Meeting of the EMBO Young Investigator Network on ecology and evolutionary biology of microbes

Book of Abstracts

5–7 October 2023

Chęciny, Poland

Organizers

Anna Karnkowska (University of Warsaw)

Rafał Mostowy (Jagiellonian University)

Kasia Piwosz (National Marine Fisheries Research Institute)

Program

Thursday, 5th of October

12:00–12:30 Registration and check-in

12:30–13:45 Lunch

13:45–14:00 Introduction from Anna Karnkowska

14:00–14:15 Introduction from Rafał Mostowy

14:15–14:45 Kasia Piwosz

Microbes in the water: who is there and what do they do?

14:45–15:20 **Invited Speaker: Tomasz Kościółek**

Modeling microbiome function and temporal dynamics

15:20–15:50 Coffee break

15:50–16:25 **Invited Speaker: Jelena Godrijan**

Unlocking the Mysteries of Coccolithophores: Exploring Their Haplo Diplontic Life Cycle and Ecological Significance

16:25–16:40 Marta Sałek

Abundance and diversity of protistan-prokaryotic interactions in freshwater lakes of the Masurian Lakeland based on single cell microbiome study

16:40–16:55 Anhelina Kyrychenko

Viruses of freshwater microbial eukaryotes

16:55–17:10 Valentina Smacchia

Single-cell transcriptomics: Establishing SPLiTseq method for protist communities

17:10–19:00 Visit to the Chęciny Royal Castle / Free time

19:00– Dinner at the restaurant Zajazd pod Srebrną Górą

Friday, 6th of October

8:30–9:30 Breakfast

9:30–10:05 **Invited Speaker: Gytis Dudas**

Towards genomic epidemiology of arthropod viruses

10:05–10:20 Vyshakh Rajachandra Panicker

Predicting Phage Depolymerases and Their Capsular Specificities Using AlphaFold 2 Structural Modeling

10:20–10:35 Wanangwa Ndovie

Extensive recent horizontal gene transfer events among distantly related phages

10:35–10:50 Janusz Koszucki

Using genome-wide associations studies to identify putative genetic determinants of phage host-range in Klebsiella

10:50–11:20 Coffee break

11:20–11:55 **Invited speaker: Bartek Wacław**

Biofilm growth and evolution - how can physics contribute?

11:55–12:10 Iván García-Cunchillos

Metabolic integration of kleptoplasts in their transition to organelles

12:10–12:25 Bogna Smug

How phages play with LEGO: studying protein-level mosaicism in phages and its evolutionary implications

12:25–12:50 Jade Leconte

Exploring genetic diversity and phage-host dynamics in the Klebsiella genus

13:00–14:00 Lunch

14:30–15:05 **Invited Speaker: Piotr Łukasik**

The evolutionary processes in insect-microbe interactions

15:05–15:20 Małgorzata Chwalińska

The usage of long, nanopore, high-quality operational taxonomic units (OTUs) to improve the taxonomic annotation of freshwater ciliates

15:20–15:35 Metody Hollender

Protist single cells picked from environmental samples allow to uncover new bacterial endosymbionts

15:35–15:50 Michał Karlicki

What after the binning? Ecogenomics of plastid genomes reconstructed from freshwater metagenomes

15:50–16:00 Closing

16:00–16:30 Coffee break

16:30–19:00 Social program / Free time

19:00–end Bonfire

Saturday, 7th of October

8:30–9:30 Breakfast

9:30–11:00 Check out

Abstracts

Microbes in the water: who is there and what do they do?

Kasia Piwosz[1], Jared Vincent Lacaran[1], Sohrab Khan[1], Uroosa[1], Cristian Villena-Alemany[2]

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Microorganisms play a key role in the environment. In my group (SepulkoLab), we focus on the effect of natural and anthropogenic changes on diversity, community composition, function and interactions of two large groups of aquatic microorganisms: bacteria and protists. I will present the current insight from my ongoing projects on 1) the food web structure of pelagic microbial food webs, 2) the effect of microplastic on microbial communities, 3) the importance of photoheterotrophic bacteria in the carbon flow and recurrent events in freshwater lakes. Our results from experiments in microcosms and observation of ecological processes in natural environments (Baltic Sea and freshwater lakes in Czechia) allowed us to refine the current models of microbial food web, and also of the phytoplankton ecology group (PEG) model, which has profound consequences for understanding of the carbon flow through the system.

Modeling microbiome function and temporal dynamics

Tomasz Kościółek[1]

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The microbiome, especially the human gut microbiome, is a diverse and dynamic ecosystem of bacteria, archaea and other microorganisms important to health. In my research I develop methods to help us better understand the microbiome on a functional level and its dynamics - how does the microbiome composition change over time. I will present my group advancements on those two fronts. First, how we use protein 3D structure information and deep learning to better functionally annotate the microbiome. Second, how we analyze the temporal dynamics of the microbiome and are able to predict its composition in the future.

Unlocking the Mysteries of Coccolithophores: Exploring Their Haplo-Diplontic Life Cycle and Ecological Significance

Jelena Gordijan[1]

[1] Ruđer Bošković Institute, Zagreb, Croatia

Coccolithophores, unicellular marine algae, exert a profound influence on seawater chemistry, driving the ocean's carbon cycle and Earth's climate through photosynthesis and calcification. An understudied aspect of their biology is their unique haplo-diplontic life cycle, wherein diploid and haploid phases independently reproduce via mitosis. These phases exhibit differences in morphology, carbon content, and ecological roles, with the haploid phase remaining poorly understood. My research focuses on elucidating the triggers governing phase transitions and looks into the ecology, physiology, and biogeography of coccolithophore life cycle phases. By investigating this neglected aspect of coccolithophore biology, I plan to expand our comprehension of their global success and their pivotal role in shaping marine ecosystems and the Earth's climate.

Abundance and diversity of protistan-prokaryotic interactions in freshwater lakes of the Masurian Lakeland based on single cell microbiome study

Marta Sałek[1], Małgorzata Chwalińska[1], Metody Hollender[1], Paweł Hałakuc[1], Michał Karlicki[1], Valentina Smacchia[1], Anna Karnkowska[1]

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The abundance, partners, and nature of protist-bacteria interactions remain largely understudied. To address this problem, we designed a study to identify interactions based on a single-cell microbiome of isolated protists from a set of lakes. We studied 150 single cells of protists, hand-picked from the samples collected from four lakes in the Masuria district (Poland). DNA from single cells was isolated, and the whole genome amplification was performed. From the studied cells, 12 sets of 3 cells per morphotype in total were selected (Dinoflagellates, Ciliates and Chrysophytes). We amplified 16S rDNA to identify symbionts: in two studied lakes the data showed dominance of Acinetobacter sp. in all of studied cells, indicating contamination during the workflow. In other two lakes we revealed presence of Rickettsiales bacteria (obligatory endosymbionts) in Dinoflagellate Ceratium hirudinella cells, suggesting first described case of bacterial endosymbionts present in this group (confirmed with data from metagenomic sequencing).

Viruses of freshwater microbial eukaryotes

Anhelina Kyrychenko[1], Anna Karnkowska[1]

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Viruses are Earth's most abundant biological entities, infecting nearly all life forms. While extensive research has focused on viruses affecting humans, animals, and plants, less is known about those infecting microbial eukaryotes such as protists. The project's overarching goal is to understand how viruses influence the freshwater protist communities and to verify their presence in selected groups of protists experimentally. To achieve this goal, we are planning to analyze the metagenomes and single-cell genomes of protists and identify a viral genetic footprint; to isolate protist viruses from freshwater environmental samples; to establish cultures of protists, with a focus on euglenozoans; to sequence candidate cultures with a confirmed viral infection and analyze the viral genomes. The resulting data will contribute to the identification and study of viruses infecting protists, including detailed analyses of infection cycles, host-virus interactions, and the evolutionary history of protists and viruses.

Single-cell transcriptomics: Establishing SPLiTseq method for protist communities

Valentina Smacchia[1], Małgorzata Chwalińska[1], Marta Sałek[1], Jordi Solana[2], Anna Karnkowska[1]

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Despite the significance of protists in various environments, their 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 cells. 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. We designed a mock community of 5 known freshwater protists species, representing diverse groups, differing in their size, cell types, and abundance in the natural environments.

Towards genomic epidemiology of arthropod viruses

Gytis Dudas[1], Joshua Batson[1]

[1] Vilnius University Life Sciences Center, Vilnius, Lituania

Metagenomic virus discovery studies over the last decade have largely overhauled our understanding of RNA virus diversity. At the same time, closely related viruses are increasingly encountered in particular host groups opening the door to phylogenetic analyses previously restricted to viruses of economically important species. I'll introduce one such mosquito orthomyxovirus which as a research system possesses novel features and exhibits signs of being able to infect vertebrates.

Predicting Phage Depolymerases and Their Capsular Specificities Using AlphaFold 2 Structural Modeling

Vyshakh Rajachandra Panicker[1,2], Janusz Koszucki[1,2], Paweł Szczerbiak[1], Aleksandra Otwinowska[3], Sebastian Olejniczak[3], Zuzanna Drulis-Kawa[3], Rafał Mostowy[1]

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[3] Department of Pathogen Biology and Immunology, University of Wrocław, Wrocław, Poland

Phage depolymerases that target Klebsiella pneumoniae capsules are highly specific. However, computational approaches to predict depolymerase specificity are largely limited. Here, we developed a novel method to identify depolymerases targeting Klebsiella pneumoniae capsules and predict their specificities using AlphaFold 2 (AF2) structural modeling. To this end, we curated two datasets: first, with depolymerases whose specificity validated via recombinant production and second, with specificity known at the genome and not at the depolymerase level. Using annotations and AF2, we pinpointed 74 promising depolymerases from 54 phage genomes. As a case study, we focused on K1 capsule type and found 3 confirmed K1-specific depolymerases in the first dataset and 5 potential ones in the second dataset. Structural analysis revealed that 4 of the latter depolymerases closely resembled the K1-specific ones. The fifth depolymerase appeared different but showed structural similarity to K3/K21-specific depolymerases, suggesting a potential specificity for these K-types.

Extensive recent horizontal gene transfer events among distantly related phages

Wanangwa Ndovie[1], Rafal Mostowy[1]

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Bacteriophages are under constant selective pressure to infect their hosts, and horizontal gene transfer (HGT) is a major evolutionary force that permits their rapid adaptation to new circumstances. Here we analyzed ~22000 phages to better understand the evolutionary dynamics of HGT in phages. We employed a novel approach called MANIAC (MMseqs Average Nucleotide Identity Calculator) on complete phage genomes to systematically identify potential pairs involved in HGT among distantly related phages. Our analysis found multiple regions of high similarity between distantly related phages, indicating frequent exchange of genes among distantly related phages. Our analysis also revealed high frequency of HGT events among temperate phages. Moreover, our analysis uncovered genes with diverse functions involved in HGT between bacteriophages demonstrating the potential for the transfer of crucial genetic elements across phylogenetically distant phages.

Using genome-wide associations studies to identify putative genetic determinants of phage host-range in *Klebsiella*

Janusz Koszucki[1,2], Vyshakh R. Panicker[1,2], Bogna Smug[1], Aleksandra Otwinowska[3], Sebastian Olejniczak[3], Louise Judd[4], Kathryn E. Holt[4], Edward J. Feil[5], Zuzanna Drulis-Kawa[3], Eduardo P. C. Rocha[6], Rafal J. Mostowy[1]

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Phages infecting *Klebsiella* pneumoniae rely on the initial binding between bacterial surface polysaccharides, like the capsule, and viral receptor-binding proteins (RBPs) like tail fibers or spikes. This highly specific interaction is a major determinant of phage host range. However, due to the immense diversity of capsular polysaccharides (K-types) and *Klebsiella*-targeting phages, a limited number of capsule-specific phage proteins have been characterized, hindering our understanding of phage host range in *Klebsiella*. To address this, we leveraged a diverse collection of 3,911 Klebsiella isolates. Utilizing genome-wide association studies, we pinpointed protein clusters in prophage regions linked to prevalent K-types, validating them by functional annotation and AlphaFold2 predictions. Identified proteins form three major groups: proteins with (1) high or (2) distant structural similarity to known *Klebsiella* phage RBPs, and (3) RBPs showing distant structural similarity to *E. coli* phage RBPs with different mechanism of receptor recognition.

Biofilm growth and evolution - how can physics contribute?

Bartek Wacław[1]

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Microbes in biofilms interact with each other and the environment in many ways, including mechanical repulsion, adhesion, and friction. In the last 10 years, these physics-like interactions have been shown to be as important for biofilm growth and evolution as biochemical interactions. In this talk, I will discuss how mechanical interactions affect the establishment probability of new genetic variants, and how the physics of a growing biofilm can be used against it to reduce the chance that an undesired variant, e.g., an antibiotic-resistant mutant, spreads in the biofilm.

Metabolic integration of kleptoplasts in their transition to organelles

Iván García-Cunchillos[1], Anna Karnkowska[1]

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Plastids in euglenids are well known to have originated in a single endosymbiotic event involving a green alga. The analysis of the plastid proteome revealed numerous metabolic novelties. Recently, the discovery of *Rapaza viridis*, showing strict kleptoplasty on a different alga, suggested an ongoing new plastid acquisition. Genomic and behavioral data demonstrated that *R*. *viridis* temporarily controls the kleptoplast and the exchange of molecules. However, we lack an understanding of the kleptoplasts integration in the *R*. *viridis* metabolism. In this project, we reconstruct the metabolic pathways in *R*. *viridis* from transcriptomic data. Besides, we integrate these results into a broader scenario, including other photosynthetic and secondarily osmotrophic euglenids.

How phages play with LEGO: studying protein-level mosaicism in phages and its evolutionary implications

Bogna J. Smug[1], Krzysztof Szczepaniak[1], Stanisław Dunin-Horkawicz[2,3], Eduardo P.C. Rocha[4], Rafał J. Mostowy[1]

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Phages exhibit remarkable genetic modularity, which allows their genomes to evolve independently and combine, resulting in astounding diversity. While genome modularity in phage populations has been studied, less is known about within-protein modularity and its impact on viral evolution. To fill this knowledge gap, here we quantified such modularity by detecting instances of domain mosaicism, defined as a homologous fragment between two otherwise unrelated proteins. We used highly sensitive homology detection to quantify protein mosaicism between pairs of 133,574 representative phage proteins and we found that domain mosaicism is ubiquitous in phage genomes, particularly in receptor-binding proteins, endolysins, and DNA polymerases. To further investigate protein mosaicism in phages, we created a database of evolutionary conserved fragments (ECFs) of phage proteins. This database contains fragments not covered by known domain databases such as PFAM or ECOD, enabling better examination of the structural architecture and mosaicism of diverse phage proteins.

Exploring genetic diversity and phage-host dynamics in the *Klebsiella* genus

Jade Leconte[1]

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The *Klebsiella* genus, renowned for its genetic and ecological diversity, thrives across various environments. Notably, the variability in bacterial surface sugars serves as an obstacle to phage infection. To overcome this challenge, phages have evolved specialized enzymes within receptor-binding proteins (RBPs) with high sugar specificity. Using a comprehensive collection of nearly 4,000 *Klebsiella* genomes, encompassing 15 species from diverse environmental niches, we conducted prophage detection and achieved high-quality functional annotation. This extensive dataset allows us to investigate variations in viral genomes and their associations with ecological and genetic diversity of *Klebsiella*. In my project, I am planning to quantify the distribution of the prophages populations across the genetic and ecological backgrounds of bacteria in which they are found, and compare it to the diversity of bacterial surface polysaccharides.

The evolutionary processes in insect-microbe interactions

Piotr Łukasik[1]

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Symbiotic microorganisms have played and continue to play a massive role in insect biology. On longer time scales, symbioses have enabled the evolution and diversification of taxa specialized on unbalanced foods such as plant sap. In the short term, they may provide dynamic protection against natural enemies and environmental stressors and facilitate adaptation to rapidly changing environmental conditions. At the same time, close long-term associations with insects have strongly affected the genomic evolution of microbes. During my presentation, I will briefly explain the functional diversity of insect-associated bacteria and some of the general evolutionary patterns observed. I will then outline two of my lab's study directions: (1) the evolutionary patterns related to nutritional heritable endosymbioses of hoppers (Hemiptera: Auchenorrhyncha), and the extreme genomic evolutionary patterns we observe; and (2) the dynamics of facultative endosymbiont infections across multi-species insect communities, and their likely roles in insect adaptation to environmental challenges.

The usage of long, nanopore, high-quality operational taxonomic units (OTUs) to improve the taxonomic annotation of freshwater ciliates

Małgorzata Chwalińska[1], Michał Karlicki[1], Valentina Smacchia[1], Anna Karnkowska[1]

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Recently metabarcoding data became our main source of knowledge about the diversity of protists. The usage of long reads can improve diversity assessment and expand our knowledge about understudied freshwater habitats. My goal was to improve the taxonomic annotation of freshwater ciliates using long, nanopore, high-quality operational taxonomic units (OTUs) and a reference tree. I analysed the samples from the December of 2022 from three ponds. The V4 fragment of the 18S rRNA gene was amplified and sequenced. As a reference tree, I used the Eukref tree of Ciliophora. I recalculated the tree with 680 high-quality whole 18S rDNA OTUs from twelve Masurian lakes, which were obtained using nanopore technology and a custom bioinformatic pipeline. Next using EPA-ng I added 158 V4 ASVs. ASVs were located in all the main groups of ciliates often together with nanopore OTUs, which shows the significant role of those OTUs in improving taxonomic annotation.

Protist single cells picked from environmental samples allow to uncover new bacterial endosymbionts

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Interactions of protists with intracellular bacteria are gaining an increasing attention, as they seem to contain vast diversity of taxa and symbioses function. Despite recent advances, identification of novel endosymbionts is still a significant challenge, as it relies on either culturing of the host or using samples rich in target host's cells. Here we present the results of genomic screening of single cells manually picked from environmental samples without enrichment or prior knowledge about interaction. Utilising the previously proposed approach, we discover a number of endosymbiotic bacteria in various protistan hosts. Curiously, we identified both known intracellular lineages in novel hosts and novel Alpha- and Gammaproteobacteria endosymbionts, including representatives of new family-level lineages. Together with obtained genomic data, this shows valuable opportunities for making the wide diversity of endosymbiotic interactions in environment more accessible to deeper inquiry.

What after the binning? Ecogenomics of plastid genomes reconstructed from freshwater metagenomes

Michał Karlicki[1], Anna Karnkowska[1]

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Ecogenomics is a term describing usage genomics of techniques to answer ecological questions about biogeography, phylogenetics and diversity of microbes. One of the most interesting applications of ecogenomics can be study plastid genomes - genetic elements crucial for performing photosynthesis in microbial eukaryotes. Here, we present preliminary results of utilizing plastid genomes obtained from metagenomes (ptMAGs) to ecogenomics. In the process of manual binning, we obtained so far around 120 ptMAGs that, we consider as a fully complete, representing all major lineages of photosynthetic microbial eukaryotes in freshwaters. Furthermore, we studied potential of ptMAGs for abundance or phylogenomic analyses. Next, we speculate that ptMAGs not only can be a good material for study phytoplankton on the level of a single lineage but due to the amount of data, we may deconvolute single genome (genomospecies) into strains and look at their genetic microdiversity within the population across time and space.

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