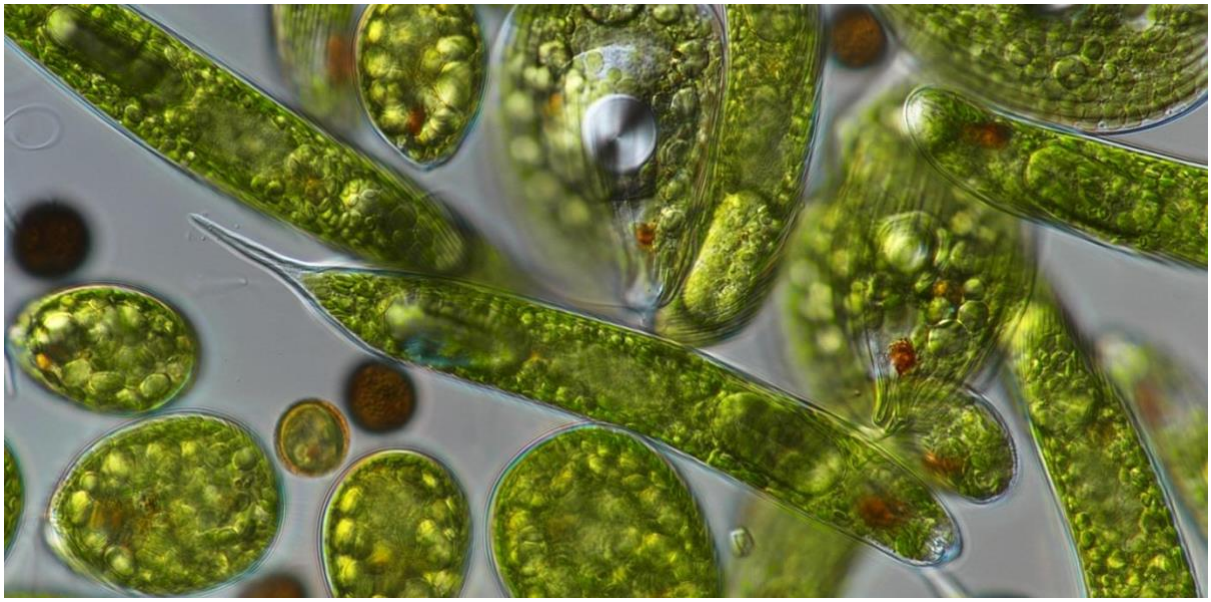
3rd Annual International Congress on Euglenoids



Book of Abstracts

17-18 July, 2023
Prague, Czechia

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Program

Monday, 17th of July

Speakers on-site, Speakers online

9:00-9:05	Welcome message
	Basic biology
9:05-9:45	**Keynote speaker - Masami Nakazawa** *Anaerobic Respiration Coupled with Wax Ester Production in Euglena gracilis*
9:45-10:05	**Michael Hammond** *Spatial proteomic analysis of the non-photosynthetic Euglena longa*
10:05-10:25	**Paweł Hałakuc** *Automatic annotation of nonconventional introns as a necessary step in the analysis of Euglena genomes*
10:25-10:40	Coffee break
10:40-11:00	**Ingmar Riedel-Kruse** *Phototaxis regulation in Euglena - from dim to bright light*
11:00-11:20	**Juraj Krajčovič** *Representatives of Euglenozoa and cyanobacteria as models to study calpain enzymes*
11:20-11:40	**Andrej Jedlička** *Unraveling meiotic genes in Euglena gracilis: Insights into protist evolution*
11:40-12:00	**Metody Hollender** *Euglenophyceae members are hosts for a new group of Legionellales bacteria*
12:00-13:30	Lunch
	Ecology and environment
13:30-14:10	**Keynote speaker - Josef Juráň** *Euglenoids' distribution: from local to global scale*
14:10-14:30	**Alicja Fells** *When biology walks into a bar – or metabarcoding studies of Euglenophyceae loricate taxa, Strombomonas and Trachelomonas*
14:30-14:50	**Jaroslav Kubín** *Dependence of diversity of colorless euglenoids on environmental factors*
14:50-15:20	Coffee break
15:20-15:40	**Chihana Toyokawa** *Exploring a resource-recycling food production system using Euglena gracilis*
15:40-16:00	**Emma Kaszecki** *Assessing the roles of microbial partners in cadmium tolerance of an Euglena mutabilis co-culture*
16:00-16:20	**Diana Lihanová** *Bacteria and fungi can supply Euglena gracilis with vitamins B1 and B12*
16:20-16:40	**Sanet Janse van Vuuren** *Blooms of Euglena sanguinea in South Africa*
16:40-16:50	EIN Science Committee Updates (Neil Hall, Michael Ginger, Paul Zimba)
16:50-17:00	EIN Annual General Meeting (Ellis O'Neil, Ross Low, ThankGod Ebenezer)
17:20	Organised transport to restaurant
19:00-22.00	Conference dinner (downtown)

Tuesday, 18th of July

Speakers on-site, Speakers online

9:00-9:05	Announcements
	Evolution
9:05-9:45	**Keynote speaker - Alastair Simpson** *Euglenid diversity and evolution; revealing the phagotrophic majority*
9:45-10:05	**Gordon Lax** *Molecular phylogenetics of the sessile phagotroph Dolium sedentarium*
10:05-10:25	**Susanne Kramer** *Convergent evolution of mRNA decapping complexes in eukaryotes*
10:25-11:00	Coffee break
11:00-11:20	**Jiří Pergner** *Unraveling the molecular mechanisms of plastid transcript 3´end modification in secondary plastids of euglenophytes*
11:20-11:40	**Anežka Konupková** *Green or white: Knock-in of HA-tags in plastid protein Derl-1 results in an unstable bleached phenotype of Euglena gracilis*
11:40-12:00	**Ryo Harada** *Evolution and switching of mitochondrion-localized DNA polymerases in Euglenozoa*
12:00-13:30	Lunch
	Translation and Commercialisation
13:30-14:10	**Keynote speaker - Masahiro Hayashi** *Heterotrophic high-density cultivation of Euglena gracilis to produce paramylon and its utilization as a raw material for some chemicals*
14:10-14:30	**Ricardo Neto** *Effects of a algae β-1,3-glucan on the incidence of tail biting in a finishing herd in France*
14:30-14:50	**Kengo Suzuki** *Exploring a resource-recycling food production system using Euglena gracilis*
14:50-15:20	Coffee break
15:20-15:40	**Yuichiro Kashiyama** *Genetic transformation of Rapaza viridis and biochemical demonstration of its kleptoplast-targeted proteins*
15:40-16:00	**Ellis O'Neill** *The complex chemistry of the alga Euglena gracilis*
16:00-16:20	Talk contest, Wrap-up
16:30	Departures

Abstracts

Anaerobic Respiration Coupled with Wax Ester Production in *Euglena gracilis*

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Euglena gracilis accumulates a storage polysaccharide called paramylon in aerobic environments. When exposed to anaerobic or hypoxic conditions, paramylon is rapidly degraded to synthesize wax esters (WE), which consist of medium-chain saturated fatty acids and fatty alcohols. In the 1980s, it was hypothesized that the acyl chain of WE is synthesized through a partial reversal of mitochondrial fatty acid β-oxidation, as it does not require ATP or malonyl-CoA. However, a detailed understanding at the molecular level was lacking.

To gain deeper insights, we conducted a reverse genetic analysis using RNA interference (RNAi) based on publicly available transcriptome information. Our investigation revealed that *Euglena* synthesizes lipids without ATP through a complete reversal of fatty acid β-oxidation, while simultaneously producing ATP via anaerobic respiration. The reverse reaction of acyl-CoA dehydrogenase (ACD), which is believed to be thermodynamically challenging in living organisms, is likely facilitated by electron transfer from the respiratory chain via electron transfer flavoprotein using rhodoquinone, as well as electron bifurcation on ACD. These mechanisms bypass thermodynamic barriers and allow for the reduction of *trans*-enoyl-CoA.

Furthermore, in the anaerobic fatty acid synthesis system, the utilization of NADH as the electron donor in complex I of the respiratory chain contradicts the known reliance on NADPH supply by pyruvate:$NADP^+$ oxidoreductase (PNO). Knockdown of PNO resulted in decreased wax ester synthesis and ATP production, highlighting the significance of NADPH supply. We identified transmembrane transhydrogenase (NNT) in the mitochondrial inner membrane as a key element responsible for the conversion between NADPH and NADH. NNT plays a crucial role in wax ester synthesis and ATP production under anaerobic conditions by facilitating the interconversion of redox coenzymes and maintaining a proton concentration gradient in the mitochondrial inner membrane.

Overall, our studies provide valuable insights into the molecular mechanisms underlying Euglena's unique lipid synthesis pathways under anaerobic conditions. These findings shed light on the physiological adaptations of *Euglena* and expand our understanding of lipid metabolism in single-celled organisms.

Spatial proteomic analysis of the non-photosynthetic *Euglena longa*

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Publicly accessible Euglenid genomes and transcriptomes posses thousands of hypothetical predicted proteins, for which no functional annotation or localization data is currently available. We employ LOPIT-DC (Localization of Organelle Proteins via Isotope Tagging through Differential Centrifugation) on secondary osmotroph *Euglena longa* for the purpose of spatially localizing unknown proteins to cellular compartments and assigning putative functions. Our efforts generated several thousand mapped proteins, which we assign to organelle regions using a selection of characterized marker proteins. We comment on the implications of new organelle constituents and shed light on the enigmatic metabolic capabilities and evolutionary adaptions of this former-phototrophic representative.

Automatic annotation of nonconventional introns as a necessary step in the analysis of *Euglena* genomes

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Euglenids exhibit unique genomic characteristics, including nonconventional introns that differ from common spliceosomal introns. These introns lack specific border sequences and have short repeats on the borders making their precise positions challenging to determine during gene annotation. To address this, we developed a statistical machine learning classifier based on genomic and transcriptomic data from three Euglena species.

Using identified key characteristics of nonconventional introns we designed a binary classifier that accurately predicted nonconventional intron positions. The classifier was integrated into a pipeline that identifies the best canonical and nonconventional position for each predicted intron. Our approach offers an effective tool for predicting nonconventional introns with high accuracy (>90%) during genome annotation. We verified predictions experimentally on a set of previously not-researched introns and genes.

Improved prediction of nonconventional introns provides better insights into euglenid genomes and their unique genetic features. Our results contribute to understanding of evolution of nonconventional introns in Euglenids.

Phototaxis regulation in *Euglena* - from dim to bright light

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How a single cellular circuit in *Euglena gracilis* can switch efficiently between positive vs. negative phototaxis under desired vs. undesired stimuli is poorly understood. We investigate multiple general switching mechanisms for microswimmer phototaxis using a biophysical modeling and experiments. We find that a photoresponse inversion mechanism is regulating Euglena phototaxis. Specifically, a light-intensity dependent transition between two flagellar beat states on the sub-second time scale ultimately generates positive phototaxis at low light intensity via a run-and-tumble mechanism vs. negative phototaxis at high light intensity via directed steering. A picture emerges where many complex Euglena behaviors over a large range of light intensities as reported in the literature can be simply explained by the selective switching over time between just two flagellar beating states that are in turn controlled by two light sensors that are responsive to stimulus decrease at low ('step-down') vs. stimulus increase at high ('step-up') light intensities, respectively. These results provide general design principles for microswimmers for simple two-state switching mechanisms to operate under both noisy and saturated stimulus conditions.

Representatives of Euglenozoa and cyanobacteria as models to study calpain enzymes

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Calpains are cysteine proteases activated by calcium ions. This large superfamily of enzymes has been increasingly studied in mammals because of their involvement in human diseases, such as muscular dystrophy, diabetes mellitus, cancer and many others. Despite the attention paid to calpain research, their exact function, origin and evolution still need to be clarified. Although calpains are primarily studied in humans, they are well evolutionary conserved and have been identified in most eukaryotes and some bacteria, but not in Archaea. Our study focuses on identifying calpains in new species of microorganisms, primarily in Euglenozoa and cyanobacteria. We show that homologs of human calpains are present in many protists and some species of cyanobacteria. Moreover, we identified new calpains specific only for Euglenozoa, and we have further studied their structure, catalytic sites, subcellular localization, and evolution. Our findings show a surprisingly high number of calpains (≥20), especially in Leishmania and Euglena species, compared to plants (1 calpain) and even humans (15 calpains). We believe that identifying new calpains and new calpain homologs in species that are less complex and easier to study can shed more light on their function and bring forward their biomedical and even biotechnological applications.

Unraveling meiotic genes in *Euglena gracilis*: Insights into protist evolution

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The evolution of sexual reproduction is intimately intertwined with the emergence of eukaryotes, with protists serving as the earliest actors in this evolutionary narrative. Among the diverse group of protists, euglenoid flagellates have garnered significant interest due to their unique gene expression regulation and evolutionary trajectory. The presence of meiotic genes within an organism's genome is a key prerequisite for sexual reproduction. In this study, we conducted in-silico analyses and initial in-vitro experiments to investigate the presence of essential meiotic genes in *E. gracilis*, a representative euglenoid flagellate. Our findings reveal that 8 out of the 9 essential meiotic genes have been identified in the genome and transcriptome of *E. gracilis*, with only one, SPO11, yet to be identified. Furthermore, phylogenetic analysis validates these preliminary results which holds great promise offering a fertile ground for further in-vitro experiments that may shed light on the origin and evolution of protists. This research contributes to our understanding of the fundamental processes underlying the emergence of sexual reproduction and provides a platform for future investigations into the intriguing world of protist evolution.

Euglenophyceae members are hosts for a new group of Legionellales bacteria

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The endosymbioses between protists and prokaryotes are highly diverse and crucial for understanding events such as eukaryogenesis or the emergence and spread of intracellular pathogens. However, they are significantly understudied, with some protist taxa often evading the attention of researchers. One such taxon is Euglenida, whose associations with intracellular prokaryotes are largely unknown despite themselves being a common element of plankton worldwide.

Here, we present a novel group of endosymbiotic bacteria harboured by four species of different Euglenophyceae. Symbioses were identified by screening metagenomic assemblies of 11 single-cells and 12 cultures from various Euglenids. The newly identified group forms a separate clade within Coxiellaceae (Legionellales) and is closely related to the known amoebal endosymbionts Ovatusbacter and Fiscibacter. Analysis of four obtained draft genomes shows typical reduction for endosymbionts, with 1,4-1,6 Mbp genome size and strongly reduced biosynthetic capabilities. Curiously, the genomes analysed show signs of inability to produce energy on their own, probably relying on the host's energy pool.

Our results show the first case of Legionellales endosymbionts associated with Euglenophyceae and corroborate the statement that Euglenids' endosymbioses are more diverse than initially thought. Additionally, since the discussed Euglenophyceae are available in culture, they are valuable for further studies.

Euglenoids‘ distribution: from local to global scale

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Photosynthetic euglenoids are a cosmopolitan group of protists, representatives of which can be found in almost all types of biotopes, including extreme ones (e.g. extremely acidic biotopes, hot volcanic mud). However, what about their distribution on local scales – in any case, we are able to observe their temporal and spatial distribution within localities – can this be reflected in our knowledge of the rarity of individual species? What vectors can play role in the spread of these microorganisms? We know that in some protist groups we observe a ubiquitous cosmopolitan distribution, but at the same time, there are examples of microorganisms with the limited global distribution – it is well-documented in chrysophytes, diatoms, ciliates or testate amoebae. If there are cosmopolitan species among the euglenoids – will it be e.g. *Phacus longicauda* or *Trachelomonas volvocina*, e.g.? Are there endemic species or species of limited distribution or is their potential existence rather a reflection of our lack of knowledge about the distribution of these algae? The aim of this keynote lecture is to summarize the current knowledge in this area based on case studies and own data, as well as to highlight some possible questions for future research on the biogeography of photosynthetic euglenoids.

When biology walks into a bar – or metabarcoding studies of Euglenophyceae loricate taxa, *Strombomonas* and *Trachelomonas*

Alicja Fells[1], Katarzyna Jankowska[1], Maja Łukomska-Kowalczyk[1], Rafał Milanowski[1], Bożena Zakryś[1]

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Though *Euglena gracilis* is a model organism, a lot is yet to be known about Euglenophycean biology and ecology. Molecular data-based taxonomy and the recent utilization of metabarcoding as well as manually-curated databases vastly aid studies on the distribution of particular taxa. NGS data obtained from a three-year environmental sampling study were analyzed using a curated sequence database of Euglenaceae loricate taxa – *Strombomonas* and *Trachelomonas*. Sampling was carried out across three regions in Poland (vicinity of the capital, Warsaw; Masuria Lakes; Northern Poland – vicinity of the Baltic Sea) from freshwater bodies (i.e. ponds). The study aims to analyze species distribution and find potential factors (e.g. season) that influence the appearance of particular loricate taxa.

Dependence of diversity of colorless euglenoids on environmental factors

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In this contribution, the results of a long-term project devoted to the ecology of colorless euglenoids will be presented – it aims to explain autoecology of freshwater heterotrophic euglenoids. Microbial diversity at 18 diverse localities was studied periodically with a special focus on heterotrophic euglenoids. Environmental parameters were measured (including chemical-physical environmental parameters, geographical and terrain parameters) together with the diversity and abundance of other protists and cyanobacteria. Of 1204 microbial taxa found, 155 were heterotrophic euglenoids. A differentiation of ecological preferences (e.g. pH, type of the biotope) of osmotrophic and phagotrophic euglenoids was observed. The major total diversity and abundance of heterotrophic euglenoids were found in biotopes with highly developed macrovegetation with pH between 7-7,5, dominated by phagotrophic species. The major diversity of osmotrophic species was found in Sphagnum-dominated biotopes with pH lower than 6. The diversity of heterotrophic euglenoids rapidly fell in pH higher than 8,5. During the survey, some rare species were found, for these species, detailed literature research was made, and all previously and currently known ecological data were summarized.

Exploring a resource-recycling food production system using *Euglena gracilis*

Chihana Toyokawa[1], Koji Yamada[1], Yoshihiro Naka[1], Toshihisa Nomura[2], Keiichi Mochida[2,3], Kengo Suzuki[1,2]

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A sustainable and stable food production system is indispensable for a long-term human residence on the Moon and Mars. To develop such system, microalgae are expected to be suitable as an organism for resource-recycling food production. Microalgae are known to proliferate by assimilating various types of nitrogen and phosphorus compounds contained in human waste and produce nutrients including protein and vitamins. Additionally, microalgae contribute to air regeneration by converting carbon dioxide generated by human respiration into oxygen through photosynthesis. Furthermore, a high biomass productivity per unit volume is expected by the flexibility of the size and form of photobioreactors. In this research, we searched microalgal species that are appropriate for the system. We examined multiple species of microalgae for their ability to proliferate using human urine or artificial urine as a primal nutrient source. Euglena gracilis is a species that has been identified as suitable for cultivation using urine. Although it does not assimilate urea, it showed fast proliferation in the urine-based medium. Additionally, we will also discuss the breeding of *E. gracilis* using genome-editing to optimize it as a strain for resource-recycling.

Assessing the roles of microbial partners in cadmium tolerance of an *Euglena mutabilis* co-culture

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Microorganisms with metal binding capability are under investigation as tools for sustainable wastewater remediation, including pollutants from mine tailing ponds. A co-culture of acid resistant, heavy metal-accumulating *Euglena mutabilis* (CPCC 657) isolated from a Canadian gold mine tailing pond was investigated for its cadmium resistance and the influence of antibiotic treatments. Unidentified fungi and bacteria were detected in the *E. mutabilis* culture, exhibiting a near-mutualistic relationship, and it was hypothesized that these partner organisms help confer the heavy metal tolerance of the co-culture. Forceful isolation of *E. mutabilis* through chemical and mechanical means proved unsuccessful as it was unable to survive separation from the partners. Manipulations of the growth conditions was used to visually examine the interactions between the organisms, which further suggested interconnectivity and dependency between partners. Since *Euglena* sp. have a demonstrated tolerance to conventional antimicrobial compounds; the isolation of the *E. mutabilis* from the co-culture was attempted using a selection of antibiotics and antifungals. Cadmium tolerance of the co-culture was assessed with and without these treatments. Updates of this investigation will be presented.

Bacteria and fungi can supply *Euglena gracilis* with vitamins B1 and B12

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Euglena gracilis can be utilized to produce biotechnologically interesting products such as feed additives, biofuels and pharmaceuticals. Since it cannot synthesize thiamine (vitamin B1) and cobalamin (vitamin B12), these vitamins must be added to media for the growth of axenic cultures. However, these vitamins are expensive and therefore, the cultivation of *E. gracilis* in big containers for biotechnological applications is limited. On the other hand, many bacteria, plants and fungi can synthesize thiamine, and some bacterial and archaeal species can synthesize cobalamin. We have shown that no addition of vitamins is required when *E. gracilis* is grown in the co-culture with bacteria Pseudobacillus badius and Lysinibacillus boronitolerans, and with the micromycete Cladosporium westerdijkiae. These results suggest that these microorganisms can produce sufficient amounts of these vitamins essential for *E. gracilis* growth. We propose that using such mixed cultures for biotechnological applications can help to lower the cost of cultivation media for euglenoid flagellates grown in big containers. Many *E. gracilis* cells in the co-culture are attached to the fungal hyphae by flagella. Such bioflocculation offers the possibility for effective harvesting of *E. gracilis* biomass.

Blooms of Euglena sanguinea in South Africa

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Blooms of *Euglena sanguinea* Ehrenberg were observed at two different locations in South Africa (Kruger National Park, Mpumalanga Province and Limpopo Province). Besides being responsible for a blood-red discolouration of the water, the species is also toxic, causing fish kills. Samples from South Africa were dominated by *E. sanguinea* and very low concentrations and diversity of other algal species were present. Microscopic investigations revealed that the size ranges and the morphology of the cells corresponded to descriptions of the species in literature. High quality micrographs, illustrating the general cell structure will be provided. Intensive literature searches allowed mapping of the worldwide geographical distribution of the species, which revealed that it was sparsely distributed throughout Africa as it was reported from only seven countries. Only one previous record of its presence in experimental ponds in South Africa exists, rendering this study a second record for its presence in the country.

Euglenid diversity and evolution; revealing the phagotrophic majority

Alastair G.B. Simpson[1], Gordon Lax[2], Won Je Lee[3]

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Almost all research on euglenids examines phototrophic forms. Yet, most of the phylogenetic diversity of euglenids consists of phagotrophs that diverged from the phototroph lineage before the endosymbiotic acquisition of plastids. They share other characteristic features, however, and are key to understanding how phototrophic euglenids evolved. Furthermore, phagotrophs are ecologically important in benthos, and exhibit their own fascinating biology (e.g. exceptionally rapid cell gliding). Historically, the diversity of phagotrophic euglenids species was represented by a small number of genera distinguished by facile morphological criteria, and very few cultures were available. Recently, the number and breadth of cultured species has been greatly improved, and single-cell molecular approaches have been applied broadly. This promises, for the first time, a natural classification of phagotroph diversity, meaningful phylogenies for euglenids, and the means to test hypotheses for the evolution of euglenid cells and genomes. Ideas that early euglenids had inflexible pellicles with few strips are strongly supported, with Petalomonadida in particular the best candidate for the deepest euglenid clade. Some phagotrophs, including many petalomonads, are small cells that can be cultivated to reasonable density. These attributes, together with them representing the greater breadth of euglenid diversity, may make them attractive candidates for genomic investigation.

Molecular phylogenetics of the sessile phagotroph *Dolium sedentarium*

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Euglenids are a species-rich group of flagellates with varying modes of nutrition that can be found in diverse habitats. Phagotrophic members of this group gave rise to phototrophs and hold the key to understanding the evolution of euglenids as a whole, including the evolution of complex morphological characters like the euglenid pellicle. To understand the evolution of these characters, a comprehensive sampling of molecular data is needed to correlate morphological and molecular data, and to estimate a basic phylogenetic backbone of the group. Unfortunately, phagotrophic euglenids remain somewhat of a novelty in euglenid research and are still undersampled. While the availability of SSU rDNA and, more recently, multigene data from phagotrophic euglenids has improved, several 'orphan' taxa remain without any molecular data whatsoever. Dolium sedentarium is one such taxon: It is a rarely-observed phagotrophic euglenid that inhabits tropical benthic environments and is one of few known sessile euglenids. Based on morphological characters, it has been thought of as part of the earliest branch of euglenids, the Petalomonadida. We report the first molecular sequencing data for Dolium using single-cell transcriptomics. Both SSU rDNA and multigene phylogenies confirm it as a solitary branch within Petalomonadida.

Convergent evolution of mRNA decapping complexes in eukaryotes

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Eukaryotic mRNA degradation is initiated by removal of the poly(A) tail by the deadenylation complex, followed by 5´-end decapping and 5´-3´ exoribonucleolytic degradation. It is the predominant and essential mRNA decay pathway in most eukaryotes. Recently, we uncovered significant divergence in mRNA degradation mechanisms in Kinetoplastea. The canonical decapping enzyme Dcp2 is absent and instead kinetoplastids rely on a distinct enzyme family, the ApaH-like phosphatase ALPH1, for decapping activity. The 5´-3´ exoribonuclease (Xrn1/XNRA), in contrast, is conserved and interacts with ALPH1. Phylogenetic reconstruction supports loss of Dcp2 and the prototypic Dcp2 complex subunits from Discoba and Metamonada, suggesting the presence of Dcp2 in the last eukaryote common ancestor. However, with the ALPH1 decapping complex appearing to be an innovation occurring early within the Metakinetoplastida, it remains to be investigated how mRNA-decapping is achieved in those organisms that are lacking both DCP2 and ALPH1. Euglenoids fall within this latter category and, with the advent of genetic manipulation in Euglena gracilis, it is now possible to experimentally assess this question. We propose to immunoprecipitate E. gracilis Xrn1 to elucidate the composition of the Euglena decapping complex and further extend our understanding of the diversity of mRNA degradation mechanisms.

Unraveling the molecular mechanisms of plastid transcript 3´end modification in secondary plastids of euglenophytes

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The secondary plastid of euglenophytes exhibits various unorthodox features. Among others, 3´polyadenylation of a small portion of plastid transcripts was documented by Zahonova et al. (2014) in *Euglena gracilis*. We aim on understanding the mechanism behind this type of modification. To this end we identified five proteins putatively involved in 3'-end modifications in *E. gracilis*. All of them are nucleus-encoded and possess a typical plastid-targeting pre-sequence. Specifically, we found: (1) poly(A)-specific ribonuclease (PARN); (2) polynucleotide phosphorylase (PNPase); (3) a homolog of the mitochondrial deadenylase (PDE12); and (4) two different members of the TRF family of ribonucleotidyl transferases (ptTNT1 and ptTNT2). Four of these genes (except for ptTNT2), were also found in *E. longa*. We have knock-outed (KO) all of the above-mentioned genes using CRISPR/Cas9 in E. gracilis. The phenotype of homozygous KO mutants in ptTNT1 or ptTNT2 was macroscopically recognizable, the cultures being yellowish. The phenotype of PNPase mutants was even more pronounced – the culture being whiteish in colour. 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.

Green or white: Knock-in of HA-tags in plastid protein Derl-1 results in an unstable bleached phenotype of *Euglena gracilis*

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The secondary plastid of *Euglena gracilis* is surrounded by three membranes. Hundreds of plastid proteins are nucleus-encoded, necessitating transport machinery for these proteins through the plastid envelope. Of the known components of the TIC/TOC (translocon of inner/outer chloroplast membrane) complexes supposedly responsible for transport across the two innermost membranes, *E. gracilis* possesses only the Tic21 subunit of the TIC complex, while the TOC complex seems altogether lost. Two candidates for a protein-conducting channel across the second membrane were found in the chloroplast proteome of *Euglena gracilis* – Derlin-like pseudoproteases Derl-1 and Derl-2.

We performed RNA interference on *E.gracilis* genes for Derl-1 and Derl-2 and obtained knock-downs with visible yellow phenotype in both cases, which points out the functional importance of these proteins for the chloroplast. Subsequently, we accomplished C-terminus HA-tagging in Derl-1 and obtained two clones – Derl-1-HA-A6 and Derl-1-HA-H5. Derl-1-HA-A6 contained one Derl-1 allele with a correctly inserted 2xHA-tag, in the other allele the C-terminus was modified. In Derl-1-HA-H5, one allele contained a correctly inserted 1xHA tag, and the other allele remained wild-type. Derl-1-HA-A6 exhibits transient bleached phenotype and growth retardation after inoculation into Cramer-Myers media. Derl-1-HA-H5 shows no differences from the wild type, neither in colour nor in growth rate.

Evolution and switching of mitochondrion-localized DNA polymerases in Euglenozoa

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The replication of mitochondrial (mt) genomes requires DNA polymerases, together 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 found that Discoba species except Euglenozoa have a novel type of mt-localized DNA polymerase that showed an evident phylogenetic affinity to alpha-proteobacterial PolI. Thus, we propose that this mt-localized DNA polymerase is the direct descendent of the PolI of the alpha-proteobacterial endosymbiont taken up by the last common ancestor of eukaryotes (LECA). Considering the finding of the novel mt-localized DNA polymerase, we will overview and discuss the evolution of mt-localized DNA polymerases during the divergence of euglenozoans.

Heterotrophic high-density cultivation of *Euglena gracilis* to produce paramylon and its utilization as a raw material for some chemicals

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For industrial production of paramylon, β-1,3-glucan accumulated in *Euglena* cells, fed-batch cultivation of Euglena gracilis was heterotrophically investigated. The optimization of culture conditions and feeding of nutrients gave the biomass and paramylon yields 108.9 g/L and 95.0g/L, respectively. Then paramylon content in the cells was reached at 87.2%.

For utilization of paramylon as bio-based plastics, it was shown that paramylon was efficiently thermoplasticized by adding acyl groups that differ in alkyl chain length. Glass transition temperature of mixed paramylon esters was higher than those of plant-based polylactic acid and petroleum-based acrylonitrile-butadiene-styrene (ABS) resin. Their thermoplasticity was equivalent to or higher than those of these reference plastics. It was shown that paramylon was thus a potential component of thermoplastic materials.

And a polysaccharide nanofiber made from paramylon fabricated. Preparation of this nanofiber primarily hinged on the bottom-up approach. First, paramylon presented in a particle with high degree of crystallization, was fibrillated to a randomly coiled polymer by dissolving the particle in a 1.0-mol/L NaOH aqueous solution. Second, the randomly coiled polymer was self-assembled into a triplex as the NaOH solution diluted. Third, a 20-nm-width nanofiber made from the triplex emerged in the solution when the NaOH solution more diluted.

Effects of a algae β-1,3-glucan on the incidence of tail biting in a finishing herd in France

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The objective of this study was to assess the impact of a immunomodulator on tail lesions. Pigs with an average age of 75 days and 30.5 kg body weight (n= 628) were allocated to one of two diets during 70 days. A control group (C) (n=316) received a commercial diet and group BG (BG) (n=312) received a diet supplemented with β-1,3-glucan (BG) (AletaÔ) (200 g/ton). Initial and final average pen body weights (BW) and pig mortalities were measured lesion assessments were carried approximately every 2 weeks. Data were analysed with proprietary statistical software using Khi-square test.

The average percentage of tail biting lesions in group C was higher than in BG for the whole duration of the trial (16.1 and 12.9 % respectively, p<0,005) and for the 3rd (15.4 and 8.33%) time point. No differences were observed on the BW or mortality, 93.0 and 94.8 kg and 0.6 and 1.3 % for the BG and C groups respectively.

Immunomodulation with an algal β-1,3-glucan can support the health of the animals through modulation of inflammatory processes that may lead to tail biting. In the conditions of this study, β-1,3-glucan resulted in a lower percentage of pigs suffering from tail biting lesions.

Potential of *Euglena gracilis* as a fermentation aid in the food field

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Euglena gracilis, is a microalga which is cultured and used as a food ingredient due to its high nutritional value. The food products using *E. gracilis* has been sold primarily in JAPAN, while it recently began to be distributed and sold worldwide. In addition to food products, a medium additive is under development using the components derived from *E. gracilis* which are known to increase the proliferation and activity of lactic acid bacteria. In this study, we investigated the effect of *E. gracilis* components on the fermentation process of tempeh, a traditional Indonesian fermented food. We analyzed the alteration of nutrient component, enzymatic activity, and the flavor of tempeh, by adding dried E. gracilis powder. The results of the component analysis showed an increase of ergothioneine, a rare and useful antioxidative aminoacid. Enzymatic activity analysis showed an increase in the activity of acidic proteases. In addition, the results of sensory tests suggested that the unique flavor of soybeans was reduced and, thus, the eating taste was improved.

Genetic transformation of *Rapaza viridis* and biochemical demonstration of its kleptoplast-targeted proteins

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Rapaza viridis distinguishes itself among kleptoplast-bearing organisms by possessing a diverse repertoire of chloroplast protein genes in its nuclear genome, obtained through horizontal gene transfer from various phototrophs. Our study aimed to biochemically validate, with the assistance of genome editing, the transport and expression of the translation products of these genes within the kleptoplasts acquired from the green alga *Tetraselmis* sp. Firstly, we selected RvRCA and RvRbcS from the group of proteins predicted to be transported into kleptoplasts, as they exhibited notably high expression levels according to quantitative transcriptome data. We confirmed their specific expression patterns by generating highly specific peptide antibodies and employing immunofluorescent microscopy to determine their intracellular localization within the kleptoplasts. Using CRISPR/Cas9 genome editing, we successfully knocked out each gene and confirmed the consequent disappearance of fluorescence. Additionally, we introduced epitope tags to these proteins and conducted observations using tag-specific antibodies. Remarkably, the RvRbcS knockout mutants demonstrated a significant reduction in chloroplast photosynthetic activity. Furthermore, we verified that the fusion of a luminescent promoter downstream of a peptide sequence upstream of the functional domain of RvRCA (presumed transport sequence) resulted in luminescence specifically located within the kleptoplasts when expressed in *R. viridis*.

The complex chemistry of the alga *Euglena gracilis*

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The metabolism of *Euglena* is extremely complicated using some unusual pathways for the synthesis of core metabolites, including with unique protein architectures. *Euglena* can also grow on a range of carbon sources, rerouting the metabolism to take advantage of different reductant levels to maintain growth. Uniquely, Euglena can lose its chloroplast and still survive, thanks to dual localisation of some pathways.

Whilst the unique carbohydrate store in *Euglena*, paramylon, has long been known, we have recently described complex surface glycans and simple N-glycans. *Euglena* can also make complex secondary metabolites, including the ichthyotoxic Euglenophycin and the newly discovered lipopeptide Euglenatide, which shows anticancer activity. For a supposedly simple organism, *Euglena* has the ability to do some complex chemistry.