

**Charles University, Faculty of Science
Univerzita Karlova, Přírodovědecká fakulta**

**Department of Zoology
Katedra zoologie**

**Ph.D. study program: Zoology
Doktorský studijní program: Zoologie**

Summary of the Ph.D. Thesis



**Anaerobic ciliates as a model group for studying the biodiversity
and symbioses in anoxic environments**

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Thesis supervisor
prof. RNDr. Ivan Čepička, Ph.D.

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ABSTRACT

Ciliates are also of the most extensively studied and diverse groups of unicellular eukaryotes, and yet, their anaerobic representatives have been largely neglected; in part due to culturing difficulties. Although all main ciliate lineages contain anaerobes, their diversity and evolution of anaerobiosis are especially poorly understood and just starting to gain attention. In fact, Ciliophora is an excellent model group to study adaptations to life in anoxia, since it, apart from the aerobic majority, includes free-living and endobiotic obligately anaerobic lineages, facultative anaerobes, microaerophiles, and microaerotolerant species. The diversity of Metopida, the free-living order of obligately anaerobic class Armophorea, has been partially revised and significantly broadened during the past years, including numerous redescription using modern methods, as well as the description of novel families Tropicdoatractidae and Apometopidae, several genera, and multiple species. Oxygen plays a crucial role in ATP production via oxidative phosphorylation that takes place in the mitochondrion in most known eukaryotes. Nevertheless, anaerobic ciliates, among many other eukaryotes that have adapted to low oxygen concentrations or even its absence, have modified their mitochondria and energetic metabolism to be able to produce energy in anoxia. Discovery of two new ciliate classes within SAL group, Muranotrichea and Parablepharisma, which form the APM clade of obligate anaerobes together with Armophorea, has allowed a complex comparative analysis of adaptations and provided insight into the evolution of transition to obligate anaerobiosis in eukaryotes. All members of APM clade host various prokaryotic symbionts, such as endosymbiotic methanogens, which represent the only known intracellular archaea. The genomic analyses of symbiotic archaea have revealed that not all endosymbionts must have undergone a significant reduction, providing an insight into the nature and evolution of symbiotic relationships of prokaryotes and anaerobic ciliates.

Keywords: anaerobiosis, anaerobic ciliates, phylogenomics, MROs, metabolism, symbioses

ABSTRAKT

Nálevníci patří k nejrozsáhlejším a nejrozmanitějším skupinám jednobuněčných eukaryot, avšak jejich anaerobní zástupci byli do značné míry zanedbáváni; částečně kvůli kultivačním obtížím. Přestože všechny hlavní linie nálevníků obsahují anaeroby, jejich diverzita a evoluce anaerobiózy jsou zvláště málo studovány a teprve začínají získávat pozornost. Ve skutečnosti je kmen *Ciliophora* vynikající modelovou skupinou pro studium adaptací na život v anoxii, protože kromě aerobní většiny zahrnuje volně žijící a endobiotické striktně anaerobní linie, fakultativní anaeroby, mikroaerofily i mikroaerotolerantní druhy. Diverzita řádu Metopida, volně žijících nálevníků ze striktně anaerobní třídy Armophorea, byla v posledních letech částečně revidována a výrazně rozšířena, včetně četných redeskripcí pomocí moderních metod, jakož i popisu nových čeledí Tropidoattractidae a Apometopidae, několika rodů a druhů. Kyslík hraje klíčovou roli při produkci ATP oxidační fosforylací, která se vyskytuje v mitochondrii ve většině známých eukaryot. Nicméně anaerobní nálevníci, mezi mnoha dalšími eukaryoty, kteří se přizpůsobili nízkým koncentracím kyslíku nebo dokonce jeho nepřítomnosti, modifikovaly své mitochondrie a energetický metabolismus tak, aby byly schopné produkovat energii i při anoxii. Objev dvou nových tříd nálevníků v rámci skupiny SAL, Muranotrichea a Parablepharisma, které tvoří APM klád obligátních anaerobů spolu s Armophorea, umožnil komplexní srovnávací analýzu adaptací a poskytl nahlédnutí do vývoje přechodu k povinnému anaerobióze v eukaryotech. Všichni členové APM klámu jsou hostiteli různých prokaryotických symbiontů, jako jsou endosymbiotičtí metanogeni, kteří představují jediná známá intracelulární archaea. Genomické analýzy symbiotického archaea ukázaly, že ne všichni endosymbionti podstupují významnou redukci genomu, a poskytují náhled do povahy a evoluce symbiotických vztahů prokaryot a anaerobních nálevníků.

Klíčová slova: anaerobióza, anaerobní nálevníci, fylogenomika, MRO, metabolismus, symbiózy

1. INTRODUCTION

1.1. Life in anoxia

Diatomic oxygen is a key molecule in energy production for most of the known complex life on Earth. Over the last five decades, the concentrations of dissolved oxygen in the ocean have dropped significantly (Schmidtko et al., 2017) and hypoxic areas in the ocean are increasing in number, extent, and persistence, forming so called 'dead zones' (Diaz and Rosenberg, 2008). Far from dead, oxygen-depleted environments teem with a wide variety of organisms. Anaerobic prokaryotes, a subject of intensive study, include bacteria and archaea that play a key role in major geochemical cycles and production of green-house gases, contributing to globally accelerating environmental changes (Smith et al., 2019). Yet, besides prokaryotes, numerous hypoxia-adapted eukaryotes are being discovered, ranging from diverse lineages of microbial eukaryotes (Fenchel, 2014; Reboul et al., 2019; Takishita et al., 2012), to multicellular and often macroscopic metazoans (Yahalomi et al., 2020). Nevertheless, our knowledge on the diversity and evolution of eukaryotes thriving in the seemingly hostile conditions, as well as their metabolic adaptations and interactions with prokaryotic communities, is scarce (Beinart et al., 2018a; Edgcomb et al., 2011b). The study of anaerobic eukaryotes is critical to our understanding of adaptations of complex life to anoxia but also to our comprehension of the advancing environmental processes, since eukaryotic anaerobes may play a more significant role in geochemical cycles than previously thought (Worden et al., 2015).

1.2. Ciliates – diversity, ecology, and classification

Ciliophora is one of the most studied groups of unicellular eukaryotic organisms, and, hand in hand, it is considered to be one of the most diverse and species-rich protist groups (Lynn, 2008; Warren et al., 2017). Ciliates contain several important model organisms (e.g., *Tetrahymena*, *Paramecium*, *Euplotes*), research of which has contributed to pivotal findings in gene function, epigenetics, modes of inheritance and sexual reproduction of eukaryotes, but also understanding function of telomeres in senescence and exposure to cancer and other severe diseases (Montagnes et al., 2012).

According to the current classification, based on both single-gene and phylogenomic analyses, the ciliates are divided into two subphyla Postciliodesmatophora and Intramacronucleata, which further split into more than 15 classes (Adl et al., 2019) and two super class level taxa – CONThreeP (compound of Colpodea, Oligohymenophorea, Nassophorea, Phyllopharyngea, Prostomatea, and Plagiopylea) and SAL (originally compound of Spirotrichea, Armophorea, and Litostomatea), as well as an independent deep lineage Protocruzica (Gao et al., 2016; Gentekaki et al., 2017). Since the establishment of the SAL clade, it has been broadened to include also lineages Cariacotrichea, Odontostomatea, and the recently discovered Muranotrichea and Parablepharisma (Fernandes et al., 2018; Orsi et al., 2012b; Rotterová et al., 2020).

Ciliates in general are of great ecological importance, playing a major role in regulating microbial populations, functioning as top predators in microbial food webs,

and being the main grazers of bacteria and protists. They contribute to decomposition of organic detritus and provide nutrition for other organisms, therefore having a considerable impact on carbon sources (Kemp, 1988). Ciliates have successfully colonized both terrestrial and aquatic diverse habitats as well as the intestinal tracts of numerous metazoans (Fenchel and Finlay, 1991), some of which are environments without oxygen, unbearable for most metazoans (Edgcomb and Pachiadaki, 2014). The ecological importance of ciliates is indisputable, yet our understanding of their role in anoxic sediments is still very limited and anaerobic ciliates remain severely understudied compared with their aerobic counterparts, including the model organisms already mentioned.

The diversity and evolution of anaerobic lineages are especially poorly understood and just starting to gain attention (Bourland et al., 2017b; Campello-Nunes et al., 2020; Fernandes et al., 2018; Omar et al., 2017; Rotterová et al., 2018, 2020). Despite the species rich Spirotrichea and Litostomatea, which are prevalently aerobic, the majority of main lineages within SAL group are anaerobes. The recent phylogenomic analysis of ciliates that included two newly discovered ciliate classes, Muranotrichea and Parablepharisma revealed they constitute a clade (APM ciliates) of obligate anaerobes with Metopida and Clevelandellida (Rotterová et al., 2020). Further analyses based on the 18S rRNA gene show there are other deep lineages within SAL super-group, the deep-sea anaerobes – Cariacotrichea, and the recently established anaerobic class Odontostomatea (Orsi et al. 2012; Fernandes et al., 2018; Rotterová et al. 2020). Overall, the SAL clade encompasses most of the diversity of anaerobic ciliates and a predominant part of its deep lineages is represented by obligate anaerobes. The striking abundance of anaerobes or microaerophiles among ciliates, particularly within SAL clade, attracts numerous questions about the number of origins of anaerobiosis within ciliates and their possible pre-adaptions to anaerobic lifestyle and likely mechanisms of transition to anoxia (Lewis et al. 2020; Rotterová et al., 2020).

1.3. Anaerobic ciliates as model organisms

Ciliates have adapted to an anaerobic lifestyle repeatedly across numerous independent lineages, which makes them an ideal model for comparing patterns of reductive evolution of mitochondrial metabolism in response to adaptation to low oxygen environments. Although researchers aspired to use ciliates as model organisms for studying anaerobiosis during the last 30 years (De Graaf et al., 2011; Fenchel and Finlay, 2010, 1991; Hackstein et al., 2001), the knowledge of anaerobic ciliates and their mitochondrial metabolism has not expanded much since, especially in comparison to other anaerobic protist groups, such as Metamonada, Archamoebae, Breviatea or Heterolobosea (Hamann et al., 2016; Karnkowska et al., 2016; Leger et al., 2017; Stairs et al., 2015). Nevertheless, anaerobic ciliates represent a great model tool for studying anaerobiosis due to their recently described easy cultivation (Rotterová et al., 2018), manipulation, their high complexity, and rich diversity. In addition, genetic manipulations and other modern methods enabling our

comprehension of adaptations to certain environmental conditions were launched, as well, on ciliates (Ruehle et al., 2016).

1.4. Mitochondrion-related organelles in anaerobic ciliates

Adaptations to the anoxic environment include transformation of mitochondria and several categories of mitochondrial derivatives have been defined early in the research on anaerobic metabolism (Müller, 1993). With further studies and discoveries of more anaerobic eukaryotes, the mitochondrial modifications among eukaryotes inhabiting oxygen-depleted environments were found to vary diversely, resulting into a broad spectrum of anaerobic mitochondrion-related organelles (MROs) (Embley, 2006; Gawryluk et al., 2016; Leger et al., 2017; Van Der Giezen, 2009). Nevertheless, complex and highly functional MROs of free-living obligate anaerobes have been studied only in a single group so far – *ciliates* (Lewis et al. 2020; Rotterová et al., 2020).

1.5. Energetic metabolism of anaerobic ciliates within SAL group

Questions concerning the production of energy without oxygen, often regarded as a key molecule for eukaryotic life (Schmidt-Rohr, 2020), raise interest in the reduction levels of energy metabolism in mitochondrial organelles (MROs) among all eukaryotic anaerobes. Although ciliates belong to the most studied protists in general, the energetic metabolism of their anaerobic representatives has been surprisingly understudied. Recently, two studies have elucidated the energetic metabolism of free-living anaerobic ciliates (Lewis et al. 2020; Rotterová et al., 2020). Several common traits were shown for Metopida and Clevelandellida (Armophorea), such as retaining the proton-pumping complex I but likely lacking functional complexes III and V of the ETC, therefore having lost the capacity for ATP synthesis via oxidative phosphorylation. In silico analyses indicate that all of them produce ATP by substrate-level phosphorylation via ASCT and SCS. The electron transport chain is probably reduced into just two steps through mitochondrial complex I and II, as almost all of the genes coding the complex I, which pumps protons out of the organelles, and complex II, that allows fumarate (electron acceptor) reduction, were identified, but genes coding complexes III, IV, and V, have not been discovered (de Graaf et al. 2011; Lewis et al. 2020; Rotterová et al., 2020).

In the recently described Muranotrichea and Parablepharisma, forming the so-called APM clade together with Metopida and Clevelandellida, genes for nuclear-encoded mitochondrial PDH were discovered, while no evidence for the presence of the usual hydrogenosomal enzyme PFO was found, leading to conclusion that PDH plays a crucial role in their hydrogenosomal metabolism (Rotterová et al. 2020). APM ciliates possess a complex nuclear encoded [FeFe] hydrogenase (Boxma et al., 2007; Lewis et al. 2020; Rotterová et al. 2020), which functions within their MROs as a sink for electrons in ETC, transferring them to protons (hydrogen ions, H⁺) and producing a molecule of H₂.

1.6. Symbioses of free-living anaerobic ciliates in SAL clade

Anaerobic ciliates are known to commonly form symbioses with various prokaryotes (Fenchel and Finlay, 1991; Orsi et al., 2012a), some even host a stable consortium of several unrelated prokaryotes within their cells (Beinart et al., 2018a; Edgcomb et al., 2011a; Takeshita et al., 2019). Majority of the identified symbionts of anaerobic ciliates were assigned to two functional types of both ecologically and economically relevant groups of prokaryotes, methanogens and sulfate-reducers (Finlay and Fenchel, 1993). Both methane producing symbionts, classified within Archaea, and sulfate reducing symbiotic bacteria utilize the hydrogen and other metabolic byproducts, such as acetate, produced by the hosts' mitochondrion related organelles (Fenchel and Finlay, 1992). The coordinated removal of waste molecules from the host cell was shown to boost the metabolic rate and effectivity of the ciliate hosts (Fenchel and Finlay 1992). The elevated effectivity of the host's metabolism may have given the ciliate an advantage in the competition in hostile anoxic conditions with limited resources. A recent hypothesis of a symbiont driven transition to obligate anaerobiosis presents this advantage as a motivation for the ciliate to reside in the anoxic environment permanently, eventually leading to the reduction of its mitochondrion and aerobic pathways for energetic metabolism (Rotterová et al. 2020).

Prokaryotic symbioses with eukaryotes are important for our understanding of genome reduction mechanisms and origins of endosymbiotic organelles, such as mitochondria. Endosymbiotic bacteria are commonly known to have a reduced genome as a consequence of their endobiotic lifestyle (Boscaro et al., 2013; Lind et al., 2018). However, in the freshwater metopid *Heterometopus* sp., the endosymbiotic methanogen might remain relatively independent, keeping its genome largely intact (Beinart et al., 2018b). Such variation might reflect either different evolutionary age of the relationships or varying levels of host specificity among the host taxa, allowing replacement of the symbiont in some cases.

The role of methane, one of the most potent green-house gases, in enhancing the greenhouse effect and accelerating climate change is apparent (Shindell et al., 2009), and therefore it is critical to better understand its sources and sinks and numerous studies pay attention to this topic. However, studies focusing on the impact of prokaryotes on such geochemical cycles mostly neglect the fact that an unknown portion of these are actually in some symbiotic relationships and their metabolism differs from the free-living prokaryotes. Thus, the involvement of prokaryotic symbionts of protists in major ocean cycles, carbon and sulfur productions and sinks is often overlooked in large scale microbial studies. Intensified study of free-living marine anaerobic protists is central to our understanding of major geochemical processes in the ocean, particularly in expanding oxygen-depleted sediments and water columns (Edgcomb et al., 2011b).

2. AIMS of the DOCTORAL PROJECT

The overall aim of the project is to considerably expand our understanding of the evolution of ciliates and microbial symbioses in oxygen-depleted environments using modern molecular methods. This would not be possible without expanding our insight into the biodiversity and phylogeny of the understudied yet ecologically important anaerobic ciliates and their prokaryotic symbionts that together play crucial role in anoxic ecosystems.

The particular aims of the doctoral project are:

- Phylogenomic analysis of ciliates including free-living anaerobes and using newly generated data. Sequencing the transcriptomes and genomes of several deep-branching lineages of anaerobic ciliates for a phylogenomic analysis based on approximately 150 genes to elucidate the evolution of anaerobiosis in ciliates and gain means for more accurate estimates of the numbers of independent anaerobiosis origins in ciliates.

- Taxonomic revision of anaerobic ciliates within SAL group with emphasis on Metopida. Mapping the diversity of anaerobic ciliates within SAL group, expanding and maintaining the culture collection of hundreds of free-living anaerobic ciliates (freshwater, marine, and brackish) isolated from anoxic sediments worldwide, analyzing the phylogenetic relationships within particular lineages using 18S rRNA gene sequences, and performing morphological characterization of cultivated anaerobic ciliates of various Metopida and newly discovered lineages within SAL clade. Formal description of newly discovered taxa.

- *In silico* prediction of pathways of energetic metabolism. Using transcriptomic and metagenomic data to characterize mitochondrial metabolism of individual anaerobic ciliate lineages and deepen our knowledge of the evolution of anaerobiosis in ciliates.

- Research of symbiotic methanogenic Archaea and unidentified bacteria associated with anaerobic ciliates, their identification and host-specificity, using methods of amplicon 16S rRNA gene and metagenomic sequencing, localization of symbionts on or within a host cell will be studied by transmission and scanning electron microscopy and CARD-FISH with group-specific probes.

3. CONCLUSIONS OF THE DOCTORAL PROJECT

We have chosen anaerobic ciliates from the SAL group as a model group for studying the evolution of anaerobiosis. To achieve goals set for the Doctoral project, we have implemented a multi-method approach, combining various molecular, bioinformatic, and microscopic methods and including diverse cultivational and physiological experiments. We have expanded and maintained the world's largest culture collection of freshwater, brackish, and marine anaerobic ciliates, established during my Master's project (Rotterová, 2015), resulting in long-term cultivation of several hundred strains of ciliate representatives from anaerobic SAL lineages together with their symbionts, with isolates obtained from anoxic sediments worldwide. We have determined the 18S rRNA gene sequences of most of the cultivated ciliates, carried out phylogenetic analyses, studied their morphology using light, fluorescence, and scanning electron microscopy with various staining and hybridization techniques. We have also studied the ultrastructure of multiple species from main SAL anaerobic lineages in transmission electron microscopy (Beinart et al. 2018; Rotterová et al. 2018; 2020).

We have used genomics and transcriptomics to analyze the phylogenomics of ciliates including anaerobic lineages and determined the phylogenomic position of the class Muranotrichea, which we have discovered, and Parablepharismaea. We used our data to *in silico* predict pathways of energetic metabolism in anaerobic ciliates of SAL clade, including the two newly discovered classes and an undescribed metopid species SK (Rotterová et al. 2020). We have broadened the known diversity of the order Metopida with a description of a novel metopid family Tropidoactinidae and characterization of its five species; a determination of the phylogenetic position of the family Apometopidae and characterization of its six species; and we have identified several armophorean clades, as well as provided a redescription and molecular characterization of several other Metopida species (Bourland et al., 2017a, 2018a, 2018b, 2020; Rotterová et al., 2018).

Furthermore, we have studied the symbionts of several species from all three lineages, analyzing their identity and diversity, host specificity, and evolution. We have characterized the genome of a methanogenic archaeal endosymbiont, determined as *Methanobacterium* sp., and hosted by the metopid *Heterometopus* sp. CSS (Beinart et al. 2018). In addition, we have determined the 16S rRNA gene sequences of endosymbionts in several ciliate strains of multiple ciliate species (Rotterová et al. unpublished data) that we found to be positive for harboring methanogens by autofluorescence of F420 coenzyme or positive for archaea and deltaproteobacterial sulphate reducers in the applied CARD FISH group specific probes and have determined their position within or outside the host cell, suggesting the association with ciliate MROs using transmission electron microscopy (Bourland et al. 2018; Rotterová et al. 2018).

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Education

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- 2015 – now Doctoral program in Zoology, Department of Zoology, Faculty of Science, Charles University. Thesis: *Anaerobic ciliates as a model group for studying the biodiversity and symbioses in anoxic environments*, supervised by prof. RNDr. Ivan Čepička, Ph.D.
- 2013 – 2015 Master's program in Protistology, Department of Zoology, Faculty of Science, Charles University. Thesis: *Morphological and molecular diversity of the free-living representatives of the family Metopidae and the discovery of a new lineage of anaerobic ciliates*, supervised by prof. RNDr. Ivan Čepička, Ph.D., MSc. degree received in 2015, *Rerum naturalium doctor* (RNDr.) in Protistology received in 2016.
- 2010 – 2013 Bachelor's program in Molecular Biology and Biochemistry of Organisms, Faculty of Science, Charles University. Thesis: *The use of DNA barcoding method in protists*, supervised by prof. RNDr. Ivan Čepička, Ph.D., BSc. degree received in 2013.

Appointments

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- 2019 – now Scientific researcher at Charles University (Czech Republic), Faculty of Science, Department of Zoology
- 2019 – 2020 Principal researcher in Charles University Grant Agency funding project nr. 116119, titled *Phylogeny and diversity of anaerobic ciliates of the class Odontostomatea (SAL, Ciliophora) and characterization of their methanogenic symbionts*.
- 2019 – 2021 Member of the research team in Czech Science Foundation (GACR) project nr. 19-19297S titled *Free-living anaerobic ciliates as a model group for studying the biodiversity and symbioses in anoxic environments*.
- 2018 – 2020 Member of the research team in Czech Science Foundation (GACR) project nr. 18-18699S, titled *Non-standard genetic codes in protists and their evolution*.
- 2015 – 2018 Principal researcher in Charles University Grant Agency funding project nr. 389915, titled *Diversity and Evolution of Anaerobic Ciliates, an ecologically important but poorly known group of protists*.

2014 – 2016 Scientific researcher at Charles University (Czech Republic),
Faculty of Science, Department of Zoology

Publications in impacted scientific journals

- see publications 1. – 10. below in chapter List of Publications

Presentations at selected international conferences from last five years

Rotterová, J., Salomaki, E., Pánek, T., Bourland, W.A., Edgcomb, V.P., Táborský, P., Žihala, D., Beinart, R.A., Kolísko, P., Čepička, I., 2019. Where oxygen is not popular – phylogenomic analysis of anaerobic ciliates (Ciliophora). VIII. ECOP (European Congress of Protistology) – ISOP (International Society of Protistologists) Joint Meeting, Rome, Italy.

Rotterová, J., Beinart, R.A., Edgcomb, V.P., Bourland, W.A., Táborský, P., Kolísko, P., Čepička, I., 2018. Phylogenomic analysis of SAL super-group (Ciliophora), including novel marine lineages of anaerobic ciliates, which host prokaryotic symbionts. ISEP (International Society of Evolutionary Protistology), Paphos, Cyprus.

Beinart, R.A., **Rotterová, J.**, Sylva, S., Seewald, J.S., Čepička, I., Gast, R.G., Edgcomb, V.P., 2017. Metabolic functioning of a ciliate-methanogen symbiosis from anoxic habitats, 6th International Symposium on Chemosynthesis-Based Ecosystems (CBE6), Woods Hole, Massachusetts, USA.

Rotterová J., Beinart, R., Edgcomb, V., Bourland W., Čepička I., 2017. Novel marine lineages of anaerobic ciliates hosting prokaryotic symbionts, XV. ICOP (International Congress of Protistologists), Prague, Czech Republic.

Rotterová J., Nováková L., Čepička I., 2015. Mapping the diversity of Metopida and revealing new marine anaerobic ciliates hosting prokaryotic symbionts, VII. ECOP 2015, Seville, Spain.

Awards

2018 – Best doctoral poster in 2018 prize awarded in competition at Department of Zoology, Faculty of Science, Charles University, Prague, Czech Republic

2018 – ISEP (International Society of Evolutionary Protistology) Travel Award from FEMS (Federation of European Microbiology Societies)

2015 – 2019 Awarded scholarship from the program for supporting talented PhD students at the Faculty of Science, Charles University (STARS)

2016 – Hlávková Foundation Endowment for an internship in WHOI, MA, USA

2016 – Mobility Fund of Charles University (MFCU) support for internship in WHOI, MA, USA (2016)

2014 – Hlávková Foundation Endowment for ISOP congress, Banff, Canada

2014 – Best conference poster at 44th Jírovec Protozoological days, Krásná, Czech Republic

Memberships in scientific organizations

Since 2018 – Czech Slovak Society of Microbiology; International Society for Evolutionary

Since 2018 – Protistology

Since 2015 – International Society of Protistologists

Since 2014 – Czech Society of Parasitology, Protozoological Section

Scientific internships and selected workshops

- 8 – 9/2019 Scientific internship in the laboratory of Dr. Roxanne Beinart, Graduate School of Oceanography, University of Rhode Island, RI, USA.
- 2/2019 Evomics Workshop on Phylogenomics, Český Krumlov, Czech Republic.
- 7/2018 OstraPy, Ostrava, Czech Republic – bioinformatics workshop focused on coding in Python language.
- 6 – 9/2016 Scientific internship in the laboratory of Dr. Virginia Edgcomb, Department of Geology & Geophysics, Woods Hole Oceanographic Institution, MA, USA.
- 7 – 8/2016 Molecular Evolution Workshop (MEW), Marine Biological Laboratory (MBL), Woods Hole, MA, USA, visiting attendance as an internship student of Woods Hole Oceanographic Institution (WHOI).
- 7 – 8/2016 Microbial Diversity Course (MDC), MBL, Woods Hole, MA, USA, visiting attendance as an internship student of WHOI.
- 7 – 8/2016 Strategies and Techniques for Analyzing Microbial Population Structures (STAMPS), MBL, Woods Hole, MA, USA, visiting attendance as an internship student of WHOI.
- 5/2014 IRCN - BC workshop on ciliate diversity and methods of ciliate taxonomy, Royal Holloway, University of London (RHUL), Egham, United Kingdom.

Conference organization and volunteer activity

- 2018 – 2019 Main organizer and head coordinator of international scientific conference 49th Jírovec Protozoological Days, 22nd – 26th of April, 2019 (Protodays 2019).

Teaching & advising activities at Faculty of Science, Charles University

- 2015 – 2019 Assistant, Lecturer, Practical Course in Protistology, Department of Zoology.
- 2019 External lecturer on Diversity of Ciliophora, Protistology, Department of Zoology.

2019 – now Advisor of Master's student MSc. BSc. Ondřej Pomahač, Thesis: *Diversity and phylogeny of metopid ciliates of the IAC group* (supervised by prof. RNDr. Ivan Čepička, Ph.D.), Department of Zoology.

2017 – now Advisor of Master's student BSc. Kateřina Poláková, Thesis: *Prokaryotic symbionts of free-living anaerobic protists* (supervised by prof. RNDr. Ivan Čepička, Ph.D.), Department of Zoology.

2015 – 2017 Advisor of Bachelor's student Kateřina Poláková, Thesis: *Diversity of scuticociliates and their symbionts* of student (supervised by prof. RNDr. Ivan Čepička, Ph.D.), Department of Zoology.

06/2017 Oponent at Bachelor's Thesis Defense of student Aneta Kubánková (supervised by doc. Mgr. Vladimír Hampl, Ph.D.), Thesis: *Prokaryotic symbionts of protists living in the intestine of wood eating cockroaches and termites*, Department of Parasitology.

Technical skills

Molecular methods of isolation, amplification, purification, cloning, and sequencing of eukaryotic and prokaryotic DNA/RNA.

Laboratory methods of cultivation of anaerobic protists and prokaryotes.

Light and fluorescence microscopy methods (CARD Fluorescence *in situ* hybridization methods; microphotography, BF, DIC, phase contrast, Nomarski, living and fixed cells observations; methods of fixing and cell staining, such as protargol-staining or silver-carbonate; image analyses).

Electron microscopy (scanning EM to study the morphology of fixed cells, transmission EM to assess an ultrastructure of fixed cells, image analysis and interpretation).

Bioinformatics methods (user level - RaxML-ng, PhyloBayes, IQ Tree, QIIME, Blast, Unix, and other tools for biological methods of sequence analyses; genomics and transcriptomics methods for phylogenomics and *in silico* predictions of metabolic pathways; primer design; R and Python language – beginner).

Language skills

Czech – native speaker

German, Spanish, Italian – semi-fluent speaker

English – near-native speaker

French, Indonesian – beginner

LIST of PUBLICATIONS

1. Bourland, W.A., **Rotterová, J.**, Čepička, I., 2017a. Redescription and molecular phylogeny of *Metopus es* Lauterborn, 1916 and *Brachonella contorta* Jankowski, 1964, based on broad geographic sampling. *Eur. J. Protistol.* 59: 133 – 154.
2. Bourland, W.A., **Rotterová, J.**, Čepička, I., 2017b. Morphologic and molecular characterization of seven species of the remarkably diverse metopid genus *Urostomides* (Armophorea, Ciliophora). *Eur. J. Protistol.* 61: 194–232.
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