleftid, an understudied group of heterotrophic protists with an unclear phylogenetic position within the Cercozoa. Earlier studies have revealed interesting morphological and life cycle characteristics within this group (Shiratori et al., 2020). Here, we expand the number of studied ventricleftid species and generate information on their molecular capabilities by sequencing their transcriptome. To characterise the morphological features and monitor the life cycle of this newly isolated species, we employed phase-contrast, fluorescence and electron microscopy. We detected 3 intriguing life history stages: a motile biflagellate cell, a crawling cell with emerged filopodia, and crawling colonies with shared filopodia that collectively contribute to their motility. Super-resolution fluorescence microscopy revealed an extracellular structure covered with 'bumps', long extrusomes, and a ventral groove from where the filopodia emerge. Extended live imaging unravelled the transitions between the three life history stages and demonstrated that colonies can form by aggregation. Preliminary data suggest that within-species interactions with dead or dying cells may trigger the transition from the motile to the crawling phenotype. Additionally, UV irradiation is sufficient to promote colony dissociation, suggesting a potential role of light in regulating this process. Electron microscopy showed that the cells within a colony are connected with thin, long protrusions that are stained with an antibody against the non-muscle Myosin IIA protein. Notably, previous studies did not detect the presence of this gene family in Cercozoa (it was found only in Amorphea and Excavata), suggesting a potentially more complex evolutionary history. Finally, ongoing transcriptomic analysis will help to resolve the species' phylogenetic position and provide insights regarding its molecular repertoire. Altogether, we have characterised the morphology and life cycle of a novel Ventrifissura species. Considering that these cells can arrange within colonies, we will continue to explore these processes in greater detail, aiming to add insights into the evolution of mechanisms for cellular aggregation and cooperation within colonies.

Biodiversity of freshwater ciliates in the Lake Weishan Wetland, China

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Ciliates are core components of the structure and function of aquatic microbial food webs. They play an essential role in the energy flow and material circulation within aquatic ecosystems. However, studies on the taxonomy and biodiversity of freshwater ciliates, especially those in wetlands in China are limited. To address this issue, a project to investigate the freshwater ciliates of the Lake Weishan Wetland, Shandong Province, commenced in 2019. Here, we summarize our findings to date on the diversity of ciliates. A total of 187 ciliate species have been found, 94 of which are identified to species-level, 87 to genus-level, and six to family-level. These species show a high morphological diversity and represent five classes, i.e., Heterotrichea, Litostomatea, Prostomatea, Oligohymenophorea, and Spirotrichea. The largest number of species documented are oligohymenophoreans. A comprehensive database of these ciliates, including morphological data, gene sequences, microscope slide specimens and a DNA bank, has been established. In the present study, we provide an annotated checklist of retrieved ciliates as well as information on the sequences of published species. Most of these species are recorded in China for the first time and more than 20% are tentatively identified as new to science. Additionally, an investigation of environmental DNA revealed that the ciliate species diversity in Lake Weishan Wetland is higher than previously supposed.

Long-read sequencing and soil eukaryote diversity

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Mapping diversity patterns across broad-scale climate gradients may predict how ecosystems will respond to climate change in the future. The soil biota, including protists, fungi and microinvertebrates, are essential components of terrestrial ecosystems but it is so far not clear how these communities will respond to climate change. By analyzing soil samples collected from various climates throughout Norway, we aim to assess how important climate is for structuring the soil biota compared to other factors, including vegetation and local soil properties. As the morphology and ecology of many of these eukaryotes make them unsuitable for direct observation or culturing, metabarcoding of environmental DNA has emerged as an alternative mapping approach in recent years. However, due to the vast genetic span of the target organisms and the varying suitability of commonly used DNA marker regions, standard short-read metabarcoding techniques sometimes fall short. To encompass a wider span of taxa and increase the phylogenetic resolution, we use long-read amplicon sequencing (PacBio HiFi) with eukaryote-specific primers to obtain continuous reads spanning a ~4500 bp region of the rDNA operon (including parts of SSU and LSU genes, and the full ITS region). Through sequencing of environmental DNA from 90 sites we recover over 12,900 OTUs, most of which belong to Fungi, Cercozoa, Metazoa and Alveolata. We present a global phylogeny for soil eukaryotes and assess how richness and community structure might vary with climatic factors, local soil properties and vegetation. Although a high gamma diversity was recovered, there were indications that long-read amplicon sequencing may target only a sub-set of the diversity in each sample, possibly restricting its usability in community ecology analyses. In light of this, we discuss the future applications of long-read amplicon sequencing in microorganism diversity research.

Nephridiophagids (Chytridiomycota) reduce the fitness of their host insects

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Nephridiophagids are unicellular fungi (Chytridiomycota), which infect the Malpighian tubules of insects. While most life cycle features are known, the effects of these endobionts on their hosts remain poorly understood. Here, we present results on the influence of an infection of the cockroach Blattella germanica with Nephridiophaga blattellae (Ni = Nephridiophaga-infected) on physical, physiological, and reproductive fitness parameters. Since the gut nematode Blatticola blattae is a further common parasite of B. germanica, we included double infected cockroaches (N+Ni = nematode plus Ni) in selected experiments. Ni individuals had lower fat reserves and showed reduced mobility. The lifespan of adult hosts was only slightly affected in these individuals but significantly shortened when both Nephridiophaga and nematodes were present. Ni as well as N+Ni females produced considerably less offspring than parasite-free (P-free) females. Immune parameters such as the number of hemocytes and phenoloxidase activity were barely changed by Nephridiophaga and/or nematode infections, while the ability to detoxify pesticides decreased. Quantitative proteomics from hemolymph of P-free, Ni, and N+Ni populations revealed clear differences in the expression profiles. For Ni animals, for example, the down-regulation of fatty acid synthases corroborates our finding of reduced fat reserves. Our study shows that an infection with Nephridiophaga (and nematodes) leads to an overall reduced host fitness.

Cleaving *Closterium*: A new feeding strategy found in the leptophryid amoebae (Vampyrellida, Rhizaria)

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The order Vampyrellida comprises predatory amoebae, which form a large and genetically diverse clade of rhizarian protists. These intricate microbes specifically prey on other eukaryotes, and there are currently four distinct feeding strategies known: free capture, protoplast extraction, colony invasion and infiltration. As part of the 'Taxon-omics' Priority Programme of the German Research Foundation, we examine the vampyrellids in a modern light and discover new lineages and their ecology. Here, we report on a new vampyrellid genus, which belongs to the family Leptophryidae and exhibits a hitherto unknown feeding strategy. This 'fifth' feeding strategy involves the phagocytotic uptake of entire cells of the desmid Closterium (Zygnematophyceae), rupturing of the algal wall inside of the food vacuole, subsequent extraction and packaging of the algal cell contents, and finally exocytosis of the emptied Closterium cells. So far, such behaviour - reminiscent of pellet-casting in owls - is unknown for vampyrellids. We will showcase the new vampyrellid amoeba and its feeding strategy by light microscopy, and present data on prey range specificity and the phylogenetic position. Our findings highlight the diversity of feeding strategies in the Vampyrellida and demonstrate how much remains to be discovered about these widespread and fascinating microbes.

ATP generation in *Paradiplonema papillatum*: A planktonic protist without the need for oxygen

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Diplonemids (Diplonemea) are a group of heterotrophic flagellates that belong to the phylum Euglenozoa, and thus are closely related to the predominantly parasitic kinetoplastid and free-living euglenid protists.Their success in the ocean may be a result of their ability to adapt to changes in the environment. The genome of Paradiplonema papillatum, the best-known representative, encodes a wide range of enzymes that enable aerobic and anaerobic metabolism. While the transcript levels of these enzymes are little affected by the oxygen level, anoxic environment causes changes in cell morphology and mobility, and redirects the metabolic pathway where ATP is synthesized. The TCA cycle appears to be crucial for substrate oxidation. P. papillatum carries the standard enzymes of the TCA cycle, but also 2-oxoglutarate decarboxylase (OGDC) and succinate-semialdehyde dehydrogenase (SSDH), which allows the cycle to participate in anabolic reactions. The electron transport chain, even when fully developed, is not always coupled to oxidative phosphorylation, but ATPase plays an important role in maintaining the mitochondrial membrane potential when nutrients and oxygen are limited.

A non-kareniacean dinoflagellate with a haptophyte-derived plastid indicates multiple tertiary endosymbioses

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Canonical photosynthetic dinoflagellates possess plastids containing peridinin, but the unarmored dinoflagellate family Kareniaceae replaced this plastid with a haptophyte-derived plastid containing fucoxanthin and its derivatives through tertiary endosymbiosis. Kareniaceans maintain only plastids without other symbiont components (e.g., nucleus and mitochondria), and their symbiotic relationship is permanent. In general, such complex evolution is believed to have occurred only once in dinoflagellates, but recently several results contradicting this view have been published. Here we show that a non-kareniacean dinoflagellate also possesses the highly integrated plastids derived from a haptophyte endosymbiont. Our dinoflagellate (Gymnodinium sp.) from the Japanese coast is an undescribed unarmored species with permanent plastids, and grew autotrophically in unialgal culture for more than 60 months with only light irradiation and nutrient addition. Morphological observations under LM, SEM, and TEM, molecular phylogenies inferred from the host/plastid rDNA and photosynthetic reaction center genes, and a pigment analysis using HPLC revealed the following features. The dinoflagellate host was closely related to the genus Gymnodinium rather than to the Kareniaceae. For instance, the dinoflagellate investigated here possessed the horseshoe-shaped apical groove, the chambers within nuclear membranes, and the fiber connecting the flagellar apparatus and the nucleus, as the previously described Gymnodinium spp. The Gymnodinium plastid was of the haptophyte type but lacked other symbiont components. The phylogenetic analyses indicated that the Gymnodinium plastid genes evolved more rapidly than the haptophyte plastid genes, as did the kareniacean plastid genes. Our results indicate that the tertiary plastid arose in Gymnodinium independently from those in the Kareniaceae. We are currently working on how many times the haptophyte-derived plastids arose by phylogenetic analyses including this undescribed dinoflagellate.

Transient and permanent residents: Examples of euglenozoan-bacterial endosymbioses

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Endosymbioses with prokaryotes are diverse and common among most lineages of protists. The nature of these relationships spans from mutually beneficial to parasitic, and from transient facultative to permanent and obligate. Among the most understudied protists in respect of their symbiotic relationships are euglenozoans, a species-rich group of flagellates, which includes kinetoplastids, diplonemids, euglenids, and symbiontids. Here, we describe two distinct types of endosymbiosis between euglenozoans and bacteria. The first case is a clear example of a permanent mutualistic relationship between a trypanosomatid and a β-proteobacterium, which supplies its host amino acids, purines, vitamins, and heme. This obligate symbiont evolved from a free-living group of bacteria and, over the long term, became integrated within the eukaryotic cell, which is manifested as complementation of metabolic pathways and the host's control over symbiont number and intracellular localization. The second, very different case is a loose association of multiple diplonemid species with representatives of three ancient, exclusively endosymbiotic clades – Holosporaceae, Rickettsiaceae (both α-proteobacteria), and Chlamydiae. These symbionts usually form only transient symbioses, frequently switch hosts, and tend to disappear from axenically cultivated strains. Although the analysis of the symbionts' genomes, as well as transcriptomes of holo- and aposymbiotic hosts, does not clearly identify the nature of this relationship, studying diplonemids in the environmental context provides clues on the possible function of symbionts.

Comparative genomics of the vampyrellid amoebae (Vampyrellida, Rhizaria)

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The vampyrellid amoebae (Vampyrellida, Rhizaria) are naked, filose amoebae that inhabit marine, freshwater and soil environments. Phylogenetic trees based on the SSU rRNA gene indicate that the vampyrellids are genetically diverse, comprising several family-level clades. The members of these clades exhibit diverse morphologies as well as interesting variations in their feeding strategies and prey range specificities. Vampyrellids are well known to attack algae, fungi and even small animals, suggesting that they play important ecological roles. Currently, we still lack genomic data of vampyrellids, which could help us understand the evolution and diversification of these rhizarian amoebae on a molecular level. Based on whole genome amplification and the short-read Illumina sequencing technology, we established a workflow for “single-cell genomics” and generated 19 draft genomes of diverse vampyrellids. These draft genomes are highly complete and contain thousands of functionally annotated, protein-coding genes per species. Here, we discuss technical aspects of our workflow and present the first multi-gene species tree for the vampyrellids based on over 200 eukaryotic marker genes. This phylogenomic approach provides robust bootstrap support for branches in the vampyrellid phylogeny that were weakly supported in previous SSU rRNA phylogenies. The resulting phylogenetic framework can now be used for more detailed studies on genome/trait evolution in these ecologically diverse protists.

Phylogenomics reveals multiple independent lifestyle transitions within early-branching fungi

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Chytridiomycota (chytrids) are early-branching unicellular fungi that feed osmotrophically via rhizoids as parasites, saprotrophs or intermediate forms. They thrive in most aquatic and terrestrial environments in all climates, can reach high abundances and fulfil important ecosystem functions. As parasites, chytrids influence the planet's carbon cycle by terminating algae blooms and they can have significant impacts on host populations causing for instance global amphibian declines. The evolutionary emergence of parasitism within the phylum has been unclear because only few parasitic lineages have ben cultured so far and genome or transcriptome data for parasites is scarce, and due to the inability of ribosomal marker genes to phylogenetically resolve the early diversification of holozoa and fungi with confidence. Here, we use culture-independent single-cell genomics and a phylogenomic approach to overcome such limitations. We provide new genomic/transcriptomic data of 29 parasitic taxa, together with a robust backbone topology for the diversification of Chytridiomycota. Our analyses reveal multiple independent lifestyle transitions between parasitism and saprotrophy among chytrids and imply a capability of the chytrid last common ancestor to parasitise on phytoplankton.

Phylotranscriptomics support a temporal constraints model of development for the dictyostelid social amoebae.

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Karl Ernst von Baer's third law of embryology posits that the earliest stages of animal development are the most conserved between taxa. However, modern studies using phylotranscriptomics have shown most animals and other complex multicellular organisms follow a pattern of maximum developmental conservation at mid-development. An initial study on the transiently multicellular dictyostelid amoebae proposed they follow neither of these patterns rather maximal developmental conservation occurs late in their development. However, due to the limited availability of genomic data from relatives of dictyostelid amoebae at the time, alternative approaches were employed in the study rather than established phylotranscriptomic methods previously used to assess developmental conservation patterns in animals, plants, and fungi. Since then, numerous genomes and transcriptomes from dictyostelids have become available. Additionally, we have sequenced the genomes of twenty-five dictyostelid amoebae that represent the known diversity of the group. With these new data we constructed a highly resolved phylogeny using 240 conserved protein coding genes. Using our new genomic data, phylogeny, and previous developmental transcriptomic data from 5 diverse dictyostelids we reinvestigated transcriptional age dynamics during development of dictyostelid amoebae using well-established phylotranscriptomic approaches. Our results demonstrate that none of the dictyostelids we sampled exhibited either a period of maximum developmental conservation at mid-development, or maximal developmental conservation late in development as previously suggested. Instead, our results support a developmental pattern that exhibits maximum conservation in early development which fits the temporal constraints model, von Baer's third law, of evolutionary conservation during development.

Microbial predators form a new supergroup of eukaryotes

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Molecular phylogenetics of microbial eukaryotes has reshaped the eukaryotic tree of life by establishing broad taxonomic divisions, termed 'supergroups', which supersede the traditional 'kingdoms' of animals, fungi, and plants, and aim to encompass the whole breadth of eukaryotic diversity. The vast majority of newly discovered eukaryotic species fall into a small number of known supergroups. Recently, however, a handful of species with no clear relationship to other supergroups have been described, raising questions about the nature and degree of still undiscovered eukaryotic diversity, and exposing the limitations of strictly molecular-based exploration. Here we report eleven previously undescribed strains of microbial predators isolated through culture that collectively form a diverse new supergroup of eukaryotes, termed Provora. The Provora supergroup is genetically, morphologically and behaviourally distinct from other eukaryotes, and comprises two divergent clades of predators — Nebulidia and Nibbleridia — that are superficially similar to each other, but differ fundamentally in ultrastructure, behaviour and gene content. These predators are globally distributed in marine and freshwater environments, but are numerically rare and have consequently been entirely overlooked by molecular diversity surveys. In the age of high-throughput analyses, investigation of eukaryotic diversity through cultivation remains indispensable for the discovery of rare but ecologically and evolutionarily important eukaryotes.

This research was supported by grants from the Russian Science Foundation, https://rscf.ru/en/project/23-14-00280/ (to D.V.T., investigation of cryptic species diversity, morphology, and 18S rRNA sequencing)," Gordon and Betty Moore Foundation (to P.J.K., https://doi.org/10.37807/GBMF9201), the Natural Sciences and Engineering Research Council of Canada (to P.J.K., Grant Number 2019-03994).

Endosymbionts in amoebae – a community approach

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Several authors have formulated the potential ecological role of microbiomes in protists, including promoting survival, providing resilience, adaptation potential, and nutrients. While research on this topic has rocketed, synthesis is limited to tabular summaries of known/presumed symbiont functions. However, some generalizations can already be made based on the morpho-ecological traits of protists examined so far: Selective Hosts (SH) are present among ciliates, diverse amoebae, flagellates, and some excavates, whereas Inclusive Hosts (IH) are found in Amoebozoa and likely, large benthic Foraminifera, with a diverse prokaryote composition variable in time. Amoebozoa studies still focus on medically important hosts and some large amoebae, whereas knowledge of rhizarian amoeba hosts remains sparse. Bacterial uptake through endocytosis is observed in all groups except for some excavates. Characterization for IH amoebae based on the hosts' morpho-ecological traits and known host-symbiont relationships can facilitate predicting the nature of novel host-symbiont systems in protist hosts. KA hosts: Large amoeboid protists with K-strategy, mostly benthic, in organically rich lakes and marine environments. rA hosts: Small amoeboid protists: r-strategy, cyst formation typical; soil, organically poor sediment and biofilm. The following hypotheses can be established for the bacterial endosymbiont communities of the above host types: In SH: either aposymbiotic or permanently with one specific symbiont; well-established role, highly evolved relationship with the host, mostly beneficial; small, phagotrophic hosts, e.g. Paulinella. In IH-KA: rarely apo-symbiotic, presence of diverse prokaryotic clades; transient prokaryotes overwhelmingly; highly evolved host-symbiont relationships missing; numerous prokaryote clades prone to endosymbiosis or pathogenesis; host species bad colonizers, in prokaryote-poor outer environment do not perform well: internal bacterial diversity reduces, pathogenic bacteria earlier suppressed become dominant. Low potential for trophic chain-mediated microbial ecosystem regulation; instead, limited to host population regulation. E.g. Arcella, large benthic foraminiferans. In IH-rA: host species good colonizers in organically poor environments; pathogenic bacteria typically occur, posing potential hazard in anthropogenic environments; high potential for trophic chain-mediated microbial ecosystem regulation; Trojan Horse effect probable. E.g. Naegleria, Acanthamoeba and other FLA. Formulating the testable hypotheses outlined above helps to facilitate the filling of knowledge gaps related to host-prokaryote symbiont systems in various clades of heterotrophic amoeboid protists.

Deciphering the evolution of the early-diverging clades of heterotrophic flagellates Apusomonadida and Ancyromonadida

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Heterotrophic flagellates are found in most eukaryotic supergroups and some of them, such as the Apusomonadida and Ancyromonadida, diverged early in evolutionary history. Consequently, they might have differentially retained some ancestral characteristics already present in the last eukaryotic common ancestor (LECA). However, to reconstruct life history traits, a robust phylogenetic backbone is required and some of these flagellate lineages await a proper phylogenetic placement. This is the case of several lineages of heterotrophic flagellates within the Opimoda (Amoebozoa, Ancyromonadida, Apusomonadida, Breviatea, CRuMs, Malawimonadida and Opisthokonta), for which the deepest relationships between lineages are not fully resolved. To address this, we have sequenced transcriptomes of fourteen recently described apusomonads (Torruella et al. 2022), seven ancyromonads (Yubuki et al. submitted) and the incertae sedis species Meteora sporadica (Galindo et al. 2022). We have included them in a paneukaryotic phylogenomic analysis focusing on free-living heterotrophic flagellates. This analysis solved the internal relationships within Apusomonadida and Ancyromonadida, as well as the relationships within other Opimoda clades, opening the doors to reconstruct ancestral states for these lineages.

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Establishing a multi-omics approach to study *Cryptosporidium parvum* in calves

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Cryptosporidium genus is comprised of protozoan parasites which infect a wide range of hosts, including humans, causing a disease called cryptosporidiosis. In cattle farms, the incidence of cryptosporidiosis results in high mortality and, consequently, is a source of considerable economic loss in the livestock industry. Cryptosporidium infection occurs through the faecal–oral route, followed by the release and propagation of the parasite inside of the gastrointestinal tract of the host. Disturbances of the normal gut microbiota have been associated with numerous diseases, however the underlying molecular and biochemical mechanisms in which C. parvum interferes with these dynamic and complex systems are still poorly understood. Hence studying the interplay between gut microorganisms and metabolites might grant new therapeutic solutions and identify potential new compounds that could be used as indicators for the presence of C. parvum. This study aimed to monitor how C. parvum infection modulates the gut microbiome and metabolome of the host. Two parallel investigations targeting an in vivo and in vitro infection using calves as an infection model were designed. The in vitro study involved the sampling and collection of fecal samples from preweaned calves in dairy farms at a singular time point. The in vivo study entailed a longitudinal study where newborn calves were selected and divided into two groups denominated infected and noninfected after half of those were experimentally infected with C. parvumoocysts. Following infection calves were followed daily and fecal samples collected for 21 days after infection. A well-established qPCR protocol targeting the C. parvum oocyst wall protein (COWP) gene fragment, was used for the detection and quantification of the parasite in both studies. In parallel, faecal samples were also collected for the 16S ribosomal RNA (rRNA) sequencing and NMR-based metabolomics for both studies. Analysis of the 16S rRNA sequencing and metabolomics data, was carried out using several bioinformatics tools. Preliminarily results from these analyses will be presented. In conclusion, this study provides an improved understanding of how C. parvum impacts the gut metabolism of the calves, by following the response to the parasite infection during the length of the disease in neonatal animals.

Independent colonizations of athalassohaline water bodies by freshwater lobose testate amoeba (Arcellinida, Amoebozoa).

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The salinity barrier is considered as one of the most important frontiers dividing biodiversity and strongly influencing the distribution of organisms. Crossing it opens the possibility for newcomer organisms to colonize new niches and diversify. In turn, these transitions require profound physiological adaptations and are therefore supposed to happen rarely in the evolutionary history of the clades. Most of these transitions have been studied in marine environments, where abiotic and biotic (i.e. competitors and predators) factors difficult transitions. Here, we explored the biodiversity of Arcellinida testate amoebae, a group present mainly in freshwater sediments and soil, in athalassohaline water bodies (i.e. of non-marine origin and with different salt concentration and composition than the sea). These saline lakes experience extreme salinity and temperature fluctuations, which makes them arguably a harsher environment than the sea from the abiotic perspective. Biotic pressures, on the other hand, may be lower due to their island-analogous nature. We combined microscopical observations, single-cell barcoding and environmental metabarcoding to explore the biodiversity of Arcellinida in different water bodies of Spain and Chile, with salinities ranging from freshwater to near saturation. In general, communities were composed of euryhaline species which were able to tolerate wide salinity ranges, sometimes from freshwater to salinities higher than seawater, in accordance to the high fluctuations present in their original environments. We also found several transitions across the salinity barrier, indicating multiple independent colonizations of the saline environment by Arcellinida from freshwater ecosystems. Since Arcellinida are physiologically able to adapt to salinities higher than those of seawater, salinity alone is not the barrier keeping Arcellinida from transitioning towards the marine environment.

Newly discovered deep lineage of eukaryotes with unusual morphology, ultrastructure, and mitochondrial genome

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The eukaryotic tree of life has been reworked multiple times since the of molecular phylogenetic era, often by discovery of novel deep branching protist lineages. A novel protist, termed SUM-K and presented here, is such a novel deep lineage. SUM-K is a morphologically inconspicuous protist related to hemimastigophoreans, and is possibly a member of a new emerging eukaryotic supergroup. The organism has two cell types, a flagellated and a “sun-like” form. The latter displays a novel type of extrusomes we have examined by electron microscopy. Deep-branching protist lineages are thought to be indispensable for unraveling the early evolution of mitochondria, because they frequently retain metabolic pathways that have been lost in model organisms. SUM-K is an another such example. It possesses an unusually complex and plesiomorphic mitochondrial genome. Moreover, SUM-K has a near complete set of ETC subunits as well as a rich repertoire of enzymes involved in aerobic and as well as anaerobic metabolism that point to a flexible energy metabolism. This is another metabolic capability often attributed to LECA, which is even more noteworthy considering that the SUM-K requires anaerobic conditions to thrive.

Apical annuli are specialised sites for post-invasion secretion in *Toxoplasma gondii*

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Apicomplexans are ubiquitous intracellular protistan parasites of animals. These parasites use a programmed sequence of secretory events to find, invade, and then reengineer their host cells to enable parasite growth and proliferation. The secretory organelles micronemes then rhoptries mediate the first steps of invasion, and both secrete their contents through the apical complex. This cell feature provides an apical opening in the parasite's elaborate inner membrane complex (IMC) — an extensive subpellicular system of flattened membrane cisternae and proteinaceous meshworks that otherwise limits access of the cytoplasm to the plasma membrane for material exchange with the cell exterior. The secretion programme after invasion drives host cell remodelling and occurs from dense granules, however, the site(s) of dense granule exocytosis has been unknown. In Toxoplasma gondii, small subapical annular structures that are embedded in the IMC are seen, but the role or significance of these apical annuli to plasma membrane function has also been unknown. Here, we determined that integral membrane proteins of the plasma membrane occur specifically at these apical annular sites, that these proteins include SNARE proteins, and that the apical annuli are sites of vesicle fusion and exocytosis. Specifically, we show that dense granules require these structures for the secretion of their cargo proteins. When secretion is perturbed at these apical annuli, parasite growth is arrested. The apical annuli, therefore, represent a second structure in the IMC specialised for protein secretion, and demonstrate that in Toxoplasma there is a physical separation of the processes of pre- and post-invasion secretion that mediate host-parasite interactions.

Revealing the kleptoplastidic symbiosis in the marine centrohelid *Meringosphaera*

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Kleptoplastidy is a type of plastid symbiosis whereby a host cell temporarily retains plastids stolen from algae prey, giving the host the opportunity to use photosynthates. This widespread biological process is found in a variety of protists as well as some animals, but has only been well-studied in a few organisms. Using a combination of culture-free methods such as environmental sampling, single-cell genomics, phylogenetics and microscopy, we investigate the enigmatic kleptoplastidic symbiosis in the globally distributed and sometimes abundant marine centrohelid Meringosphaera. Meringosphaera harbors plastids of Dictyochophyceae origin and shows evidence of genetic integration of these plastids by endosymbiotic gene transfers (EGTs) of plastid-associated genes. At least two distinct groups of Dictyochophyceae prey have been detected, which seem to be specific for different Meringosphaera hosts. Here we show that Meringosphaera is phylogenetically diverse and sequences are found in metabarcoding datasets spanning marine coastal and oceanic environments. Environmental sampling and application of catalysed reporter deposition-fluorescence in situ hybridization (CARD-FISH) assays suggest seasonal plastid switching for one of the Meringosphaera groups, but the identity of its plastids during the spring months is currently unknown. We will present the results of new analyses of 12 recently obtained single-cell assembled genomes (SAGs) from samples collected in May 2022 on the west coast of Sweden, to determine the identity of these plastids. We will also confirm the presence of the Dictychophyceae prey in the environment, by performing double CARD-FISH on environmental samples, targeting both the Meringosphaera host and the Dictyochophyceae plastids. With these new results, we will be closer to understanding the complexity of this kleptoplastidic system and therefore its potential as a model to study the dynamics of organelle evolution.

Gametocyte-specific factor 1 (GTSF1) is required for genome rearrangement of *Paramecium tetraurelia*

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In animal germline, Piwi-interacting RNA (piRNA) pathway represses transposable elements at transcriptional and post-transcriptional levels to safeguard the genome integrity. Gametocyte-specific factor 1 (GTSF1) is a conserved factor of the piRNA pathway that has been demonstrated to interact with Piwi and involve in transposon silencing in mammals and insects, however, whether GTSF1 also exists in unicellular organisms is unknown. Here, we identified the homolog of Gtsf1 in Paramecium tetraurelia (PtGtsf1) and revealed it interacts with Ptiwi09 and PRC2 complex and loss of PtGTSF1 derepressed transposons and impeded transposon and transposon derived DNA elimination. Like mouse and insects, PtGTSF1 participates in the piRNA-like pathway, but it affects the degradation of piRNAs rather than the biogenesis. As a result of piRNA disturbance, PtGTSF1 deficiency increased the intensity of H3K9me3 and H3K27me3 in maternal and progeny macronucleus. Furthermore, PtGTSF1 deficiency perturbed the degradation of domesticated endonuclease PiggyMac and introduced massive DNA breakage in the progeny macronucleus. Our results indicate GTSF1 exists in ciliate protozoa and is a key factor in their piRNA-like pathway.

Functional division of two distinct methyltransferase complexes for eukaryotic DNA N6-adenine methylation

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DNA N6-adenine methylation (6mA) is lately rediscovered as a potential eukaryotic epigenetic mark in eukaryotes. We and others previously reported that 6mA in unicellular eukaryotes, catalyzed by the MT-A70 family methyltransferase AMT1 (adenine methyltransferase 1) and its orthologues, displayed an enrichment at the 5' end of the gene body. However, the molecular mechanism directing the specific genomic distribution of 6mA remains elusive. Here, we characterize two mutually exclusive yet functionally cooperative 6mA methyltransferase complexes in the ciliate Tetrahymena thermophila. One is responsible for the high 6mA methylation at the 5' end of genes via transcription-associated pathways and the other is involved in low 6mA deposition by linking with replication. These two mechanisms provide a double security system and thus ensure 6mA methylation homeostasis.

The biology of peritrichs, including some historical perspectives

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The peritrichs (subclass Peritrichia) are characterized by their conspicuous oral ciliature comprising the haplokinety and the polykinety which wind counterclockwise around the border of the peristome before descending into the infundibulum, and a reduced somatic ciliature comprising a subequatorial trochal band that is permanently ciliated on mobile species but is temporarily ciliated on the dispersal stage of sessile species. Peritrichs are classified into two orders: the Sessilida, which are free-living (although many are epibiotic) and typically sessile, attaching to their substrate either via a scopula or its derivatives (e.g., stalk, lorica), although some are always free-swimming; and the Mobilida, which are obligate parasites that are mobile but attach temporarily to their host via a distinctive adhesive disc or holdfast. The Sessilida are the larger of the two groups comprising over 800 nominal species, compared to about 280 species of mobilids. The taxonomy of sessilid peritrichs is traditionally based on their morphology, in particular their lifestyle (solitary vs. colonial), features of the cell or zooid (e.g., shape, pattern of the silverline system), and features of attachment organelles (stalk contractile vs. non-contractile; lorica with vs without valves). In the past two decades, however, molecular phylogenetics has led to a significant re-evaluation of peritrich systematics. In this talk I will give a brief introduction to sessilid peritrichs, including their morphology and some aspects of their biology.

Assessing the impact of past environmental changes on marine protist biodiversity using sedimentary ancient DNA

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Arctic marine ecosystems are highly sensitive to climate change and are currently being altered by increasing water temperatures, changes in sea ice conditions and anthropogenic stressors. These rapid changes will inevitably have profound effects on biodiversity and productivity. However, so far, our knowledge on the cumulative impact of these changes on protist communities remains limited, despite their important roles in food webs and nutrient cycling. In order to understand ongoing and future changes in Arctic ecosystems and the resilience of protist communities, it is essential to assess their response to past changes in environmental conditions. So far, such studies were limited to lineages with a fossil record (e.g. Foraminifera), leaving an incomplete picture of the remaining protist diversity. We are applying sedimentary ancient DNA sequencing as a new tool for reconstructing past changes in protist communities in relation with past environmental changes. We are focusing on marine sediment cores from the shelf areas of the Nordic Seas and assess environmental and biodiversity changes throughout the last 10,000 years. Here, we present the first results from a sediment record from a coastal area of northern Svalbard. We extracted ancient DNA and trace a wide range of protist taxa through time to estimate past changes in diversity and productivity. These paleogenomic data are compared to other paleo-proxies, changes in sea ice cover and water temperature to identify drivers of biodiversity change.

This work is supported by the Norwegian Financial Mechanism for 2014-2021, project no 2019/34/H/ST10/00682.

Mortality rates of planktonic ciliates

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Despite their early use as indicators of water pollution more than 100 years ago and Gause's seminal experimental investigations on predator-prey dynamics with Didinium and Paramecium in the 1930s, free-living ciliates have been used less frequently as model organisms in ecological and evolutionary studies than in other biological disciplines. In recent years, genetic and experimental studies with aquatic ciliates tackled macroecological concepts and increasingly used ciliates to model ecological properties concerning biodiversity, biogeography and global warming. Modell predictions depend on the realistic parameterization of the key processes. Like all organisms, ciliate population sizes are controlled by their resource-dependent growth rates and by losses imposed by predators and parasites. A recent meta-analysis demonstrated that top-down control is common in many lakes, but predation pressure in the central parts of the ocean is too low to keep ciliate growth rates in check. In offshore regions, resources are too scarce to promote fast ciliate growth. Ciliate growth rates become negative if the food supply falls below a species-specific critical threshold and the populations decline. Maximum (specific) mortality rate (δmax) at zero food levels is the counterpart of specific growth rates (rmax) achieved under optimal, food-replete conditions. In contrast to rmax, δmax has only sporadically been investigated for aquatic ciliate species. Using a meta-analysis, I will demonstrate that δmax of planktonic ciliates are positively related to rmax but appear unaffected by temperature and habitat (marine vs freshwater). The median δmaxof cyst-forming ciliates is twofold higher than that of those unable to encyst. Probably due to the paucity of available data, these differences are statistically insignificant. I will discuss the open questions and main conclusions from these findings for future scenarios (global warming).

Spatial distribution and self-organized pattern formation in single-species systems – experiments with ciliates

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Dispersal is a central process for natural populations, as it connects local to spatially structured populations. It is a crucial factor for the spatial functioning of (meta)populations and (meta)communities and can have fundamental effects on ecological and evolutionary dynamics. The complexity of the dispersion process results in many nonlinear interactions and the induction of self-organized patterns. Although studies showed that even single-species systems can exhibit nonlinear behavior, the causes and consequences of nonlinear dynamics and their relation to spatial distributions are still unknown. Working with a chamber of interconnected habitat patches, automated microcosm experiments measuring spatial distribution were performed using ciliates (e.g. Tetrahymena) as model organisms. The fully automated setup allows a measurement of the abundances for each habitat patch every few minutes and allows us to determine peaks in abundances and self-organized pattern formations. Here, we show that even single-species experiments can not only fluctuate in their total abundance, they also show a complex distribution behavior resulting in the formation of self-organized patterns. Studying these patterns and their nonlinear behavior gain insights into how ecosystems function and how they can be managed to promote conservation and sustainability. Nonlinearity should be considered as an important phenomenon in cell biology and single-species dynamics and also, for the maintenance of high biodiversity in nature, a prerequisite for nature conservation.

Characterization of a eukaryotrophic flagellate representing a novel major lineage within Stramenopila

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Eukaryotrophic protists are ecologically significant and possess characteristics key to understanding the evolution of eukaryotes. These predators remain poorly studied, in part, due to the complexities of maintaining predator-prey cultures. Isolate SC is a free-swimming biflagellated eukaryotroph with a conspicuous ventral groove, a trait observed in distantly related lineages across eukaryote diversity. Di-eukaryotic (predator-prey) cultures of isolate SC were established by single-cell isolation using three marine algae (Isochrysis galbana, Guillardia theta, and Phaeodactylum tricornutum) as prey. Growth trials showed that the studied clone grew particularly well on G. theta, reaching a peak abundance of 1.0 x 105 ± 4.0 x 104 cells ml-1. Small-subunit ribosomal DNA phylogenies infer that isolate SC is a stramenopile with moderate support; however, it does not fall within any well-defined phylogenetic group, including environmental sequence clades (e.g. MASTs). In our current work, multigene phylogenetics is employed to better resolve the placement of isolate SC. Electron microscopy shows traits consistent with stramenopile affinity, including mastigonemes on the anterior flagellum, and tubular mitochondrial cristae. Isolate SC may represent a novel major lineage within stramenopiles and be important for understanding the evolutionary history of the group. While heterotrophic stramenopile flagellates are considered to be predominantly bacterivorous, eukarytrophy may be relatively widespread among this assemblage.

Evolution of splicing in *Pseudoloma neurophilia*: exploring the most reduced spliceosome

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Microsporidia are obligate intracellular parasites that have the most reduced eukaryotic genomes. With this reduction has come the loss of most, if not all, introns. This loss of introns has led to extensive reduction in the molecular machine that catalyzes their removal from pre-mRNA, the spliceosome, which is composed of five snRNAs (U1, U2, U4, U5, and U6) and upwards of 200 proteins. We analyzed the purportedly intron-lacking genome of the microsporidian Pseudoloma neurophilia and identified two introns. These two introns are spliced at high levels, despite an extremely reduced spliceosome. Indeed, with only 14 associated proteins, the spliceosome of P. neurophilia is the most reduced functional spliceosome known. The proteins that are retained are divergent from canonical orthologs. Highlighting this divergence, the central spliceosomal protein, Prp8, which is derived from the proteinaceous component of self-splicing introns, lacks functional domains in P. neurophilia that are highly conserved across the diversity of eukaryotes, even in other microsporidia. We identified candidates for all five snRNAs in P. neurophilia. All but the U2 snRNA are extensively diverged from canonical orthologs, likely as a result of the loss of protein interacting partners. Despite this divergence, the U1 and U2 snRNAs have motifs that are predicted to interact with intronic motifs beyond any previously described interaction, highlighting the importance of RNA-RNA interactions in the absence of proteins in the splicing reaction of P. neurophilia. The spliceosome in P. neurophilia is retained to splice a mere two introns and, with the loss of associated proteins and the reliance on RNA-RNA interactions, functions in a manner more reminiscent of presumed ancestral splicing.

Spatial proteomics: the dark side of the method

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Various techniques enable us to study specific proteins and their interactions, obtain a proteomic profile of the entire cell, and determine the precise subcellular localization of proteins through spatial proteomics (SP) or organelle proteomics. Assigning the position of proteins within the cell is a crucial step in uncovering their functions and binding partners. SP aims to capture the localization of all proteins expressed in a given cell type or culture. This approach has been applied to extensively studied model organisms such as yeast, various types of HeLa cells, cancer cell lines, and Toxoplasma, as well as non-model organisms like Paratrimastix. In contrast to traditional organellar gradients that focus on a limited number of fractions, SP allows us to comprehensively study the entire proteome. Thus, it appears to be an ideal tool for investigating various organisms and cell lines. However, what are the limitations and challenges one might encounter? The critical step in any SP analysis is effectively disrupting the cells while preserving organelle integrity. This is where most of the issues arise. Determining the optimal level of cell lysis is crucial—whether the cells are underlysed, overly lysed, or properly lysed. We have attempted SP on several organisms and developed species-specific cell lysis protocols followed by differential centrifugation. Each organism was lysed under different conditions, and the lysates were subjected to a series of spins to separate subcellular particles based on their sedimentation rate. For Paramecium tetraurelia, the cell lysis and fractionation protocols were relatively straightforward to develop, yielding excellent data for several organelles. However, with Tetrahymena thermophila and Cafeteria roenbergensis, the lysis protocols proved to be more challenging. Preliminary results indicate that most organelles and their associated proteins sedimented in the initial spins, making it difficult to distinguish between them. However, we observed a consistent pattern of ribosomes being distinctly separated, even under suboptimal lysis conditions. While SP is a useful tool to rapidly obtain cell biology information about most organisms with annotated genomes, there are challenges that need to be addressed along the way.

High quality genome assemblies of six protists provide insights into giant virus and host defence mechanisms

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Protists can host a wide variety of giant viruses, with different effects on host cell populations. To fend themselves from harmful and often lytic infections, protist have evolved different defence strategies. One of these strategies is in the form of integration of endogenous viral elements in their genomes. These elements potentially allow us to track past infections and some may serve as an antiviral defence system. In turn, viruses have evolved different counter-defence strategies. To better understand the evolution of defence mechanisms on a genomic level, we combined long- and short read data, to generate high quality genome assemblies for six protists in two different taxonomic clades (Amoebozoa and Discoba). Analyses of their genomes show striking difference in codon usage preferences, which in some cases is opposite to the codon usage preferences of giant viruses replicating in these hosts. Contrary to most viruses, giant viruses encode tRNA and other translation-related genes, potentially compensating for codon usage differences with their hosts. Yet, instead of this compensation our computational analyses suggest that for some giant viruses the viral tRNAs can upregulate the expression of host genes, targeting diverse host proteins for proteasome-mediated degradation. Manipulation of the host protein ubiquitination machinery to overcome the host cell defence mechanisms is a strategy that several viruses use. However, instead of encoding specific proteins to target specific pathways, we suggest that giant viruses can modify host expression levels through viral tRNA-mediated codon usage adaptation as a more general counter-defence mechanism to cellular responses in different hosts. The high quality genomes presented here, set a gateway for experimental studies to deeper understand the interplay between defence and counter-defence of giant viruses and their hosts.

Lateral gene transfer into the “BRC”, a deep-branching group related to fornicate metamonads

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The supergroup Metamonada encompasses a variety of both free-living and parasitic protists that inhabit highly diverse anaerobic environments. They are notable for the diversity of mitochondrion-related organelles (MROs) that house metabolic and biogenetic systems that have been acquired through lateral gene transfer (LGT) events. Recent investigations of metamonad genomes have also noted an absence of genes encoding DNA segregation and processing proteins and suggested that alternative systems must exist to carry out these essential functions. Investigations of additional diverse metamonad genomes are crucial to clarifying the patterns and processes by which their extremely divergent biochemistries and cell biologies evolved. To this end, we have recently characterized the genomes of five newly-discovered free-living metamonad flagellates – the “BRC” – that form a deeply-branching clade sister to the Fornicata, a group containing diplomonads, retortamonads and Carpediemonas-like organisms. To understand the dynamics of BRC genomes, we assembled a phylogenomic pipeline to screen their predicted proteomes for laterally transferred genes from prokaryotes. BRC protein sequences were first clustered into orthologous groups (OGs) that were then used search for homologous proteins within public sequence databases, including predicted proteomes of variety of other metamonads. Phylogenies of the OGs were then screened for evidence of potential prokaryote-eukaryote transfer events yielding a total of 2462 out of 4570 OGs that were flagged for potential LGTs. Using functional assignment annotations, we narrowed this list to 68 phylogenies for manual curation. Amongst candidate LGTs, we found proteins involved methionine metabolism, nucleotide synthesis, and DNA methylation. The most remarkable of these potential LGTs are several giant non-ribosomal peptide synthetase (NRPS) proteins of 13,820 and 10,218 amino acids each. These NRPS are predicted to form small bioactive peptides that are typically involved in antimicrobial defense or iron scavenging, and have previously only been found in bacterial, fungal and a handful of other eukaryotic lineages. These investigations help to clarify the biochemical needs and functions of these small anaerobic flagellates, as well as illuminate potential molecular interactions with other microbes within their environments.

There and back again, the genomics of free-living diplomonads

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Diplomonads are flagellated protists that inhabit oxygen-poor environments and lack conventional mitochondria. They are classified within Fornicata and include both, free-living organisms that inhabit freshwater and marine anoxic sediments (e.g., Hexamita inflata), and host-associated organisms that reside in the intestinal tract of animals, including humans (e.g., Giardia intestinalis). Several medically and economically important diplomonads have been more intensely studied and there are three host-associated diplomonad genomes that have been fully sequenced and well annotated (G. intestinalis, G. muris, Spironucleus salmonicida). However, there is severe lack of data from free-living lineages. Interestingly, the free-living diplomonads are nested within the host-associated species in the phylogenetic trees. This placement suggests that they might represent secondarily free-living lineages that evolved from parasitic ancestor. Such reversal to free-living lifestyle is considered an extraordinarily rare event as endobiotic species commonly undergo adaptations to the host environment and at the same time loose genes for pathways whose products can be scavenges from the host. Loss of these pathways is very hard to surpass obstacle. Here we used combination of a deep sequencing by short reads generated by Illumina HiSeq X-ten, and lower-coverage long reads generated by Oxford Nanopore MinION to sequence the genomes of three free-living diplomonads (Novel diplomonad lineage Ghost, Hexamita sp. SM and Trimitus sp. IT1). Additionally, we performed gene predictions using Augustus and initial comparative analyses with the genomes of G. intestinalis, G. muris, and S. salmonicida genomic data to explore genome evolution and mechanisms of transition between lifestyles within diplomonads.

Mitochondrial RNA editing in ascetosporean amoebae

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Ascetosporea is a subgroup of Endomyxa and all species are parasites of marine invertebrates. Any cultures have not been established and their complete life cycles were not understood. In the previous studies, the cells of Mikrocytida, which is an ascetosporean subgroup, were isolated from the infected oysters/crabs and analyzed. They showed that their mitochondria were reduced to mitochondrion related organelles and their organellar genome was lacking. In the present study, we established two axenic cultures of Paradinida, which is another subgroup of Ascetosporea. DNA-seq on them reveled that they possessed ca. 20 kbp of circular organellar genomes, respectively. However, their genes were fragmented by the insertions of stop codon, suggesting that they were either pseudogenes or involved in RNA editing. As reconstructed genome sequences were compared with their RNA-seq data, massive RNA editing, i.e., substitutions from adenosine and cytidine to inosine and uridine, respectively, was confirmed. Many of the editing sites were shared between two paradinid cultures, but strain-unique editing sites were also detected. The gene sequences after the editing seemed operative and they were involved in electron transfer system. Hence, the mitochondria of Paradinida are not functionary reduced, unlike Mikrocytida. Further, we also detected adenosine deaminase acting on RNA (ADAR), which is a key enzyme of A-to-I substitution, from paradinids as well as other several protists. As we analyzed its localization in paradinids' cell using the commercially available anti-human-ADAR antibody, they were clearly and specifically localized in their mitochondria. Since ADAR was thought to be unique in metazoans and function in nucleus for a long period, it was surprising and speculated that ADAR may have originated in the last eukaryotic common ancestor. ADAR of paradinids may have a function to mask some lethal substitution in their mitochondrial genomes, which may help their diversification.

RNA editing in non-model trypanosomatids

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Telomeres are physical ends of linear eukaryotic chromosomes, composed of DNA repeat arrays of variable lengths and sequences, and associated proteins. In most cases, telomeres undergo gradual shortening with each round of cell division. Trypanosomatidae is a family of obligatory parasitic flagellates with a single large mitochondrion. Numerous Leishmania and Trypanosoma spp. cause severe human diseases. Telomeres of trypanosomatids were mainly studied in a model species, T. brucei. They are characterized by the canonical DNA sequence, presence of t-loops, association with capping protein complexes, and dependence on TbTERT, and increase in length by ~ 10 bp per generation until reaching an equilibrium. Deletion of TbTERT leads to a progressive telomere shortening. When telomeres become critically short, they get stabilized in the TERT-independent manner. Notably, deletion of TbKu proteins resulted in telomere shortening over several passages in vitro. The studies in Leishmania spp. are more controversial. While telomeres of most Leishmania spp. increase in length over time, the same was not observed for L. mexicana after 100 passages in vitro. The set of identified telomere-associated proteins in Leishmania sp. only partially overlaps with that of T. brucei indicating intrinsic differences between these genera of Trypanosomatidae. Here, we investigated telomeres in trypanosomatids and report that they dramatically different in length, even between closely related species. Among analyzed species, they ranged from 250–1,000 bp for Crithidia fasciculata to 800–33,500 bp for Vickermania ingenoplastis. This does not correlate with phylogenetic positions of given species, since telomeres of even closely related species significantly differ. We have previously demonstrated that ablation of either LmKu70 or LmKu80 resulted in telomere elongation in L. mexicana. This was unexpected because ablation of these proteins in T. brucei has an opposite effect. Our data indirectly suggest that short telomeres in L. mexicana are maintained in both TERT-dependent and TERT-independent ways, somewhat reminiscent of the situation observed in T. brucei when its telomeres become critically short. Indeed, ablating LmxTERT on either the wild type or ΔKu80 backgrounds in L. mexicana resulted in telomere shortening and a marked change to a discrete telomere pattern, respectively.

On the relationship between protist metabarcoding and protist metagenome-assembled genomes

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Current studies focused on protist communities are based largely on marker gene metabarcoding and whole genome analysis through metagenomics. Gaining a deeper understanding of the correspondence between the data produced by these two approaches has the potential to reveal the advantages and disadvantages inherent in each technique and to integrate information between datasets. We investigated the correspondence between V9 metabarcoding OTUs from the 18S rRNA gene (V9 OTUs) and metagenome-assembled genomes (MAGs) from the Tara Oceans expedition (2009-2013) and made an attempt to match them based on their relative abundances across samples. We evaluated the performance of several methods for detecting correspondence between features in the compositional dataset and developed a series of controls to filter artefacts of data structure and processing. After selecting the best-performing correspondence metrics, ranking the V9 OTU-MAG matches by their proportionality/correlation coefficients and applying a set of selection criteria, we identified candidate matches between V9 OTUs and MAGs. Our findings suggest that V9 OTUs and MAGs of some taxa can be successfully matched with one another, in which one MAG and one V9 OTU likely represent the same biological entity. However, there are also scenarios in which:

1. A single MAG matches many V9 OTUs;

2. A single V9 OTU matches many MAGs;

3. A set of V9 OTUs matches a set of MAGs.

Notably, different MAGs within the same genus do not necessarily all belong to the same scenario; that is, some MAGs from a genus might match only a single OTU, whereas other MAG/OTU matches might fall within one of the 3 aforementioned non-1-to-1 scenarios. We applied thorough procedures to filter potential artefacts, permitting an interpretation of the complex relationships between MAGs and V9 OTUs in terms of the genomic and ecological diversity of the underlying biological entities that they represent. These results illustrate that it may be possible to relate the OTUs of metabarcodes with OTUs of metagenome-assembled genomes from the same samples, but that the correspondence between the two datasets can be more complex than a direct 1-to-1 OTU match.

Physiological and transcriptomic responses of mixotrophic *Ochromonas* to light regimes and prey availability

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Mixotrophs are very common in aquatic ecosystems, and they can grow autotrophically, mixotrophically, and heterotrophically. Light and prey availability are the most important factors affecting their conversion of nutritional modes, however, how the mixotrophs respond to the changes in these two-resource availability and regulate nutritional strategy is poorly understood. This study investigated the phenotype, photophysiology, and transcriptome responses of the common mixotrophic organism Ochromonas. Results showed that food addition promoted that mixotrophic and heterotrophic Ochromonas grew at a significantly higher rate and reduced chlorophyll contents. Cell size of the mixotroph Ochromonas was influenced by their nutritional status and prey size. Additionally, structural equation model analysis suggested that mixotrophic Ochromonas altered their ingestion and photosynthesis in response to shift of light and prey availability mainly through stimulation effect of prey uptake on rapid growth as well as enhanced photosynthetic efficiency in combination with reduced thermal dissipation. At the transcriptional level, the differentially expressed genes of mixotrophic Ochromonas at different nutritional conditions were mainly related to photosynthesis, mRNA regulation, photosynthetic antenna proteins, carbon sequestration, glycolysis/gluconeogenesis, and phagosomes. In food presence, mixotrophic Ochromonas reduced the food intake and digestion activities through nutrient and energy sensing driven by AMPK, and reduced the synthesis of substances involved in photosynthesis. In light absence, mixotrophic Ochromonas positively enhanced food intake and digestion processes. In addition, these regulatory processes were also linked to their responses to environmental signals. In food presence, mixotrophic Ochromonas regulated catabolism and anabolism through an accurate mRNA regulatory mechanism, such as reducing the intake of nutrients through the pathway of plant-pathogen interaction, and weakening their photosensitivity of biological clock in circadian rhythm. In summary, the multiple regulatory pathways of mixotrophic Ochromonas at the transcriptional, physiological, and population levels reflect their highly efficient nutrient metabolism strategies in response to changes in food and light conditions, which enables them to better adapt to changes in complex environments.

A holistic approach to inventory the diversity of mobilid ciliates (Protista: Ciliophora: Peritrichia)

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Ciliates of the order Mobilida are obligate symbionts of a broad spectrum of invertebrates and vertebrates. Rigid structures of their adhesive disc, which serves for attachment to the host, are traditionally used for species identification. Using a holistic morpho-evo-eco approach, we attempted to address whether (1) also host organisms and their life histories represent important features in the mobilid taxonomy, (2) morphological similarities estimated from characters of the adhesive disc correspond to genetic similarities, and (3) which molecular markers are reliable DNA barcodes in the mobilid taxonomy. Multidimensional analyses revealed that morphometric data of the adhesive disc are not sufficient to delimit closely related species that are, however, phylogenetically and ecologically distinct. Out of the seven tested molecular markers, only the mitochondrial 16S and COI genes were found to be reliable DNA barcodes allowing unambiguous delimitation of also closely related species. Stochastic mapping suggested that higher taxonomic groups and life histories of host organisms also represent important extrinsic characteristics of mobilid species, especially when they are put in the phylogenetic context. Links of ciliates with freshwater fishes and with marine mollusks were recognized as statistically significant correlates during the mobilid evolution. The new standard in the inventory of the diversity of mobilid ciliates is exemplified by a holistic characterization of two species associated with aquatic mollusks, Trichodina unionis and T. baltica.

Abstracts:
Poster Presentations
(in alphabetical order)

Genomes of diverse non-model Trypanosomatidae

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Trypanosomatidae represents one of the most evolutionary successful groups of parasitic protists, remarkable for its adaptability to a wide range of vectors and hosts. They are subdivided into 19 monoxenous (one host) and 5 dixenous (two hosts genera. This diversity of life strategies makes Trypanosomatidae a good model to study the evolution of parasitism. However, the genomic information for many of these “obscure” clades was lacking. Despite the fact that monoxenous trypanosomatids represent a majority of known species, representatives of 8 monoxenous genera have never been analyzed on either genomic or transcriptomic level, and for many groups only one representative has been sequenced. This resulted in poorly resolved trees and uncertain position for many groups. A robust and definitive phylogenetic analysis of Trypanosomatidae demands complete sequencing data. In this regard, we assembled short-reads DNA sequences for 21 new trypanosomatid species, including 6 Wallacemonas spp., 5 Trypanosoma spp., 2 Crithidia spp., 2 Herpetomonas spp., Zelonia costaricensis, Vickermania spadyakhi, Jaenimonas drosophilae, Sergeia podlipaevi, Borovskyia barvae, and Obscuromonas modryi. In addition, we also assembled a transcriptome of Lafontella sp. using short-reads RNA-seq data. All the genomes and a transcriptome were assembled using 3 strategies. The size of the assembled genomes ranges from 20.3 Mb (N50 59.1 Kb) of T. scelopori to 35.4 Mb (N50 118.6 Kb) of C. brevicula and 35.3 Mb of Z. costaricensis. Completeness scores of BUSCOs and KAT (k-mer analysis) vary from 95.4% and 96.7% to 100% and 99.22%, respectively. Notably, these assemblies have a very low percent of gaps and errors testifying to their quality. We hope that these genomes will provide a valuable resource for the scientific community involved in studies of trypanosomatid biology.

Putative MutS2 homologs in algae: More goods in shopping bag?

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Bacterial MutS2 proteins, which are members of the diverse MutS family, can specifically bind to branched DNA structures (such as Holliday junction and D-loop) in an ATP-dependent manner and, having the ability to endonuclease activity, cleave them. This can lead to suppression of recombination, as has been demonstrated for Helicobacter pylori and Thermus thermophilus, although there is evidence that MutS2 promotes homologous recombination in Bacillus subtilis. Homologs of the MutS2-coding genes were previously found in the genomes of plants and some green algae, and their plastid origin was suggested. However, their actual diversity within eukaryotes remained unknown. We revealed amino acid sequences identified as homologous to MutS2 protein in photosynthetic organisms from various microalgal groups across the eukaryotic tree of life – in glaucophytes, chlorophytes, rhodophytes, haptophytes, cryptophytes, dinoflagellates, photosynthetic colpodellids, chlorarachniophytes, and ochrophyte species. We analyzed the domain composition and features of specific motifs in these sequences and tested them for the presence of a signal peptide. Four groups of MutS2 homologs can be distinguished according to their primary structure – MutS2 homologs with the full set of specific domains and motifs, MutS2-like sequences without endonuclease domain, MutS2-like sequences without DNA association domain, and putative homologs with only a complete ATPase domain. We also investigated the phylogeny of eukaryotic and bacterial MutS2 homologs. All the eukaryotic sequences are divided into two clusters. One of them includes sequences belonging to Archaeplastida species and one subset of haptophyte homologs, and the second one – sequences belonging to CASH lineages, i.e., cryptophytes, alveolates, stramenopiles, and haptophytes, and to chlorarachniophytes. The cyanobacterial MutS2 cluster together with the first group that is consistent with plastid origin. Unexpectedly, amino acid sequences belonging to myxococcal bacteria show phylogenetic affinity to the CASH-including group with strong support. The observed clustering does not fit into a clear division of eukaryotes into lineages with “red” and “green” plastids but seems to be in favor of the genetic contributions from multiple endosymbionts before and for permanent plastid (“shopping bag” model of plastid evolution) and of red–green mosaicism in algae with complex plastids.

Supported by RSF, project 22-74-10097.

Protist:bacteria metabolic associations might be pervasive in the Breviatea

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Living without oxygen is a daunting prospect for organisms. The endpoint of our metabolism as aerobes is ultimately electrons, in order for our metabolism to move forward we deposit these electrons to oxygen, which acts as the final electron acceptor. However, some organisms - especially anaerobes - can cooperate with other organisms whereby the metabolism of one partner serves as an electron acceptor for the other partner in a process known as syntrophy. These interactions are common in prokaryotes and also occur between prokaryotes and eukaryotes. Previous research has demonstrated a symbiotic relationship between the anaerobic breviate protist Lenisia limosa and the bacterium Arcobacter sp. EP1, where both partners benefit through hydrogen transfer. The extent of this relationship in other members of the Breviatea remains unknown; however it is essential to document it if we are to understand the impact of these symbiotic associations in oxygen minimum zones (OMZ). To address this knowledge gap, we generated high-quality metagenome-assembled Arcobacter genomes from four previously undescribed breviate isolates, using both short-read and long-read metagenomes. Using Fluorescence in situ hybridization, we explore the cell:cell contact of breviates and Arcobacter species under different conditions. Serendipitous observations of older cultures and preliminary FISH analyses demonstrate that other prokaryotes might also colonize breviate cells suggesting that other electron sharing symbioses could be at play in our mesocosms. Mining publicly available data revealed that Arcobacter and breviate-related sequences are present in the same environmental sequencing projects, which strengthens the hypothesis that this interaction may be common in Breviatea. By using comparative genomics, we aim to gain insights into the evolutionary history of syntrophy across the Breviatea phylum. This will allow us to better understand how these two complex, independent cellular systems co-evolved to form potentially symbiotic relationships in OMZs.

Paramecium species in an acid rain-recovering lake

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In the early 1970s, acid deposition became a serious issue to New York's waters and forests. The primary sources of acid rain were sulfur dioxide (SO_2) and oxides of nitrogen (Nox) from the combustion of fossil fuels that would result in deposition downwind from the source. Ecosystems such as the Adirondack Mountains, Catskills and the Hudson highlands were very susceptible to the effects of acid deposition. The US Federal legislature passed the Clean Air Act amendment (CAAA) of 1990 and, in 1995, implemented limitations of sulfur dioxide emissions from coal-fired power plants upwind of northeastern North America. Since then, lakes in New York have started seeing some recovery in their ecosystems such as viable conditions for fish survival. However, we know less about the recovery or shifts in community structure of protists such as Paramecium. In this project, we have been examining the genotypes, growth rates, and morphology of Paramecium cells isolated from Lake Awosting in New Paltz. Lake Awosting is a previously low-pH lake (~4) and in the reovery process now, approaching neutral in recent years. It also has a simplified fishless food web. We have found both divergent and conserved strains of Paramecium in Lake Awosting, including Paramecium bursaria, P. putrinum-related, and P. dubosqui-related strains. The isolates of the latter two species may represent species not in the databases currently. We have also found that cells from Lake Awosting are severely growth-impaired in neutral pH media. We have been comparing this species composition and growth rates with strains isolated from Mohonk Lake, a nearby lake that never underwent acid rain- associated pH changes.

Morphological redescription of three *Sonderia* species (Ciliophora; Plagiopylea; Sonderiidae) based on Korean populations

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Ciliate populations collected from Korea were studied morphologically with modern methods. As a result, they were identified as three species of the genus Sonderia Kahl, 1928. Among them, two species were consistent with the previously described species (Sonderia vorax Kahl, 1928 & S. steini Li et al., 2022), but some characteristics were not found in previous descriptions. The remaining one is very similar to the species of Sonderia macrochilus Kahl, 1930, however significant differences between them were found. Therefore, we called it as Sonderia cf. macrochilus. Here, we redescribe and compare the three species, focusing on the new characteristics found: relatively small body size, the present of one suture on the ventral and the dorsal sides, and relatively less number of somatic kineties in the Korean population of S. vorax. Interestingly, the Korean population of S. steini harbors longitudinal rows of cortical granules, a long cytopyge that covers about 50% of body length, and two contractile vacuole pores present on the dorsal side. Sonderia cf. macrochilus can be recognized by an oral opening located slight distantly from the anterior cell margin and elevated mid-region of the buccal cavity resembling S. macrochilus Kahl, 1930. However, this Korean population possesses long needle-like extrusomes embedded in the cytoplasm, which are significant characteristics to discriminate S. macrochilus Kahl, 1930, and S. paramacrochilus Li et al., 2022.

Phylogenomics and genomic metabolic features of gastrointestinal symbiotic ciliates of herbivorous mammals (Ciliophora, Trichostomatia)

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Ciliates within the subclass Trichostomatia represent most of the microbiome biomass in the gastrointestinal tracts of herbivorous mammals and take part in several metabolic pathways of their hosts. We have expanded the genomic data for this subclass with seven new genomes from capybara symbionts of the families Cycloposthiidae and Pycnotrichidae. With our data, we investigated both the genomic and metabolic features of the subclass and reconstructed its evolutionary history with a phylogenomic approach. The predicted genomes are highly AT-rich (GC content of 21.09 - 25.46%) and their sizes vary from 68-174 Mb, as estimated from 15,336 – 174,045 contigs assembled (N50 of 816 – 24,041 bp), exhibiting a completeness of 56.7 - 96.4, as indicated through BUSCO analyses. To investigate the metabolic features of trichostomatids, we selected one genome per subfamily/family included here (Entodiniinae, Diplodiniinae, Ophryoscolecinae, Cycloposthidae, Isotrichidae, and Pycnotrichidae) and predicted (Augustus and Augustus_Braker) and annotated their genes (eggNOG_mapper and dbCAN). 1,931 ~ 12714 genes were predicted, and 473 ~ 4911 genes were annotated. Considering eggNOG annotations, most of the annotated genes are related to "Signal Transduction", suggesting intense interaction of these organisms with other microbial taxa and with the gastrointestinal environment. On the other hand, dbCAN annotations showed that trichostomatid groups display distinct functional profiles. Ophryoscolecids and cycloposthiids are essentially fibrolytic, with a large enzymatic framework, active in structural carbohydrates. Isotrichids are amylolytic, adapted to digest non-structural carbohydrates, and Muniziella cunhai has several enzymes to degrade microbial cell wall constituents, such as chitin and peptidoglycan, suggesting predation on bacteria and fungi. Our phylogenomic reconstruction recovered, with full support, Trichostomatia and all the five studied families as monophyletic groups. Noteworthy, our intrafamily phylogenetic relationships have greater support compared to previous studies.

A new symbiont ciliate of freshwater bivalvia, *Conchophthirus* n. sp. (Ciliophora: Scuticociliatia) from South Korea

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A New Symbiont Ciliate, Conchophthirus n. sp. was discovered in freshwater Bivalvia (Anodonta (Sinanodonta) woodiana (Rea, 1834)) from Buyeo-gun South Korea. The species was described based on live and stained specimen observations, and small subunit ribosomal RNA gene (18S rDNA) analysis. Conchophthirus n. sp. is characterized by the following features: body size 74–98 µm × 40–57 µm in protargol preparations; 4–6 polykineties, 6–10 vestibular kineties; one ellipsoid macronuclear nodules and one or two micronuclei; 20-29 thigmotactic kineties; 71-100 somatic kineties. Conchophthirus n. sp. is distinguished from C. anodontae by the number of vestibular kineties (6–10 vs. 10–15). In phylogenetic tree, Conchophthirus n. sp. is nested with other congeners.

New contributions to the taxonomy and phylogeny of the genus *Condylostoma* (Alveolata, Ciliophora, Heterotrichea)

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The genus Condylostoma Bory de St. Vincent, 1824 is one of the most species-rich among heterotrichs, being widely distributed in brackish and marine environments, and including organisms living in periphyton, benthos, and psammon. However, Condylostoma species identification remains challenging due to insufficient original descriptions, few reliable distinguishing characters, and overlapping features between different species. This study presents an updated revision of the genus based on descriptions of four species, including eight populations collected from China, using both morphological and molecular methods. The main findings are as follows: (1) about 40 nominal species and 100 populations are reviewed, leading to the identification of 31 valid species; (2) keys, synonyms, geographic distribution and amended/improved diagnoses of all valid species are provided; (3) the kinetosomes of ciliature adjacent to the distal end of the paroral membrane show two patterns (blocky arrangement and stripy arrangement), which serve as important key features; (4) 18S rRNA gene-based phylogeny shows that blocky pattern differentiated only once, while stripy pattern differentiated twice in the evolutive history of the group; (5) cryptic species are detected and proposed for the first time to form the Condylostoma curvum species complex. This work provides a valuable taxonomic reference for further studies on this genus and highlights the need for a reliable and comprehensive key to identify the boundaries between species.

Bunch formation of *A. castellanii* infected with Marseilleviridae virus

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Marseilleviridae is a family of large double-stranded DNA viruses, which have a highly complex genome of ~360 kb and a large particle size of ~250 nm. Marseilleviridae infect protists, such as Acanthamoeba castellanii. These viruses are currently divided into five lineages, A-E. In Marseilleviridae, only lineage B Marseilleviridae viruses have been reported to induce host Acanthamoeba cells to form aggregations called "bunches". The bunch formation of the infected host cells is inhibited by galactose (Aoki et al., 2021). The bunch formation may be mediated through cell-cell interactions involving a galactose recognition process, which is supported by the members of Marseilleviridae containing a galactose-binding protein (GBP) gene in their genomes. The alignment of the amino acid sequences of several marseillevirus GBP proteins revealed differences in the respective N-terminal regions between lineage B and lineage A Marseilleviridae viruses. In this study, Serial slice images of bunched Acanthamoeba cells caused by infection with lineage B hokutovirus were acquired by using serial-block face SEM (SBF-SEM). these images were reconstructed 3D and visualize the distribution of virus particles in the bunches of Acanthamoeba cells and investigate the relationship between these characteristics. The results showed that hokutovirus particles were distributed locally in the interfaces between the viral infected Acanthamoeba cell, indicating that bunch formation was caused by intercellular interaction through virus particles. This provides insight into the mechanism of bunch formation. Furthermore, 3D reconstruction of tokyovirus, which belongs to lineage A Marseilleviridae virus, by using cryo-electron microscopy single particle analysis (cryo-EM SPA) showed that tokyovirus has a “cap" structure on top of the major capsid protein (MCP) that composes the outer capsid shell. The “cap” structure above the MCP trimer was suggested to be a ~14 kDa glycoprotein by PAS staining. These results suggested that the glycosylated cap structure on the MCP trimer occur the bunch formation of virus-infected Acanthamoeba by binding the viruses to the Acanthamoeba cell. the cap structure may differ between lineages, which determines the presence or absence of bunch formation.

Morphology, morphogenesis, and molecular phylogeny of a new species in the genus *Aspidisca* (Protozoa, Ciliophora) from South Korea

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The morphology and molecular phylogeny of a new Aspidisca species, discovered in the eastern coast of South Korea, were investigated. In this study, we investigate the living cells, silver-stained specimens and the nucleotide sequence of the small subunit rRNA gene. The new Aspidisca species is characterized by having a small body size, a distinct peristomial spur on the left margin, seven frontoventral cirri in “polystyla-arrangement”, and a unique arrangement of anterior portion of adoral zone of membranelles, i.e., anteriormost membranelle distinct separated from the other three membranelles. Because of this character, it can be easily distinguished from congeners. Phylogenetic analyses also support the establishment of a new species.

Metabarcoding analysis to detect protozoa and helminths in wild animals in South Korea

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Four species of wild animals, including Prionailurus bengalensis euptilurus, Nyctereutes procyonoides koreensis, Hydropotes inermis argyropus, and Sus scrofa coreanus, have been found to harbor potential infectious agents such as helminths and protozoa. Therefore, analyzing the presence of infectious agents in these wild animals is crucial for monitoring and controlling the spread of such pathogens. This study collected fecal samples from 51 wild animals inhabiting the mountains of Yangpyeong in Gyeonggi Province, Hoengseong in Gangwon Province, and Cheongyang in Chungcheongnam Province, South Korea. The fecal samples were analyzed using 18S rRNA sequencing to identify the parasite species infecting these wild animals. The results of the investigation revealed the presence of nematodes such as Metastrongylus sp., Strongyloides sp., Ancylostoma sp., and Toxocara sp. in the four wild animal species. In addition, Platyhelminthes such as Spirometra sp., Echinochasmus sp., Alaria sp., Neodiplostomum sp., and Clonorchis sp., as well as protozoa including Entamoeba sp., Blastocystis sp., Sappinia sp., Isospora sp., Tritrichomonas sp., Pentatrichomonas sp., and Cryptosporidium sp. were also detected. Therefore, continuous monitoring of intestinal helminths and protozoa in these wild animal species is necessary for controlling the spread of parasites.

Freshwater protist diversity analysis using long nanopore 18S rDNA amplicons reveals hidden diversity of 'excavates'

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Eukaryotic metabarcoding studies based on short 18S rDNA amplicons revealed an unprecedented diversity of protists in marine environments. Short amplicons, however, underestimate the diversity of some groups, like 'Excavates', and are not sufficient for phylogenetic reconstructions. Oxford Nanopore technology opened new possibilities for the analysis of diversity. This fast and efficient method is mainly used for genome sequencing, but the long reads also give advantages in the case of amplicon sequencing. Long amplicons have better taxonomic resolution and provide more phylogenetic information but are more biased due to the high error rate of the sequencing technology. Their usage is also tricky because of the lack of established pipelines for creating representative sequences and limited databases, which are especially insufficient for freshwater species. Our goal was to use long, nanopore, high-quality operational taxonomic units (OTUs) to close the gap in the knowledge of freshwater protistan diversity. We analysed the environmental samples from the summer of 2020 from twelve lakes in the Mazurian region (Poland). Then, the whole 18S rRNA gene was amplified and sequenced using a MinION device. Using our custom bioinformatic pipeline, we obtained 46 to 550 Illumina-like quality OTUs per sample and their abundances. Obtained OTUs belong to all the main groups of Eukaryotes with the domination of dinoflagellates, ciliates, cryptophytes and perkinseans, which are mostly unknown and could be potential parasites of freshwater invertebrates. Taxonomic composition looks similar to that obtained using Illumina V4 18S rDNA amplicons but is enriched with the 'Excavates' like metamonads and discobans, including diplonemids. Out of 3545 OTUs, 2782 were classified at the identity levels between 98% and 85%. We identified 39 OTUs of 'Excavates' with high confidence and found also Jakobida-like sequences, however, with lower identity levels to any known taxa. Our results indicate that it is possible to obtain, MinION high-quality OTUs, which represent protists' composition equally well as Illumina's amplicons. Many of the obtained sequences don't have identical representatives in the databases and possibly represent currently unknown organisms. Our long OTUs are a valuable addition to the freshwater protists databases and allow further phylogenetic reconstruction.

Unveiling the role of microtubule organizing centers (MTOCs) evolution in protists' diversity

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The appearance of a microtubule-based cytoskeletal system represents a milestone event in eukaryotes' evolution, redefining fundamental cellular features such as motility, sensing, morphology, and division. Despite its importance, the crucial transition remains largely unknown: what was the ancestral cytoskeleton structural configuration and its primary function, how it gave rise to the present-day diversity, and what were the drivers shaping this phenomenon? Many of these outstanding questions may be answered by analyzing the extant diversity of MicroTubule Organizing Centers (MTOCs), the entities providing the foundations of the microtubular cytoskeleton and defining its spatial arrangement. Our work aims to reconstruct the cytoskeletal evolutionary framework by determining MTOCs' morphological variations towards a wide array of protists sampled from eukaryotic lineages of particular interest. Electron and fluorescence microscopy will provide the structural insight of these fundamental cytoskeletal organizers necessary to infer ancestral features, associated functions, and evolutive pathways. The first part of this work will be integrated with a bioinformatic approach to decode the morphological and molecular constraints shaping MTOCs' evolution. After developing a pipeline of conserved centrosomal proteins, predicted protein structures from the AlphaFold2 database will be aligned using tools such as Foldseek to debunk MTOCs' evolution and obtain additional insights. Overall, this work may provide precious intel regarding the evolution of a fundamental cellular component and hopefully uncover fundamental forces driving cellular evolutionary events.

Proteomics of the zoospore-to-vegetative cell transition in the thraustochytrid *Aurantiochytrium limacinum* (Labyrinthulomycota) reveals putative constituents of the bothrosome and ectoplasmic network

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Thraustochytrids (a group of Labyrinthulomycota) are osmoheterotrophic marine protists that use a typical stramenopile zoospore stage to disperse to new habitat. Settled zoospores transition to a surface-associated vegetative growth phase by producing the ectoplasmic network (EN), formed by branching extensions of the plasma membrane and elaborated from a unique organelle known as the bothrosome or sagenogenetosome. We took a proteomics approach to gain insight into the composition and evolutionary origins of the EN and sagenogenetosome by examining changes in protein composition between swimming zoospores and growing vegetative cells of Aurantiochytrium limacinum ATCC MYA-1381 as the EN developed 2 to 8 hours after settlement. We detected 3580 proteins (~25% of the predicted proteome), and Principal Components Analysis of the detected proteins showed the biggest differences between zoospores and 2-hour-old settled cells. 410 proteins were differentially expressed (DE) in settled cells compared to zoospores, with 239 relatively more abundant in zoospores and 171 relatively more abundant in settled cells. k-means clustering put the differentially expressed proteins into five clusters, two containing proteins with relatively highest abundance in swimming zoospores and the other three containing proteins with relatively highest abundance after 2 to 4 hours, 4 to 6 hours, or 6 to 8 hours. Functional categories significantly enriched in swimming zoospores included proteins associated with cilia and intracellular trafficking as well as signal transduction, while functional categories significantly enriched after settlement included proteins associated with transcription and translation. Additionally, abundance of proteins associated with lipid transport and metabolism declined while proteins associated with amino acid transport and metabolism increased after settlement. Several different subsets of DE proteins were examined for proteins potentially involved in the structure/function of the sagenogenetosome and EN. Among candidate proteins lacking obvious orthologs outside labyrinthulomycetes were a very long PT (proline-threonine) repeat protein and a set of unusual coiled-coil proteins. Our results reveal a potential set of candidates for proteins that may be expressed in the sagenogenetosome and/or EN. These findings provide insights into the mechanisms underlying the life cycle of Aurantiochytrium limacinum and contribute to a better understanding of the physiology of this marine protist.

Diversity survey of endosymbionts associated with Polycystinea (Radiolaria) from the Sargasso Sea

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Polycystinea is a subgroup of Radiolaria (supergroup Rhizaria) which include silica skeleton-bearing amoebae that exist throughout the global ocean. Surface-water polycystines host marine phytoplankton as photosymbionts, which are mostly identified with microscopy as the dinoflagellate Brandtodinium nutricula, from the order Peridiniales. However, sparse reports use molecular evidence to identify the photosymbiont. Here I present a diversity survey of polycystine endosymbionts using 18S V4 rRNA gene metabarcoding on single radiolarian cells from the Sargasso Sea. While many polycystines contained B. nutricula, solitary polycystines from the Sargasso Sea were associated with a wide diversity of dinoflagellates from Dinophyceae, Suessiaceae, and Syndiniales (MALVs). In addition to dinoflagellate symbionts, Sargasso Sea polycystines also associated with possible photosymbionts from the group Chlorophyta. Further analyses and benchwork will be aimed at supporting these findings.

Rumen ciliates (Ciliophora, Trichostomatia) in Brazilian domestic cattle feed diets with crescent urea levels

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Ciliates in the subclass Trichostomatia (Ciliophora, Litostomatea) were found in large amounts colonizing the gastrointestinal tracts of several herbivorous mammals. In ruminants, they are found in the rumen and participates in various processes in the digestive metabolism of their hosts, degrading structural and non-structural carbohydrates and acting as bioindicators, showing the balance and proper functioning of ruminal activities. Since their discovery, rumen ciliates of domestic cattle were reported in various geographic locations and several host species. However, until now there is only one taxonomic inventory of ciliates associated to Brazilian cattle. Urea and other non-protein nitrogen (N) compounds are used in ruminant diets more than a century and were considered a relevant economical replacement for vegetable and animal proteins. Despite the large knowledge about urea supplementation, there is no reports on the effects of this compound on rumen ciliate species. The present study aimed to access the ciliate composition, relative abundance, richness, and density of rumen ciliates of Brazilian cattle feeding diets supplemented with crescent urea levels. Gastrointestinal samples were obtained from four Holstein x Gir cattle (Bos primigenius taurus L. x Bos primigenius indicus L.). Across all treatments analyzed, one subclass, two orders, four families, 11 genera and 32 species of ciliates were identified. The composition and species richness varied among the four treatments evaluated. However, the total ciliate density was not affected by the experimental diets used ($p>0.01$). The species Entodinium dalli was identified for the first time in Brazil and the species Holophryozoon bovis, described by Jirovec (1933) in domestic cattle in Russia was morphologically characterized in the present study based on live observations and staining by Lugol's and MFS solutions. These evidences highlight the peculiarities of the ciliatofauna of Brazilian trichostomatids. The present study performs the second taxonomic inventory in Brazilian domestic cattle, 36 years after the first report by Dehority (1986). The urea diets tested proved to be an efficient and low-cost alternative compared to commercial protein sources.

Polycycla (Poljansky, 1951) mobilines from South African holothurian hosts

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Polycyclids (Mobilida: Urceolariidae) are symbiotic peritrichous ciliates, endozoic to the gut of some holothurian hosts. Originally described as the genus Urceolaria, studies on polycyclids are limited with the most recent publication dating from 1966. Currently Polycycla is a sister clade to the genera Urceolaria and Leiotrocha within the family Urceolariidae, mostly due to the presence of an adhesive disc that is notoriously challenging to prepare for microscopy. To date no standard morphological method for species discrimination has been suggested and it is uncertain whether the genus is monotypic or not. For this study, polycyclids from three South African holothurian hosts, Roweia frauenfeldi frauenfeldi, Pentacta doliolum and Hemiocnus insolens were isolated for morphological and phylogenetic investigation. Scanning electron and light microscopy revealed peculiar morphological traits uncharacteristic of the mobilines, but usually associated with members of the order Sessilida. These traits include the ribbon-shaped macronucleus, the presence of scopular cilia on the thigmotactic aboral adhesive disc and a robust myoneme network. The denticle ring of the adhesive disc, present in all mobilines, is similar to other genera of the Urceolariidae, but is made up of more numerous and reduced plates with minimal articulation planes. Historically, systematic placing of this group has been challenging, but in the light of the morphological differences between Polycycla and the other families (including Urceolariidae) within Mobilida, the present study proposes that this genus be removed from the family Urceolariidae and placed back into the nomen nuda family Polycyclidae, as originally proposed by Johnston.

Unrolling of R-bodies isolated from the *Paramecium* endosymbiont *Caedimonas varicaedens*

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A wide variety of proteobacteria contain R-bodies, which are usually observed as coiled proteinaceous ribbons, about 500 nm in width. They were first observed in bacterial Caedibacter sp., which live endosymbiotically in paramecia. Under specific conditions, such as a decrease in local pH, the R-bodies rapidly and reversibly transform their coiled resting state into a long rod, often more than 10 µm in length. Unlike the R-bodies in Caedibacter taeniospiralis, which are well-characterized and unroll consistently in response to pH changes, our data shows that the R-bodies of Caedimonas varicaedens react in a more complex way. Our C. varicaedens strain is an endosymbiont living in the macronucleus of Paramecium caudatum. Here we present light and electron microscopic observations of the unrolling of R-bodies isolated from that strain by a novel procedure and compare these to a morphological model created using open-source software.

New Arcellinida metabarcoding protocol

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In the last few years, there has been an increase in the number of studies using environmental DNA sequencing to assess diversity in ecosystems. The quality of this assessment depends on the taxonomical precision barcoding markers are able to achieve. Ideally, metabarcoding should rely on species level discrimination to achieve maximum accuracy, which implies using fast evolving marker genes. Unfortunately, finding conserved regions suited for primer design in these genes is not straightforward. Therefore, a multiple primers approach can be required to characterize the diversity of ancient eukaryotic groups. Arcellinida is one of these ancient groups, whose origins may be found in the Neoproterozoic era. These organisms are well-known for being excellent bioindicators for ecosystem health, and developing a metabarcoding protocol that captures all their environmental diversity and allows species level discrimination has obvious advantages. Our aim in this work is to expand a recently published metabarcoding protocol (González-Miguéns et al. 2022), in which the infraorders Longithecina and Hyalospheniformes are less efficiently amplified. We designed several primers and tested their efficiency on five soil and sediment samples from Bulgaria and one sample from Madrid. Each sample has been observed in visu to characterize the diversity in situ. In parallel, we amplified each of these environmental DNA samples (with each primer designed here plus the primer in González-Miguéns, 2022) and sequenced them using Illumina Mi Seq. We expect to obtain similar or higher diversity with the molecular analysis than in visu and to create a protocol that could be used in bioindication, biogeography, ecology or systematics-studies.

Evidence for a recent horizontal gene transfer from a close relative of *Paramecium* killer bacteria to *Blepharisma*

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In our recently published genomes of Blepharisma stoltei (Seah et al. 2023; Singh et al. 2023), we found a gene pair with a high sequence identity to the reference genome of the alphaproteobacterium Caedimonas varicaedens (Suzuki et al. 2015). This obligate intracellular bacterium is known for conferring the "killer trait" to host paramecia (Preer et al. 1974) but has not been described in Blepharisma before and is absent from our lab cultures. A strain of C. varicaedens has, however, been described in a Euplotes species (Boscaro et al. 2019), showing a potential for wider distribution across the ciliate tree. The genes we found are transcribed and poly(A)-tailed like conventional eukaryotic genes, and are also encoded on long reads containing an internally eliminated sequence characteristic of Blepharisma germline DNA. So, it appears they are present in the Blepharisma genome, rather than an artefact from contamination or misassembly, and are therefore a product of a relatively recent horizontal gene transfer. Of the two genes, only one (lpxC), homologous to a key enzyme in the production of endotoxin A in gram negative bacteria, is full length and might therefore still be functional. Aside from an lpxB homolog independently acquired earlier in its evolutionary history, Blepharisma lacks the remaining enzymes of the pathway and thus would be incapable of producing the toxin. We are currently exploring whether exposure to infected Paramecium biaurelia cultures can lead to infection of Blepharisma with Caedimonas.

Anti-amebic activity and mechanism of action of synthetic pyrazolines

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Entamoeba histolytica infects 55 million people worldwide and causes 55-100 thousand fatalities every year. Current treatment drugs (e.g. paromomycin) work either at the level of the intestinal lumen (where trophozoites proliferate via cell divisions) or on the invasive trophozoites that have penetrated the gut or colonized internal organs (e.g. metronidazole). Some of these drugs generate high toxicity in patients, trophozoite resistance in vitro, and are mutagenic in laboratory animals. As anaerobic pathogen, E. histolytica trophozoites obtain energy by fermenting glucose to ethanol. The bifunctional Entamoeba histolytica alcohol dehydrogenase 2 (EhADH2), an essential enzyme in this pathway, catalyzes the conversion of acetyl Co-A to acetaldehyde and from acetaldehyde to ethanol. Four series of novel 2-pyrazolines (4-8 / series) were synthesized and evaluated for their ability to inhibit trophozoite growth and enzymatic activities. The four series differed in the substitution arrangement of the pyrazoline ring: series 1a (1,3-diaryl-1-carbamoyl-2-pyrazoline), series 1b (3-aryl-1-propylcarbamoyl-2-pyrazoline), series 2 (1,3,4-triaryl-1-carbamoyl-2-pyrazoline), and series 3 (1,3,5-triaryl-1-carbamoyl-2- pyrazoline). Series 1a, 1b, and 2 compounds were distinctively effective at inhibiting growth and ALDH/ADH enzymatic activities based on the position/presence of halogens on the aromatic rings, specific heteroatoms (thiocarboxamide vs. carboxamide). Series 3 had no inhibitory properties implying that bulkiness at pyrazoline C5 position could affect enzyme binding. Inhibitor 3-(4-chlorophenyl)-1-(4-bromophenyl carboxamide)-2-pyrazoline (series 1a#4) revealed the lowest IC50 (5.04 µM) and Ki values (9.11 µM -ADH-; 11.24 µM -ALDH). These results suggest pyrazolines are potential anti-amebic drugs with comparable efficiency to metronidazole and known mechanism of action.

Identification of key enzymes for starch synthesis in chromerids and Apicomplexa

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Starch and glycogen are the two most common forms of storage polysaccharides in living organisms and define two states of the same type of polysaccharide. Despite being both composed of α-1,4-linked and α-1,6-branched glucose chains, they have different physicochemical characteristics due to the different distribution of branching points. Starch is crystalline and allow to store huge quantity of glucose, but is only found in eukaryotes derived from plastid endosymbiosis and is linked to the appearance of Archaeplastida, and has for now always been linked to the presence of specific enzymes. However, starch accumulation also occurs in some Alveolata, such as Dinoflagellates, Chromerids or Apicomplexan parasites, which arose from other endosymbiosis involving not a cyanobacteria but an eukaryotic alga. Until now, functional analysis was mainly focused on Chloroplastida and led to the identification of key enzymes, like the isoamylase required for starch crystallization, or some specific starch synthase for initiation. Comparative analysis performed in silico of metabolisms in different eukaryotic lineages reveals that many eukaryotes and especially Chromerids and Apicomplexa do not contain sequences encoding those types of enzymes. Moreover, it is still unclear if the initiation of starch synthesis involves a specific starch synthase as in plants. To identify the enzymatic mechanism in those organisms, a collection of 95 Chromera velia UV mutants deficient in starch synthesis was generated. Targeted sequencing highlighted mutants containing distinct alleles altered in a different gene acting on carbohydrate. Among these, an allelic series of an atypical protein-encoding gene, found only in starch accumulating Chromerids and Apicomplexan, shows drastic deficiencies in starch or soluble polysaccharides production in mutants impacted in this enzyme, suggesting a potential defect in the initiation of storage polysaccharide synthesis. Further structural characterization of soluble polysaccharides accumulated by these mutants reveals they adopt an atypical structure. Finally, while its function remains unknown, preliminary works to define the enzymatic and physiologic activities of the identified protein suggests its potential role during the very first steps of polysaccharides synthesis, and in C. velia viability under certain conditions.

Unveiling the role of microtubule organizing centers (MTOCs) evolution in protists' diversity

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The appearance of a microtubule-based cytoskeletal system represents a milestone event in eukaryotes' evolution, redefining fundamental cellular features such as motility, sensing, morphology, and division. Despite its importance, the crucial transition remains largely unknown: what was the ancestral cytoskeleton structural configuration and its primary function, how it gave rise to the present-day diversity, and what were the drivers shaping this phenomenon? Many of these outstanding questions may be answered by analyzing the extant diversity of MicroTubule Organizing Centers (MTOCs), the entities providing the foundations of the microtubular cytoskeleton and defining its spatial arrangement. Our work aims to reconstruct the cytoskeletal evolutionary framework by determining MTOCs' morphological variations towards a wide array of protists sampled from eukaryotic lineages of particular interest. Electron and fluorescence microscopy will provide the structural insight of these fundamental cytoskeletal organizers necessary to infer ancestral features, associated functions, and evolutive pathways. The first part of this work will be integrated with a bioinformatic approach to decode the morphological and molecular constraints shaping MTOCs' evolution. After developing a pipeline of conserved centrosomal proteins, predicted protein structures from the AlphaFold2 database will be aligned using tools such as Foldseek to debunk MTOCs' evolution and obtain additional insights. Overall, this work may provide precious intel regarding the evolution of a fundamental cellular component and hopefully uncover fundamental forces driving cellular evolutionary events.

Ecology of Vampyrellida: Broad insights from meta-analyses of molecular environmental surveys

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Vampyrellida (Endomyxa, Rhizaria) is an interesting and understudied group of free-living, predatory amoebae. They are often large and voracious, feeding mostly on other eukaryotes, and thus may play important roles in the microbial food web. Phylogenetically they form a clearly monophyletic lineage composed of at least eight clades, with the families Leptophryidae, Vampyrellidae, Placopodidae, Sericomyxidae and a number of incertae sedis groups, some of which are only known by environmental sequences. Even if vampyrellids are rarely abundant in the environment, they are widely distributed and occur in marine, freshwater and terrestrial habitats. Hence, a global view of their distribution and detailed ecology is desirable. For this aim, we established a bioinformatic pipeline to recover vampyrellid 18S rRNA gene sequences from public environmental molecular studies, and infer the ratio of each taxon in the major biomes. This will provide a first glimpse into the worldwide distribution of these ecologically diverse amoebae. In addition, these data might help search, isolate and describe the species so far only known by their sequences.

Bridging biology and physics – *Paramecium bursaria* as a model ambassador

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Paramecia, like many protozoa, exhibit a predominantly predatory lifestyle, a key factor in the control of microbial biomass in ecosystems. In cell and molecular biology as well as genetics and epigenetics, Paramecium is used as model organism due to its easy cultivation and established genomics. Additionally, its huge variety of naturally occurring diverse intracellular symbionts makes it a suitable model in symbiosis' research. In our project, we demonstrate Paramecium as model organism that obeys a random walk. Random walks are ubiquitously present in all natural sciences and are used as simple models for Brownian motion, e.g., to describe diffusion of pollutants in the air, to model motile bacteria, and to describe the travel of data in the world-wide web. We focus on Paramecium bursaria, known for its symbiotic lifestyle with photosynthetically active endosymbiotic algae and photoaccumulative behavior. Assessing its swimming behavior in well-defined specific environments, we test multiple potentially influencing parameters such as nutritional condition, symbiotic status, and different illumination scenarios. We describe our implemented experimental environment, and explain our methods of measuring and analyzing trajectories of individual cells as well as of their mean squared displacement (MSD; squared average distance a particle moves within a certain time). We confirm Paramecium as a simple model suitable to verify predictions in statistical physics, active soft matter, and ecosystem modeling. This project will further facilitate predictions of Paramecium's swimming behavior, an aspect highly relevant with respect to its feeding strategy, response to disturbances, and distribution.

Unraveling cryptic speciation within *Ceratiomyxa fruticulosa*

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Morphology and ultrastructure have long ruled for determining species identification. For protists with few visual morphological characters, molecular methods alleviated much of the difficulty with species determination; however, it sometimes reveals unexpected degrees of divergence between organisms with identical morphologies. This is the case with the species complex Ceratiomyxa fruticulosa, and appears to be consistent with an event of cryptic speciation. Past molecular work with C. fruticulosa found a very large genetic distance between different isolates, but little work has been done to follow up on this discovery. We have sampled and sequenced many new isolations of C. fruticulosa from geographically distinct locations within the continental United States. Single-gene 18S SSU RNA gene trees with these new isolates support the idea of cryptic speciation within this complex, and multi-gene phylogenomics using as many as 240 genes are underway to grant stronger support to this hypothesis. Ceratiomyxa was long considered to be a member of the Myxogastrid amoebae based on their similar fruiting behavior, but molecular phylogenetics revealed that they belong to a sister group of fruiting amoebae, Protosporangiida. Aside from Ceratiomyxa, the other members of Protosporangiida display simpler single-celled non-plasmodial fruiting behavior. The elaboration of Ceratiomyxa species and their behaviors works to bridge the gap in understanding between microscopic simple fruiting amoebae and the macromycetozoa, including Myxogastria and Dictyostelia.

Description of new *Lambornella* (Tetrahymenidae, Oligohymenophorea, Ciliophora) and *Lambornella*-like isolates from Mexico

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The genus Lambornella (Tetrahymenidae, Oligohymenophorea, Ciliophora) currently includes three species. Two of them, L. stegomyiae and L. clarki, are parasites of mosquito larvae and can be found in tree holes filled with water, while L. trichoglossa is a free-living ciliate that was collected from the water tanks of Brazilian and Central American bromeliads. Historically, there is a confusion in discriminating between Lambornella and Tetrahymena, and while morphologists insist on the validity of the genus (Foissner, 2003), molecular phylogenetic data, though very incomplete, suggest that the Lambornella genus may be invalid (Strüder-Kypke et al., 2001). We will present morphological and molecular data characterizing several free-living Lambornella isolates collected in Mexico from bromeliad water tanks. We will also describe a Lambornella-related ciliate isolated from the river waters at the Pacific coast in Mexico. This ciliate shares main morphological features characteristic to Lambornella, but, apparently, it is not a typical Lambornella from morphology. Moreover, it may come in two different morphological forms, both free-living and actively swimming. Precise systematic attribution of this ciliate remains to be clarified.

Diversity of protist communities in the extreme environments revealed by metabarcoding

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The reports describing diversity of protists in the extreme environments are still rare. In general, the data on protists inhabiting such extreme niches as hot springs or oligotrophic waterbodies in deep caves are very scarce. We performed the 18S rRNA gene metabarcoding of protist component of the biofilms growing along a hot stream in the aquatic system of the Stolbovskie thermal springs (Kunashir Island, the Kurils, Russia) and of the microbial communities from the underground lakes of Shulgan-Tash cave (South Ural, Russia). In parallel diversity of prokaryotes was assessed. Six biofilms of different structure and color were sampled along the hot stream at the Stolbovskiye springs with neutral waters. One biofilm developed at temperature 79-81 ℃, two at temperature about 69 ℃, the rest grew at lower temperatures. In all biofilms the amplicon sequence variants (ASV) attributed to Ciliophora were the most abundant, among them anaerobic ciliates from genus Trimyema were numerous, followed by Oxytricha and some scuticociliates related to Cinetochilum. The representatives of Cercozoa (heterotrophic flagellates) were also characteristic for all biofilms, but only in biofilms with lower temperature they were dominant together with representatives of Sagenista and conose amoebae. In Shulgan-Tash cave the water samples and biofilms were collected in the underground lake at 450 m from the cave entrance in aphotic conditions at 6 ℃. The eukaryotic ASV, unexpectedly, appeared to be highly diverse and were assigned to twenty phyla of protists. Heterotrophic organisms belonging to Fungi, Pseudofungi (classes Pirsonia and Oomycota), Cercozoa, Ciliophora, and lobose amoebae were the most abundant. However, the phylum Ochrophyta represented mainly by classes Chrysophyceae (phototrophic and heterotrophic flagellates) and Bacillariophyta (diatoms) was also rather abundant. We isolated some protists from those samples and established the clonal cultures that were further identified by the 18S rRNA gene sequencing. They included heterotrophic flagellates Vivaspumella sp., Heteromita sp., Goniomonas sp., Neocercomonas jutlandica, a ciliate Anoplophrya lumbrici, as well as amoebae Parafumarolamoeba stagnalis and Naegleria polaris.

We acknowledge the Science Resource Center “Persistence of microorganisms” of the ICIS, UrB RAS.

Supported by the Kazan Federal University Strategic Academic Leadership Program (PRIORITY-2030).

A first look into protists communities and related environmental drivers along a surface water - groundwater gradient in the Danube wetlands of Vienna

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Although huge in dimension, groundwater ecosystems are hardly investigated in terms of microbial and metazoan communities. This is partly because they are hidden and often difficult to access. Moreover, abundance of organisms in groundwater is generally low. The lack of light and moderate import of organic matter from the surface put organisms under the challenge of energy limitation. While there is a steady growing number of studies on groundwater prokaryotic communities as well as on groundwater fauna (meio- and macrofauna), microeukaryotes, in particular protozoa, have received minor attention. Protozoa, and when including microeukaryotes originating from nearby surface waters we may speak about 'protists', are for sure the quantitatively dominant eukaryotes in the subterranean hydrosphere and believed to play a critical role in biogeochemical cycling of matter and in groundwater food webs. Samples collected monthly from an oxbow lake and the adjacent shallow aquifer were analyzed for their protist communities by means of extraction of environmental RNA (active fraction of community) and amplicon sequencing of the ribosomal 18S marker gene. Our preliminary analysis focused on compositional differences of protist communities in surface water and groundwater, as well as their seasonal dynamics. As expected, a substantial difference between surface water and groundwater communities is observed. Moreover, community data from molecular analysis were put into context with the prevailing environmental conditions, revealing different key drivers such as redox conditions and nutrients availability for surface water and groundwater protists, respectively.

A well-established symbiosis between *Dictyostelium giganteum* and a chlamydial symbiont under the influence of recurring multicellularity

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The soil amoeba Dictyostelium discoideum is one of the best-known protist model systems and especially famous for its so-called social life cycle, a conserved mechanism found in all dictyostelid species. During this life cycle, tens of thousands of cells aggregate and develop multicellular structures, eventually leading to the formation of durable spores. Under the influence of this behavior, complex interaction patterns between D. discoideum and the associated bacterial community have emerged. Only recently, a high prevalence of chlamydiae was described in dictyostelid spores isolated from diverse soil samples. Besides the well-known human pathogen Chlamydia trachomatis, this bacterial phylum comprises a vast diversity of strictly intracellular bacteria found in terrestrial and aquatic environments and associated with diverse eukaryotic hosts. A common feature of these environmental chlamydiae is the characteristic biphasic life cycle, in which they temporarily exit the host cell to infect new hosts. Here, we report on the successful isolation of a strain of Dictyostelium giganteum infected with a chlamydial symbiont identified as a member of the family Rhabdochlamydiaceae. Using fluorescence in situ hybridization and digital droplet PCR, we found that the chlamydial symbiont is primarily vertically transmitted through the social life cycle of D. giganteum. To our surprise, we found no evidence for the extracellular phase observed in all other chlamydiae described so far. These findings are further underpinned by genome sequencing and the lack of otherwise conserved chlamydial genes essential for extracellular survival. Horizontal transmission is, however, still possible but is apparently limited to the aggregating stages of the D. giganteum life cycle. We hypothesize that the chlamydial symbiont has adapted to the social life of its dictyostelid host during extended periods of co-evolution. The availability of related dictyostelid-chlamydiae symbioses, provides the unique opportunity to study convergent evolution and the adaptation to recurring multicellularity in dictyostelid hosts.

Standing above the rest: The unique behavior of new thecamoebids

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Members of the Sappinia genus (Thecamoebida, Discosea, Amoebozoa) are primarily terrestrial amoebae characterized by multiple nuclei (often in pairs), a protective mucoid coat (glycocalyx) to prevent desiccation, and ridges along their cell body sides. Although their cell surface closely resembles their sister group, the Thecamoeba genus, they lack dorsal longitudinal folds. Sappinia pedata was first described by P. A. Dangeard (1896) based on observations from cow dung, and was distinguished by its unique "standing" behavior where the cell attaches to a substrate and extends most of its mass into the open air. This position can be maintained for hours or even days on completely dry substrates. In 1902, Edgar Olive suggested the existence of at least two species of standing Sappinia on various animal dung, with one being larger and the other smaller. Despite the lack of new information on Sappinia or standing behavior for over a century, advances in phylogenetics and microscopy in the early 2000s led to further research. In 2007, Brown et al. molecularly confirmed, described in detail, and standardized the strain Sappinia pedata. In this study, we report a new species of Sappinia discovered on cow dung in Mississippi, and a new species of Thecamoeba from soil confirmed through phylogenetics of the 18S rRNA gene and morphometrics. Our findings support Olive's hypothesis of two distinct Sappinia species on dung. Moreover, we investigated the standing behavior mechanism using fluorescent microscopy in the new Sappinia species, revealing that the primary structural component relies heavily on localizing actin to the stalk. While standing behavior was previously only observed in Sappinia pedata, our research on Sappinia platani and the new Thecamoeba species confirms that this behavior is more prevalent within the Thecamoebida family.

A new group of endosymbiotic Legionellales of Euglenophyceae identified via metagenomic analyses

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Protists are known to form a range of symbioses with prokaryotes. They exhibit high diversity of the involved taxa and the mechanisms of interaction. The protist-prokaryotes endosymbioses are crucial for understanding events such as eukaryogenesis or the emergence and spread of intracellular pathogens. However, they are significantly understudied in contrast to prokaryotic symbionts of animals, which are less diverse but were the main focus of research on endosymbionts for a long time. Therefore, our main source of knowledge about the intracellular lifestyle in prokaryotes is not fully representative. Despite increasing attention to protist endosymbioses, a comprehensive picture of the theirdistribution across various hosts is lacking. Common challenges are the inability to culture the host and uneven investigation of known taxa. Here, we present a novel group of endosymbiotic bacteria harboured by members of Euglenophyceae (Euglenozoa). We used screening of over 20 metagenome assemblies originating from various single cells or cultures of Euglenida in search for endosymbionts' genomes. The newly identified group forms a separate clade in Coxiellaceae (Legionellales) and is related to known amoebal endosymbionts Ovatusbacter and Fiscibacter. Analysis of four draft genomes originating from different Euglenophycean hosts shows typical reduction for endosymbionts, with 1,4-1,6 Mbp genome size and strongly reduced biosynthetic capabilities. Curiously, the genomes analysed also show signs of inability to produce energy on their own, with incomplete glycolysis, TCA, and oxidative phosphorylation pathways while possessing genes coding for ATP/ADP translocase. Therefore, this group probably relies on the host's energy pool directly. Our results show the first case of Legionellales endosymbionts associated with phototrophic Euglenids and expand significantly their range of known associations with non-amoeboid/flagellated protists. Additionally, since the discussed Euglenophyceae are available in culture, they may be a promising object for further studies and comparison with other endosymbiosis cases.

How did you get here? Protozoan diversity from freshwater volcanic crater lakes in the South Pacific

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Understanding the geographical dispersal and ecological adaptation of microbial species is key to reconstructing the evolution of life. However, our current understanding of these processes is based largely on studies of interconnected aquatic environments. These data are difficult to use to infer patterns of adaptation as rapid colonisation precludes local adaptation, making adaptive events difficult to detect. In order to resolve this, we need to study multiple aquatic ecosystems that are isolated yet comparable to one another. To this end, we conducted an expedition to the volcanic crater lakes of Samoa and Wallis and Futuna to genetically characterize the eukaryotic microbes living in these environments. These lakes formed independently of one another, are separated by thousands of kilometres of ocean, and are largely inaccessible. The formation of the islands can also be reliably dated, which is important for understanding the timescale of ecological events. Here, we present early findings from our expedition, including environmental characterizations of the lakes, microscopic data of identified species, and preliminary genetic data and phylogenetics. We propose that these lakes represent a unique environmental model for understanding the evolution and dispersal of microbial eukaryotes.

Impact of taxon sampling on the internal relationship of Archaeplastida by nuclear gene-based phylogenomic analyses

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Archaeplastida is defined as a taxonomic assemblage comprising Chloroplastida, Rhodophyta plus non-photosynthetic lineages related to Rhodophyta, and Glaucophyta, namely the descendants of the eukaryote that uptook and transformed a cyanobacterial endosymbiont into a primary plastid. Both nuclear and plastid gene-based phylogenomic analyses supported the monophyly of Archaeplastida with high statistical support. However, it has been known that the phylogenetic relationship among Chloroplastida, Glaucophyta, and Rhodophyta inferred from nuclear genes and those from plastid genes were incongruent. The nuclear gene-based phylogenomic analyses put Rhodophyta at the base of the Archaeplastida clade. On the other hand, the plastid gene-based analyses inferred Glaucophyta as the first branch in Archaeplastida. In this study, we analyzed a phylogenomic alignment of approximately 300 nuclear genes to evaluate the relationship among Chloroplastida, Rhodophyta plus its non-photosynthetic relatives, and Glaucophyta. The new phylogenomic alignment contains (i) 45 taxa that cover the diversity of Archaeplastida and (ii) 12 non-photosynthetic members of Diaphoretickes that include six members of Pancryptista as the outgroup. The analyses conducted here consistently excluded the possibility of Chloroplastida as the earliest branch in Archaeplastida. We here demonstrate that taxon sampling greatly impacts the relationship among Chloroplastida, Rhodophyta plus its non-photosynthetic relatives, and Glaucophyta. Thus, nuclear gene-based phylogenomic analyses are insufficient to infer the earliest branch in Archaeplastida with confidence.

Taxonomy of new non-photosynthetic species of the genus *Poterioochromonas* (Chrysophyceae) based on morphological and molecular evidence

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The genus Poterioochromonas, established by Scherffel in 1901, is characterized by spherical cell having two unequal flagella, a greenish yellow-brown plastid and a conical lorica attached to the substratum by a stalk. The key characteristics to distinguish the genus Poterioochromonas from Ochromonas-like chrysophytes are the biflagellate housing a conical or cup-shaped lorica with a narrow stalk and their mixotrophic nutritional mode. To date, only three mixotrophic species bearing plastids have been reported in the genus Poterioochromonas: Poterioochromonas malhamensis, P. nutans and P. stipitata. In this study, we report the first discovery of non-photosynthetic Poterioochromonas species, revealing an independent loss of plastid in the genus. To fully understand the taxonomy of the new non-photosynthetic species, we performed a molecular phylogenetic analysis based on a concatenated dataset and observed morphological features using light and electron microscopy. For the phylogenetic analysis, we used a combined dataset from four gene sequences: nuclear small subunit (SSU), large subunit (LSU) rDNA and plastid LSU rDNA, rbcL gene. The molecular data divided the strains into two major clades according to the presence or absence of a plastid, including two new non-photosynthetic species, each with unique molecular signatures for nuclear SSU rRNA from the helix 10 of the V1 region, the helix E23-1 of the V4 region. The non-photosynthetic Poterioochromonas species lack an eyespot and plastid, and were housed in a lorica composed of a cup, stalk and foot. One new species produced stomatocyst in culture conditions, and their ultrastructure of true complex collar showed unique characteristics. Here, we provide new insight into the species diversity within the genus Poterioochromonas and propose two new species based on both morphological and molecular evidence.

Morphology and molecular phylogeny of two planktonic hypotrichous ciliates (Ciliophora, Hypotrichia) from China

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The morphology and molecular phylogeny of two planktonic hypotrichous ciliates Pelagotrichidium sinica n. sp. and Psilotrichides hawaiiensis Heber et al., 2018, from Shanghai, China were investigated based on living morphology, infraciliature, and small subunit (SSU) rDNA sequence data. Pelagotrichidium sinica n. sp. is distinguished from its congener, P. faurei (Tuffrau, 1972) Jankowski, 1978 by the smaller size, much pointed body rear end and fewer frontal cirri and ventral rows. Shanghai population of Psilotrichides hawaiiensi matches well with the other two American populations, but with a slightly higher number of adoral membranelles and cirri. In the molecular phylogenetic trees based on SSU rDNA sequences, the position of P. sinica n. sp. is rather poorly resolved. The family Spirofilidae is not a monophyletic group based on the limited representative taxon sequences. As more species in Spirofilidae are described and sequenced in the future, more convincing phylogenetic topology may be obtained.

Giardia intestinalis – unique model for understanding the post – translational protein transport pathway into the endoplasmic reticulum membrane

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The anaerobic protist Giardia intestinalis is the most common intestinal parasite of human. In addition, its unique cellular structures and the phylogenetic position makes G. intestinalis an excellent model for the evolutionary cell biology. We focus on the characterisation of the GET pathway which plays a key role in the post-translational insertion of tail-anchored (TA) proteins to the endoplasmic reticulum. The TA proteins such as SNAREs and various membrane receptors are transported post-translationally via a protein cascade that begins with Sgt2, which transfers TA-protein to the Get4-Get5 complex. TA-protein is then handed over to Get3 which serves as an ER - membrane targeting factor passing the TA-protein to the membrane embedded Get1-Get2 complex, which acts as the insertases. So far, we have identified all the components of the GET pathway in G. intestinalis and proposed their conserved role in post-translational transport. We used pull-down and subsequent mass spectrometry analyses to detect their interaction partners and CRISPR/Cas9 method to describe their function. In addition, the identification of the GET pathway components in G. intestinalis showed the involvement of Bag6 homologue, which was so far known only in metazoan species. Detailed characterisation and crystal structures of G. intestinalis Get3 greatly contributed to our understanding of the Get3 catalytic cycle during the TA-protein transport. Our current data show that G. intestinalis-GET pathway involves a new giardia-specific protein that has no homology to any known protein family. Moreover, we examine the role of GiGet1 as the universal protein insertase in the ER that functionally took over the EMC3 and TMCO1 insertase which are present in vast majority of other eukaryotes. Our results suggest that the GET pathway is highly conserved and essential eukaryotic invention.

Structure and composition of cyst envelopes of vampyrellid amoebae (Vampyrellida, Rhizaria) with special emphasis on chitinous substances

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The vampyrellid amoebae (Vampyrellida, Rhizaria) are naked, filose amoebae that feed on other eukaryotes. They are found in a variety of habitats, including freshwater, marine and terrestrial systems. All vampyrellid amoebae show a common life history, which involves the alternation between a motile trophozoite and an immotile but metabolically active digestive cyst. We provide a close look at the structure and chemical composition of the digestive cysts over a broad range of vampyrellid species. The cysts of some species display a remarkable, blue autofluorescence demonstrated by fluorescence microscopy and λ-scans with a confocal laser scanning microscope. Furthermore, we provide evidence for the presence of chitin and chitosan in the digestive cyst walls with fluorescent probes. However, the detectable amount of these two biopolymers differs clearly among the ten studied vampyrellid species. Additionally, we discovered two undescribed structures in the digestive cysts of Leptophrys vorax: 1) a chitin-rich layer surrounding the ingested prey in the food vacuole, and 2) chitin-rich lines that run around the digestive cysts. The function of these structures is not yet known.

Metabarcoding of bacteria, protozoa, and helminths in the gut of *Apodemus agrarius*

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The striped field mouse (Apodemus agrarius) is a common type of wild rodent found in Korea. Wild rodent feces are known to carry various pathogenic bacteria and parasites. The aim of this study was to rapidly detect bacterial, protozoa, and helminths pathogens in the feces of wild mice using next-generation sequencing (NGS)-based metabarcoding analysis. We conducted 16S/18S rDNA-targeted high-throughput sequencing on fecal samples from A. agrarius (n = 48). The taxa of bacteria, protozoa, fungi, and helminths in the feces were identified. There were no statistical differences in microbial richness (number of operational taxonomic units) and Shannon diversity index between the spring and fall. The following helminths were detected in the cecum samples: Heligmosomoides sp., Syphacia sp., Hymenolepis sp. and Raillietina sp. The following protozoa were detected in the cecum samples: Tritrichomonas sp., Monocercomonas sp., Giardia sp., and Eimeria sp. Additionally, Lactobacillus gasseri and Lactobacillus intestinalis were found to be more abundant in Heligmosomoides sp.-infected mice than in those not infected by this parasite. These results highlight the advantages of the application of NGS technology in monitoring zoonotic disease reservoirs.

Pirsonia catenata sp. nov. (Pirsoniales, Pirsonea), a parasitic nanoflagellate infecting the marine centric diatom *Coscinodiscus radiatus* from coastal waters of Korea

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A novel nanoflagellate species, Pirsonia catenata sp. nov. infecting the marine centric diatom Coscinodiscus radiatus was isolated from the southern coastal waters of Korea and described based on light and electron microscopy and molecular sequences. The parasite zoospore had an ovoid cell shape with two apically inserted, unequal flagella and the cell size ranged 5 to 10 µm in length and 2 to 7 µm in width. The parasite invaded into the host cytoplasm using a pseudopod, which becomes the trophosome and then lost the flagella shortly. The zoospore body remained outside the host cell, which is called auxosome and is connected to the trophosome by thin cytoplasmic strands. The trophosome gradually become larger as it phagocytizes the host cytoplasm, but does not fuse with other adjacent trophosomes. The auxosome has a globular shape and grows up to 14 µm in diameter before it divides. The auxosome divided six to seven times, while still connected to the trophosome, forming 32-64 loosely chained secondary auxosomes. The lastly formed secondary auxosome had two short flagella and became the flagellate mother cell (FMC). The FMC detached from the auxosome chain and divided once or twice to produce mature zoospore. It takes about 9 hours from the primary auxosome division to the production of mature zoospores. To determine the host range of the parasite, 9 genera and 15 species of marine centric diatoms were applied to infect with the parasite. Of these, P. catenata infected only Actinocyclus sp. except for the primary host C. radiatus . Molecular phylogeny inferred from SSU rRNA revealed that all Pirsonia species including the novel parasite species formed a monophyletic group with high statistical supports and P. catenata was closely related to P. diadema. The genetic distance of Pirsonia species showed low intraspecific (0-0.07%) and interspecific variation (0.40-1.69%) based on SSU rRNA gene sequences (1516bp). The genetic distance between P. catenata and P. diadema represent 0.40%. Analysis of the ITS2 secondary structure revealed that the secondary structure of P. catenata sp. nov. differed from that of P. diadema with four h-CBCs.

Detection of parasites and blood-meal hosts of tsetse flies from Tanzania using metagenomics

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Tsetse flies are the vectors for humans, wild animals, and livestock and can cause trypanosomiasis. Amplicon deep sequencing of the 12S ribosomal RNA (rRNA) gene can detect mammalian hosts, and the 18S rRNA gene can be used to detect eukaryotic pathogens, including Trypanosoma spp. Tsetse flies were collected from the Serengeti National Park (n=48), Maswa Game Reserve (n=42), and Tarangire National Park (n=49) in Tanzania in 2013. Amplicon deep sequencing targeting 12S rRNA and 18S rRNA genes was performed to screen the blood-feeding sources of tsetse flies and eukaryotic parasites in tsetse flies, respectively. 12S rRNA gene deep sequencing revealed that various mammals were blood-feeding sources of the tsetse flies, including humans, common warthogs, African buffalos, mice, giraffes, African elephants, waterbucks, and lions. Genes of humans were less frequently detected in Serengeti (P=0.0024), whereas African buffaloes were found more as a blood-feeding source (P=0.0010). 18S rRNA gene deep sequencing showed that six tsetse samples harbored the Trypanosoma gene, which was identified as Trypanosoma godfreyi and Trypanosoma simiae in subsequent ITS1 gene sequencing. Through amplicon deep sequencing targeting the 12S rRNA and 18S rRNA genes, various mammalian animals were identified as blood-meal sources of tsetse flies and two Trypanosoma spp. were detected. It may provide essential data for formulating better strategies to control African trypanosomes.

Changes in marine bacterial community in response to parasite-induced dissolved organic matter from the harmful dinoflagellate *Akashiwo sanguinea*

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Amoebophrya ceratii species complex is a widespread endoparasite that infects a variety of harmful bloom-forming dinoflagellates. The parasite eventually kill the host by the mature parasite trophont emerging from the host with the release of host materials including dissolved organic matters (DOM) into water. The parasite-induced DOM released from the host provides complex organic matter to bacterioplankton in marine ecosystem. Here, we isolated the dinoflagellate Akashiwo sanguinea infected with Amoebophrya in coastal water of Korea during the dinoflagellate bloom event and established the host and parasite system as culture. To investigate experimentally how parasite-induced DOM affects the community structure and diversity of marine bacterioplankton and to identify the most responsive bacterial taxa, infected and uninfected A. sanguinea cultures were incubated with coastal bacterioplankton for 20 days. In infected treatments, the abundance of the host A. sanginea declined sharply at day 4 when mature parasites began to emerge from the host, whereas the host abundance in uninfected controls exponentially increased by day 8. After emergence of the parasite from the host, DOC concentrations in infected treatments increased from day 5 to day 8, with bacterial abundance peaking on day 6. Compared to the uninfected controls, alpha diversity indices decreased over time in infected treatments as a single bacterial taxon (OTU001, identified 99.5% with Ichthyenterobacterium magnum), belonging to the class Flavobacteriia, predominated up to 50% of sequence read abundance. Our results demonstrated that parasite-induced DOM can be rapidly utilized by certain specialized bacterial taxa and change the diversity and structure of bacterial community.

A biological indication of a tintinnid species, *Tintinnidium primitivum*, of the Yellow Sea Bottom Cold Water

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Tintinnid ciliates, having distributional sensitivity to hydrological changes, have been applied as a useful tool to track the movements of water masses. The Yellow Sea Bottom Cold Water (YSBCW) is one of the most prominent summer oceanographic phenomena in the Yellow Sea (YS), a semi-closed marginal sea of the western Pacific. To examine the potential of tintinnid species as a biological indicator of the bottom cold water, the spatial distributions of the ciliates were investigated in the southeastern YS during the summers of 2010 and 2020. A dominant species, Tintinnidium primitivum, was found in the bottom cold waters in both years. The temperature and salinity (T-S) diagram for the dominant species distribution was plotted against the T-S range for the YSBCW at low temperatures between 6 ℃ and 12 ℃ and salinity between 32 and 33. Tintinnidium primitivum as a potential indicator species was seasonally monitored to trace vertical mixing along the 35°N line from 2019 to 2022. A bottom-oriented distribution was observed in every season except for winter. Winter vertical mixing was supported by water temperature and salinity profiles and the homogenous distribution of T. primitivum throughout the water column at Stns. 35-11 and 35-13. The absence of this species in response to the difference in the low-salinity surface water mass at Stn. 35-15 during winter indicated the invasion of shallow water mass from China. Using the sensitive indicator species, horizontal movements of the YSBCW from the 32°N to the 37°N were also monitored in the central trough of the YS. Distribution of the species showed southward movement in spring whereas its distribution extended toward the northern area of the 37°N in autumn. Therefore, the species sensitivity to hydrological changes can provide a valuable insight into the vertical and horizontal migration of the YSBCW. Detail distributional information on T. primitivum accumulated through further spatial and temporal investigations in the YS can contribute toward T. primitivum serving as an alternative tool when the water mass of the YSBCW cannot be clearly tracked by T-S measurement owing to water mixing and diffusion.

Protistan plankton communities and the influence of a branch of Kuroshio in the northeastern East China Sea during the late spring

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The marine ecosystem in the northeastern East China Sea is ecologically important and is impacted by warm water derived from the Kuroshio Current. Nonetheless, relatively little is known about the spatial variation of protist communities and the factors that regulate them in this ecosystem during the spring season. Here, we investigated protistan community structures using a complementary approach combining 18S rRNA gene amplicon sequencing and light microscopy cell counts at nine stations from the northeastern East China Sea to the west of Jeju Island. The vertical profiles of physicochemical properties revealed that the Jeju Warm Current water mass, flowing from the southeast towards the northwest, created a thermohaline front dividing the region in two. These two regions had similar planktonic biomass, but the protistan communities differed significantly: dinoflagellates accounted for higher proportions of the protistan communities in the warm and saline waters, while the relative abundances of diatoms and picochlorophytes were higher in the low-density water of the western stations. Furthermore, higher species richness and Shannon Diversity Index values in the warm and saline waters suggest that the Jeju Warm Current, a branch of the Kuroshio, increases protistan taxonomic diversity in the northeastern East China Sea during the late spring. Seed populations of harmful algal bloom-causing species were discovered in the warm and saline water originating from the Kuroshio, which is particularly important as it indicates that these waters could introduce harmful species that may spread to the Yellow Sea and Korea Strait. Taken together, the study suggests that potential changes to the current systems in the region could dramatically alter the structure of its protistan community.

Exploring the mitochondrial genetic code diversity

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During translation, the ribosome uses the genetic code consisting of nucleotide triplets to assemble a functional protein. However, this code is not invariant – one eukaryotic organism might even employ different codes in its nucleus, mitochondria, and plastids. A particularly intriguing aspect is the relationship between the usage of codons as translation terminators and the composition of release factors which recognise them. Typically, three stop codons are present in a genetic code: UAA, UAG and UGA. In mitochondria, their recognition is carried out by mitochondrial release factors mtRF1a (UAA and UAG) and mtRF2a (UAA and UGA). The former has been independently lost from different eukaryote lineages, underpinning reassignment of UGA as a sense (usually tryptophan) codon, whereas an unprecedented case of mtRF1a loss recently uncovered by our lab relates to a stop-to-sense reassignment of UAG. Furthermore, our team has already shown a correlation in changes in stop codon recognition, including sense-to-stop-reassignments, with mutations of specific amino acids in mitochondrial release factors in the green algal order Sphaeropleales and the stramenopile group Labyrinthulea. However, a comprehensive picture of mitochondrial genetic code diversity and evolution is lacking due to both lack of analyses of existing sequence data and poor sampling of particular eukaryote lineages, such as Rhizaria or Amoebozoa. We have initiated a systematic exploration of mitochondrial genetic code diversity and the underlying molecular mechanisms, such as alterations to the mitochondrial release factors across the eukaryote phylogeny. In multiple cases of protist taxa with no mitochondrial genome sequence available, we could obtain information on their mitochondrial genetic code by analysing sequences of mitochondrial transcripts extracted from transcriptome assemblies. As a result, we have discovered multiple new cases of stop-to-sense, sense-to-stop, and sense-to-sense codon reassignments in mitochondria as well as new independent instances of mutated mitochondrial release factors. These correlate with specific mitochondrial genetic code changes, reinforcing the notion of the significance of the mutations in altered termination codon recognition. Specific examples will be discussed.

More than a weed: Utilizing the diversity of vannellid amoebae to uncover the developmental and evolutionary secrets of protostelid amoebae

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Complex behavior and developmental strategies are found throughout the Eukaryotic Tree of Life. Sporocarpic fruiting is a unique developmental stage that only occurs within several Amoebozoan lineages in which a single amoeba produces a single spore atop an acellular stalk. Of these lineages, Vannella fimicola, of the Vannellidae family, is the only species observed to exhibit this developmental stage. Despite their wide ecological distribution and obvious evolutionary significance, little work has been done to classify the true diversity and elucidate deeper evolutionary relationships of Vannellid amoebae. Here we show the first sequenced genome of V. fimicola using MinION and Illumina sequencing technologies that will serve as preliminary genomic data for understanding protostelid development and comparative genomics of Vannellid amoebae. Using synchronized cultures, transcriptomic data from trophic, pre-spore, and fruiting amoebae can be mapped to the draft genome to understand the structure and function of genes involved in this characteristic developmental stage. Comparative genomics of V. fimicola other sporocarpic amoebae will better elucidate the function and evolutionary history of genes shared by protostelid amoebae. Comparative genomics of candidate Vannellid amoebae will be employed elucidate the evolutionary history of environmental transitions, acquisition of unique developmental strategies, and species divergence within the group.

Two new species candidates of anaerobic ciliates genus *Tropidoatractus* (Ciliophora, Armophorea, Metopida, Tropidoatractidae) based on morphology and molecular phylogeny

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The morphology of two new species candidates collected from Jeju Island and Ulsan, South Korea are characterized using live observation and silver-stained preparations. Tropidoatractus n. sp. 1 characterized as small to medium size cell, 65–93 × 25–35 µm in vivo; ellipsoidal shape with wide anterior and tapered posterior; ventral side depressed and left side twisted towards right side; translucent pipe-like structure lined longitudinally in the cytoplasm; dome brim formed a beak; 7–8 rows of colorless cortical granules (CG) located between somatic kineties (SK); SK number 11–14; false kinety number in perizonal stripe (PS) rows 23–29. Tropidoatractus n. sp. 2 characterized by small size cell, 52–71 × 20–30 µm in vivo; elongate ellipsoidal shape with dome brim formed a snout; aggregated granules present in the anterior end; CG absent; SK number 15–17; false kinety number in PS rows 17–25. Both species share similar characters such as single globular to ellipsoidal macronucleus (Ma) located in the anterior part covered by endosymbiont bacteria; single globular micronucleus (Mi) attached to the Ma; contractile vacuole (CV) terminally; caudal cilia (CC) conspicuously longer than ordinary cilia; adoral membranelles (AM) number 12–14; Paroral membrane (PM) in diplostichomonad type. Tropidoatractus n. sp. 1 differs from Tropidoatractus n. sp. 2 and T. spinosus (Kahl, 1927) by the body shape (an ellipsoidal shape with an wide anterior and tapered posterior vs. elongated ellipsoidal shape vs. elongate, ellipsoidal with elongated spine), CG (present vs. absent vs. undescribed), pipe-like structure in the cytoplasm (present vs. absent vs. absent), dome brim shape (beak vs. snout vs. snout), SK number (11–14 vs. 15–17 vs. 12–13), CC (present vs. present vs. absent), and PM type (diplostichomonad vs. diplostichomonad vs. stichomonad). According to phylogenetic analyses inferred from SSU rDNA sequence data, Tropidoatractus n. sp. 1 candidate grouped with its congeners and cladded with T. spinosus (MG896717). However, it has shown 91.11% sequence similarity with T. spinosus, having 110 nucleotides differences. Based on the molecular analyses and significant morphological differences with its congeners, we suggest that two Korean populations should be Tropidoatractus new species.

Divergent ERMES complex is conserved in *Trichomonas vaginalis*

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Communication is a key process of life manifestation including intracellular interactions between organelles. One of the most studied interactions involves these between ER and mitochondria via ER-mitochondria encounter structure (ERMES). ERMES consists of four core proteins (Mmm1, Mmm2/Mdm34, Mdm12, and Mdm10) and is involved in the trafficking of phospholipids between ER and mitochondria, calcium signaling, mitochondrial dynamics, DNA inheritance, and mitophagy. Although, initially proposed as a fungal complex, later searches across eukaryotic genomes revealed the presence of ERMES orthologs in Ameobozoa, Discoba, Glaucophyta, and Metamonada. Our investigation focused on investigations of divergent ERMES homologs in organisms possessing anaerobic mitochondria (hydrogenosomes and mitosomes) with a focus on Metamonada. We identified all four ERMES components in Anaeramoeba flameloides and three components (Mmm1, Mmm2, and Mdm12) in all the members of Trichomonadidae and Tritrichomonadidae with lineage-specific expansion of Mmm1 in all parabasalid species. However, in Fornicata and Preaxostyla no ERMES components are present. ERMES was found only in free-living Carpediemonas. We experimentally investigated ERMES of the human parasite T. vaginalis. Immuno-fluorescence microscopy of the ERMES components shows expected localization of Mmm1 in ER, while Mdm12 and Mmm2 were partially localized to hydrogenosomes. Pull-down assays of the ERMES complex components identified a parabasalid-specific Porin2 as a possible substitute for the missing Mdm10 component of ERMES. In silico modeling of ERMES complex in T. vaginalis showed a formation of quasi-continuous hydrophobic tunnel of TvMmm1-Mdm12-Mmm2, which is consistent with its involvement in phospholipid trafficking. Overall, this study delivers a closer understanding of the connection between ER and hydrogenosomes.

Predation by *Acartia* on the heterotrophic dinoflagellates *Gyrodinium* spp.

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This study aimed to determine the ingestion rate of Acartia spp. (A. hongi and A. omorii) feeding on three Gyrodinium species: G. jinhaense, G. dominans, and G. moestrupii. The results showed that the maximum ingestion rates of Acartia spp. on G. jinhaense and G. dominans were similar, at 3,680 and 3,480 ng C predator-1d-1, respectively. However, the maximum ingestion rate on G. moestrupii was much lower, at 1,600 ng C predator-1d-1. This may be due to larger size and faster swimming speed of G. moestrupii. At the prey concentrations of 50 and 100 ng C mL-1, the ingestion rates of Acartia spp. on G. jinhaense, G. dominans, and G. moestrupii were higher than those on the heterotrophic dinoflagellates Pfiesteria piscicida and Luciella masanensis but lower than those on Stoeckeria algicida and Oxyrrhis marina. Thus, the three Gyrodinium species are moderate prey for Acartia spp. and contribute to the differential feeding of the copepods.

Comparative mitochondrial genomics of Synurales (Chrysophyceae)

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The Synurales is a photoautotrophic group in chrysophytes, which is unicellular or colonial flagellate algae covered by delicate siliceous scales and bristles. To understand mitochondrial genome evolution of synuralean algae, we sequenced the mitochondrial genomes of nine species from the three genera Mallomonas, Synura and Neotessella, and analyzed comparative genomics of eleven mitochondrial genomes including previously published chrysophycean species Synura synuroidea and Chlorochromonas danica. The mitochondrial genomes were similar in terms of their general architecture and gene content. The genome size ranged from approximately 35 kbp to 53 kbp. The total number of genes including RNAs ranged from 62 to 75 genes. They shared a core set of 2 rRNAs, 23~ 29 tRNAs, and 34 protein coding genes. The synuralean mitochondrial genomes retained almost all genes found in many other eukaryotes. However, all succinate dehydrogenase subunits (sdh genes) are missing, but secY gene is presented in synuralean mtDNAs, except for Neotessella volvocina. The mitochondrial gene order re-arrangements were detected in each genome, but moved together in the context of 6 synthetic blocks. The mitochondrial genes of Neotessella volvocina were scrambled in gene arrangement, and encoded group II introns in the cob gene, as well as trans-spliced cox1 gene. We performed a comparative analysis of genome structure and gene re-arrangements and investigated the phylogeny of mitochondrial genes among the chrysophytes. Our results provide important insights into the evolution of mitochondrial genomes and fine-scale dynamics within chrysophytes.

Trichomonas vaginalis-secreted lipid mediator LTB4 induces chemokine IL-8 production via dynamin-mediated endocytosis of LTB4 receptor BLT1 and phosphorylation of NF-kB

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Trichomonas vaginalis is a protozoan parasite that causes the most common non-viral sexually transmitted infection worldwide. The host immune response to T. vaginalis involves the chemokine IL-8 production at the site of infection. Previously, we reported that LTB4 contained in T. vaginalis-derived secretory products (TvSP) play an essential role in IL-8 production in human mast cell line (HMC-1 cells) via LTB4 receptor BLT1- or BLT2-mediated NF-kB activation. Dynamin, a GTPase, has been known to be involved in endocytosis of surface receptors for signaling of production of cytokine or chemokines. Here, we investigated the role of dynamin in the BLT-dependent IL-8 production induced by T. vaginalis-secreted LTB4. When HMC-1 cells were transfected with BLT1 or BLT2 siRNA, TvSP-induced IL-8 production was significantly inhibited compared to results for cells transfected with control siRNA. Pretreatment of HMC-1 cells with MßCD, an inhibitor for lipid rafts formation, inhibited TvSP-induced IL-8 production. In addition, pretreatment of HMC-1 cells with dynasore (an inhibitor of dynamin) reduced IL-8 production induced by TvSP or LTB4 (positive control). TvSP- or LTB4-induced phosphorylation of NF-kB was strongly inhibited by pretreatment with dynasore. Next, we checked the kinetics of BLT1 migration to the cell surface or intracellular site by using flow cytometry. In contrast to finding that BLT2 was expressed only at the cell surface in unstimulated state, BLT1 was expressed only within intracellular site. After exposure of HMC-1 cells with TvSP or LTB4 for up to 60 min, BLT1 translocated from intracellular compartments to the plasma membrane within 30 minutes. At 60 min after stimulation with TvSP or LTB4, BLT1 migrated from the cell surface to the intracellular areas. Finally, we investigated whether internalization of BLT1 induced by LTB4 can be mediated by dynamin-dependent endocytosis. Pretreatment of HMC-1 cells with dynamin-2 siRNA perfectly prevented internalization of BLT1 induced by LTB4. By using co-IP experiments, we found that dynamin-2 was physically interacted with BLT1 at 60 min after stimulation with LTB4. These results suggest that T. vaginalis-secreted LTB4 induces IL-8 production in HMC-1 cells via dynamin-mediated endocytosis of BLT1 and phosphorylation of NF-kB.

NOX2-derived ROS-dependent calpain activation is involved in human hepatoma cells (HepG2) death induced by *Entamoeba histolytica*

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Background: Entamoeba histolytica is an enteric tissue-invasion protozoan parasite that causes amoebic colitis and occasionally liver abscess in humans. Amoebic contact to host cell can induce the host cell death through the activation of calpain. In addition, Entamoeba can trigger the generation of reactive oxygen species (ROS) via NADPH oxidase (NOX) system. Our previous study showed that NADPH oxidase (NOX) 1-derived ROS generation is closely related to colon epithelial cell death induced by Entamoeba. However, it is unknown whether Entamoeba-induced ROS generation is responsible for death of hepatocytes and which NOX isoform is in charge of ROS production in HepG2 cell death induced by E. histolytica. Methods and Results: In this study, when HepG2 cells were co-incubated with amoebic trophozoites, DNA fragmentation was remarkably increased compared to results for cells incubated with medium alone. In addition, HepG2 cells adhered to amoebic trophozoites showed strong DCF fluorescence light, suggesting that intracellular ROS is highly accumulated within host cells incubated with E. histolytica. To address whether NOX-derived ROS can be responsible for HepG2 cell death by E. histolytica, we checked the inhibitory effect of diphenyleneiodonium chloride (DPI) or NOX specific inhibitors on Entamoeba-induced cell death. Pretreatment of DPI or NOX2 specific inhibitor of HepG2 cells was reduced the ROS generation and calpain activation induced by Entamoeba. Entamoeba-induced LDH release of HepG2 cells was effectively inhibited by pretreatment with DPI or NOX2 specific inhibitor. In addition, NOX2 silenced host cells using NOX2 siRNA efficiently reduced the Entamoeba-induced calpain activation and LDH release of HepG2 cells. Conclusions: These results suggest that NOX2-derived ROS-mediated calpain activation plays an important role in the HepG2 cell death induced by E. histolytica.

Preliminary morphological and molecular analyses on free-living ciliates from South Africa

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Free-living ciliates from South Africa are practically unknown: at present there is no literature available about their biodiversity or species description in that Country. The aim of the present work is to provide preliminary results regarding the multidisciplinary description of some free-living ciliate species from South Africa, as a first step in filling the gap of knowledge in this field. The samples come from a variety of environments, both brackish and freshwater, collected during a sampling campaign in August-September 2022. A mixed approach has been chosen to describe the retrieved species, using traditional morphological staining techniques, such as Silver impregnation and Feulgen reaction, together with sequencing of the 18S rDNA. The study also involves the investigation of symbionts in these ciliate species, using electron microscopy (TEM and SEM) and fluorescence in situ hybridization (FISH) in order to provide a description, as much exhaustive as possible of them. The ciliate species analyzed so far belong to Euplotes, Frontonia, Stentor as well as others genera and constitute a very first attempt to filling the gap of knowledge about free-living ciliates from South Africa.

Deciphering deep phylogenetic relationships among eukaryotic supergroups with improved gene and taxon sampling

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Understanding the relationships between all known forms of life remains a major challenge. In the case of eukaryotes, morphological and ultrastructural characteristics permitted to infer the monophyly of a number of phyla. However, the relationships among them and the discovery of an increasing diversity of unicellular species (protists) of difficult taxonomic affiliation vividly encouraged the use of molecular data (especially protein-coding genes) to reconstruct the eukaryotic Tree of Life (eToL). This shift in core data, accompanied by the increasing use of phylogenomics (i.e., analysis of multiple gene concatenations), made possible the classification of eukaryotic diversity into major lineages and even into a small number of so-called supergroups (e.g., Amorphea, SAR). However deep relationships (i.e., how these supergroups are related to one another) are not consistent among studies, especially with the discovery of new major 'orphan' eukaryotic lineages like Hemimastigophora or Provora. In this work, we aim at clarifying such deep relationships among the major eukaryotic lineages using both Maximum Likelihood and Bayesian Inference with improved taxon and gene marker sampling. We gathered an unprecedented collection of 647 eukaryotic proteomes spanning the entire characterized diversity and reconstructed their phylogeny based on 303 BUSCO proteins, supposed to have been well conserved and vertically inherited. We used a two-step approach where we first inferred the relationships within large eukaryotic clades, before using those results to constrain the eToL and focus on resolving the deep relationships between these clades. Here, I will present what insights into early diversification of eukaryotes were brought by this original approach.

Comparative transcriptome and antioxidant biomarker response reveal molecular mechanisms to cope with zinc ion exposure in the unicellular eukaryote *Paramecium*

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The development of industry has resulted in excessive environmental zinc exposure which has caused various health problems in a wide range of organisms including humans. The mechanisms by which aquatic microorganisms respond to environmental zinc stress are still poorly understood. Paramecium, a well-known ciliated protozoan and a popular cell model in heavy metal stress response studies, was chosen as the test unicellular eukaryotic organism in the present research. In this work, Paramecium cf. multimicronucleatum cells were exposed in different levels of zinc ion (0.1 and 1.0 mg/L) for different periods of exposure (1 and 4 days), and then analyzed population growth, transcriptomic profiles and physiological changes in antioxidant enzymes to explore the toxicity and detoxification mechanisms during the zinc stress response. Results demonstrated that long-term zinc exposure could have restrained population growth in ciliates, however, the response mechanism to zinc exposure in ciliates is likely to show a dosage-dependent and time-dependent manner. The differentially expressed genes (DEGs) were identified the characters by high-throughput sequencing, which remarkably enriched in the phagosome, indicating that the phagosome pathway might mediate the uptake of zinc, while the pathways of ABC transporters and Na+/K+-transporting ATPase contributed to the efflux transport of excessive zinc ions and the maintenance of osmotic balance, respectively. The accumulation of zinc ions triggered a series of adverse effects, including damage to DNA and proteins, disturbance of mitochondrial function, and oxidative stress. In addition, we found that gene expression changed significantly for metal ion binding, energy metabolism, and oxidation-reduction processes. RT-qPCR of ten genes involved in important biological functions further validated the results of the transcriptome analysis. We also continuously monitored changes in activity of four antioxidant enzymes (SOD, CAT, POD and GSH-PX), all of which peaked on day 4 in cells subjected to zinc stress. Collectively, our results indicate that excessive environmental zinc exposure initially causes damage to cellular structure and function and then initiates detoxification mechanisms to maintain homeostasis in P. cf. multimicronucleatum cells.

Keywords: Antioxidant enzymes; Molecular mechanisms; Paramecium; Transcriptome; Zinc

Distribution patterns of ciliate diversity in the South China Sea

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Ciliates are abundant microplankton that are widely distributed in the ocean. In this study, the distribution patterns of ciliate diversity in the South China Sea (SCS) were analyzed by compiling community data from previous publications. Based on morphological identification, a total of 592 ciliate species have been recorded in the SCS. The ciliate communities in intertidal, neritic and oceanic water areas were compared in terms of taxonomy, motility and feeding habit composition, respectively. Significant community variation was revealed among the three areas, but the difference between the intertidal area and the other two areas was more significant than that between neritic and oceanic areas. The distributions of ciliates within each of the three areas were also analyzed. In the intertidal water, the community was not significantly different among sites but did differ among habitat types. In neritic and oceanic areas, the spatial variation of communities among different sites was clearly observed. Comparison of communities by taxonomic and ecological traits (motility and feeding habit) indicated that these traits similarly revealed the geographical pattern of ciliates on a large scale in the SCS, but to distinguish the community variation on a local scale, taxonomic traits has higher resolution than ecological traits. In addition, we assessed the relative influences of environmental and spatial factors on assembly of ciliate communities in the SCS and found that environmental selection is the major process structuring the taxonomic composition in intertidal water, while spatial processes played significant roles in influencing the taxonomic composition in neritic and oceanic water. Among ecological traits, environmental selection had the most important impact on distributions.

Evolution of a fibre-forming citrate synthase

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Previous studies showed Tetrahymena thermophila's mitochondrial citrate synthase (CS) to form fibrous structures of size comparable to intermediate filaments, putatively involved in conjugation and metabolic regulations. We aimed to unravel the evolutionary processes that lead to the emergence and the recruitment of such structures for additional functions. Using a combination of experimental biochemistry, ancestral sequence reconstruction, and in vivo and in vitro microscopy, we discovered that in vitro fibre formation is triggered by a specific chemical moiety shared by MES and other buffers, and such structures are not directly involved in sexual reproduction or regulatory functions during starvation. Surprisingly ordered fibres are observed in the last common ancestor of Tetrahymenidae but yield amorphous aggregates in other extant and ancestral proteins. Furthermore, we show how MES-triggered high-complexes formation is a feature present only in the eukaryotic version of this enzyme, while bacterial homologs show no propensity for aggregation. The amount of experimental evidence provided strongly argues against adaptative roles gained by this protein during evolution and shows how complex, ordered structures can emerge neutrally from simple components with no selectable function.

Oxygenic or anoxygenic? *Spirostomum teres* feeding preferences

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In a monomictic hyposaline crater lake Alchichica (Puebla / Veracruz, Mexico), the pelagic / anoxic hypolimnion population of Spirostomum teres has been investigated as a part of the long-term ciliates' monitoring since 2003. Recently, climate changes in the lake region have been producing the prolongated S. teres occurrence period, but due to the more pronounced anoxia, typical microaerophilic/ anaerobic species are gradually disappearing from the anoxic hypolimnion. Spirostomum teres feeding upon picocyanobacteria (PICY) was periodically analysed using fluorescently labelled Synechococcus sp. (FLB); during 2017-2019 we were looking for possible photosynthesizing symbionts' retention, which was observed in the case of unidentified cyanobacterium, deposited within 24 hours in quite well defined ubication within the cell (as reported in ECOP Roma 2019). However, dilution experiments performed with 20 µm screen harvested S. teres suspended in prey-free water revealed also curious behaviour of ingested purple anoxygenic photosynthetic sulphur bacteria (APB; molecularly identified as Thiocapsa sp.), which were not either digested or deposited in the same place as picocyanobacteria. To evaluate the feeding budget of S. teres, biomass of both cyanobacteria and Thiocapsa sp. were evaluated using image analysis (ImageJ). If the known median clearance rate of S. teres upon PICY (2000 nL/cell.h) is applied on APB feeding, the same organic biomass (approx. 10 pg/cell.h) could be ingested in the layers were PICY and APB coexisted. However, analysing the relation between the ciliate and its food biomasses (redundancy analysis, RDA) was shown that upon the present PICY and APB biomasses the ciliate was driven on the opposite directions: abundance of S. teres was increasing along with PICY while decreasing with higher APB abundances. Based on the observations, S. teres was frequently described as a bacteria and purple sulphur bacteria eater in the literature, but our observations have proven that among its prey, PICY might play very important role both as a prey and a possible symbiont. Even though the ingested APB have been seen notoriously in the ciliate cells, if they served as an important feeding source for the ciliate and/or they were used within the symbiotic pathways, has not been solved, yet.

Gregarines from tenebrionid beetles of the Atacama Desert

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Gregarine apicomplexans, a group of single celled organisms, inhabit the extracellular spaces of most invertebrate species. The nature of the gregarine-host interactions is not yet fully resolved, mutualistic, commensal and parasitic life forms have been recorded. In the extreme arid environment of the Atacama Desert, only a few groups of invertebrates such as darkling beetles (Tenebrionidae), were able to adapt to the harsh environment. These beetles, unable to fly and hence limited in their distribution, host gregarines and therefore provide the unique opportunity to study co-evolutionary diversification processes. Several species of darkling beetles formed spatially separated populations due to geological and meteorological events. Our study aims to enlarge the morphological and molecular (rRNA) data on gregarines from the Atacama Desert and adjacent area with focus on the co-evolution of host-endosymbiont. We sampled separated populations of beetles, in particular from the genus Psectrascelis and Scotobius to compare the diversification of gregarines within a population and between populations.

An overview of the amoebae genera diversity in Mexico

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Amoeboid protists that possess pseudopods are included in Amoebozoa and Rhizaria. Although amoebae play an important role in the ecosystems, they have received insufficient attention. To show an update of the genera of amoebae reported in Mexico, we performed the search by consulting all available literature. We grouped the data of amoebae as naked and testate, and eumycetozoans. We obtained 56 genera of amoebae reported in 33 different environments represented by temporal and permanent freshwater habitats and soil, 27 are genera of naked and 29 of testate amoebae, and 44 genera of eumycetozoans corresponding to 40 myxomycetes and four dictyostelids inhabiting mainly in decaying wood from different type of forests. Naked amoebae include free-living, potential pathogens, and parasites. The records correspond to 27 of the 32 states of the Mexican Republic, showing an insufficient knowledge of these protists in Mexico. Our data demonstrated the importance of continuing research on this group.

Keywords. Naked amoebae, testate amoebae, myxomycetes, dictyostelids, Mexico.

Epibiotic ciliate communities from a crayfish cultivated in artificial ponds in Southern Mexico

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The artificial ponds traditionally used for rustic aquaculture to grow edible crayfish provide suitable conditions for the establishment of epibiotic ciliates communities on the external surfaces of crayfish. As the protist diversity has been poorly studied in Southern region of Mexico and to assess the importance of artificial ponds, we analyzed the diversity of sessile ciliates hosted by Procambarus (Austrocambarus) in a farm, located in Tziscao, Chiapas, Mexico. We obtained some crayfish during the dry and humid season of the years 2014 and 2015 by manual sampling. Live observation using bright field microscopy, and silver impregnations were carried out for species identification. We identified ten species of peritrichs belonging to the genera Cothurnia, Epistylis, Opercularia Vorticella, and Zoothamnium, and three suctorians of the genera Tokophrya and Trichophrya. They were observed in almost all regions of the crayfish´s body. We concluded that the traditional practice of crayfish production in farms enhance the establishment of ciliated communities on these crustaceans. Acknowledgements: to Posgrado en Ciencias Biológicas, UNAM, and Comisión Nacional de Ciencia y Tecnología (CONACYT) for grant number 481159.

Developing gregarine apicomplexans as aquatic symbiosis model systems

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The phylum Apicomplexa consists mainly of parasitic protists, some of which are the causative agents of potentially fatal infectious diseases in humans such as malaria (caused by Plasmodium) and cryptosporidiosis (caused by Cryptosporidium sp.). Despite the intensive study of medically important species, many aspects of apicomplexan biology and evolution remain unknown. The apicomplexans evolved from algal ancestors and some still retain a cryptic plastid, the apicoplast. It is still unknown how the evolution from a free-living algal lifestyle to the parasitic lifestyle occurred, but the sequencing of genomes and transcriptomes and comparative analyses will aid in our understanding of the evolutionary past of the phylum Apicomplexa. In general, all Apicomplexa are referred to as being obligatory parasitic, but the large group of gregarine apicomplexans infecting a wide range of freshwater, marine, and terrestrial invertebrates has been shown to fall across the spectrum of symbiosis (mutualistic to parasitic). Studying the effects of gregarines on their hosts is currently challenging and requires sacrificing the host to verify gregarine infection then isolation of gregarines under a microscope. This technique comes with the caveat that early-stage infections can be difficult to determine using microscopy alone. The unique position of gregarines and close relatives in the Apicomplexa makes them ideal candidates for a model system to study the evolution of aquatic symbiosis. We present here the foundations of an in-vitro axenic culture system for gregarines that is animal and tissue free. This system could provide the building blocks to further study the evolution of aquatic symbiosis within the gregarines and potentially the wider Apicomplexa.

Entamoeba histolytica: Investigation of the lipopeptidophosphoglycan (LPPG) surface antigen and the monoclonal antibody EH5

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Entamoeba histolytica, a protozoan parasite, inhabits the human colon and causes amoebic dysentery and liver abscess. Its surface, which interacts strongly with the colonic environment, is densely covered with molecules either binding to glycans as the Gal/GalNAc lectin or heavily glycosylated as the lipopeptidophosphoglycan (LPPG). The expression of LPPG is upregulated during invasion into the bloodstream and furthermore, it is strongly antigenic. The monoclonal antibody EH5, which binds to LPPG, was able to protect immunocompromised mice against amoebic liver abscess. LPPG is a complex GPI-(glycosylphosphatidylinositol)-linked protein with O-glycosyl side chains containing α-1,6-linked glucose, resembling dextran. Whereas much of the glycan structure is known, its core structure, hypothetically a polypeptide, has remained elusive so far. Moreover, the solvents to extract LPPG from the amoebae are either complex or dangerous like hot phenol. We therefore attempted to improve these methods using a series of aqueous mixtures with alcohols of varying hydrophobicity. We discovered that 2-butanol saturated water was the best agent to extract LPPG from E. histolytica trophozoites. We then subjected the extracted LPPG to various cleaving agents and used SDS-PAGE and western blots with antibody EH5 to test what was happening to the antigen. Surprisingly, the extracted LPPG was not susceptible to degradation by most proteases with the possible exception of pronase. In contrast, the enzyme dextranase or the compound scandium triflate (scandium(III) trifluoromethanesulfonate) destroyed the LPPG epitopes. This led to the finding that obviously our antibody EH5 binds to the dextran side chains of the LPPG. This was further tested and dextrans were able to inhibit the binding of antibody EH5 to LPPG both in western blots and immunofluorescence microscopy. In addition, a commercial anti-dextran antibody also stained E. histolytica trophozoites. In spite of this progress, the nature of the LPPG core structure has remained elusive.

Supported by grant P31793 from the Austrian Science Fund (FWF)

Graphical tool for manual editing and annotation of sequence sampling in phylogenetic datasets

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The assembly of single gene datasets for phylogenetic reconstruction has become a very common task in evolutionary and comparative analyses. It is therefore important that there are software tools available to facilitate this process. The remarkable rise in the amount of available sequence data over the last few decades often makes assembling single gene phylogenetic datasets a very tedious task, as many thousands of sequences can be easily recovered from public databases for any particular gene, and a subset of these must be selected to create a dataset with balanced taxon sampling. Moreover, it is often important to balance the sampling of several different paralogs of the studied gene family or of protein sequences with slightly different domain structures. It is very common for researchers to perform multiple rounds of manual modification of the dataset to obtain optimized sequence sampling. We developed Taxus – an application that allows users to visualize the phylogenetic tree and edit sequence sampling through clicking or using keyboard shortcuts – which allows for direct removal or annotation of sequences to generate modified datasets. This software has entered the stage of public beta-testing and is available on GitHub for Windows, Linux, or MacOS.

Does *Blastocystis* shape the gut microbiota? A case study in a group of mothers and children volunteers from the Zanzibar Archipelago

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Most of the gut microbiome research focus on bacteria, neglecting the impact of fungi and intestinal opportunists/parasites, that include protozoa and worms. We are currently analysing the associations between nutritional habits, gut microbiome, and opportunists/parasites in a group of volunteers characterized by a peculiar diet, mostly based on carbohydrates and fish, living in the two main islands of the Zanzibar Archipelago (Tanzania). Faecal samples were collected during different seasons from 120 pairs of mothers and children of age between 18 months and 3 years. Volunteers declared rare cases of diarrhea and more than one third of the children showed wasting and/or stunting. Microbiome structure and predicted functions are under analysis using the 16S rRNA genes and whole shotgun metagenomic sequencing on the DNA extracted from the samples. The presence of helminths, known to be endemic in Zanzibar, was investigated by microscopical and molecular analysis, while Blastocystis was detected at molecular level by PCR. Among helminths, the most frequent was Trichuris trichiura that has been previously demonstrated to have an impact on the bacterial gut microbiota in the same population (Chen et al. Parasites and Vectors, 2021). The T. trichiura frequency was around 18% in mothers and 25% in children, with differences related to the seasons. Blastocystis was present in 40% of the mothers and 38% of the children. Intriguingly, the co-infection by Blastocystis and T. trichiura was detected in only three pairs. Ongoing investigations are revealing a role of Blastocystis in shaping the bacterial gut microbiota. In addition, correlations of Blastocystis presence or absence with nutritional habits, such as a balanced or an unbalanced diet, are currently studied.

Reinvestigation of two poorly known metopid ciliates: *Metopus major* and *M. pellitus* by Kahl, 1932 from South Korea (Ciliophora, Armorphorea)

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The complex of Metopus contortus including M. major, and M. pellitus was described by Kahl almost a century ago. Even though two species have been accepted as valid species by several investigators, there are no detailed descriptions, and diagnoses to separate them. Thus, the morphology and phylogeny of two metopids, collected from marine habitats in South Korea, were reinvestigated using live observation, protargol impregnations, SSU rRNA sequencing. The Korean population of M. major Kahl, 1932 is distinguished from M. contortus sensu strictus in terms of larger body size 140–180 × 40–60 µm in vivo, a greater number of somatic kineties about 50 longitudinal rows, adoral zone of polykinetids about 75 membranelles. Interestingly, the somatic cortical of M. major is composed of polykinetids adjacent to di-, tri-, tetrakinetids. M. pellitus Kahl, 1932 has smaller body sizes 60–75 ×25–35 µm in vivo, fewer somatic kineties, and adoral zone of polykinetids (24 and 22, respectively) than that M. contortus s.str. and M. major, cell surface covered by ectosymbiotic bacteria, and long caudal cirri up to 70% of body length. The molecular phylogeny based on SSU rRNA gene sequences corroborates the morphological identification of two species and their positions in the phylogenetic tree. These two species and their congeners are grouped together as a clade with full support (100 ML/1.00 BI), in which M. contortus s.str. is a sister group of M. major with strong support (99 ML/0.99 BI).

Search for the new group-specific V9 hypervariable region metabarcoding primers

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High-throughput amplicon sequencing is nowadays a powerful tool for uncovering diversity and ecology in all types of habitats. The 18S rDNA variable regions are most commonly used for this purpose. With our group focusing on the evolution and diversity of the Euglenozoa and Metamonada clades (formerly the Excavata supergroup), we attempted to design group-specific metabarcoding primers for the hypervariable region V9 and compare them with the general V9 primer. The study aimed at investigating the effect of primer efficacy on free-living freshwater anaerobic protists harvested from various habitats from the SOOS national reserve in the western part of Bohemia and nearby areas. The methodology involved sequencing protocol suggested by Earth Microbiome Project (Golay barcode system), clustering using Swarm and PR2 (version 4.14.0) as a reference database. The results show relatively high specificity in the case of the Euglenozoa primer as 46,3 % and 45,2 % of reads, after filtering of low-quality data, represented Kinetoplastea and Euglenida, respectively. On the other hand, the specificity of Metamonada primer pair was low and Metamonada represented only 45 % of reads. Amoebozoa and Alveolata species were abundant in this set and they represented 16,9 % and 28,8 % of reads, respectively. Altogether we have detected 213 Euglenozoa and 79 Metamonada OTUs in the samples indicating high diversity of both groups in these environments.

The metabarcoding of bacteria and protozoa of domestic pigeons in Seoul, Korea

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In Korea, feral pigeons pose significant public health risks because they carry various zoonotic pathogens; however, few studies have examined the status of pathological processes associated with pigeons in South Korea. Therefore, this study used 16S and 18S rRNA amplicon sequencing to detect possible bacterial and parasitic pathogens to assess the current risk of zoonosis in Seoul, South Korea. Pigeon faecal samples (n = 210) obtained from 24 public sites in and near Seoul were collected and their DNAs were extracted for the amplicon sequencing. The most predominant bacteria at the genus level was Lactobacillus spp., with an average relative abundance of 55.2%.Potentially pathogenic bacteria were also detected in the faecal samples; Campylobacterwas found in 19 samples from 13 regions, Listeriaceae was found in seven samples, and Chlamydiawas found in three samples from two regions. Protozoa were also detected; The most predominant protozoa at the genus level was Eimeria, that was found in 100 samples. Isospora was found in 54 samples. Principal coordinate analysis and permutational multivariate analysis of variance revealed a significant difference in bacteria and parasites composition between the regions with and without the homeless individuals. Linear discriminant analysis effect size showed that Streptococcus, Pseudomonas and Cyclospora were significantly abundant in the regions with homeless people. Overall, this study identified various potentially pathogenic bacteria and protozoa in pigeon faeces at public sites in South Korea. This study provides important information for public health strategic planning and disease control.

Endosymbiotic relationship between the heterotrophic ciliate *Paramecium bursaria* and the green algae *Chlorella variabilis* under starvation

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Endosymbiotic relationships are widespread in Eukaryota. Some are based on photosynthesis, and eventually led to emergence of eukaryotic algae and plants very long ago. In many photosynthesis-based endosymbioses, such as those seen in green paramecia and corals, a heterotrophic host cell grazes microbial prey, and the resultant nitrogen-containing wastes are supplied to a photosynthetic (algal) endosymbiont. The endosymbiont, in turn, provides photosynthate to the host. This association is often observed in oligotrophic environments, suggesting that it is advantageous in such conditions. Many studies on photosynthesis-based endosymbiosis have been conducted using cultures supplemented with, very high concentrations of organic compounds and/or abundant prey. However, such analyses likely overlook the nature of endosymbiotic associations and their responses to environmental changes. To overcome this issue, we have been examining the endosymbiotic relationship between the ciliate Paramecium bursaria (host) and the green alga Chlorella variabilis (symbiont), in an inorganic medium, and its responses to prey starvation which often occurs in nature. In the light, even without any prey, both the host and the endosymbiont kept their numbers for 21 days, regardless of the availability of external nitrogen sources. In contrast, in the dark, the host digested the endosymbiont and started to die in 14 days. When either the host or the endosymbiont was grown in monoculture, the symbiont cells bleached and then died in a nitrogen-depleted medium, and the host without the symbiont did not survive, regardless of presence or absence of external nitrogen sources. Besides, we performed RNA-seq analyses of the host and the endosymbiont on the 3rd day of the starvation. The results showed that the external inorganic nitrogen sources have some effect on endosymbionts but little effect on the survival of the endosymbiont and the hosts. These results suggest that the symbiosis is beneficial under starved and nitrogen-depleted condition for both Paramecium host and Chlorella endosymbiont as long as light for photosynthesis is available.

Two new species of the eupelagonemids

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Eupelagonemids are a group of heterotrophic flagellates belonging to Euglenozoa. They are abundant in our oceans, especially in the aphotic zone, suggested by large numbers of environmental sequences, and were initially referred to as Deep Sea Pelagic Diplonemids clade I (DSPDs I). SSU ribosomal gene trees have confirmed that they are a diverse group with short branches, implying a slow rate of evolution or a more recent diversification. However, despite their dominance in the deep ocean, only one species has been described (Eupelagonema pacifica) and a study investigating single-cell amplified genomes produced data of low quality. Here we report two new species of eupelagonemids isolated from a depth of 300m on the Central Coast of British Columbia, Canada. The cells are loricated with a short protrusion on the apex. SSU rDNA phylogenies show they each represent a different clade of eupelagonemids, distinct from E. pacifica. Our multigene analyses place the eupelagonemids within Hemistasiidae, which suggests that we need more data from both eupelagonemids and hemistasiids to understand their evolutionary relationship.

Genetic manipulation of *Micromonas* - New tools for a marine model alga

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Marine protists play a fundamental role in ocean ecosystems and the earth's climate system, participating in major elemental cycles. Despite their importance, our current understanding of the physiology of marine protists is still patchy. It mostly relies on comparison of their functional proteins to homologs in a few model taxa that do not necessarily represent overall protist diversity or ecological relevance in the marine environment. However, over the recent years multiple efforts were undertaken to develop new model systems from all major marine protist lineages. Recently, tools have been developed which open possibilities for genetic manipulation of Micromonas, a phototrophic marine picoeukaryote related to plants, which thrives globally and can dominate the pico-size fraction. By delivering foreign DNA into the cell of Micromonas, we successfully observed expression of transgenes such as eGFP and Luciferase, which is a crucial step for implementing the latest approaches to genetic engineering, such as CRISPR/CAS9. Ultimately, our study aims to modify the phenotype of Micromonas by targeting ecologically relevant genes to study the physiology of these marine protists. Extension of these efforts to a polar alga Micromonas polaris will enable the first genome manipulation of an environmentally relevant polar picoeukaryote and unlock the genetic basis of the physiology of this important organism. Here, we will report on our latest results and advancements on Micromonas genetics.

Characterisation of a novel pan-microalgal chloroplast ATP-binding protein

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The chloroplasts of algae are supported by complex networks of nucleus-encoded proteins, many of which may not have direct homologues in plants. Understanding the role of this chloroplastic “dark matter” is fundamental to understanding the diversification of algal life, and the photosynthetic activity of the modern ocean. Recently, we identified the presence of a distant homolog of the F-type ATP synthase α subunit - that we hereby name "xATPA" – in the chloroplast of the euglenid Euglena gracilis. Its gene has been subsequently identified in several distant microalgae principally containing primary green and secondary (and higher) red chloroplasts. Surprisingly, xATPA seems to be absent from other photosynthetic organisms like plants, primary red algae and cyanobacteria, but present in the labyrinthulomycetes. These results suggest that xATPA has a complex evolutionary history marked by horizontal gene transfers, and is strongly associated to algal phytoplanktonic communities. In contrast to canonical plastidial ATPA, which is plastid-encoded, xATPA is a nuclear-encoded protein. GFP localisation of xATPA in the model diatom Phaeodactylum tricornutum indicates it is chloroplast-targeted. Structural predictions suggest that xATPA is able to bind NTPs thanks to a P-loop motif, but lacks the interacting domain with the β subunit, and is unlikely to participate the plastidial F-type ATP synthase complex. Using both experimental and data analysis approaches, we outline preliminary efforts to understand the function of xATPA in microalgae. We have generated four P. tricornutum xATPA knock-out mutants using the CRISPR-Cas9 system, and also analysed the expression trends of xATPA in the wild from the Tara Oceans dataset. Mutant lines do not exhibit significant growth defaults when cultured in conditions associated with xATPA expression in the wild. We have also used publicly available transcriptome data to retrieve genes co-expressed with xATPA in P. tricornutum and to define its physiological role. These analyses show that xATPA has a circadian expression that reaches its maximum around sunset, and is expressed along with genes involved in transcription and translation processes. Further steps will involve xATPA purification, and in vitro assays in order to identify the molecular mechanisms underlying xATPA functions.

Towards deciphering the molecular mechanisms of RNA 3'-end modification in secondary plastids of euglenophytes

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The secondary plastid of euglenophytes exhibits various unorthodox features. Among others, using the model representative Euglena gracilis, 3' polyadenylation of a small portion of plastid transcripts was documented by Zahonova et al. (2014). This is so far the only documented case of transcript polyadenylation in secondary plastids. In our project we aim on understanding the molecular mechanism underlying this type of modification of Euglena plastid transcripts. We identified four proteins putatively involved in 3'-end modifications in the experimentally determined plastid proteome of E. gracilis (Novak Vanclová et al., 2020) and one more putative mRNA 3'-end modifying enzyme in the E. gracilis transcriptome assembly. Each of the four candidate proteins is nucleus-encoded and possesses a typical plastid-targeting pre-sequence. At the same time putative cytoplasm/nucleus-targeted versions of these proteins were identified, with one exception encoded by separate (paralogous) genes. Specifically, we found: (1) poly(A)-specific ribonuclease (PARN), in which the plastid-targeting pre-sequence is added via alternative splicing; (2) polynucleotide phosphorylase (PNPase); (3) a homolog of the mitochondrial deadenylase (phosphodiesterase) PDE12; and (4) two different members of the TRF family of ribonucleotidyl transferases (ptTNT1 and ptTNT2). Orthologs of four of these genes, the exception being ptTNT2, were also found in the transcriptome assembly of the non-photosynthetic sibling E. longa. Using a CRISPR/Cas9-based methodology we targeted all five genes in E. gracilis. The phenotype of homozygous knock-out (KO) mutants in ptTNT1 or ptTNT2 was macroscopically recognizable, the cultures being yellowish instead of green. The phenotype of PNPase mutants was even more pronounced – the culture being almost whiteish in colour. Interestingly, homozygous KO mutants in PDE12 or PARN, or KO mutants in the control GSL2 gene did not exhibit such phenotype. Moreover, analysis of ptTNT1 and ptTNT2 KO mutants showed that they are not producing chlorophyll and are not able to perform photosynthesis in vivo. ptTNT1 and ptTNT2 thus seem to play a role in proper function of the E. gracilis plastid, probably by affecting plastid transcript turnover. Work is underway to test the hypothesis that 3'-termini of plastid transcripts are specifically affected in ptTNT1 and ptTNT2 KO lines.

Evidence and distribution of various *Chlorovirus* (Phycodnaviridae) strains in Lake Mondsee (Austria) infecting endosymbiotic green algae

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Members of the genus Chlorovirus are double-stranded DNA viruses and belong to the family Phycodnaviridae. They infect endosymbiotic green algae, which were isolated from several strains of the ciliate Paramecium bursaria and the heliozoan Acanthocystis turfarea. Three groups of viruses have been found so far and they are highly host-specific: NC64A viruses infect Chlorella variabilis, SAG viruses Chlorella heliozoae, and Pbi viruses Micractinium conductrix. These viruses have a worldwide distribution and can be found in high concentrations in almost any water body. In contrast to the worldwide distribution of these viruses, the host algae are only known as endosymbionts with restricted distribution (Chlorella variabilis: Paramecium bursaria species-complex; Micractinium conductrix: Paramecium bursaria species-complex, Coleps viridis) and have never been found free-living so far. However, during monitoring of the biodiversity of phytoplankton and ciliate communities, we studied the occurrence of the three viral groups in Lake Mondsee. For these tests, we used several reference strains of the host algae and incubated them with sterile-filtered lake water originating from different depths in the lake. In all tests, we observed the lysis of the host algae, which indicates that all three viral groups and probably also their host algae are present in Lake Mondsee.

This study was funded by the Austrian Science Fund (FWF): project P28333-B25.

Phyloplast: A plastid database with novel signal sequence prediction tool and a suite of programs for phylogenetic analyses

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The development of molecular ecology and innovations in DNA sequencing technologies have produced an overwhelming amount of data on various eukaryotic lineages, including those with plastids. The data can be an invaluable and rich source of information about the past endosymbioses and the mechanisms behind plastid establishment. However, the knowledge is widely dispersed across many databases and publications, not systematically elaborated and often unreviewed. Therefore, we constructed Phyloplast - a newly established database that provides a comprehensive and up-to-date resource for researchers working on plastid genomics. The Phyloplast includes sequence data (Gene, Protein and RNA) of over 10000 plant species, including land plants and algae, providing a high-quality, curated dataset from both complete and partial plastid genomes. The database also delivers information on biological processes, molecular function and cellular components, all gathered as Gene Ontology resources, newly created systematics compiled with NCBI taxonomy, predicted protein localisation inside plastids, giving researchers a powerful tool for exploring the evolution and function of plastid and its proteins. Additionally, the database provides links to other relevant databases, such as the NCBI and UniProt, to facilitate cross-disciplinary research. The data in Phyloplast is organised in a way that makes it easy to search and analyse, and it is regularly updated with new sequences as they become available. Moreover Phyloplast includes a range of tools for analysing and visualising plastid sequence data as well as a novel predictor tool that utilises machine learning algorithms to predict the plastid protein localisation.

Protist of the year 2023: Colony-forming ciliate *Ophrydium versatile* (Peritrichia)

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Protists belong to the most important living organisms of our planet. They are often not visible to the naked eye, but play an important role in natural nutrient cycles. To give these microorganisms recognition, the German Society of Protozoology (Deutsche Gesellschaft für Protozoologie; DGP, www.protozoologie.de) announced the "Protist of the Year" since 2007. For this year 2023 Ophrydium versatile was selected. This ciliate belongs to the Peritrichia, subgroup Vorticellidae, and forms colonies from a few millimeters up to 15 centimeters in diameter, which therefore are visible to the naked eye. Striking is the green color that is caused by green algae, which are living in symbiosis with Ophrydium. The green colonies occur regularly in lakes in spring. Ophrydium versatile is an indicator for very good water quality. The large "mucilage balls" host very often other ciliates, protists and hydrozoans (Hydra), as well as different algae, and form therefore their own biotopes. It is characterized by the following features:

- Single cells are 300-600 x 20-40 µm in size, more than 10-times longer than wide.

- Single cells are vase-shaped, contracted almost spherical, ellipsoid or cylindrical.

- Macronucleus (large nucleus) filiform.

- One contractile vacuole for osmoregulation.

- Oral apparatus anterior, with undulating membrane, which surrounds 1.5 time the oral apparatus.

- Each cell forms a gelatinous matrix. The colony grows by these to a large mucilage, which adheres on substrates.

- Each cell contains hundreds of unicellular spherical green algae, which were often named as Chlorella vulgaris.

Apofrontonia jejuensis n. sp. (Ciliophora, Oligohymenophorea), a new marine ciliate from Jeju Island, South Korea

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The assignment of the genus Apofrontonia to the family Frontoniidae was made based on two diagnostic features: the closely arranged kinetal rows in the peniculi and vestibular kineties located on the right side of the vestibular cavity's opening. The first phylogenetic analysis of this genus relied solely on the 18S rRNA gene, which was limited not only by the absence of other gene sequences from other species within the genus but also from the Peniculia as a whole. However, the combination of morphological and molecular data obtained from the species A. dohrni suggested the establishment of a new Peniculida family to accommodate the genus Apofrontonia. Nevertheless, due to the limited taxon sampling, the genus Apofrontonia was provisionally considered as incertae sedis within the Peniculida order. Apofrontonia jejuensis n. sp. was recently discovered in a coastal water sample of Jeju Island, South Korea. Apart from possessing the typical genus-specific features, this species exhibits a fibrillar system associated with the oral ciliature, likely linked to nematodesmata-like structures, as observed in Frontonia species. This study increases the taxon sampling, offers further insights into the morphological variability of the genus Apofrontonia, and provides additional molecular evidence for its distinction from the genus Frontonia.

Sessile ciliates colonizing the hindgut of higher termites

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While most studies on the eukaryotic gut microbiota of termites have focused on the symbiotic flagellates of lower termites, our knowledge on sessile peritrich ciliates in the gut of higher termites (Termitidae) is limited. The only species described, Termitophrya africana from Jugositermes tuberculatus (Apicotermitinae), was documented merely as a drawing, and the observation of ciliates with similar morphology in other Apicotermitinae remains undocumented. Here, we investigated the presence of sessile ciliates in a broad range of higher termites using an SSU rRNA-based approach. Phylogenetic analysis revealed that all sequences formed a monophyletic group in the radiation of Peritrichia (Sessilida), with Epistyliidae and Opisthonectidae as closest relatives, supporting previous affiliations by morphologic features. They were detected exclusively in samples of Astalotermes, Jugositermes, and Phoxotermes, albeit not in all species, and were specific for their respective host. Their presence outside the subfamily Apicotermitinae could not be confirmed. A detailed investigation of morphology and ultrastructure of the sessile ciliates from J. tuberculatus was done by light, scanning, and transmission electron microscopy. Typically, expanded individuals of the contractile Termitophrya cells are elongate and continuously thicken towards the attached posterior end. Cilia arrange in a small anterior ring and circle down into a canal-like, counter-clockwise spiraling buccal cavity (infundibulum). A posterior scopula equipped with short cilia attaches the cell to a substratum via a sticky secretion. We found two Termitophrya morphotypes, one with and one without rod-like ectobacteria, and a morphologically distinct morphotype with a short anterior region clearly demarcated from the broader, barrel-like cell body, which is in agreement with the presence of three closely related phylotypes. Certainly, the occurrence and diversity of peritrichs in higher termites is underestimated and requires more investigation.

The activity of PHMB and other guanidino containing compounds against *Acanthamoeba* and other ocular pathogens

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In recent years, a rise in the number of contact lens users in the UK and worldwide coincided with an increased incidence of microbial keratitis. The aim of this study was to investigate the antimicrobial activities of polyhexamethylene guanidine (PHMG), polyaminopropyl biguanide (PAPB), and guazatine in comparison to the common contact lens disinfectant constituent, polyhexamethylene biguanide (PHMB), thereby identifying compounds that show potential for the treatment of microbial keratitis and for the inclusion in multi-purpose solutions (MPS). The study involved at first determining the minimum concentrations of these compounds against a broad range of organisms, including Acanthamoeba castellanii, Acanthamoeba polyphaga, Staphylococcus aureus, Pseudomonas aeruginosa, and Candida albicans. Then using these concentrations, the rate of kill for these compounds against each organism was investigated using the time-kill method. This study demonstrated that PHMG, PAPB, and guazatine are equal in activity to PHMB against Acanthamoeba trophozoites and cysts. All compounds demonstrated significant antimicrobial activity against trophozoites of both Acanthamoeba species resulting in a 2–2.6 log10 reduction in viability in comparison to the control ($p < 0.001$) at 6 h, which is the standard disinfection time for a contact lens solution. However, there was no significant difference between PHMB, PHMG, PAPB, and guazatine at this 6 h time point ($p > 0.05$). With S. aureus, PAPB and PHMB were the most active. With P. aeruginosa, PHMG showed the lowest MIC, and guazatine the lowest MBC. Finally, with C. albicans, PHMG was the most active, with an MIC/MBC. PHMG and PAPB are also equal in activity to PHMB against S. aureus and P. aeruginosa, whereas PHMG shows significantly better activity than PHMB against C. albicans ($p < 0.001$). To our knowledge, this is the first study to demonstrate the effectiveness of PHMB, PHMG, PAPB, and guazatine against Acanthamoeba and other ocular pathogens. As alternatives to PHMB, these compounds warrant further investigation for inclusion in contact lens solutions and for the treatment of keratitis.

Nitric oxide signaling controls collective contractions in a colonial choanoflagellate

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Although signaling by the gaseous molecule nitric oxide (NO) regulates key physiological processes in animals, including contractility, immunity, development and locomotion, the early evolution of animal NO signaling and its role in the last common ancestor of animals remains unclear. In great part, this is due to incomplete characterization in early-branching animals and to a lack of studies in close animal outgroups. To reconstruct the role of NO in the animal stem lineage, we set out to study NO signaling in choanoflagellates, the closest living relatives of animals. In animals, NO produced by the nitric oxide synthase (NOS) canonically signals through cGMP by activating soluble guanylate cyclases (sGCs). We surveyed the distribution of the NO signaling pathway components across the diversity of choanoflagellates and found three species that express NOS, sGCs, and downstream genes in the NO/cGMP pathway. One of these, Choaneoca flexa, forms multicellular sheets that undergo collective contractions controlled by cGMP. We found that treatment with NO induces sustained contractions in C. flexa by signaling through the sGC/cGMP pathway. Biochemical assays show that NO directly binds C. flexa sGC1 and stimulates its cyclase activity. The NO/cGMP pathway acts independently from other inducers of C. flexa contraction, including light, mechanical stimuli, and heat. The output of NO signaling in C. flexa – contractions, resulting in a switch from feeding to swimming – resembles the effect of NO in sponges and cnidarians, where it interrupts feeding and activates contractility. This research may provide insights for reconstructing the biology of the first animals and the evolution of NO signaling.

In vivo evaluation of bioenergetic parameters in heat-stressed *Cassiopea*

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The symbiotic partnership between cnidarians and dinoflagellates from the family Symbiodiniaceae constitutes the basis of remarkably diverse ecosystems. This tight association between the cnidarian host and the intracellular photosymbionts displays a complex energetic metabolism, involving respiration from both partners and photosynthesis from the dinoflagellate symbionts. Despite the major importance of these two critical processes, their interplay and regulations remain poorly studied. Abiotic factors can unsettle the symbiotic balance, leading to the collapse of the partnership and threatening the survival of entire ecosystems. Among them, the rise in sea water temperature is getting more and more concerning as global warming takes place. To address this topic, our first approach consisted in subjecting Cassiopea, an established model organism for photosynthetic jellyfish, to a mild hyperthermic stress for 28 days. Despite an increase of 6°C of the water temperature, the stressed jellyfish kept on growing over the experiment, their symbiont density stayed constant and their photosynthetic capacity was barely impacted (Fv/Fm, rETR-PSII, rETR-PSI, oxygen exchanges). The measurement of the level of pigments in Symbiodinium cells hints at an adaptation of the photosynthetic apparatus to heat stress, with the slight increase of the amount of total pigments per cell and the transient rise in the deepoxidation of xanthophylls. Despite the minor impact of heat stress on the photosynthesis of symbiotic Cassiopea, an increased respiration along with a rise in bell pulsation rate were observed in the heat-stressed population. To dig more into the cellular scale and understand the factors that ensure photosynthesic stability, we are repeating this long-lasting heat stress experiment, collecting samples to carry out bottom-up proteomics analysis. The production of reactive oxygen species is also under investigation, along with the fatty acid composition of both symbiotic partners. All in all, our work aims at taking advantage of the biophysical and spectroscopic techniques in the context of the mutualistic partnership between cnidarians and dinoflagellates, as well as its disruption. Our results show the tolerance of Cassiopea andromeda jellyfish and their dinoflagellate symbionts to a long-lasting hyperthermic stress.

Specimens of common Baltic ciliates fixed with acid Lugol's solution - photomicrographs

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This poster presents light micrographs of pelagic ciliates from the surface and the near-bottom waters of the southern Baltic Sea sampled during different studies. All specimens were fixed with acid Lugol's solution, which made some features of taxonomic importance impossible to assess. Thus, not all specimens are identified to the generic or species level. Most of the images presents ciliates from the following orders: Prostromatida, Haptorida, Oligotrichida, and Choreotrichida. However, specimens from other orders are also presented. Both naked and loricate ciliates are shown.

A dead end for both partners: Hyperinfection with Ca. *Gortzia yakutika* in *Paramecium nephridiatum*

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Ciliates often form symbiotic associations with other microorganisms, some species being more prone to such interactions than others. Paramecium nephridiatum can harbor a plethora of bacterial endosymbionts, predominantly, cytoplasmic ones. A strain of P. nephridiatum (BMS 21-9-1) isolated in 2021 from a pool in the intertidal zone on the Srednyi island (the White Sea, Kandalaksha Bay, Chupa inlet) was found to be infected with two species of endosymbiotic bacteria, one residing in the host cytoplasm and the other one in the macronucleus (Mac). Living cell observations performed with DIC demonstrated heavy infection of the host Mac. The species identity of the host was proved by sequencing of its COX I gene. Molecular cloning, sequencing of the endosymbiont SSU rRNA gene and fluorescence hybridization in situ with the species-specific oligonucleotide probes showed that the macronuclear endosymbiont was a Holospora-like bacterium Ca. Gortzia yakutika (Holosporales), while the bacterium inhabiting the host cytoplasm was Ca. Megaira venefica (Rickettsiales). Transmission electron microscopy revealed both, reproductive and infectious forms of Ca. G. yakutika in the host Mac. Occasionally, the infectious forms were surrounded by some fibrous material of unknown nature suggesting degradation of the bacteria. In some ciliates, invaginations of the macronuclear envelope containing multilamellar bodies were observed, presumably, implying expulsion of the decaying bacterium from the nucleus into the cytoplasm for final destruction. Heavy macronuclear infection and bacterial degradation repressed host cell proliferation, leading to rapid strain extinction.

Supported by RNF grant #22-24-00335.

The first arctic rhizochromuline: Morphology, ultrastructure, and position in the evolutionary tree of Rhizochromulinales (Ochrophyta, Dictyochophyceae)

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Rhizochromulinales is an understudied and relatively obscure order of ochrophyte algae from the class Dictyochophyceae. The order comprises two genera: Rhizochromulina and Ciliophrys. Today, the genus Rhizochromulina includes a single validly named species – Rhizochromulina marina: a unicellular amoeboid alga capable of forming flagellate cells. However, there are numerous strains and environmental sequences, designated as Rhizochromulina sp. or R. marina, but their morphology, ultrastructure, and phylogeny are not sufficiently described. Biogeography of the genus is understudied as well: rhizochromulines from the Indian, Southern, and Arctic Oceans are unknown. In August 2019 we isolated the first rhizochromuline from an arctic habitat: a supralittoral rock pool on the island of Ryazhkov (Kandalaksha Gulf of the White Sea, Murmansk oblast, Russia). After the cell culture was obtained, we revealed that amoeboid cells of the organism form a biofilm on the bottom of a culture vessel, where they are embedded into a transparent gel-like matrix. Moreover, neighboring cells are often connected to each other via branching pseudopodia, thus forming a meroplasmodium. Transmission electron microscopy, immunofluorescence staining of tubulin and phalloidin labeling of actin showed that these pseudopodia contain both microtubules and short actin filaments. We also managed to develop a protocol of induction of amoeba-to-flagellate transformation in this microalga: it turned out that the amoeboid cells transform into flagellates upon a prolonged mechanical disturbance (agitation at 200 rpm for 20 – 24 h). During the reverse transformation, flagellate cells can shed or retract flagella. The results of morphological, phylogenetic, and haplotype analyses coupled with the 18S rDNA alignment examination indicated that the arctic strain is most closely related to the type strain of R. marina. At the same time 18S rDNA sequences of early branching off rhizochromulinids differ significantly from the sequence of the studied microalga, suggesting a high divergence at a genus level.

Composition of telomeric proteins in Blastochritidia nonstop

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Protein complexes at the telomeric ends are important for their maintenance and regulation. Recent data demonstrated diversity in telomeric length in different species of trypanosomatids (Polaková et al., 2021). In addition, the composition of a telomere-associated complex in Trypanosoma brucei revealed a set of 20 conserved proteins putatively associated with telomers in trypanosomatids (Reis et al., 2018). Two of these proteins (KU70 and KU80) are conspicuously missing in the genus Blastocrihidia, while telomeres in these species do not appear to be affected. In this work, we analyzed the composition of the telomere-associated protein complex in B. nonstop, a recently described member of this genus, in which all 3 stop codons were recoded to encode amino acids (Kachale et al. 2023).

Does salinity matter? Ciliates in the southern Baltic

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Although marine benthic protist communities are assumed to play an essential role in terms of the microbial food web and to have severe influence on the composition and dynamics of this ecosystem, sufficient data on the marine benthos are still rather scarce. One of the most diverse and ecologically important groups of unicellular eukaryotes is the phylum Ciliophora. On the poster, the benthic ciliate diversity of the Oderbank in the southern Baltic Sea is addressed by characterizing new species which were found during a sampling campaign in summer 2021, where - among others - members of the genii Euplotes and Aspidisca could be found. Based on SSU rDNA sequences, a description of the affiliated morphology received through different staining techniques (e.g., Protargol, DAPI and dry silver nitrate stain) as well as scanning electron microscopy, respectively, was done. One Euplotes and one Aspidisca species could be described as new species. Furthermore, the assignment to a genus level based on taxonomic analysis was done for all six isolated strains with based on molecular analyses. In some cases, the obtained ciliate phylogenies revealed close relations to sequences isolated under similar habitat and environmental conditions. Interestingly, some ciliate strains showed affiliations to anaerobic genotypes, that should be further investigated during ongoing studies of the MGF-Baltic Sea project, which currently investigates the impact of trawling fishery on the benthic ecosystem of the Baltic Sea. On the poster, we will present results of this study concerning the occurrence of the species found under different salinities as well as results on metabarcoding analysis. We hope to add valuable information on a small fraction of the benthic protist community in the Baltic Sea and support future studies on ciliate diversity and function.

Genome of a Ca. *Megaira* endosymbiont from the cercozoan amoeba *Rhogostoma pseudocylindrica*

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"Candidatus Megaira" (Alphaproteobacteria: Rickettsiales) are intracellular, symbiotic bacteria of different eukaryote lineages, including ciliates and microalgae. The limited genomic data available thus far suggest that Ca. Megaira is actually a diverse, family-level clade. One member of the clade, designated "Ca. Megaira telluris", is found in a thecate amoeba Rhogostoma pseudocylindrica (Rhizaria: Cercozoa: Thecofilosea), which belongs to a genus that is abundant in soil, where it is often associated with plants, but also occurs in wastewater. Here we report an effectively complete metagenome-assembled genome (MAG) for "Ca. Megaira telluris", comprising a single circular contig of 1.32 Mbp with high estimated completeness (97%, CheckM2). Like other Ca. Megaira, this genome encodes a complete nonoxidative pentose phosphate pathway but no complete vitamin biosynthesis pathways and is hence unlikely to be a nutritional symbiont. Seven putative plasmids totaling 613 kbp with similar GC% and coverage to the main genome were also assembled, which encode genes for mobile elements, conjugative transfer, and a predicted biosynthetic cluster for non-ribosomal peptides. These functions may mediate host-symbiont interactions while being readily gained or lost because they are encoded on plasmids. We hence speculate that horizontal gene transfer of interaction factors may underlie the association of Ca. Megaira species with various unrelated eukaryotic host lineages.

Effects of warming on the protist communities in the Southwestern Sea of Korea during the late spring

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The Southwestern Sea of Korea (northeastern East China Sea) is one of the regions where seawater temperature rises rapidly in the world, thereby vulnerable to ocean warming and some waters have already become subtropical. The marine ecosystem of the region is also directly affected by a branch of the Kuroshio warm Current. To understand the effects of future warming in the spring season of the waters (i.e., 17–19 °C) on changes in protistan communities of the region, we collected natural waters at two independent stations [i.e., E42—high N and low P (HNLP) and indirectly- and E46—directly-affected by warm waters at the sampling period] and incubated each community under three different temperature conditions (i.e., ambient T, +2, and +4 °C). Chlorophytes, which showed a high initial proportion in the community of the HNLP region (i.e. E42), were rapidly decreased over time. Similarly, the proportion of diatom community at E42 decreased regardless of temperature differences, but that of E46 which is abundant by Thalassiosira-like diatoms showed a positive correlation with temperature, supported by the changes of other variables including an increase in chlorophyll-a and a decrease in Si[OH]4 concentrations. Interestingly, rising water temperature and phosphorus deficiency did not constrain the proportions of dinoflagellates at both incubations, suggesting that their phagotrophic ability may be an important adapting strategy in changing marine environments. In conclusion, the results of the study suggest that the effect of warming on the protistan community in the Southwestern Sea could be strongly regulated by local differences in oceanographic conditions. Most importantly, our study is one of the rare attempts to examine the influence of potential warming on the protist community during the spring season in oligotrophic offshore waters.

Absence of spliceosomal introns in the mixotrophic ciliate *Mesodinium rubrum* - how did they disappear?

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Mesodinium rubrum is a free-living mixotrophic ciliate that causes red tides in coastal regions. The ciliate steals plastids (kleptoplasty) after engulfing the cryptophyte alga Teleaulax amphioxeia, exploiting its photosynthesis. M. rubrum also steals the cryptophyte nucleus (karyoklepty), which remains transcriptionally active for an extended time. The stolen nucleus (kleptokaryon) is essential in regulating plastid activity. We sequenced and assembled the somatic and germline genomes from M. rubrum nuclei purified by fluorescence-activated flow sorting. We also generated poly(A) RNA-seq to guide protein-coding gene prediction. We observed no M. rubrum genes with spliceosomal introns. In contrast to other ciliates, we found M. rubrum lacks all spliceosomal snRNAs and most core spliceosomal proteins. We believe the M. rubrum somatic genome assembly is relatively complete since most RNA-seq maps to it (91.44%) and most ribosomal proteins are present. M. rubrum thus appears to be the first known “free-living” ciliate that has lost the spliceosome complex and introns. It also appears M. rubrum lacks the key protein involved in nonsense-mediated decay of mRNAs, Upf1, which surveys unspliced introns. In contrast, organisms known to have lost most introns are typically obligate parasites, and the losses are related to genome minimization. We consider multiple possible evolutionary forces that may have contributed to intron loss in M. rubrum.

Identification and analysis of cilia-associated gene families in *Euplotes amieti* (Ciliophora, Hypotrichida)

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Ciliates are important model organisms for protein composition and assembly regulation. To systematically analyze the structure and function of ciliopathic genes in Euplotes amieti. In this study, Next-generation sequencing technology was used to sequence the macronuclear genome of Euplotes amieti, and a total of 372 cilia-associated genes were identified from the macronuclear genome of Euplotes amieti using BLASTP. These gene sequences are highly homologous between Euplotes octocarinatus, Stylonychia lemnae, Oxytricha trifallax, Paramecium, and Tetrahymena. They are classified into kinases, dyneins, flagellin, tubulin, and autophagy-related proteins by NR function annotation according to their protein sequences. A total of 3 conserved domains were predicted by Pfam domain annotation. GO analysis showed that these genes were mainly enriched in tubulin binding, cilia assembly, and microtubule base movement. The biological signaling pathway was enriched in adenine ribonucleic glycoside biosynthesis, glycolysis/gluconeogenesis, Wnt signaling pathway, and AMPK signaling pathway, which indicated that cilia-associated genes in ciliates also play an important role in DNA duplication, energy metabolism, communications between ciliates and morphogenesis. In addition, 5 hub genes IFT172, TTC21B, CEP290, ACTB, and DYNC1H1 are highly correlated with ciliopathies, which may have a negligible function in regulating ciliogenesis in ciliates.

Morphological reconstruction during cell regeneration in the ciliate *Spirostomum ambiguum*

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When the ciliate Spirostomum ambiguum is transected into two pieces, both fragments regenerate and proliferate in the same manner. In the anterior fragment, which lost its contractile vacuole after transection, a new contractile vacuole forms at its posterior end, while the contractile vacuole of the posterior fragment remains unchanged. When the cell was transected into three pieces, new contractile vacuoles always formed at the posterior ends of both the anterior and middle fragments. The anterior-posterior axis of S. ambiguum was maintained after transection, indicating the existence of a mechanism that maintains this axis. Morphological restoration was also observed when only the apical portion was transected to cut out small fragments that did not contain the macronucleus. These results suggest that Spirostomum undergoes morphological repair after transection without requiring new gene expression, and that the mechanism for maintaining the anterior-posterior axis is also maintained without new gene expression. To understand the mechanism of cell regeneration and the maintenance of the anterior-posterior axis, it is essential to investigate the repair of the cleavage surface after fragmentation. Therefore, we utilized scanning electron microscopy to observe the changes in the shape of the cleavage surface of S. ambiguum during the regeneration process. First, to separate one cell into two, a thin tapered nylon fiber was applied from above to apply pressure to the cell. Subsequently, we fixed the cells using the newly-deviced water freeze-drying method, which produces fewer artifacts than the conventional chemical fixation and drying methods. Electron microscopic observation revealed that within tens of seconds after transection, the cleavage surface was covered with a cilia-free membrane that prevented the outflow of the cytoplasmic contents. The surface of the transected area then rounded with time, and cilia developed at the edges of the cilia-free area, after which the entire cleavage surface was covered with cilia, thus completing the repair of the transected area. The new cilia on the surface of the transected area appeared to be extensions of existing ciliary rows that had entered the cilia-free membrane.

Characteristics of a novel encystment-inducing pheromone released from the ciliated protozoan *Colpoda cucullus*

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When the vegetative cells of the terrestrial ciliated protozoan Colpoda cucullus sense signals that indicate forthcoming desiccation in the habitat, they rapidly transform into resting cysts that are resistant to dehydration, high temperature and freezing. In our laboratory, the encystment can be induced by the addition of Ca2+ into the overpopulated vegetative cells or the rapid increase of temperature. Recently, we found that the encystment-induced vegetative cells of C. cucullus release a certain pheromone that has an ability to induce the encystment to other cells. We named the pheromone “encystment-inducing pheromone (EnIP)” and characterized it in this study. Overpopulated vegetative cells gradually encysted in ion-free ultrapure water without any stimulation. When we added the external solution of the encysted cells to the vegetative cells at low density, the encystment was markedly induced without any additional stimulation. These results suggest that the encystment-induced vegetative cells released EnIP, which triggers encystment in other cells. Further investigations revealed the characteristics of EnIP as follows; 1) The encystment-inducing effect of EnIP occurs in a concentration-dependent manner. 2) EnIP loses its encystment-inducing activity after 1 day and 2 days. 3) EnIP is released within a couple of hours after vegetative cells were incubated at high density. 4) The release of EnIP is suppressed by an exocytosis inhibitor (colchicine). 5) EnIP loses its encystment-inducing activity by the heating at 70°C for 20 min. 6) EnIP loses its encystment-inducing activity by treatment with proteolytic enzymes (0.1 mg/ml pepsin and 0.01 mg/ml trypsin). Based on these evidences, we concluded that the encystment-induced vegetative cell of C. cucullus releases a protein acting as a pheromone with encystment-inducing activity for other cells.

Endemics ciliates of phytotelma exhibit high macroevolutionary rates

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The phytotelma is a microcosm formed by plant structures that accumulate water and organic matter, such as bromeliad tanks. This microhabitat harbors a great diversity of prokaryotes and eukaryotes, with ciliates being the most abundant eukaryotes. However, knowledge about phytotelma ciliate communities is still shallow and incomplete, especially when it comes to ecological aspects, such as endemism, occupation of this niche, the transitions between the different habitats explored, and their evolution routes. Seeking to better understand the processes related the diversification of the endemic ciliates of tanks bromeliads, our objective was to infer the time of origin and the mode of evolution of the endemic ciliates of the phytotelma, as well as delimit their dispersal routes until arrival in the phytotelma environment. Our results showed that the last common ancestor of the Ciliophora phylum occupied a habitat like the current marine/brackish environments, and that the arrival to phytotelmata habitats is a recent occurrence in the evolution of the phylum. Although recent, the acquisition of this habitat occurred numerous times and independently over evolutionary time, being the origin of endemic ciliates after the appearance of bromeliads, with origin around 90 million years. Our data also revealed that the arrival of the ciliates to the phytotelma occurred through four distinct routes: (i) from a marine/brackish ancestor, (ii) a freshwater ancestor, (iii) a terrestrial ancestor, or (iv) a symbiotic ancestor. In addition, we noticed a significant increase in the diversification rates of ciliates endemic to bromeliad phytotelma. Several hypotheses ecology, morphology and evolution can explain the evolutionary success of endemic ciliates of phytotelmata, however the scarcity of studies with this group can make it difficult to define complex hypotheses that explain such a process. This further emphasizes the need to dedicate efforts to develop ecological and molecular studies with this group.

Ancient protein resurrection of the Arf1/6 progenitor: “Jurassic Park-ing” a 2 billion year old protein

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Arf GTPase proteins are crucial mediators of material transport in eukaryotic cells. Two ancient related proteins, Arf1 and Arf6, act at sites internal to the cell and at the cell surface respectively, interacting with discrete effectors. However, the evolutionary steps leading to these two proteins are shrouded beyond the deepest event horizon reconstructable by standard molecular evolutionary techniques. Using the latest bioinformatic and synthetic biology tools, we are able to reconstruct and ultimately resurrect the Arf1/6 common ancestor. The reconstructed sequence allows for the calculation of its structural features and predictions to be made of its cellular localization, which will be tested with a resurrected protein. These findings will shed light on the steps and mechanism by which cellular transport evolved and ultimately about the fundamental process by which cells evolve. At the European Congress of Protozoology(ECOP), I will present a poster of the findings of the structure analysis of my reconstructed sequence. From the domain analysis, I will be able to comment on the localization and function of the Arf1/6 ancestor as it relates to contemporary Arf 1 and 6 proteins. Furthermore, I will make inferences on the mechanism of its evolution and lay out the rationale for further testing using ancient protein resurrection(APR).

Intracellular pigments and morphological changes as a stress response in a mesophilic *Ancylonema* species (Zygnematophyceae)

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Unicellular zygnematophytes with a rather simple cell morphology ("saccoderm desmids") have been shown to vary greatly in terms of ecology and cellular adaptations. Several saccoderm desmid species live in terrestrial habitats, which are characterised by low water availability, high solar radiation and low nutrient supply. Currently, the genus Ancylonema contains two cryophilic species, A. alaskanum and A. nordenskioeldii, both exclusively known from glacial ice. These species also produce an intracellular, red pigment, a purpurogallin derivate, which has been suggested to act as a sunscreen. Here, we present two new strains of the genus Ancylonema isolated from peat bogs in Germany. They also exhibited red vacuolar pigments when found in nature, and might represent a new mesophilic species. While the cryophilic species are difficult to cultivate, the new strains grow fast and to high densities with standard cultivation techniques - an ideal opportunity to study Ancylonema's reactions to environmental factors in the lab. We performed different light and nutrient deficiency experiments and found that the vacuolar pigments could be induced by high photosynthetically active radiation, ultraviolet A and ultraviolet B radiation. However, it appeared that the nutrient composition of the medium was another important factor: Nutrient limitation alone could lead to slightly pigmented cells. Furthermore, we managed to induce the production of zygotes, which complements our morphological knowledge about the new mesophilic Ancylonema species.

Single-cell transcriptomics: Establishing splitseq method for protist communities

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Despite the significance of protists in various environments, their direct role in those communities and gene expression profiles remain understudied. The single-cell RNA sequencing (scRNA-Seq) allows high-throughput analyses of the physiological state of individual cells and has been successfully used for diverse animals and different cell types. However, the use of this technology in protistology studies is challenging due to the lack of a protocol developed for microbial eukaryotes and the vast diversity of cell types among protists. The split-pool ligation-based transcriptome sequencing (SPLiT-Seq), based on the combinatorial barcoding of RNA directly into permeabilized cells, allows the parallel RNA sequencing of many cells with single-cell resolution. We attempt to optimize the ACME protocol in combination with the FACS sorting and SPLiT-Seq method to obtain scRNA-Seq data from protist cells. To accomplish this task, we designed a mock community of 10 known freshwater protists species, representing diverse groups, differing in their size, cell types, and abundance in the natural environments. The main challenge is to isolate single cells from mixed populations in a high-throughput manner, without lysis of the cells and influencing their expression profiles. We selected the TRIzol RNA extraction method after the comparison of several kits, because of its high performance and the highest level of RNA integrity. Six species presented optimal growth performance and maintenance of RNA integrity after isolation and were selected for further analyses using the ACME workflow. All species were well-fixed and preserved their cell integrity. The quality of the RNA was evaluated via TAPEstation. Except for Micractinium conductrix and Cyclotella cryptica, the RIN values and the gel RNA pattern showed preservation of RNA integrity, and the results before and after ACME fixation were comparable. Currently, we are testing the efficiency of the protocol for the permeabilization of the cells, toward the next step of mRNA barcoding. Subsequently, we plan to select a final mock community and mix it in different variants to recreate the consortia present in the environment. To validate our approach, we will compare the obtained transcriptomic results with complementary genomic, metatranscriptomic, and amplicon data from the same mock community.

Research and development of a novel biological water quality index based on Arcellinida using metabarcoding techniques

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The development of human activities is having an increased impact on freshwater ecosystems, in particular under arid climates. Bioindication is nowadays a method of choice for monitoring the environmental quality of these ecosystems. In Spain, several biomonitoring protocols have been developed and integrated into the legislation, like the IBMWP based on visual observation of macroinvertebrates. However, these protocols are tedious and lack of taxonomic accuracy, which in turn decreases their sensitivity; moreover, most have been developed for lotic systems and are not suited for lentic water bodies. It is necessary to search for a new category of biological indicators present in all lentic environments, which are easy to sample and sensitive enough to provide easy-to-interpret results. Furthermore, metabarcoding based protocols are sensitive, fast, relatively cheap and reproducible; they do not rely on taxonomists expertise. In this project, we are developing and applying a biomonitoring protocol based on Arcellinida (=lobose testate amoebae) metabarcoding. Arcellinida are abundant in inland water bodies, and are ecological specialists that have a fast reaction time to perturbations, which makes them excellent bioindicators. The objective of this project is the development of a new protocol for rapid and reliable monitoring of water quality in lentic systems, such as large river axes, natural lakes, brackish lagoons and reservoirs. We will apply this protocol to various river basins in Southern Spain based on Arcellinida communities' composition revealed by metabarcoding. We will develop environmental quality indices based on machine learning, aiming at incorporating this methodology into Spanish legislation to evaluate environmental health of freshwater lentic ecosystems, which are currently threatened.

New evidence of consistency between phylogeny and morphology for two large taxa in ciliated protists, the subclasses Oligotrichia (Protista, Ciliophora)

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A vast majority of marine planktonic ciliates are comprised of oligotrichs and choreotrichs, which are two subclasses of class Spirotrichea. In terms of morphology, oligotrichs are characterized by having ventrally opened oral membranelles while choreotrichs have closed oral membranelles, with many species having a lorica over their cell surface. Further phylogenetic analyses using SSU rDNA sequence information reveal oligotrichs and choreotrichs as individual monophyletic clades, and Lynnellida as an intermediate group which is basal to either choreotrichs or oligotrichs. However, the current phylogenetic assignments of oligotrichs and choreotrichs are inconsistent with previous results based on morphological features, probably hindered by the limited molecular information from single gene locus. Here we provide 55 new sequences from three gene loci (SSU rDNA, ITS1-5.8S-ITS2 rDNA, LSU rDNA) and make a more comprehensive phylogenetic reconstruction, leading to consistent observation between morphological taxonomy and an updated phylogenetic system for oligotrichs and choreotrichs. With the addition of data from ciliature patterns and genes, the phylogenetic analysis of the subclass Oligotrichia suggests three evolutionary trajectories, among which: 1) Novistrombidium asserts an ancestral ciliary pattern in Oligotrichia; 2) the subgenera division of Novistrombidium and Parallelostrombidium are fully supported; 3) the three families (Tontoniidae, Pelagostrombidiidae and Cyrtostrombidiidae) all evolved from the most diverse family Strombidiidae, which explains why strombidiids consistently form polyphyletic clades. In the subclass Choreotrichia, Strombidinopsis likely possesses an ancestral position to other choreotrichs, and both phylogenetic analysis and RNA secondary structure prediction support that tintinnids may have evolved from Strombidinopsis. Our results propose an updated hypothesis for the evolutionary history of oligotrichs and choreotrichs based on new evidence obtained by expanding sampling of molecular information across multiple gene loci

Integrative approach on key freshwater ciliates of the genus *Urotricha* (Alveolata, Ciliophora, Prostomatida)

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Species of the ciliate genus Urotricha are key players in freshwater plankton communities. In the pelagial of lakes, about 20 urotrich species occur throughout an annual cycle, some of which play a pivotal role in aquatic food webs. In ecological studies, urotrich ciliates are usually merely identified to genus rank and grouped into size classes. This is unsatisfying considering the distinct autecological properties of individual species and their specific spatial and temporal distribution patterns. As a basis for future research, we characterized in detail several common urotrich morphotypes using state-of-the-art methods. We applied an integrative approach, in which morphological studies (in vivo observation, silver staining methods, scanning electron microscopy) were linked with a molecular approach (SSU rDNA, ITS-1, ITS-2, hypervariable V4 and V9 regions of the SSU rDNA). We shed light on the diversity of urotrich ciliates as well as on their global distribution patterns, and annual cycles. Additionally, we coupled individual species occurrences and environmental parameters, and subsequently modeled the distribution and occurrence, using logistic regressions. Furthermore, for one strain, we ascertained the optimal cultivation media and food preferences. Thereby, our comprehensive view on these important freshwater ciliates that frequently occur in environmental high throughput sequencing datasets worldwide will allow future studies to better exploit protistan plankton data from lakes.

A deeper investigation in microsporidian diversity

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Microsporidia comprise a microbial eukaryotic group of obligate intracellular parasites that are related to fungi and are well known for their rapid rates of evolution and extensive genome reduction. This has resulted in a group of organisms with extremely compacted genomes and unique protein families. Only a few hundred species of microsporidia have been fully described despite thousands being predicted to exist. Two likely explanations for this shortfall are the divergence and poor understanding of microsporidian genomic sequences and host sampling bias. Microsporidia possess highly divergent 18S rDNA sequences that cannot be detected via conventional eukaryotic 18S primers and due to additional variation within Microsporidia, there is currently no true universal primer set that can detect all clades. I will first identify primer sets that are efficient at discerning microsporidian clades. Then I will survey diverse sets of potential animal and protistan hosts, as well as environmental samples, using these primers. Once identified, discovered species will be sequenced and annotated and their ultrastructure and host-parasite pathology documented through imaging. The ultimate goal of this research is to identify and describe new species of microsporidia to better understand their evolutionary history, diversity, and relationships with their hosts.

Genomic characterization of eight peritrich ciliates (Peritrichia: Sessilida), with new phylogenomic insights

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The subclass Peritrichia is a well-studied group with uncertain internal relationships. Recent studies have shown that most of the order Sessilida families are not monophyletic. Genomic investigations can provide light over these questions, however, the amount of genomic data currently available for this order is still limited. This study presents the results of a phylogenomic investigation containing eight new genomic sequences from peritrich ciliates. After genome assembly, we obtained eight peritrich new genomes with 2,827 ~ 79,758 contigs and N50 length of 1,644 ~ 56,289. The genome size (29.4 ~ 88.2 Mb; completeness_BUSCO_Alveolata_odb10 45.6 ~ 74.9) and GC content (25.45 ~ 45.59 %) were similar to those observed in other peritrich genomes available in public repositories. Among the eight new genomes presented here, we have the first genomic records for the Lagenophryidae and Opisthonectidae families and the first genomic record for the genus Platycola. As a result of the phylogenomic analyses, we found several similarities in comparison to previous phylogenetic analyses. The Vorticellidae family is polyphyletic and closely related to the representative of the Opisthonectidae family. The Zoothamniidae family is also polyphyletic, being divided by representatives of the Epistylididae family, which is polyphyletic as well. The representative of the Platycola genus was grouped with the other representatives of the Vaginicolidae family, which was identified as monophyletic. A variation from the expected was the position of the Lagenophryidae family representative, which positioned as the sister group of the clade containing Zoothamniidae and Epistylididae representatives, rather than with the clade that accommodates other loricate genera, belonging to the Vaginicolidae family. The phylogenomic analysis presented here revealed several polyphyletic families, confirming previous findings utilizing 18S-rRNA and other genetic markers. However, the position of the Lagenophryidae representative was unexpected, highlighting the need for further genomic investigations.

It takes some guts: A termite-protist co-speciation analysis using long read amplicon sequences

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The protist community within termite hindguts, which aids in the digestion of nutrient-poor wood materials, was established between 140 to 170 million years ago. The eusocial nature of termites within colonies meant that the transfer of protists became vertical, from parent to offspring through proctodeal trophallaxis. Because of this close relationship, protists co-diversified with their hosts, splitting into new lineages over time. Today there are about 800 protist-dependent termite species, each harboring its own unique cocktail of protist symbionts; however, the true description of the protist communities and level of co-diversification of these two groups is unknown due to the lack of data. While the amount of molecular data from termite-associated protists is increasing every year, it is acquired either from hand-picked single cell ribosomal small subunit (SSU) sequences, or whole gut short read SSU amplicon sequencing. The former provides more molecular information to infer phylogenies, while the latter provides a better inference of each termite species' whole gut community but without supported phylogenies. To mitigate the shortcomings of both techniques, long read amplicon sequencing was done to provide phylogenetically informative molecular data from the termite whole gut protist community. The whole ribosomal SSU from 31 termite species' symbionts were sequenced from whole gut communities and supported phylogenies were inferred, presenting a more in-depth examination of the protist diversity found within these insects.

Long-read metagenomics of microbial communities in boreal forest soils

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Boreal forests contain a large part of the terrestrial carbon globally, and the majority of this carbon is sequestered belowground. However, processes related to carbon cycling and sequestration belowground, and which organisms are mediating these processes, are still poorly understood. Anthropogenic activity, such as forestry, may affect these processes and the involved organisms, but the consequences are still largely unknown. In the EcoForest project, we aim to investigate how large-scale transformation of boreal forests affects biodiversity, carbon stocks, and ecological processes, by comparing near-natural mature Norway spruce forests with mature spruce forests regrown after previous clear-cutting. Long-read sequencing techniques are becoming increasingly powerful tools to investigate uncultured microbial communities, for instance fungal and protist communities in soils. Longer reads, higher throughput and sequence quality potentially allow for a higher coverage of more and larger genomes, and better functional and taxonomic annotation of specific taxa. In the experiment presented here, we will investigate which microorganisms are potentially affecting carbon fluxes from soils collected in near-natural and previously clear-cut forests, using long-read metagenomics and respiration measurements. We have performed monthly in situ soil respiration measurements of carbon dioxide (CO2). Heterotrophic respiration and fluxes of N2O and CH4 have also been measured during lab incubation of soil samples. To investigate the functional potential of the microbial community, we will extract total DNA from the soil samples and sequence the metagenomes using PacBio Revio, as well as target the eukaryotic and prokaryotic community using short-read metabarcoding through Illumina sequencing. Using this experimental setup, we will be able to directly link the microbial community and their functional potential to CO2 fluxes in boreal forests, as well as investigate potential changes caused by clear-cut forestry.

Interstitial environment of the Danube: Home for an assemblage of rare filose testate amoebae

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The non-silted, sandy sediments of the Danube River harbor a diverse community of freshwater, psammophilic, and psammobiont testate amoebae (TA). Among them, psammobiont species such as Corythionella and Psammonobiotus have been identified, whereas additional rare species, like Cyphoderiacalceolus and C. myosurus, have been classified as psammophilic based on their previously known habitats. A third group of filose TA, including Cyphoderialaevis and Paramphitremalemanense, are occasionally found in microhabitats with slightly higher organic content. This unique TA assemblage is highly dependent on the water regime of the Danube and has not been observed elsewhere in the same composition. Recent biogeographic data on C. calceolus and C. myosurus suggest a glacial speciation event with a center in the Swiss Alps, when large river basins of the Rhone, Rhine, and Danube were more connected through groundwater filtration. To date, all the mentioned species are restricted to Europe (C. calceolus and C. myosurus) and partly to North America, exhibiting a distinct geographic distribution. Further research on nutrient-poor sediments in the area may reveal more about this fragile assemblage.

Blastocystis under One Health - COST action

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Blastocystis is the most prevalent intestinal protist colonizing the gut of at least one billion people. Emerging data indicates a higher prevalence in animals, both endotherms and ectotherms. A high proportion of carriers are asymptomatic. Despite a century of study, pathogenicity of Blastocystis remains controversial. Currently, at least 32 genetic subtypes (STs) exist. Of these, ST1-ST9, ST10, ST12, ST16 and ST23 have been found in humans, while the rest have been isolated only from non-human hosts. Information on prevalence, geographic distribution and host specificity of STs is incomplete. Significant gaps also exist on environmental presence of Blastocystis. Collectively, this paucity of data blurs the Blastocystis landscape considerably. Thus, a COST action "Blastocystis under One Health (CA21105)" was conceptualized to bring together experts from various fields to: (1) Support advancement of Blastocystis research by bringing together professionals from various disciplines and countries; (2) Foster information sharing on current methodologies, especially in the areas of subtyping, host-Blastocystis-microbiome interactions and Blastocystis-omics; (3) Promote capacity building via a transdisciplinary network of international collaboration; (4) Open avenues of communication with veterinarians, physicians and general public. By the end of this Action, participants will be able to: (i) Apply state-of-the-art tools for molecular identification of Blastocystis; (ii) Harmonise methodologies for subtyping Blastocystis and identifying its role within the gut; (iii) View Blastocystis under One Health approach; (iv) Generate novel hypotheses to test role of Blastocystis in the gut ecosystem, health and disease.

Bioimaging in *Tetrahymena* by glucose analogs labeled at the C-1 or C-2 position with a fluorescent dansylamino group

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Glucose is the most important energy source in all organisms; however, our understanding of the pathways and mechanisms underlying glucose transportation and localization in living cells is incomplete. Here, we prepared two glucose analogs labeled with a dansylamino group at the C-1 (1-Dansyl) or C-2 (2-Dansyl) position; the dansyl group is a highly fluorescent moiety that is characterized by a large Stokes shift between its excitation and emission wavelengths. We then examined the cytotoxicity of the two glucose analogs in the ciliated protozoan Tetrahymena thermophila. In T. thermophila, both glucose analogs had no negative effects on cell growth. fluorescence microscopy revealed that the glucose analogs localized throughout the cytoplasm and nucleus. The glucose analogs were also observed to accumulate in the phagosomes and form a crystal-like structure that emitted strong fluorescence, which was not observed in the fixed cells. When India ink was applied to T. thermophila at the same time as the glucose analog, India ink and the fluorescence were observed in the same phagosome. This result indicates that a crystal-like structure exists within phagosome. We also found that swimming speed was comparable in media containing non-labeled glucose or one of the glucose analogs, which not only provided more evidence that the analogs were not cytotoxic in these cells, but also that the analogs were not affect the ciliary motion. Together, the present results suggest that the glucose analogs have low toxicity and will be useful for bioimaging of glucose-related systems. The advantage of our fluorescent glucose analogs is that we can observe the glucose in living cells as fluorescence easily without using special observation equipment, and they also have little cytotoxicity. Disadvantages are that yield of synthesis is low and it is not known whether cells recognize it as a nutrient glucose and take them up.

Mutual benefits from the symbiotic coexistence between bipolar *Euplotes* cells and *Parafrancisella* bacteria

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Ciliates are common carriers of cytoplasmic bacteria, however little is known about the molecular basis of the symbiotic relationships with their host. Working on interbreeding bipolar (Arctic and Antarctic) populations of the ciliate Euplotes nobilii, members of these populations were found to stably host Parafrancisella γ-proteobacteria. These bacteria (originally isolated from an Antarctic population of another Euplotes species, E. petzi) are phylogenetically related to pathogenic Francisella species which are well known for their capacity to colonize eukaryotic cells, causing animal diseases known as francisellosis. The finding that Parafrancisella/Euplotes associations are common in polar marine environments suggested that both microbial partners benefit from their stable partnership. To inquire into mutual advantages, we carried out a genomic analysis of E. nobilii and its Parafrancisella symbionts. In the E. nobilii genome, no gene was detected encoding methionine sulfoxide reductase of type A (MsrA), an enzyme which is essential to face damages from oxidative stress imposed by the high (saturated) oxygen concentrations of the polar sea waters. At the same time, the Parafrancisella genome revealed genes encoding MsrA sequences endowed with a N-terminal signal peptide for the secretion into the host's cytoplasm, and the effectiveness of this secretion was further supported by the identification of a complete gene set coding for the so-called 'Type VI Secretion System' that many Gram-negative bacteria use to transfer their proteins into target cells. On the other side, in the Parafrancisella genome no gene encoding enzymes involved in the biosynthetic pathways of cysteine, lysine, methionine, and threonine was detected, implying that Parafrancisella cells rely on their E. nobilii host to obtain these four essential amino acids.

This research was financially supported by the Italian 'Programma Nazionale Ricerca Antartide' (PNRA18_00152)

Unexpected survival of various groups of benthic protists at anaerobic conditions

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Globally occurring “dead zones”, areas of hypoxic or anoxic conditions, have been linked with anthropogenic influences such as surface runoff and increased nutrient influx and are nowadays occurring more frequently, especially in coastal areas. It is therefore crucial to better understand the effects of permanent or temporary anoxic conditions on organisms of the microbial food web. During investigations on the vertical distribution of benthic protist genotypes using metabarcoding techniques and live-counting, several groups were registered for which it was not yet known, whether they are able to be active in the anaerobic sediment that they were originally registered in. Knowledge on the survival and activity of many protist groups under anoxic conditions, including survival strategies of the different species, remains very limited. For instance, it is not known, whether species found at anaerobic conditions were transferred to anaerobic sediment layers via bioturbation or human activity (e.g. bottom trawling), or whether they have certain adaptations that allow an active or passive survival at such unfavorable conditions. For this reason, experiments have been carried out, in which many different strains were exposed to anaerobic conditions for a period of 72 hours. The goal of these experiments was to investigate the survival potential of different heterotrophic protists, especially kinetoplastids, chrysomonads, choanoflagellates and others from marine, brackish, and freshwater habitats. For every protist strain, three parallels were exposed to anoxic conditions using a system which reliably establishes anaerobic conditions within a few hours. Results from these experiments demonstrated that numerous species from different phylogenetic lineages were able to survive a 72h exposure at these conditions. Of the strains capable to survive the anoxic conditions, most were active again a few days after removing them from the anaerobic jar, some survived in the cyst stage, and others were observed active immediately or within two hours after removal. Among these were choanoflagellates (e.g. Enibas tolerabilis), different genotypes of Rhynchomonas and Neobodo as well as some colourless chrysomonads (e.g. Paraphysomonas spp.). The physiological mechanisms of survival of these protist strains may be different for the various protist groups and are open to further studies.

Combining morphological and molecular data to explain the history and evolutionary pattern of problematic taxa in the genus *Frontonia*

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The genus Frontonia has been a subject of debate concerning its monophyly. This study evaluates the genus Frontonia and its evolutionary patterns by analyzing phylogenetic data, SSU-rRNA secondary structures, morphological characters, and divergent times. This study shows that several helices are significant characters for the genus Frontonia, such as helix H9, E23_12, H29, and H43, which are suggested to be synapomorphic characters for the members of the genus Frontonia, and helix E23_14 which is an apomorphic character for each Frontonia group in the phylogenetic tree. Based on molecular clock analyses, the diversification of genus Frontonia may have started in the Paleozoic era, subsequently going through the famous mass extinctions that persisted into the Mesozoic era and began to diverge into member of the genus Frontonia that exist today. Group I appeared around 172 Mya, group II appeared around 83 Mya, group III appeared around 115 Mya, and group IV appeared in 190 Mya. Based on ancestor state analysis genus Frontonia tends to maintain similar morphological trait even though they are living in different habitat, this finding shows on evolutionary pattern of CV related to habitat. Ancestors of genus Frontonia are assumed to have one CV and living in brackish habitat, which later evolved to two CV in marine habitat (Group III), but this adaptation did not occur in other groups because the genus Frontonia (Group I, II, and IV) maintained a single CV even though they evolved from brackish into freshwater or marine.

Spatial and vertical distribution of pelagic nanoflagellate and nanoamoeba genotypes in relation to benthic and pelagic gene libraries of the Atlantic Ocean

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Protists are among the most diverse eukaryotic organisms on Earth. Even in the deep sea, they can maintain a high level of genetic diversity despite the challenging environmental conditions such as high pressure levels, the absence of light, low temperatures and low nutrients. Recent studies have shown that benthic protist communities of different deep-sea basins vary significantly from each other, sometimes even endemic distribution patterns have been recorded. However, it is still unknown whether deep-sea communities can be exchanged via the pelagial. The research question of the present study was whether deep-sea genotypes can be found in the water column or whether a distribution via the pelagial is negligible. We used the chance to take samples from the pelagial of the North Atlantic deep sea with special emphasis on the vertical and horizontal distribution of protist communities. Samples were taken during the cruise of R/V Maria Sibylla Merian (MSM 112/2) from November through December 2022. Cultures were established from samples collected from surface to abyssal depth down to 4000m. In addition, potential transport of protist communities by surface floating brown algae (Sargassum) was investigated. Bentho-pelagic coupling maybe a general phenomenon for protists in the size range of nanofauna (1-20 µm). The work included the isolation, cultivation, phylogenetic and morphological analysis of heterotrophic nano-protist communities. First results indicate that, for instance, among the percolozoans, only typical pelagic taxa were found, while benthic taxa were missing in the pelagial though high amounts of marine snow were present in all samples. And even completely new taxa never reported from deep-sea libraries before were found. Among other taxa reported were cercozoans, bicosoecids and amoebozoans etc. A comparison of taxa identified in the pelagic samples with those recorded in deep-sea benthic and pelagic genotype libraries will be given and discussed.

A new phylogenomic dataset for pinpointing the root of the eukaryote tree

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The resolution of a robust eukaryote Tree of Life (eToL) has been a long-standing goal in biology; however, a fundamental problem remaining is the uncertainty surrounding the position of the root of the tree, where the last eukaryotic common ancestor (LECA) initially diversified. Previous attempts to root the tree through phylogenomic methods have been hindered by the unavailability of molecular data across the full breadth of eukaryotic diversity, the lack of phylogenetic signal retained on the billion-year timescale, and inadequate evolutionary models. Recent advances in phylogenomic modelling and newly available molecular sequence data has made these types of analyses more plausible, and a new dataset has been developed for rooting the tree using mitochondrial proteins of alphaproteobacterial origin. Diverse eukaryotic proteomes were mined for marker genes that formed a monophyletic group with alphaproteobacteria in phylogenetic analyses, and taxa were selected based on the availability of both their nuclear and mitochondrial genomes. The dataset includes super-group level diversity that was previously unrepresented, such as Hemimastigophora. Single gene trees were iteratively constructed and examined to exclude contaminants, paralogs, and lateral gene transfer events prior to creating a concatenated phylogenomic supermatrix. State-of-the-art evolutionary models that account for phenomena known to confound phylogenomic analyses are being tested and will be used to estimate the root of the tree in both maximum likelihood and Bayesian frameworks. Additionally, the support for alternative root positions will be assessed under these models and compared to the optimal root found from this analysis. Identifying the root of the tree will be fundamental to understanding the traits present in LECA and will provide a robust framework for understanding eukaryotic diversity and the evolutionary processes underpinning its emergence.

Morphological plasticity and species determination: A case study from *Pseudokeronopsis erythrina* Chen et al. 2011 (Ciliophora, Hypotrichia, Urostylida).

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The two populations of urostylid species Pseudokeronopsis erythrina Chen et al., 2011 were isolated from a brackish industrial active sludge of Cuoiodepur wastewater treatment plant (WWTP) in Pisa, Italy and a nutrient-rich freshwater, the East Lake, Wuhan, China. Complete investigations were made with emphasis on these two populations' morphology, especially plasticity and phylogeny. Compared with the neotype morphologically described population, the Italy population of P. erythrina shows great variation in terms of frontal area, number of left marginal rows and dorsal kineties. Likewise, the number of left marginal rows anlagen and dorsal kineties anlagen (DKA) are also variable, e.g. some extra small anlagen that give rise to the formation of the left marginal rows are present; two DKA produced from one dorsal kinety. While the Wuhan population resembles the neotype population except for having one more dorsal kinety. The existence of giant individuals is present in both populations. We posit that the Wuhan population is the intermediate form between neotype and Italy populations. And, the high plasticity of Italy population provides a clue for species determination. Preliminary phylogenetic analysis based on 18S rRNA gene is also presented in this work.

Morphology and molecular phylogeny of the semiterrestrial ciliate *Euplotes baugilensis* n. sp. (Protozoa, Ciliophora)

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Euplotes baugilensis n. sp. was discovered in a temporary puddle formed after rain in a footpath on a mountain, South Korea. A pure culture was maintained and the cells were examined through live observation, protargol and “wet” silver nitrate staining methods, scanning electron microscopy, and the analysis of the 18S rRNA gene sequence. Morphologically, Euplotes baugilensis n. sp. is characterized by a small body size on average 49 × 31 µm in vivo, 34—45 × 19—26 µm (on average 39 × 22 µm) after protargol impregnation, outline asymmetric oval due to slightly obliquely truncated to left anterior end. 6 dorsal and 3 ventral ridges. Macronucleus inverted hook-shaped. 22—27 adoral membranelles; 9 fronto-ventral cirri (cirrotype-9) and a reduced, non-ciliated cirrus V/2 [note that the reduced, non-ciliated, frontoventral cirrus (basal plaque) is not counted]. Usually 8 dorsolateral kineties, of which the middle kinety composed of about 10—31. Dorsal silverline system of double dargyrome (double-patella-I). The molecular analysis using 18S rRNA gene sequences showed that E. baugilensis n. sp. is most closely related to E. curdsi (a sequence identity of 96.8%). Here, we provide a morphological description of the new species with microphotographs and a phylogenetic tree to show its position within the genus Euplotes.

Morphological characteristics and phylogenetic position of *Eudubosquella* species infecting dinoflagellates

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The genus Eudubosquella, one of several described genera within the syndinean dinoflagellates, exhibits considerable morphological and developmental diversity. Of the 10 formally described Eudubosquella species, seven infect tintinnid ciliates, one aloricate ciliates, and two athecate dinoflagellates. Here we report intracellular parasites, tentatively identified as Eudubosquella species, that infect the dinoflagellates Cucumeridinium coeruleum, Gyrodinium cf. ochraceum, and two unidentified species of Gyrodinium from coastal waters of Busan, Republic of Korea during summer to fall of 2019-2022. We compared morphological and molecular data for our four novel Eudubosquella species with data available for other Eudubosquella species that infect dinoflagellates and tintinnids. Species infecting dinoflagellates showed diverse morphological characteristics in shield patterns in the mature trophont, tomont shape, and sporogenesis. In SSU rRNA gene phylogeny, our four novel parasites spread out across syndinean dinoflagellate Group I, occurring in two different clades and a new lineage, whereas Eudubosquella species infecting tintinnids grouped together in a subclade within syndinean Group I clade 4. Our novel parasite from C. coeruleum was morphologically and developmentally quite similar to E. melo and E. nucleocola, the only described species of Eudubosquella that infect dinoflagellates. These three species differ from those infecting tintinnids in at least four significant ways: shield structure in the mature trophont, presence of fibrous bundles between the shield and nucleus, apparent absence of a lamina pharyngæ, and process of parasite egress. These findings indicate that parasites morphologically identifiable as Eudubosquella species are paraphyletic, thus indicating a need to revise the genus Eudubosquella. Such revision should encompass construction of a new genus to include our Eudubosquella sp. from C. coeruleum, E. melo, and E. nucleocola.

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