

# 4<sup>th</sup> Workshop of European Centre of Ichthyoparasitology

Venue: International environmental educational, advisory and  
information centre of water protection Vodňany  
23–25 November 2015



Šárka Mašová, Zuzana Kobíková & Milan Gelnar (Eds.)  
Masaryk University  
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# Preface

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Milan Gelnar

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**Research group of Ass. Prof. Milan Gelnar - Department of Botany and Zoology, Faculty of Science, Masaryk University, Brno**

<i>Michal Benovics</i>	<i>Maria Lujza Kičinjaová</i>	<i>Chahrazed Rahmouni</i>
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**Research group of Prof. Tomáš Scholz - Institute of Parasitology, Academy of Sciences of the Czech Republic, České Budějovice**

<i>Pavla Bartošová-Sojková</i>	<i>Astrid Holzer</i>	<i>Kateřina Leřtinová</i>
<i>Jan Brabec</i>	<i>Inga Meyer-Wachsmuth</i>	<i>Sneha Patra</i>
<i>Itziar Estensoro</i>	<i>Aneta Kostadinova</i>	<i>Jana Zikmundová</i>
<i>Simona Georgieva</i>	<i>Olena Kudlai</i>	
<i>Ashlie Hartigan</i>	<i>Roman Kuchta</i>	

**Research group of Dr. Pavel Jurajda - Institute of Vertebrate Biology, Academy of Sciences of the Czech Republic, Brno**

<i>Zdeněk Adámek</i>	<i>Pavel Jurajda</i>	<i>Veronika Nezhybová</i>
<i>Michal Janáč</i>	<i>Iurii Kvach</i>	<i>Markéta Pravdová</i>
<i>Karel Janko</i>	<i>Markéta Ondračková</i>	<i>Jan Röslein</i>

# Programme

## Monday, November 23

From 16:00	Arrival and accommodation
19:00	Dinner

## Tuesday, November 24

8:00- 8:30	Breakfast
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### Workshop opening

8:30	<b>Gelnar:</b> Introduction and general programme information
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### Diversity of parasites and molecular phylogenetics

8:45	<b>Tyml:</b> Free-living amoebae as a model group of organisms
9:00	<b>Holzer:</b> Deciphering the coevolutionary history of myxozoans and their hosts
9:15	<b>Meyer-Wachsmuth:</b> Myxozoan diversity as revealed by eDNA
9:25	<b>Patra:</b> Temperature-dependent proliferation of blood stages of <i>Sphaerospora molnari</i> in common carp ( <i>Cyprinus carpio</i> ) determined by real-time PCR
9:35	<b>Estensoro:</b> Motility of <i>Sphaerospora molnari</i> blood stages in common carp: actin involvement
9:45	<b>Bartošová-Sojková:</b> Epicellular Apicomplexans: Parasites “On the Way In”
10:00	Coffee Break

### Diversity of parasites and molecular phylogenetics

10:30	<b>Přikrylová:</b> The attractiveness of the continents beginning from “A” for studies of various monogenean parasites
10:45	<b>Seifertová:</b> Incorporating molecular data in systematics of selected African fish parasites
11:00	<b>Francová:</b> Dactylogyrids (Monogenea: Dactylogyridae) parasitizing African catfishes (Siluriformes)
11:10	<b>Jirsová:</b> Monogenea: From sequences to molecules...
11:20	<b>Vorel:</b> “Omics” and population genetic tools applied on selected species from class Monogenea
11:30	<b>Dávidová:</b> Diversity of African parasitic copepods (Crustacea)
11:40	<b>Sponsor panel I - presentations</b>
12:10 14:00	Lunch

### Diversity of parasites and molecular phylogenetics

14:00	<b>Kuchta &amp; Kostadinova:</b> Institute of Parasitology – Research outlines for 2015 and plans for 2016 (Roman: Cestoda, Monogenea, Acanthocephala, Nematoda; Aneta: Trematoda)
14:20	<b>Kuchta:</b> Molecular phylogeny of the Bothriocephalidea (Cestoda): molecular data challenge morphological classification
14:30	<b>Leštinová:</b> Comparative study of the eggs morphology of human diphylobothriids (Cestoda)
14:40	<b>Georgieva:</b> Molecular taxonomy of <i>Diplostomum</i> spp. (Digenea: Diplostomidae) from freshwater fishes in China
14:50	<b>Kudlai:</b> Molecular identification of metacercariae of <i>Diplostomum</i> spp. in fishes from the River Danube
15:00	Coffee Break

### Host-parasite relationships and molecular interactions

15:30	<b>Gettová:</b> How can we better understand host-parasite interactions?
15:45	<b>Rahmouni:</b> A phylogenetic perspective on species diversity: <i>Cichlidogyrus</i> (Dactylogyridae) parasitizing Lake Tanganyika cichlid tribes
15:55	<b>Pakosta:</b> Evolution of diploid-polyploid <i>Carassius auratus</i> complex
16:05	<b>Benovics:</b> Host-specific parasites as indicator of evolution and historic dispersion of their hosts: model <i>Dactylogyrus</i> (Monogenea) and cyprinid fish in Perimediterranean area
16:15	<b>Krasnovyd:</b> Influence of life traits and hybridization on parasite community structure in roach ( <i>Rutilus rutilus</i> ) and common bream ( <i>Abramis brama</i> ) hybrids
16:25	<b>Sponsor panel II - exhibition stands</b> + Coffee Break

### Evolutionary epidemiology and behavioral ecology of parasites

17:25	<b>Jurajda:</b> Successful biomanipulation of reservoir fish communities may have long-term consequences for fish parasite populations
17:40	<b>Kvach:</b> Methodological issues affecting the study of fish parasites
17:50	<b>Nezhybová:</b> Metazoan parasites of African annual killifish (Nothobranchiidae)
18:00	<b>Janáč:</b> Effect of exotic species on host-parasite interactions

### Social evening

19:00	Buffet dinner
24:00	

### Wednesday, November 25

8:00	Breakfast and departure
10:00	

# Abstracts

- Abstracts in the following section are sorted alphabetically according main author.
- To locate a particular author, please see the index of authors (sorted according author's surname) in the end of the abstract booklet.

# Environment quality and natural food exploitation at feeding sites in a carp pond

Z. Adámek, M. Mrkvová, J. Zuka, L. Mikl, L. Šlapanský, P. Jurajda

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The history of common carp (*Cyprinus carpio* L.) has a long tradition in the Czech Republic and until the beginning of 20th century, pond fish stocks were set up on low fish density and higher species diversity. However later on, pond fish stock density and subsequently higher production have been considerably increased in the frame of intensification of agriculture, including fish culture. The intensification of pond aquaculture led to principal changes not only in production, but also in conditions of fish pond farming. Currently, the effort for introduction of ecological approaches in fish pond culture is increasingly more obvious. Semi-intensive methods, based on the combination of exploitation of natural food resources, supported by supplementary feeding, are recently the widely applied technology in carp pond culture being simultaneously also acceptable and nature friendly way of fish production.

Natural diet of two- and three-year-old common carp consists to lesser extent of zooplankton, and preferably of zoobenthos. The new approaches to supplementary carp feeding in ponds arise the necessity of new information about carp behaviour and response upon supplied feeds. It was known in the fish pond farming industry that sufficient amount of food supplied in the form of feeds may alter the fish behaviour in the pond environment. It can be envisaged that carp, as any other higher animals, can learn themselves where they can easily get the food and have no necessity of any more significant relocations. Hence, an increased fish concentration on feeding sites leads to lowered attendance and utilisation of cheaper but higher-quality natural food resources.

The study was performed on the South-Moravian carp ponds. On-site monitoring of environmental parameters was performed and the samples of zooplankton and macrozoobenthos were collected at the feeding sites and outside them in 15 replicates at monthly intervals. At the same time intervals, the behavioural response upon the feed (cereals) application was performed with 44 carp individuals using the telemetric methods.

With respect to carp response upon feed application, four principal behavioural patterns could be distinguished:

- (1) staying almost continuously at the feeding sites and just sporadically outside (41% ind.)
- (2) spreading evenly over the pond area (40 – 67 ha) regardless the time of feeding (24% ind.)
- (3) just on the area (5 – 24 ha) outside the feeding site (20% ind.)
- (4) on the feeding sites during the feeding and outside on the whole pond area in the meantime (15% ind.).

In conclusion, it is obvious that the spatial distribution of carp stock in a semi-intensive pond is not uniform during the growing season – fish prefer the pond areas which are nearby or belong to the feeding sites (ca. 2/3 of the pond area). Fish captured on a feeding site were bigger and had higher fat content compared to those occurring on marginal sites distant from the feeding site. Oxygen concentrations on the feeding sites often drop below the levels of carp physiological optimum shortly after feed application (< 3 mg.l<sup>-1</sup> O<sub>2</sub>) whilst outside the feeding sites they did not drop below 4 mg.l<sup>-1</sup> O<sub>2</sub>. Fish on the feeding sites consumed almost exclusively provided cereals whilst those outside the feeding sites consumed mainly zooplankton. However, due to increased fish concentration on feeding sites and/or nearby, zooplankton represents a rich but insufficiently employed food resource in a carp pond.

## Future plans for 2016

To consider possible relationships between irregular distribution of fish in a pond and its consequences for the infestation by *Anodonta woodiana* glochidia and their spreading with fish transfers and stocking.

## Acknowledgement

This study was partly supported by ECIP (European Centre of Ichthyoparasitology); Centre of Excellence Program of the Czech Science Foundation; project No. P505/12/G112).

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# Epicellular Apicomplexans: Parasites “On the Way In”

Pavla Bartošová-Sojková

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The Coccidia and the Cryptosporidia infecting cold-blooded vertebrates, especially those of fish, represent poorly studied groups of parasites. As they possess several unique morphological and molecular features, their scrutiny can help us better understand the evolution of Apicomplexa. We reviewed the modes of interactions, invasion mechanisms and metabolic adaptations of the epicellular apicomplexans with their cold-blooded hosts as well as mapped characters of host-parasite interactions on the 18S rDNA-based phylogenetic tree especially focused on coccidians and cryptosporidians from poikilotherm hosts [1].

Apicomplexans exhibit a remarkable diversity in the strategies they use for acquisition of nutrients, invasion, and interaction with host cells. Merozoites of the epicellular species infecting poikilotherms reside in a host-derived envelope, i.e. parasitophorous sac in cryptosporidians or parasitophorous vacuole in epicellular coccidians. This envelope adopts diverse morphologies (several monopodial, one multipodial type of host-parasite interfaces) as these parasites likely rely on various modes of nutrient uptake.

Mechanisms of host invasion by the epicellular piscine cryptosporidians and coccidians are similar at the beginning whereas they differ by formation of the feeder organelle in cryptosporidians and monopodial/spider-like stages in coccidians at the final stage of invasion. RON (rhoptry neck) and AMA (apical membrane antigen) proteins seem to represent crucial molecular tool for the parasites “on their way in” to the host cell as confirmed by the recent RNA-seq analyses of the epicellular coccidian *Goussia janae* [2].

This parasite possesses enzymes implicated in most of the central carbon metabolism, resembling the versatile metabolic capabilities of the intracellular coccidian *Toxoplasma gondii* rather than the reduced ones of the epicellular *Cryptosporidium* spp. These examples highlight potential differences in the nutrients available in each niche and specific adaptations of the parasite to thrive in its environment.

Phylogenetic analyses revealed that epicellular parasitism most probably evolved convergently in (1) gregarines + poikilotherm and homeotherm cryptosporidians and in (2) piscine + reptile coccidians, as an adaptation to colonization of different cell types and tissues.

## Future plans

Inclusion of the epicellular apicomplexans into comparative genomic analyses holds a promise of identifying previously unknown pathways by which these protists execute their manipulative tricks – by subversions of host cellular functions and by metabolic adaptations to the environments they encountered.

## Acknowledgement

These studies have been supported by ECIP (European Centre of IchthyoParasitology); centre of excellence program of the Czech Science Foundation; project No. P505/12/G112)

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# Host-specific parasites as indicator of evolution and historic dispersion of their hosts: model *Dactylogyrus* (Monogenea) and cyprinid fish in Perimediterranean area

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Dispersion of cyprinid fish is enigmatic and topic of many recent discussions. Even if their dispersion is limited to rivers, this highly diversified fish family inhabits significant part of the world. The special interest is devoted to the distribution of cyprinids around Mediterranean Sea, where endemic genera are “trapped” on their specific peninsulas. The studies focusing on the historical biogeography and phylogeny of these genera can resolve the question of cyprinid speciation during Pleistocene era.

Several biogeographical scenarios were proposed to explain dispersion and recent distribution of cyprinid fish species from perimediterranean area. The first one represents the dispersal model according to which cyprinid dispersion occurred via rivers or during Lago Mare phase in the Miocene era. Another one is vicariant model, where speciation occurred by geographical isolation. However, differentiation of cyprinid fish is far more complex and it seems that it cannot be explain by using only one dispersion model.

Parasitism is one of the most popular life strategies among living organisms. Their impact on other living species, especially on their hosts, is almost always significant and therefore hosts have to develop morphological or immunochemical defense mechanisms. In this occasion the parasite is under pressure to adapt to these defense mechanisms of host. Otherwise it has to switch to new host or face extinction. If the parasite is adapting, then host-parasite interactions evolve through arm races.

The host specificity of fish parasites seems to be the most important parasite characteristic with respect to how we can improve our understanding of fish biogeography. If the host specificity of a parasitic group is high, we can expect that evolution of hosts and their parasites are intimately linked. In this case the study of parasite phylogeny may represent the helpful tool to investigate the historical biogeography of primarily freshwater fish during Pleistocene era, where the greatest adaptive radiation and geographical changes occurred.

For our study the most suitable parasite model represents the *Dactylogyrus* genus. These parasites belonging to Monogenea exhibit a high species richness and also high host specificity – the majority of the species show a preference for a single host species or closely related host species. Even within the same host they are usually restricted to several microhabitats. *Dactylogyrus* parasites are also known for their large morphological diversity with regard to many shapes of the attachment organ. Shape of this organ is usually applied as the species specific trait for monogenean identification.

The aims of this study are following: (1) to analyze the diversity and the degree of endemism of parasite communities with the special focus on gill ectoparasites of *Dactylogyrus* genus, (2) perform the phylogenetic reconstruction of the endemic *Dactylogyrus* species collected from their endemic cyprinid hosts in the perimediterranean area and (3) perform the cophylogenetic analysis to test the hypotheses of biogeographical dispersion scenario.

## Future plans

Analyze the recent diversity of *Dactylogyrus* parasites in endemic cyprinid fish species of the Mediterranean area.

Perform the phylogenetic reconstructions, using molecular data from cyprinid fish species and *Dactylogyrus* specimens as well.

Analyze the patterns and processes of speciation in *Dactylogyrus* and in endemic Mediterranean cyprinid fauna using cophylogenetic approach.

Use the *Dactylogyrus* of different highly diversified cyprinid genera as a tool for testing patterns and processes linked to the evolution and biogeography of endemic cyprinid hosts.

## Acknowledgment

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## Parasites from African Vertebrates (freshwater fish and turtles)

R Blazek<sup>1,2</sup>, I Cepicka<sup>3</sup>, N Dvorakova<sup>4,5</sup>, M Gelnar<sup>1</sup>, W Gibson<sup>6</sup>, ML Kicinjaova<sup>1</sup>, MA Qablan<sup>5,7</sup>, E Rehulkova<sup>1</sup>, P Siroky<sup>4,5</sup>

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Four new and four previously described species of *Annulotrema* were collected from the gills of four species of African tetras from the Lake Turkana, Kenya: *Annulotrema alestesnursi* Paperna, 1973 from *Brycinus nurse*; *A. ansatum* n. sp., *A. besalis* similar to Rehulkova, Musilova and Gelnar, 2014, *A. bipatens* n. sp., *A. cucullatum* n. sp., *A. nili* Paperna, 1973, and *A. pontile* n. sp. from *Hydrocynus forskahlii*; and *A. elongata* Paperna and Thurston, 1969 from *Alestes baremoze* and *Alestes dentex*. *H. forskahlii* is a new host record for *A. besalis*. The findings of *A. besalis* and *A. elongata* in Kenya represent a new locality records for these helminths. *A. besalis*, *A. elongata* and *A. pontile* n. sp., share the same type of male copulatory organ, which may indicate a close relationship among these species.

Little is known about host specificity, genetic diversity and phylogenetic relationships of African turtle trypanosomes. Using PCR targeting the SSU rRNA gene, we detected trypanosomes in 24 of 134 (17.9%) wild caught African pelomedusid turtles: *Pelusios upembae*, *P. bechuanicus*, *P. rhodesianus* and *P. subniger*. Mixed infection of *Trypanosoma* species was confirmed by PCR in three specimens of *P. upembae*, and in one specimen each of *P. bechuanicus*, *P. rhodesianus*, and *P. subniger*. Microscopic examination of stained blood smears revealed two distinct forms (broad and slender) of trypomastigotes. The broad form coincided in morphology with *T. mocambicum* Pienaar, 1962. Accordingly, we have designated this form as the neotype of *T. mocambicum*. In phylogenetic analysis of the SSU rRNA gene, all the new turtle trypanosome sequences grouped in a single clade within the strongly supported “aquatic” clade of *Trypanosoma* species. The turtle trypanosome clade was further subdivided into two subclades, which did not correlate with host turtle species or trypanosome morphology. This study provides the first sequence data of *Trypanosoma* species isolated from freshwater turtles from tropical Africa and extends knowledge on diversity of trypanosomes in the Afrotropical zoogeographical realm.

### Plans for the next years

In 2016, besides possible field trips for collection of new ichthyo-parasitological material, we wanted to finish determination of fish samples from lake Turkana (Kenya) and publish obtained data from other expeditions.

### Acknowledgement

This study was supported by ECIP (European Centre of IchthyoParasitology); centre of excellence program of the Czech Science Foundation (project No. P505/12/G112)

### References 2015

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Dvorakova N, Cepicka I, Qablan M, Gibson W, Blazek R, Siroky P (2015). Phylogeny and morphological variability of trypanosomes from African pelomedusid turtles with redescription of *Trypanosoma mocambicum* Pienaar, 1962. *Protist* (accepted)

# Molecular genetics revealing new diplozoid species and coevolutionary relationships between parasites and their hosts

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Monogeneans of the Diplozoidae family are interesting parasites on the gills of cyprinid fish. Nowadays, they are commonly identified by combining classical morphometric approach (using measurements of the size and shape of sclerotized parts of adults' attachment apparatus) and molecular methods enhancing the species discrimination by highly conclusive genome characterization. By combining these two techniques, we already described new parasite species and disclosed interesting aspects in taxonomic division of diplozoid monogeneans [1, 2]. However, very little is known about details of phylogenetic relationship between these parasites and their hosts. So far only several preliminary unpublished studies were performed [2, 3]. Increasing amount of molecular data available for diplozoons and increasing number of newly described taxa allow us to study this co-phylogeny in more details.

During years 2009-2014 we sampled tens of fish across several continents and dissected them to record parasites. Adults or diporpa stages of diplozoids found on the gill apparatus of fish (106 specimens in total) were fixed and stored in absolute ethanol at 4°C. For morphometric description, one opisthaptor of each specimen was cut off, soaked in 10% SDS, washed in distilled water, mounted on a microscope slide and fixed with GAP. A light microscope equipped with DIC, digital image analysis system and drawing attachment was used. For molecular analysis, gDNA was extracted and along with routinely analyzed ITS2 rDNA marker, two more genome rDNA regions were amplified and characterized by sequencing. To characterize hosts, GenBank data of cytochrome b gene were obtained for particular fish species and included into analyses. For computation of evolutionary distances between relative diplozoid species, for phylogeny reconstruction and evaluating evolutionary history, common statistical methods were used (model test and ML phylogeny approach in MEGA5). For reconstruction of co-phylogeny relationships, basic molecular tools (Jane, TreeMap, Copycat) were used.

Results of molecular-genetic analyses distinguished several of our parasite samples from the other valid species in genus *Paradiplozoon* and supported their uniqueness. To explain the resulting phylogenetic division, we took into account the geographical origin of the samples and host-parasite cophylogeny. The preliminary results confirm highly significant relationship between studied parasites and their hosts and help understand their evolution and speciation. Future plan for this study is to publish obtained results as soon as possible.

## Acknowledgement

The study has been supported from the project no. GBP505/12/G112 from the Grant Agency of the Academy of Sciences of the Czech Republic.

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# Diversity of African parasitic copepods (Crustacea)

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Copepods are commonly associated with microscopic components of marine and freshwater zooplankton. It is estimated that about half of the known copepod species live in symbiotic associations, of which parasitic species dominate [1]. The complexity of parasitic copepod's adaptations to the parasitic way of life usually positively correlates with of their evolutionary association with their hosts [2]. Diversity of parasitic copepods parasitizing African freshwater fishes is relatively high, where approximately 50 species are recently known. Up to now, more than 90 species of African freshwater fishes sampled in Kenya (Lake Turkana), Sudan (White Nile and Blue Nile River), Senegal (Gambia River), Burundi and Democratic Republic of the Congo (Lake Tanganyika) and Morocco were investigated. During 2015 the determination of remaining groups of ergasilid copepods was terminated (except for Lake Tanganyika). Species determination based on morphometric methods revealed 23 different parasitic copepod species classified into 7 genera: *Ergasilus* (8 species), *Lamproglena* (7 species), *Lamproglenoides* (1 species), *Lernaea* (3 species), *Opistholernaea* (2 species), *Lernaeogiraffa* (1 species), *Dysphorus* (1 species). Molecular identification based on two nuclear markers (18S and 28S) supported morphometric differentiation. In total, 15 different 18S and 17 different 28S rDNA sequences of parasitic copepods were newly identified. Our results have proven that the phylogenetic relationship among genera of parasitic copepods correspond with already published cladistic analysis of the Lernaeidae copepods [3].

The future plans include further molecular and morphological assessment of the novel collected material of parasitic copepods and new phylogenetic analysis of African parasitic copepods based on nuclear (18S, 28S) and mitochondrial (COI) markers including sequences of parasites distributed worldwide.

## Acknowledgement

This study was supported by ECIP (European Centre of Ichthyoparasitology); centre of excellence program of the Czech Science Foundation (project No. P505/12/G112)

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# Diversity of basal apicomplexans from marine invertebrates

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Apicomplexa is a large group of unicellular parasites that infect various animals. Majority of investigations concern to the apicomplexans causing harmful diseases of human and domestic animals (malaria, eimeriosis, toxoplasmosis, cryptosporidiosis, etc.), and less attention is paid to the ancestral groups, such as protococcidia, agamococcidia, eu-, archi-, and blastogregarines. The vast investigations dealt mostly with morphology of trophozoites, oocysts, and gametocysts, some of them were performed at electron microscopic level. Nowadays few investigations that combine morphological and molecular-phylogenetic approaches were published [1, 2]. We investigated several representatives of basal lineages of Apicomplexa using the light and electron microscopy, and molecular-phylogenetic methods for several conservative genes.

In our study we focused on description of the new species of eugregarine from Antarctic littoral amphipod. Mostly syzygies were observed, all of them were caudo-frontal. The syzygy partners were elongated and slightly curved. The contact between them looked simple. Both cells in syzygy had a granular cytoplasm and a transparent layer at the cell periphery. Electron microscopic observations revealed that parasites are covered with a typical alveolate pellicle, i.e. plasma membrane is underlain by the inner membrane complex. The pellicle forms characteristic epicytic folds that run longitudinally from one to another end of the parasite. Preliminary molecular-phylogenetic analysis of 18S rRNA gene sequence obtained from this gregarine confirm that this parasite belongs to eugregarines. Further analysis showed (as it was expected) its affiliation to the superclade Cephaloidophoroidea, described previously, which unites eugregarines from various marine and fresh-water crustaceans [1].

Other outcomes from 2015:

New electron microscopic (TEM and SEM) data concerning parasites (*Selenidium* sp., *Siedleckia nematoides*, *Urospora ovalis* and *U. trivisiae*) from marine polychaetes were obtained. In cooperation with A. Valigurová, M. Kováčiková (MUNI, Brno), Russian colleagues T.G. Simdyanov (MSU, Moscow) and G.G. Paskerova (SPbSU, Saint-Petersburg) these data will be used to prepare publications.

Involvement in preparation of several publications: in collaboration with AV and MK [3], and with Russian colleagues [2, 4].

Active participation in conference: Students in Polar Research, April 20-22, Brno, Czech Republic [5, 6]

## Future plans for 2016:

Analyses of selected apicomplexans (eugregarines, archigregarines, agamococcidia) using the methods of electron microscopy, freeze-etching, direct and indirect immunolabelling. Developing of protocols for molecular analyses for different genes.

## Acknowledgement

This study was supported by project № **GBP505/12/G112** from the Czech Science Foundation.

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# Free-living amoebae as potential pathogens of fish

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In the history of salmonid aquaculture, virus and bacterial diseases have been notorious to influence the economy of fish production. Along with these, several diseases caused by eukaryotic microorganisms, such as the whirling disease and proliferative kidney disease, were important almost to the same degree. Currently, also free-living amoebae are recognized as important eukaryotic agents responsible for losses in both seawater- and freshwater-reared salmonids. As against Amoebic gill disease (AGD) of marine fish, Nodular gill disease (NGD) caused by free-living amoebae in rainbow trout has received less attention.

Our recent study targeted to aetiology of Nodular gill disease (NGD) of farmed rainbow trout revealed an intravital colonization of fish gills by the testate amoeba *Rhogostoma minus* Belar, 1921. *Rhogostoma* infection was found in all fish examined microscopically. In contrast, naked amoebae related to fully developed NGD lesions were found in a minority of examined fish. They belonged to four genera, *Acanthamoeba*, *Vermamoeba*, *Naegleria* and *Vannella*. The results obtained in this study contribute to the mosaic of findings that –contrary to Amoebic gill disease (AGD) of marine fish– turn attention to the possibility of heterogeneous, amoeba multi-species and multifactorial aetiology of NGD.

Direct diagnosis of *Naegleria* sp. in rainbow trout gill lesions, frequent findings of these amoebae in asymptomatic fish and the life cycle with actively moving stages support naegleriae as strong candidates among the prospective agents of NGD. Therefore, we analysed a large set of ITS sequences of *Naegleria* strains isolated from fish gills (with 18 new and 16 previously published sequences, the most numerous set of sequences studied so far). Study of phylogenetic relationships of these 34 gill-isolated *Naegleria* strains assigned them to 5 nominal species. Two strains isolated from gills of *Oncorhynchus mykiss* with histologically confirmed signs of NGD were shown to be phylogenetically closely related/identical with *Naegleria dobsoni*.

## Plans for the future research:

Identification of an enigmatic free-living amoeba isolated from NGD-positive rainbow trout collected in an outbreak of this disease in Italy.

All reasonable effort will be made to collect additional material for NGD studies.

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**Dyková I., Týmľ T.** 2015: Testate amoeba *Rhogostoma minus* Belar, 1921 associated with nodular gill disease of rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Journal of Fish Diseases*. doi: 10.1111/jfd.12384 [IF<sub>2014</sub> = 2.056; 13/133 (Q1) in Veterinary Sciences]

**Dyková I.:** Amoebic infections in freshwater salmonids – a challenge for basic and applied research in ichthyoparasitology. Manuscript (14 pp. text, 5 plates of figures), based on an invited contribution to the Fish Veterinary Society Annual Conference 2015, Edinburgh, UK, 24–25th March 2015, has been prepared on request of Fish Veterinary Society and will be published in *Fish Veterinary Journal*.

## Diversity of free-living amoebae

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Free-living amoebae (FLA) are currently considered to be a heterogeneous assemblage of microeukaryotes belonging to five supergroups. For a long time they have been studied using traditional culture-dependent methods based on the morphology of trophozoites and eventual cyst stages. More recent studies introduced a modified culture-dependent approach that integrates morphological study with molecular methods and phylogenetic analyses of selected molecular markers. Thanks to this approach the accuracy of generic and species delineation of FLA improved, the assemblages of amoebae that share evolutionary history could be defined, and the significance of data from a variety of ecosystems increased.

A new amoebozoan species, *Vermistella arctica* n. sp., was described from marine habitats in the central part of Svalbard archipelago. This is the first report on Arctic amoebae belonging to the genus *Vermistella* Moran and Anderson, 2007, the type species of which was described from the opposite pole of the planet. Psychrophily proved in the new strains qualified the genus *Vermistella* as a bipolar taxon. Molecular phylogenetic analyses based on 18S rDNA and actin sequences did not show any affinity of the genus *Vermistella* to *Stygamoeba regulata* ATCC® 50892™ strain that was listed as a member of an *incertae sedis* genus within Rhizopoda. A close phylogenetic relationship was found between *Vermistella* spp. and a sequence originating from an environmental sample from Cariaco basin, the largest marine permanently anoxic system in the world.

Reconstruction of multigene phylogeny of five amoeboid organisms of different origin (isolated from fish organs, soil and digestive tract of earthworm) that share unique microscopical and ultrastructural features including type and arrangement of mitochondrial cristae revealed that they belong to the same single-genus *Copromyxa* clade. The position of *Copromyxa cantabrigiensis* (syn. *Hartmannella cantabrigiensis*) within this genus is strongly supported, a new name, *Copromyxa expectata* n. nom. (syn. *Vexillifera expectata*) justified, and a new species, *Copromyxa laresi* n. sp. described. Evaluation of ultrastructural data indicated possibility that another limax amoeba, *Cashia*, also belongs to the *Copromyxa* clade.

### Plans for the future research

Identification of vahlkampfiid amoebae isolated from Arctic and Antarctic regions.

Characterization of a novel generic-level lineage of Tubulinea.

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Tým T., Kostka M., Ditrich O., Dyková I. 2015: *Vermistella arctica* n. sp. nominates the genus *Vermistella* as a candidate for taxon with bipolar distribution. *Journal of Eukaryotic Microbiology*. doi: 10.1111/jeu.12270 [IF<sub>2014</sub> = 3.217; 37/119 (Q2) in Microbiology]

Kostka M., Lares-Jiménez L.F., Tým T., Dyková I.: *Copromyxa laresi* n. sp. and molecular phylogeny of *Copromyxa* (Amoebozoa): support for *Copromyxa cantabrigiensis* and for prospective transfer of *Cashia limacoides* into *Copromyxa*. (Submitted to *Journal of Eukaryotic Microbiology*) [IF<sub>2014</sub> = 3.217; 37/119 (Q2) in Microbiology]

# Free-living amoebae as hosts of prokaryotic organisms

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The search for symbiotic associations of free-living amoebae with prokaryotes continues to be an integral part of our study of FLA. This approach is motivated by the fact that amoebae play an important ecological role as predators of microbial communities, serve as niche for bacterial replication and among their endosymbionts harbour prokaryotes pathogenic for vertebrates. The study of ultrastructure, which is a regular part of characterization of newly isolated strains, facilitates selection of symbiotic associations worth of detailed studies. In the last two decades, numerous reports described obligate intracellular symbionts in freshwater amoebae whereas marine species were rather neglected.

Currently, bacterial endosymbionts, tentatively named '*Candidatus* *Occultobacter vannellae*' and '*Candidatus* *Nucleophilum amoebae*' were found in newly isolated marine amoebae. One amoeba isolate is related to *Vannella*, a genus common in marine habitats, the other represents a completely novel lineage in the Amoebozoa. The endosymbionts showed only low similarity to known bacteria (85-88% 16S rRNA sequence similarity) but together with other uncultured marine bacteria formed a sister clade to the *Coxiellaceae*. Using fluorescence *in situ* hybridization and transmission electron microscopy, identity and intracellular location of both symbionts could be confirmed; one was replicating in host-derived vacuoles, whereas the other was located in the perinuclear space of its host cell. The new isolates represent easily maintainable model systems and pave the way for further studies on marine associations between amoebae and bacterial symbionts.

A novel chlamydial endosymbiont tentatively named '*Candidatus* *Neptunochlamydia vexilliferae*' was found in a *Vexillifera* strain isolated from a Tyrrhenian coastal lake. Transmission electron microscopy in combination with fluorescence *in situ* hybridization was used to prove the intracellular localization of these bacteria representing the first strain of marine chlamydiae that can be stably maintained alongside with their host in a laboratory culture.

## Plans for the future research

Identification of endocytobiont found in ZER3 strain of *Rhogostoma minus* isolated from *Oncorhynchus mykiss*.

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# Motility of *Sphaerospora molnari* blood stages in common carp: actin involvement

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The myxozoan parasite *Sphaerospora molnari* is the causative agent of gill sphaerosporosis in common carp (*Cyprinus carpio*). Its route of infection is still not well established, since the portal of entry into the host is unclear. However, pre-sporogonic stages proliferate in and spread with the blood stream throughout the body and reach the gills, their target organ where sporogenesis takes place. Blood stages of *S. molnari* in severely infected carp fingerlings can be found in heavy vascularised host tissues like gills, kidney, swim bladder and rete mirabile [1]. In fresh blood or tissue squashes, such stages present a swinging or dancing movement with rapid changes in direction. It is generated by the rapid formation and re-absorption of crests and folds on the primary cell surface. Our final purpose in this study is to decipher, whether this peculiar movement is used by the parasite as immune evasion strategy from the host phagocytes.

Previous studies on myxozoan motility demonstrated the involvement of actomyosin machinery due to its strong phalloidin staining at active sites [2]. The actin-specific motility inhibitor Cytochalasin D is able to impede the movement of *S. molnari* blood stages at extremely low concentrations. Nevertheless, specific actin stain by Phalloidin or common antibody immunolabelling was unsuccessful. *S. molnari* transcriptomic data showed the existence of two actin isoforms, one of which is very derived and highly overexpressed. Following the sequence of *S. molnari*'s derived actin isoform, synthetic peptides were produced and used as immunogen to obtain rabbit polyclonal antibodies (SmPab). Immunolabelling was then studied on the parasite's blood stages.

*S. molnari* blood stages, freshly obtained from infected carp, were paraformaldehyde-fixed on SuperfrostPlus microscope slides, permeabilized with Triton X100 and incubated with the anti-actin SmPab. Indirect labelling with a fluorescent-conjugated secondary antibody and counterstaining with DAPI were performed. An intense immunodetection of the derived actin form was observed in the primary cells of unicellular and multicellular parasite blood stages, under confocal microscope.

For TEM immunodetection, fresh parasite stages in blood pellet were paraformaldehyde/glutaraldehyde-fixed, gelatine-embedded, incubated in sucrose and nitrogen-frozen. Cryosections were then incubated with the anti-actin SmPab and immunogold-labelled. Immunogoldparticles were localized by TEM specifically in the cytoplasm of primary cells. The high specificity of the new anti-actin SmPab was demonstrated. With this useful tool we could observe the high expression of the derived actin isoform in *S. molnari* blood stages, which is responsible for their peculiar motility.

## Future plans

*In vitro* phagocytosis assays with carp head kidney phagocytes are currently being performed in order to assess a differential response towards normal parasite blood stages, compared to Cytochalasin D-inhibited ones.

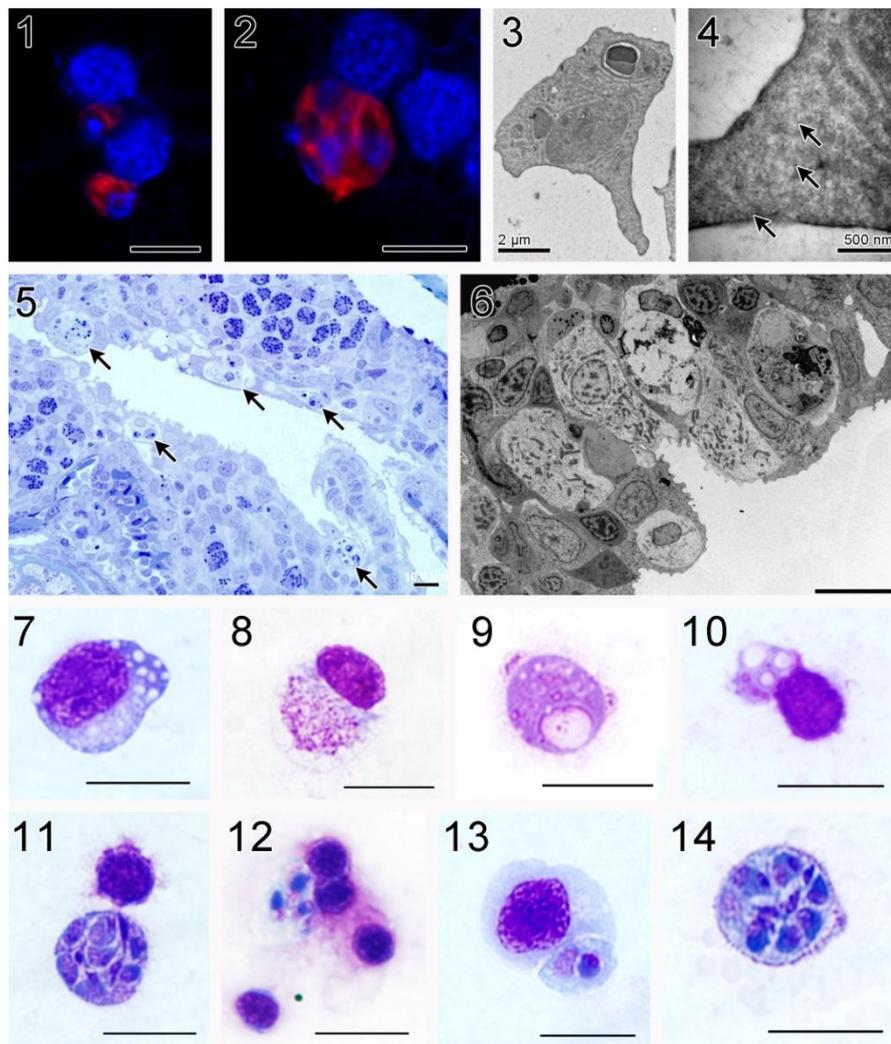
## Acknowledgement:

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**Figure 1.** *Sphaerospora molnari* blood stages immunolabelling with the specific SmPab in confocal microscopy: goat anti-rabbit\*Alexa594 (red) (1, 2) and in TEM: 6 nm immunogold particles (arrows) (3, 4). *S. molnari* infected gill epithelium with phagocytes containing cell debris (arrows) (Toluidine blue 5, TEM 6). *In vitro* assay: common carp phagocytes (7-10) and their interactions with *S. molnari* blood stages. Scale bars = 10  $\mu$ m, unless differently indicated.

# Nematogalectins: New markers for myxozoan phylogeny

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Current myxozoan phylogeny relies on single SSU rDNA or concatenated analysis of SSU + LSU rDNA and EF2 genes. These markers, however, are not sufficient to reconstruct the pattern of some early branching nodes that are important for the understanding of the main phylogenetic trends of Myxozoa. These weakly supported nodes show unstable branching patterns or large polytomies. Furthermore rDNA markers are prone to artefacts of phylogenetic reconstruction such as “Long branch attraction (LBA)”.

Multigene analysis with large taxon sampling will resolve most of the polytomic phylogenies as observed in other biological groups and provide greater stability in myxozoan interspecies relationships. We thus used publically available genomic/transcriptomic data as well as our own *Sphaerospora molnari* transcriptome to select new candidate genes that are phylogenetically informative and suitable for myxozoan phylogenetic reconstruction.

During genome/transcriptome mining we selected the following myxozoan genes for further analysis: V-ATPase, PAX-B (paired box), Elongation Factor 1 $\alpha$ , PGD (6-phosphogluconate dehydrogenase), SNF (sans fille), TPI (triosephosphate isomerase), as well as **nematogalectins** previously identified in Myxozoa [1] and known as taxon (cnidarian)-specific genes.

We designed specific primers for Nematogalectin A and Nematogalectin-related genes in order to amplify these genes from variety of myxozoan species to access the suitability of these markers for resolving myxozoan phylogeny.

The phylogenetic tree based on two nematogalectin genes corresponds to known rDNA-based myxosporean phylogeny (Fiala et al. 2015). We have revealed high phylogenetic informativeness per site of these two markers using the PhyDesign program. Furthermore, comparison of branch length of nematogalectins A and SSU rDNA based trees revealed balanced branches in nematogalectins A in contrast to long branches in SSU rDNA tree. Therefore, nematogalectin A-based phylogeny should not be sensitive to LBA and provides us a more reliable phylogenetic reconstruction. Multigene analysis of SSU rDNAs, EF2 and nematogalectins resulted in a well resolved myxozoan tree with highly supported nodes [2].

## Future plans:

- i) To continue in amplification of nematogalectins of wide range of myxozoans to cover all myxozoan taxonomic groups
- ii) To amplify other genes selected by genome mining (V-ATPase etc.)
- iii) To perform a large multigene analysis to better assess the myxozoan phylogeny
- iv) To reconstruct a cnidarian evolution using newly obtained myxozoan data to clarify the myxozoan evolution

## Acknowledgement:

This study was supported by ECIP (European Centre of IchthyoParasitology); Centre of Excellence Program of the Czech Science Foundation; project No. P505/12/G112.

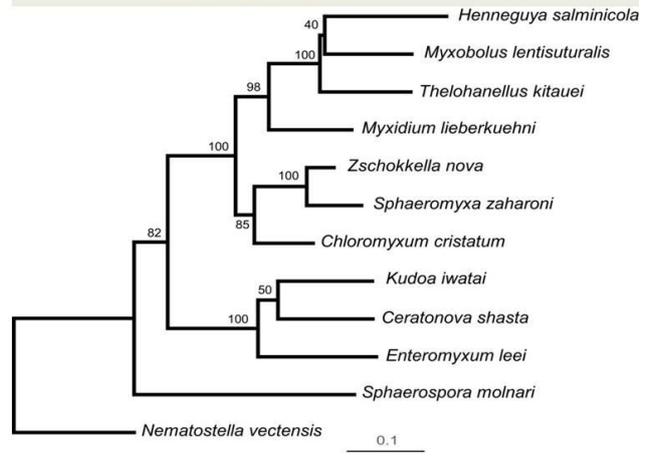
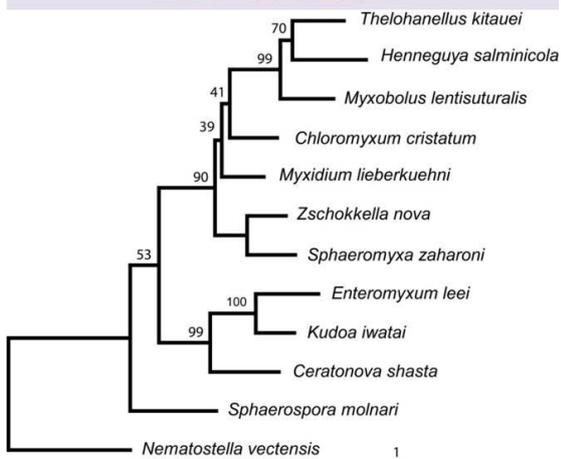
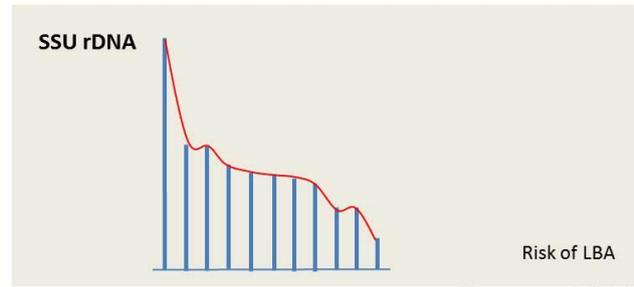
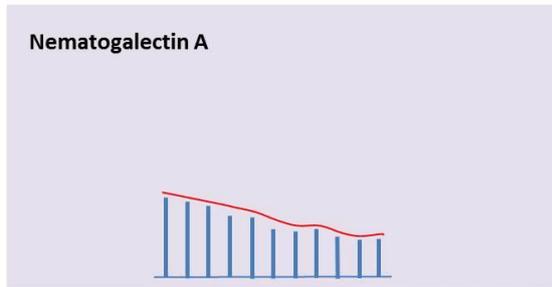
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### Branch lengths comparison



# Dactylogyrids (Monogenea: Dactylogyridae) parasitizing African catfishes (Siluriformes)

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Survey on dactylogyrids parasitizing catfishes was carried out in White Nile and Blue Nile, Sudan, in 2014. Dactylogyrids were isolated from 12 siluriform species, belonging to six families (Bagridae, Clariidae, Claroteidae, Malapteruridae, Mochokidae and Schilbeidae). Dactylogyrid species of the following genera were found: *Bagrobdella*, *Protoancylodiscoides*, *Quadriacanthus*, *Schilbetrema* and *Synodontella*. The taxonomic evaluation has been made on the basis of morphology, and also the partial 28S rDNA (LSU) sequences have been analysed for a purpose of species identification and evaluation of the phylogenetic positions of representatives of dactylogyrids infecting African catfishes within the family Dactylogyridae (**Fig. 1**) [1] (also see abstract of MS).

Detailed taxonomic evaluation of monogeneans belonging to *Quadriacanthus* revealed the presence of five species (one new) from the gills of 4 species of catfishes: *Quadriacanthus bagrae* Paperna, 1979 from *Bagrus bajad* and *Bagrus docmac* (Bagridae); *Quadriacanthus aegypticus* El-Naggar & Serag, 1986, *Quadriacanthus clariadis* Paperna, 1961, and *Quadriacanthus numidus* Kritsky & Kulo, 1988 from *Clarias gariepinus* (Clariidae); and *Quadriacanthus mandibularis* n. sp. (**Fig. 2**) from *Heterobranchus bidorsalis* (Clariidae). The new species resembles *Quadriacanthus thysi* N'Douba, Lambert & Euzet, 1999, but clearly differs from this species by having a male copulatory organ with spoon-like base, broad copulatory tube with subterminal flap, and basally articulated accessory piece with constricted medial part and hook-shaped terminal portion [2].

## Future plans

1. morphological and molecular characterization of *Bagrobdella* spp., including one new species
2. processing the material collected in Senegal (dactylogyrids of siluriforms) and during other campaigns

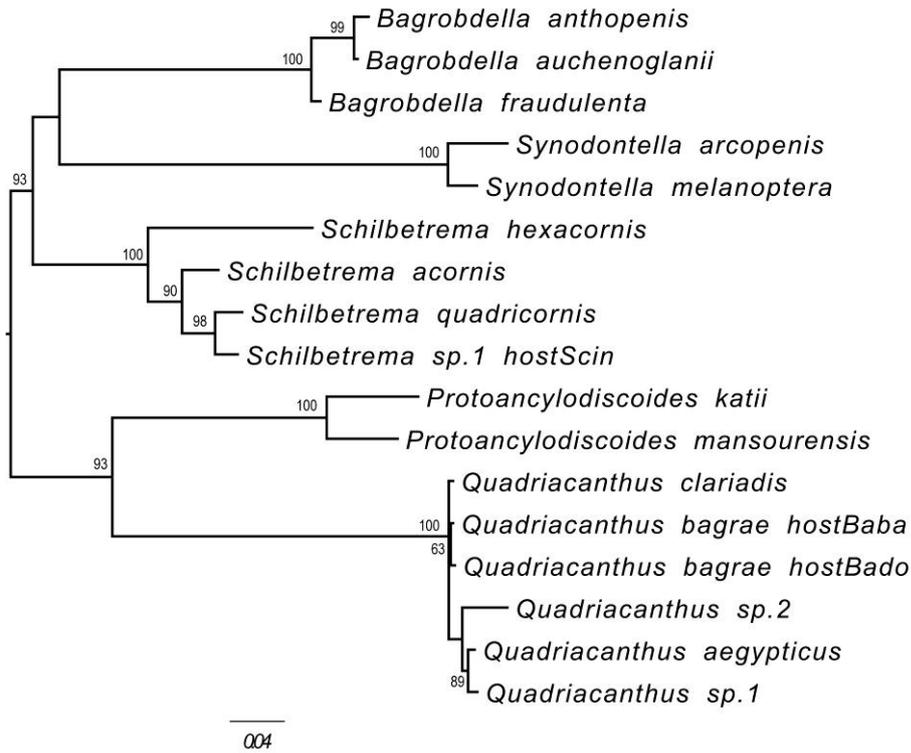
## Acknowledgement

The studies have been supported from the project no. **GBP505/12/G112** from the Grant Agency of the Academy of Sciences of the Czech Republic.

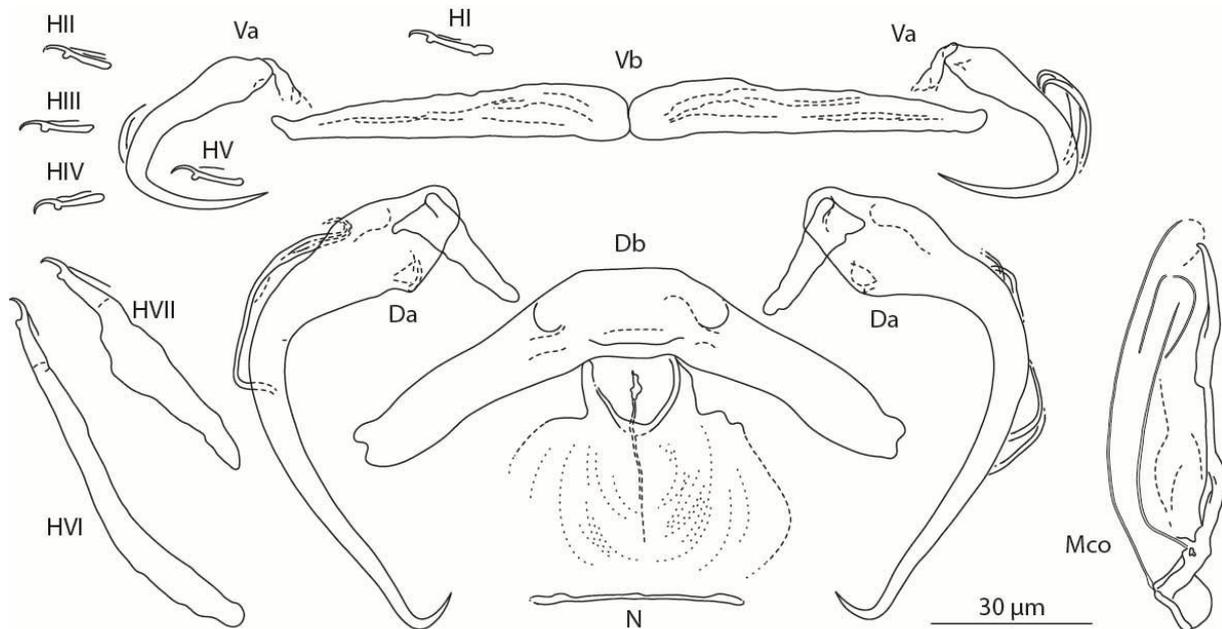
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**Figure 1:** Molecular phylogeny of dactylogyrids parasitizing African catfishes. Unrooted maximum likelihood (RAxML) tree of partial nuclear LSU rDNA shows the phylogenetic relationship of dactylogyrid species parasitizing African catfishes. Bootstrap values were calculated with the rapid bootstrap method with 1,000 replicates. Support is shown for nodes with >50% bootstrap support.



**Figure 2:** *Quadriacanthus mandibularis* n. sp. (Va) ventral anchor, (Vb) ventral bar, (Da) dorsal anchor, (Db) dorsal bar, (N) needle-like structure, (HI-HVII) hooks, (Mco) male copulatory organ.

# Molecular taxonomy of *Diplostomum* spp. (Digenea: Diplostomidae) from freshwater fishes in China

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Species of *Diplostomum* von Nordmann, 1832 represent widely distributed fish pathogens with high impact on wild and farmed freshwater fish populations. The problematic species identification of the metacercariae impedes the assessment of their diversity, distribution and host ranges. In spite of their wide geographical distribution within the Palaearctic, *Diplostomum* spp. are poorly studied in Asia. In order to clarify the taxonomy, phylogenetic relationships and host and geographical distribution of *Diplostomum* spp. in Asia, we performed a large-scale host sampling and phylogenetic analysis together with detailed morphological examination of the metacercariae of *Diplostomum* from freshwater fishes in ten localities in China.

A total of 45 fish species belonging to eight families (Bagridae, Catostomidae, Channidae, Cobitidae, Cyprinidae, Gobiidae, Percichthyidae and Siluridae) was sampled opportunistically from eight freshwater bodies (six lakes, a fish farm and a reservoir) in China during 2010–2015. Only lens-infecting specimens of *Diplostomum* were recovered from 31 fish species, predominantly cyprinids. Morphological characterisation of the metacercariae was based on fixed specimens prior to sequencing. Molecular identification was achieved *via* barcoding (sequences for the *cox1* mitochondrial gene). Analyses of the novel *cox1* dataset revealed the presence of five genetically distinct lineages of *Diplostomum* in the fish populations studied in the freshwater habitats in China; their genetic diversity was assessed and visualised using haplotype networks.

Integrated morphological and molecular approaches to species delineation provided evidence for the presence of at least five species of *Diplostomum* in China. We provide the first morphological characterisation and description of the metacercariae of these *Diplostomum* spp. This integrated approach will provide a baseline for species identification of these important fish pathogens and will advance further studies on the distribution of *Diplostomum* spp. in Asia.

## Plan for 2016

- (i) Publication of the present results;
- (ii) Molecular and morphological characterisation of adult diplostomids from various fish-eating bird hosts from Europe;
- (iii) Molecular characterisation and phylogenetic relationships of metacercariae of *Diplostomum* spp. from various fish populations in Europe;
- (iv) Molecular characterisation of strigeoid cercariae from Central Europe;
- (v) Diversity of digeneans in sparid fishes along the Algerian coasts of the Western Mediterranean;
- (vi) Molecular characterisation of digeneans in Antarctic fishes.

## Acknowledgement

This research was supported by ECIP (European Centre of Ichthyoparasitology); Centre of Excellence programme of the Czech Science Foundation (Project No. P505/12/G112).

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# How can we better understand host-parasite interactions?

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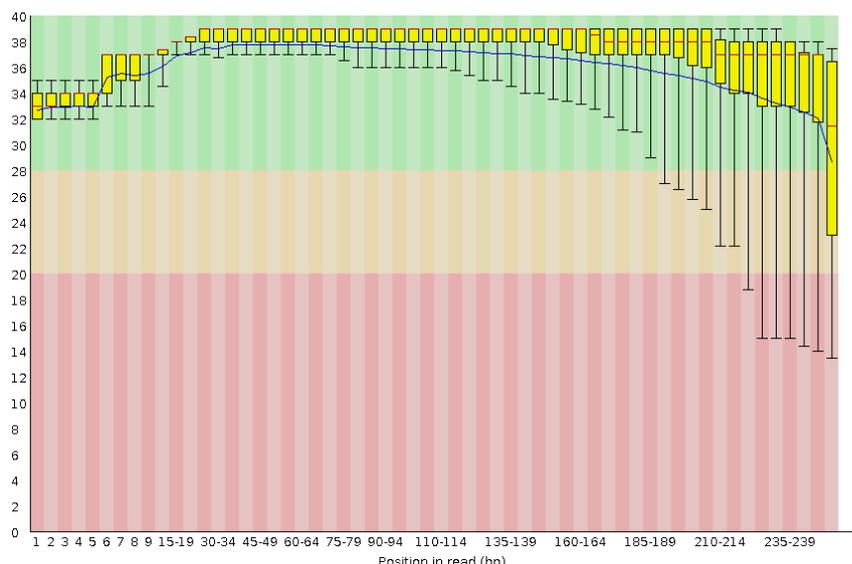
Host-parasite interactions are formed in time as a result of the complex co-evolutionary processes between the two interacting species, i.e. host and its parasite. Defense mechanisms which are involved in host-parasite interactions are genetically encoded in the genomes of the both host and parasite. Therefore, the recent growth of interest in studying the genome-for-genome interactions has become critical to understand the host-parasite interactions at the molecular level. As a result, there has been recently a boom in the genome and transcriptome characterization of the both parasite and host species which may help us in better understanding of gene's structure and function as well as their possible role in host-parasite interactions.

In our study, we firstly aimed to study *de novo* the transcriptome (a set of genes transcribed in a given tissue under specific conditions) of the two non-model fish species - *Barbus barbus* and *Barbus meridionalis*. Together, four *B. barbus* and *B. meridionalis* individuals were collected on the Pertuis and Céze Rivers (southern France). The mRNA was obtained from the different tissues of each individual (i.e. brain, fin, liver, spleen, intestine and gills) to compare tissue-specific expression patterns. Subsequently, cDNA libraries were prepared for both Illumina MiSeq a HiSeq platforms. So far, high data quality sequences were obtained from Illumina MiSeq platform (Figure 1).

Using the knowledge of the transcriptome structure in the *Barbus* fishes will help us to select different genes that play potentially an important role in the defensive mechanisms of the host immune system. Moreover, comparison of the expression level of these genes in link with the different level of parasite infection is planned for the near future. However, this will be only one piece of puzzle to better understand the host-parasite interactions since there is a need to explore the genetic mechanisms for parasite strategy to infect and survive in its host.

## Acknowledgement:

LG and AVŠ were financially supported by ECIP - Centre of excellence, GACR No P505/12/G112.



**Figure 1:** Example of the quality scores across all bases (Illumina, MiSeq)

# Deciphering the coevolutionary history of myxozoans and their hosts

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The combination of exceptionally high species diversity, extreme differences in the degree of host specificity and a complex life cycle raises many questions about the underlying mechanisms triggering speciation and evolution of the Myxozoa. That parasites and their hosts coevolve is a powerful concept with broad theoretical and applied implications. On a basic level, the term ‘coevolution’ is used to describe the extent of congruence of host and parasite phylogenetic trees. In the present study, we used all SSU rDNA sequence data of actinosporeans and their definitive invertebrate hosts as well as of myxosporeans and their intermediate vertebrate hosts to assess the extent of codivergence, i.e. parallel speciation in host and parasite lineages, by examining the degree of congruence between their phylogenies. Using event-based and global fit methods (TreeMap, Jane, ParaFit), we investigated the origin of current associations and determined whether they arose by colonization (host switching) or by descent. We detected strong cophylogenetic signal between myxozoans and their invertebrate hosts (Fig. 1), with regard to the deeper branches of the tree [1]. In contrast, we demonstrate that cospeciation with fish hosts happened more recently and resulted in several host-associated species complexes. We also found phylogenetically conserved host switching to mimic the phylogenetic signature of cospeciation, confounding topology-based programs. We speculate that myxozoans first had a direct life cycle involving only invertebrate hosts and that the acquisition of vertebrate intermediate hosts happened later [2] and was the outcome of the distinct success of alternative transmission and dispersion strategies, resulting in massive speciation events in fish.

## Future plans:

Use more comprehensive data sets of host genes for cophylogenetic analysis (Copycat program)

BLAST published genomes and transcriptomes of myxozoans of all major clades for a specific set of housekeeping genes and use PCR and DNA sequencing of the ones not available in online databases.

Use this dataset to date myxozoan evolution and cospeciation events in a molecular clock analysis of myxozoans and all known hosts as well as related animal groups using the BEAST (Bayesian Evolutionary Analysis Sampling Trees) platform

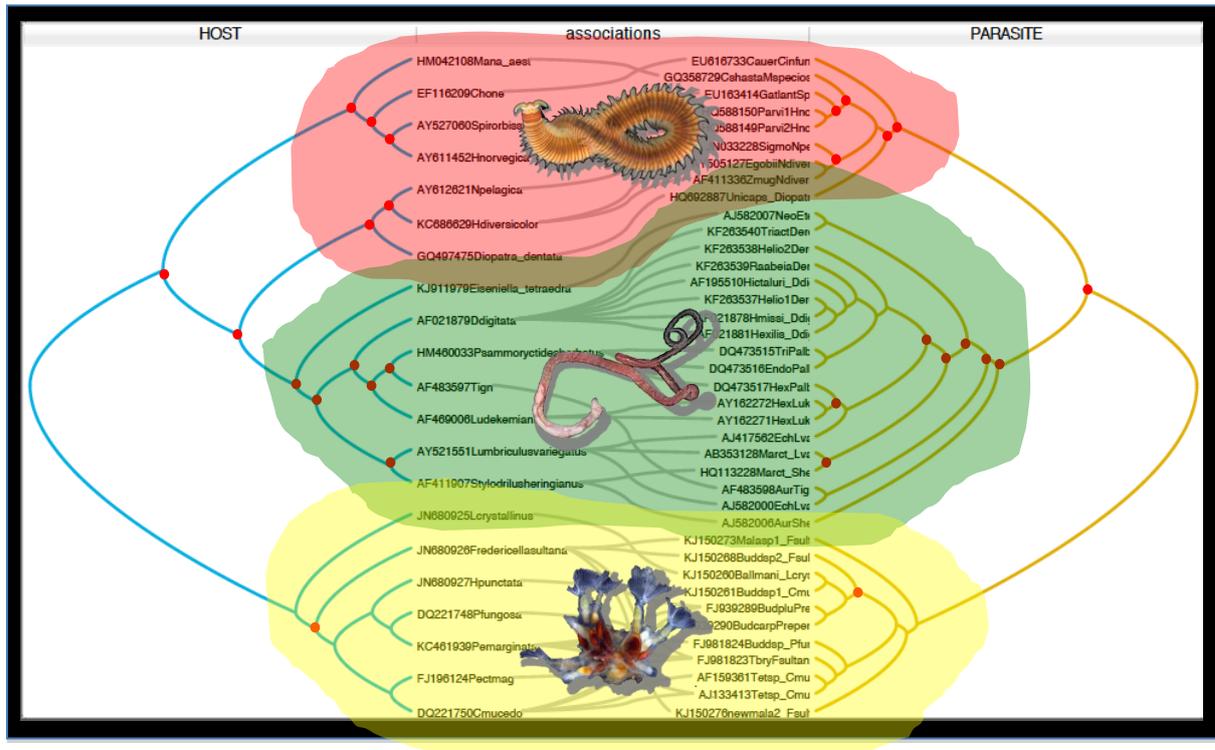
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This study was supported by ECIP (European Centre of IchthyoParasitology); centre of excellence program of the Czech Science Foundation; project No. P505/12/G112)

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**Figure 1.** Cophylogeny of myxozoans and their invertebrate hosts as (Oligochaetes, polychaetes and bryozoans) estimated by TreeMap 3.0 (event-based method) with number of cospeciation events=15 (red dots),  $p=0.001$  (highly significant cophylogeny).

# Cystatin: the cysteine peptidase inhibitor from *Eudiplozoon nipponicum* (Monogenea)

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*Eudiplozoon nipponicum* (Monogenea, Diplozoidae) typically inhabiting the gills of carp (*Cyprinus carpio*) is widely distributed Eurasian blood-feeding ectoparasite.

The foregoing research of diplozoid Monogenea has been predominately oriented towards understanding morphology and ecology of particular species. Nevertheless there is a lack of information regarding functional molecules synthesized by this taxonomic group.

Among important molecules of various parasitic species belong cystatins (inhibitors of cysteine peptidases). Besides regulation of endogenous processes in parasite bodies they play a substantial role e.g. in manipulation of the host immune system and/or regulation of blood digestion process.

The aim of our experimental work is to reveal the presence, structure and function of *E. nipponicum* cystatin DNA/protein molecules using bioinformatic, molecular and proteomic methods.

The gene coding cystatin of *E. nipponicum* was expressed in heterologous *E. coli* (BL21) system using pET19b expression vector. IPTG induced expression (22 °C, 6 hours) was analyzed on SDS-PAGE and soluble recombinant cystatin of *E. nipponicum* was purified on Ni-NTA agarose column. Production of cystatin was verified by both mass spectrometry (Orbitrap) and Western Blot using His-tag specific antibodies.

Activity of recombinant inhibitor was tested by inhibitory assay using recombinant *E. nipponicum* cathepsin L and fluorogenic substrate Z-FR-AMC.

Soluble recombinant cystatin was used for immunization of ICR mice. The polyclonal anti-cystatin sera reacted specifically with the crude extract of *E. nipponicum* and recombinant cystatin of *E. nipponicum* (Western blot).

## Future plans

Anti-cystatin antibodies will be used in immune-histochemical localization study of cystatin on cryosections of *E. nipponicum*. Comprehensive inhibitory assays will be performed to assess the inhibitory properties of *E. nipponicum* cystatin.

## Acknowledgement

The study have been supported from the project no. GBP505/12/G112 from the Grant Agency of the Academy of Sciences of the Czech Republic.

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Ilgová J., Gelnar M., Kašný M. 2015. Cystatin: the cysteine peptidase inhibitor from *Eudiplozoon nipponicum* (Monogenea). In 9<sup>th</sup> International Symposium of Fish Parasites.

## Effect of exotic species on host-parasite interactions

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Exotic hosts can act as reservoirs for native parasites, potentially threatening native hosts ('parasite spillback'). Alternatively, if it is an unsuitable host (i.e. the parasite is attracted to the host but fails to develop), it can act as an infection sink, reducing the pool of parasites infecting native hosts ('dilution effect'). Presence of an exotic host may also provide a novel resource for exotic parasites, introduced by a previously established exotic species ('invasional meltdown').

In our studies, we focus on fish-glochidia (parasitic larvae of unionid mussels) interactions in a model lowland river system previously dominated by cyprinids and unionids (duck mussel, *Anodonta anatine*, and swollen mussel, *Unio tumidus*). Since invasion by both a fish (round goby, *Neogobius melanostomus*) and mussel (Chinese pond mussel, *Sinanodonta woodiana*) species, round gobies have quickly come to dominate in the system.

Our studies show that round goby is an attractive host for glochidia of several mussel species, including those of the invasive Chinese pond mussel, with even young-of-the-year (YOY) gobies commonly infected. This is in sharp contrast with YOY cyprinids (Šlapanský et al. 2015). Our pilot study demonstrated that glochidial infection was non-lethal for gobies, with even YOY mortality apparently unaffected. Not only do gobies dominate the host assemblage, therefore, they are also the most attractive host. The way in which mussels utilise this dominant resource could prove decisive as regards competition between the different mussel species.

Our ongoing experiments, conducted in cooperation with the Czech University of Life Sciences in Prague (K. Douda), aim to identify differences in the suitability of gobies as regards development of glochidia from the various mussel species. The experiments will show (a) whether gobies are suitable hosts (compared to native hosts) and whether high infection rates result in a dilution effect or parasite spillback; and (b) whether the invasive mussel is more effective in utilising the new host (i.e. regarding completion of development), thus providing evidence for the invasional meltdown/resistance hypotheses.

### Future plans

We plan to continue the compatibility experiments in 2016, with subsequent data analysis and manuscript preparation.

### Acknowledgement

This work was supported by ECIP (European Centre of IchthyoParasitology) under the Czech Science Foundation's Centre of Excellence Program, Project No. P505/12/G112.

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## Monogenea: From sequences to molecules...

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In a first part of our pilot study of monogeneans molecules we adopted the NGS techniques in order to get the high quality “monogenean-polyopisthocotylean genome/transcriptome matrix”. In a second part of our study this platform was used for the identification of the monogeneans’ dominant protein molecules followed by their further molecular/biochemical characterization. For this purpose we adopted *Eudiplozoon nipponicum* as a experimental model species; the representative of blood-feeding monogeneans (family Diplozoidae) of fresh water fishes, the ectoparasite from the gills of *Cyprinus carpio*.

The bioinformatic analyses of genomic sequence data were performed; the first genome assembly of 98 mil of *E. nipponicum* DNA (3 libraries) sequence reads was done and the calculation of genome size was initiated. Up to now, the low coverage (6%) was reached and therefore the distorted number of bases of whole *E. nipponicum* genome (400 Gb) was estimated.

By adoption of homology searches of RNA 158 753 271 bases/223 887 transcripts the 9757 contigs (>1 kb) were assembled and particular protein molecules in *E. nipponicum* transcriptome data were identified, e.g. peptidases/inhibitors; 29 contigs of cysteine peptidases (e.g. cathepsin L) and 7 contigs of their inhibitors (e.g. cystatins); 12 contigs of serine peptidases (e.g. cathepsin A) and 7 contigs of their inhibitors (e.g. serpin).

Employing biochemical, proteomic and molecular tools, we found that cysteine peptidase activities prevailed in soluble protein extracts and excretory/secretory (E/S) products of *E. nipponicum* and the major part of activity was related to cathepsin L-like. Mass spectrometry revealed several tryptic peptides in E/S products matching to two translated sequences of cathepsin L genes. The dominance of cysteine peptidases of cathepsin L type in *E. nipponicum* resembles the situation in, e.g., fasciolid trematodes. The cathepsin L3 was cloned and expressed in both bacterial and yeasts expression systems. The recombinant enzyme was purified on Ni-NTA agarose column and the experiments focused on its molecular/biochemical properties were started.

The gene coding cysteine peptidase inhibitor - cystatin of *E. nipponicum* was cloned and expressed in *E. coli* (BL21) system using pET19b expression vector. The yield of expression was analyzed on SDS-PAGE and recombinant cystatin was purified on Ni-NTA agarose column. Production of cystatin was confirmed by mass spectrometry and Western Blot (using His-tag specific antibodies). Activity of recombinant inhibitor was tested by inhibitory assay using recombinant *E. nipponicum* cathepsin L and fluorogenic substrate Z-FR-AMC.

The experiments focused on cloning and expression of two *E. nipponicum* serine peptidases (cathepsin A, Prolyl endopeptidase) have been started.

Two genes coding serpins, were expressed in *E. coli* system (BL21 strain) in combination with synthetic genes, optimised for this system, using pET-22B(+) expression vector. IPTG induced expression (30°C, overnight) was analyzed on SDS-PAGE and soluble recombinant serpins of *E. nipponicum* was partially purified using Ni-NTA agarose beads. Successful production of serpins was verified by mass spectrometry and Western blot using His-tag primary antibodies.

Two other protein families of potential anticoagulants – annexins and Kunitz-domain proteins were discovered. The selected recombinant Kunitz-domain gene was cloned into the pET-22B(+) expression vector and successfully expressed in *E. Coli* (BL 21 strain). Soluble recombinant Kunitz-domain protein of *E. nipponicum* was purified by using Ni-NTA beads and its presence confirmed by Mass spectrometry and Western Blot.

### Future plans

- Finish the genome project of *E. nipponicum* and publish a draft genome.
- Finish the transcriptome project of *E. nipponicum*
- Perform the detail biochemical analyses of expressed recombinant molecules (*E. nipponicum* cathepsin L, cystatin, cathepsin A, prolyl endopeptidase, serpin, kunitz-domain proteins)

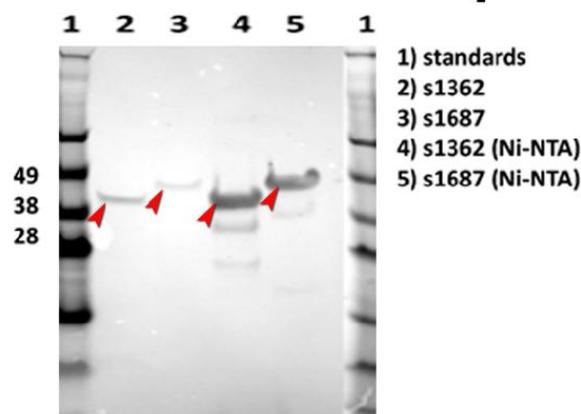
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## Recombinant serpins



# Successful biomanipulation of reservoir fish communities may have long-term consequences for fish parasite populations.

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Biomanipulation, a commonly applied measure for improving water quality in recreational and drinking water reservoirs, involves adjustment of fish community structure through removal of early-stage planktonophagous fish and perch eggs, along with removal of adult planktonophagous fish during spawning. Predatory fish are also stocked to increase juvenile mortality of the target species. As a result, the development of filtering zooplankton should increase, along with overall water quality. The Institute of Vertebrate Biology (IVB) is monitoring a number of such studies in the Czech Republic, both as regards feasibility and in relation to parasitological studies.

Biomanipulation at a 259 ha reservoir near Brno (Jurajda et al. 2015a) was only partially successful as adult bream numbers were reduced but small cyprinid numbers increased. This was partly due to low numbers of predatory fish, despite stocking, due to an increase in angling pressure. Biomanipulation at the 66 ha Hamry drinking water reservoir (no recreational pressure, angling prohibited) was more successful, due in part to a lack of recreational and angling pressure (Jurajda et al. 2015b). However, while reduced cyprinid biomass resulted in a continuous increase in zooplankton density and biomass, phytoplankton dynamics appeared to be dependent on additional factors, such as nutrient input (esp. phosphorus).

In addition to its intended effect on water quality, however, successful biomanipulation of reservoir fish communities may also have unexpected long-term consequences on fish health. Parasitological investigations at multiple reservoirs in cooperation with colleagues from the Veterinary and Pharmaceutic University in Brno (E. Jelínková) have indicated that ergasilosis incidence (caused by the parasite *Ergasilus sieboldi*) often increases following biomanipulation as zooplankton (including the nauplius and copepodite stages of arthropod parasites) populations increase due to lowered predation.

Our results have shown that biomanipulation is unlikely to prove successful in the long-term in waters with high recreational pressure. On the other hand, even apparent success in the absence of such pressure can have unforeseen consequences on long-term fish health.

## Future plan for 2016:

- We will continue the biomanipulation of fish stocks at the Hamry reservoir and monitoring of external impacts on water quality.
- We will widen our monitoring of the relationship between parasites, fish density and fish community composition.
- We will compare our results from Hamry and Brno with other biomanipulation projects at reservoirs under differing levels of recreational pressure.

## Acknowledgement:

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# Dactylogyrids from African tetras – morphological and molecular analyses

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African tetras (Characiformes: Alestidae) are known to harbor monogenean species belonging to three dactylogyrid genera, i.e. *Annulotrema* (39 species), *Characidotrema* (10 species) and *Afrocleidodiscus* (1 species). Our survey of monogeneans found on the gills of 4 species (3 genera, i.e. *Alestes*, *Hydrocynus* and *Brycinus*) of tetras from Lake Turkana, Kenya, revealed the presence of four new and four previously described species of *Annulotrema*: *A. alestesnursi* Paperna, 1973 (from *Brycinus nurse*); *A. ansatum* n. sp., *A. besalis* Řehulková, Musilová and Gelnar, 2014, *A. bipatens* n. sp., *A. cucullatum* n. sp., *A. nili* Paperna, 1973, and *A. pontile* n. sp. (from *Hydrocynus forskahlii*); and *A. elongata* Paperna and Thurston, 1969 (from *Alestes baremoze* and *Alestes dentex*). *Annulotrema elongata* was re-described on the basis of new material from *A. baremoze*. *Hydrocynus forskahlii* was found as a new host for *A. besalis*. The findings of *A. besalis* and *A. elongata* in Kenya represent a new locality records for these helminths [1]. During taxonomic evaluation of monogeneans collected from the gills of *Hydrocynus vittatus* from Lake Kariba, Zimbabwe, 4 species of *Annulotrema* and 1 species of *Afrocleidodiscus* were found. Three of the dactylogyrid species are probably new to science [2].

Morphological analysis of sclerotized structures of the *Annulotrema* species examined suggests that there are at least 3 morpho-groups within these parasites, with regard to the basic structure of the male copulatory organ (MCO). The division into three groups based on morphological criteria was also supported by the results of the phylogenetic analysis using 28S rDNA sequences [3] (Fig.1). However, a wider range of sampling from other species of the Alestidae will be needed to evaluate the phylogenetic importance of the morphology of the MCO in order to elucidating relationships among species within dactylogyrids parasitizing African tetras.

## Future plans

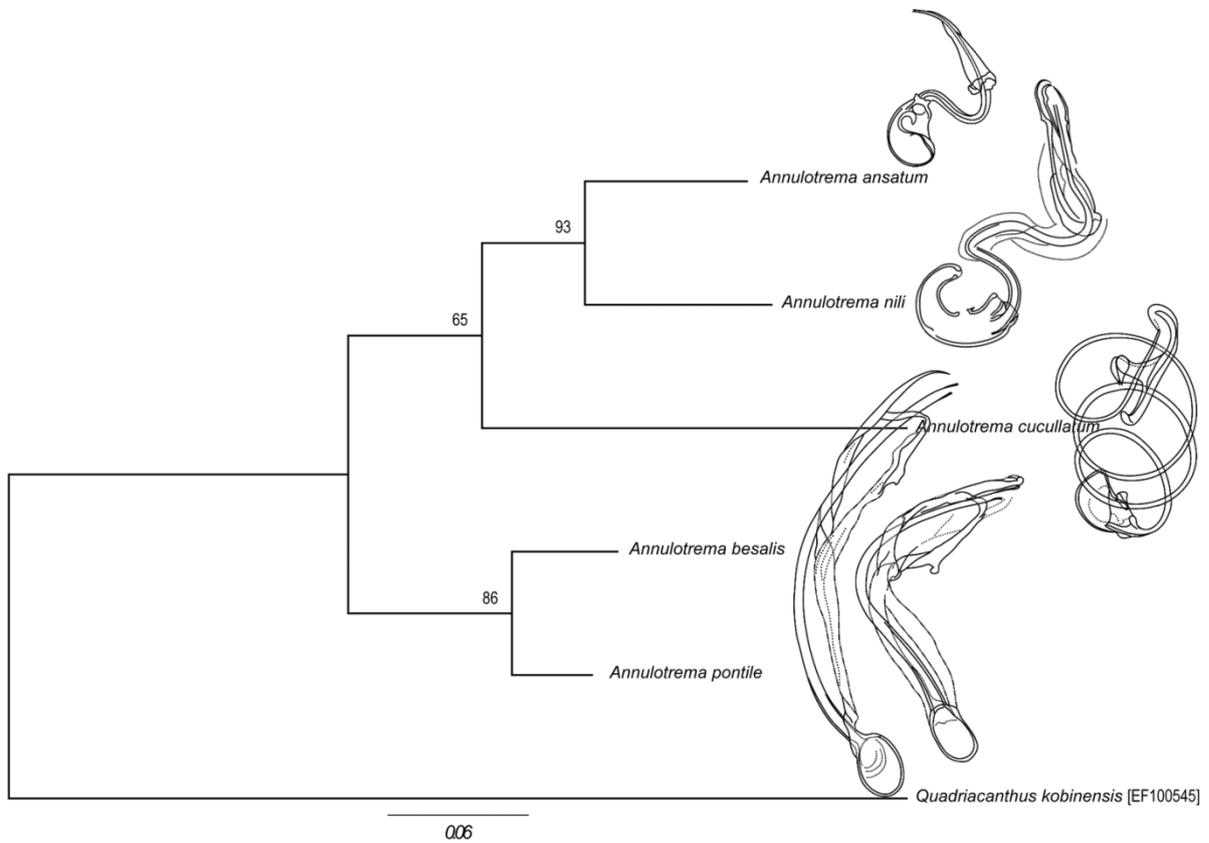
1. To submit a paper, tentatively titled: "Dactylogyrids (Platyhelminthes: Monogenea) from the gills of *Hydrocynus forskahlii* (Characiformes: Alestidae) from Kariba lake, Zimbabwe, with description of two new species of *Annulotrema* and one new species of *Afrocleidodiscus*" (Maria Lujza Kičinjaová, Maxwell Barson, Milan Gelnar and Eva Řehulková).
2. To complete processing of DNA samples of *Annulotrema* spp. from Kenya, South Africa and Sudan and perform molecular analyses clarifying phylogenetic relationships among the examined *Annulotrema* spp.
3. Parasitological sampling of African tetras from Cameroon and specimen preparation for study of structural and molecular diversity is planned for February.

## Acknowledgments

This research was supported by the Czech Science Foundation No. P505/12/G112 (ECIP). A special thank is due to Iva Příkladová for collecting monogeneans and/or their hosts from Zimbabwe and South Africa.

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**Figure 1:** Molecular phylogeny of *Annulotrema* spp. parasitizing *Hydrocynus forskahlii* estimated by Maximum Likelihood using partial sequences of the 28S rRNA gene. All species were newly sequenced for this study. The phylogenetic analysis revealed that species with the same “type” of MCO cluster together. Bootstrap support values are given above branches.

# Monogeneans of deepwater cichlids in Lake Tanganyika: What is the real host range of *Cichlidogyrus casuarinus*?

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Lake Tanganyika contains unique species flocks of vertebrate and invertebrate taxa and harbors the most diverse cichlid species assemblage of all African lakes [1]. While the first report about parasites in Lake Tanganyika is dated to 1927, parasites have been overlooked for many decades. This makes it an unrivalled study area in the field of biodiversity and investigations of parasite evolution processes and mechanisms. Whereas in littoral Tanganyika cichlids the host specificity of *Cichlidogyrus* species (Monogenea, Dactylogyridae) is relatively strong [2], previous results confirmed the reduction of this pattern in the deepwater habitat [3]. According to the aforementioned study, representatives of *Cichlidogyrus casuarinus* infecting bathybatine cichlid hosts within the genera *Hemibates* and *Bathybates* are truly conspecific. So, we asked the question whether the other deepwater cichlid species are infected by the same monogenean species?

In our study we examined and analyzed monogeneans occurring in two deepwater cichlids species, *Trematocara unimaculatum* and *Benthochromis horii* which are representatives of two different tribes (Bathybatini and Benthochromini, respectively). Samples originated from one locality in the north part of the Lake, near Bujumbura city. A morphological approach focusing on parasites' sclerotized structures was used to compare and describe the collected *Cichlidogyrus* species.

The inventory of monogeneans occurring in Lake Tanganyika was enriched by two new *Cichlidogyrus* species, described based on 30 parasite individuals from each host species. Hence, the host range of *C. casuarinus* does not cover even all Bathybatini tribe members. Moreover, the results indicate that *Cichlidogyrus casuarinus* is morphologically more similar to *C. nshomboi* described from *Boulengerochromis microlepis* compared to *Cichlidogyrus* sp. n. collected from *Trematocara unimaculatum*. Based on morphological comparison, the monogeneans therefore do not reflect the phylogenetic positions of their hosts considering the situation of *Trematocara unimaculatum* and *Boulengerochromis microlepis*. Other investigations including DNA analysis are needed to uncover the co-phylogenetic history of this system.

## Future plans

The pelagic fish stocks in Lake Tanganyika are far less species-rich but economically and ecologically more important than the littoral communities. In the next years, we will focus on inventorying the parasite fauna of pelagic latids and clupeids in Lake Tanganyika and relate it to host condition. Parasite evolutionary and demographic history, gene flow and host range within and between host species will be quantified in the light of our current understanding of the host stocks. Since one of these species was introduced into Lakes Kivu and Kariba, the results will be compared to their parasite communities, incorporating an aspect of exotic species biology into this project.

## Acknowledgement

This research was supported by project no. GBP505/12/G112 from the Grant Agency of the Academy of Sciences of the Czech Republic.

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# Developmental and evolutionary biology of the moon jellyfish

## *Aurelia aurita* senses

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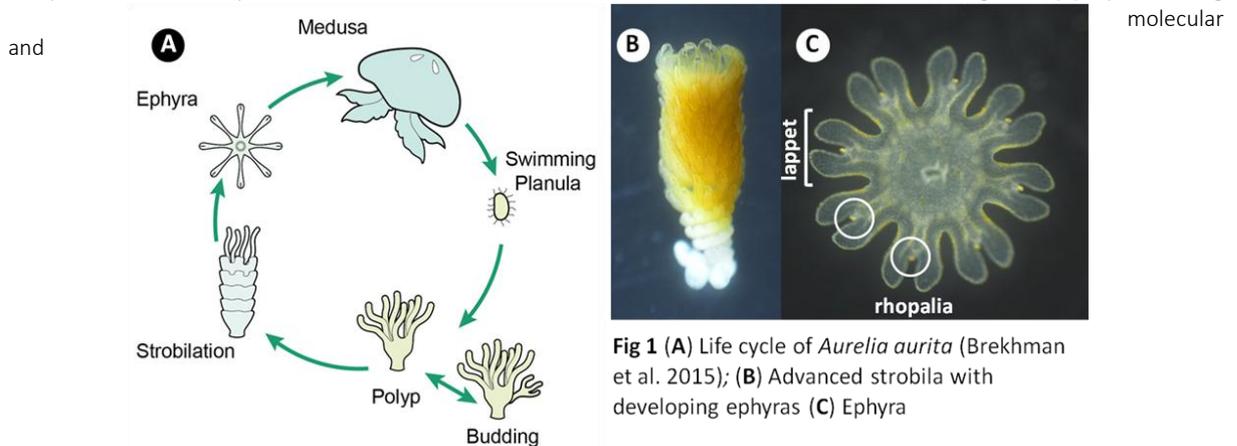
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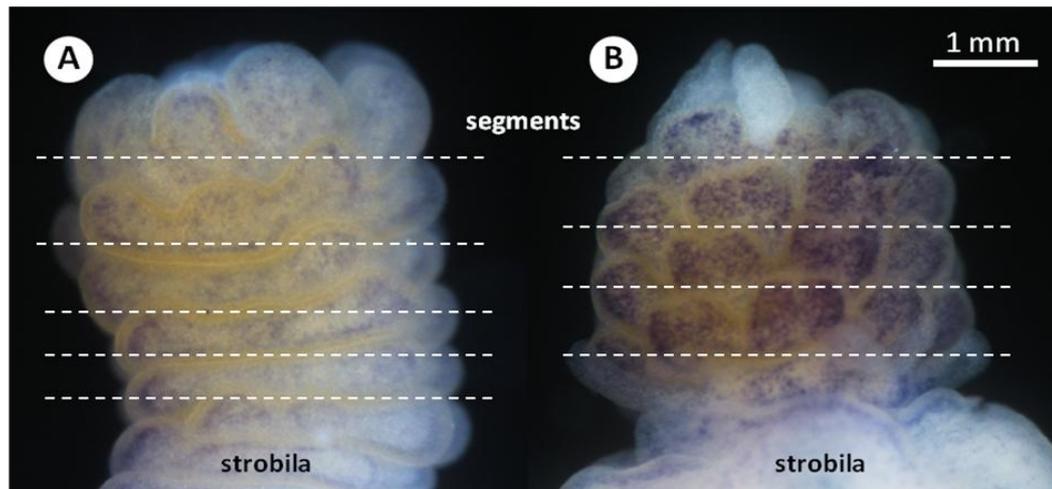
The Cnidaria, one of the most basal animal phyla, evolved nearly 700 million years ago, the period when ancestors to most extant bilaterian phyla arose from a common hypothetical ancestor [1]. In recent years, interest in the use of cnidarians as model organisms has grown, particularly with the goal of understanding the evolution of Bilateria. Cnidarians split into two major lineages: Anthozoa (corals and sea anemones) and Medusozoa (Hydrozoa, Cubozoa, and Scyphozoa), both display a tremendous degree of developmental plasticity. Beside the traditional cnidarians groups a new cnidarians member was assigned – the Myxozoa, microscopic parasitic cnidarians infecting invertebrates and vertebrates and causing high economical losses in aquaculture. Cnidaria have complex life cycles, with the bilateral benthic polyp stage being regarded as the ancestral cnidarian stage, after which radially symmetrical medusa evolved in the branch leading to Medusozoa [2]. In cnidarians missing the medusoid stage in their life cycle the polyp completes the sexual reproduction. In all other cnidarians, sexual reproduction only occurs at the medusa stage and the polyp serves as the main form for asexual reproduction. Medusozoans consist of two morphologically disparate generations with three well-defined life stages – planula, polyp and medusa. The generation of medusae occurs in fundamentally different ways in different medusozoan taxa. In Scyphozoa - the medusa phase is the dominant stage of the life cycle and the polyp's asexual proliferation results in new polyps or in the production of dozens of juvenile medusae (so-called ephyra) in a repeated segmented process, formed by apical metamorphosis of the polyp, called strobilation (Fig. 1) [3].

During the strobilation process the medusa photosensing structure begins to differentiate. Located at the medusa bell margin sensory structure called rhopalia is involved in mechanosensation, photosensation and gravity sensation, possibly representing an early stage of the vertebrate eye evolution (Fig. 1C). The widespread jellyfish *Aurelia aurita*, for which comprehensive transcriptomic data and various molecular tools are available, is used as a model organism [4]. By combining



bioinformatic approaches, the expression patterns of candidate photosensory genes such as retinol dehydrogenase (*rdh8*), atonal homolog (*Atohl*), bahr-like 2 homeobox protein (*bahr*) and dorsal root ganglia homeobox (*drg*) will be examined during strobilation and medusa formation using qPCR and in situ hybridization, and functionally analysed using RNAi.

Development of rhopalia is related to the Notch signalling pathway (unpublished, preliminary data). Notch signalling is evolutionarily conserved and operates in many cell types and at various stages during development. Our preliminary data (Fig. 2) indicates that the regulation of the rhopalia development is mediated by the Notch pathway, as Notch presenilin inhibitor (DAPT) affected the development of the lappets and the synthesis of the rhopalia structure during strobilation. In addition, the synthesis of nematocysts, which are part of the neurogenic system, was inhibited by DAPT (Fig. 2). However, the strobilation process by itself is not inhibited and a truncated ephyra is released. The inhibition of the Notch interferes with the ephyra synchronization of its pumps frequency and its normal swim (unpublished, preliminary data).



**ig 2** In-situ hybridization of minicollagen in strobila of *Aurelia aurita*; **(A)** Notch inhibition by DAPT, inhibits minicollagen gene expression in nematocyst, part of the neurogenic system; **(B)** Control strobila with high minicollagen expression; Preliminary data

F

Importantly, the Notch signalling pathway affects cell differentiation not only across a broad range of cell types in a single organism but also at different stages during the cell lineage progression [5]. The Notch pathway is related to neurogenesis in all Metazoa. Unravelling the rhopalia molecular machinery may provide a new insight into the evolution of sensory mechanisms. Thus, cnidarian photoreceptor cells may represent a very early stage of evolution leading to the vertebrate eye. As jellyfish are supposed to be the closest free-living relatives to parasitic Myxozoa, I will explore the molecular background of their sensory structures to better understand host recognition and subsequent finding infection site identification mechanisms of Myxozoa.

#### Future plans:

- i) To characterize the spatial and temporal expression of rhopalia sensory genes.
- ii) To determine the role of Notch signaling pathway in rhopalia development.
- iii) To study the function of selected rhopalia genes.
- iv) To analyse the phylogenetic position of rhopalia sensory genes and their counterparts known from Bilateria.

#### Acknowledgement:

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# Institute of Parasitology – Trematode team: Research outlines for 2015 and plans for 2016

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During 2015 the research of the trematode team (Simona Georgieva, Olena Kudlai, Anna Faltýnková and Miroslava Soldánová) was focused on the two modules of the project: 1 (Diversity of parasites) and 2 (Phylogenetics and molecular taxonomy). Our results within **module 1** include descriptions of 4 new species and redescriptions of 2 species as well as descriptions of the cercariae of 8 species and metacercariae of 3 species; all of these are characterised molecularly (5 publications; 3 in *Systematic Parasitology* [1–3], 1 in *Parasitology Research* [4] and 1 in *Folia Parasitologica* [5]). Co-authorship: A. Kostadinova (4 papers), S. Georgieva (3 papers); O. Kudlai (2 papers); A. Faltýnková (2 papers); M. Soldánová (1 paper).

**Research during 2016** will be focused on: (i) Digenean diversity in fishes from the Neotropical and Ethiopian regions; (ii) Digenean identification and parasite community diversity patterns in deep sea fishes in the Mediterranean; (iii) Digenean diversity in sparid fishes along the Algerian coasts of the Western Mediterranean; (iv) Molecular and morphological characterisation of digeneans in Antarctic fishes; (v) Molecular and morphological characterisation of strigeoid cercariae from Central Europe; and (vi) Bird schistosomes in planorbid snails from the River Ruhr drainage in Germany.

The research within **module 2** resulted in a paper (under review) in *International Journal for Parasitology* (co-authorship: O. Kudlai and A. Kostadinova [6]) which provides the first molecular phylogeny of the superfamily Echinostomatoidea with novel taxonomic and systematic decisions for a range of groups with species utilising fish as second intermediate (genera *Echinochasmus*, *Microparyphium*, *Stephanoprora*, *Petasiger*, *Paryphostomum*, *Isthmiophora*, *Ribeiroia*) and definitive hosts (*Caballerotrema*).

**Research during 2016** will be focused on: (i) Molecular and morphological characterisation of adult diplostomids from various fish-eating bird hosts from Europe (Iceland and Czech Republic); (ii) Molecular characterisation and phylogeny of larval *Diplostomum* spp. in fishes from Asia (China); (iii) Studies of the population genetic structure in *D. spathaceum* and *D. pseudospathaceum*; (iv) Morphological and molecular characterisation and phylogenetic relationships of metacercariae of *Diplostomum* spp. from various fish populations in Europe (Czech Republic, Hungary, Italy and Slovakia).

## Acknowledgement

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# Motility in phylogenetically distant groups of gregarines (Apicomplexa) parasitising marine hosts

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One of the most medically and economically important group of parasites belongs to the phylum Apicomplexa. Gregarines, relatively large parasitic protists inhabiting the intestine, coelom or extracellular space of invertebrates' hosts, represent an early emerging group within this phylum. Especially marine gregarines possess several plesiomorphic characteristics retained from the most recent ancestors of all apicomplexans. Motility in these parasites usually differs from the substrate-dependent gliding generally described in apicomplexan zoites, the so called "glideosome" concept [1]. In gregarines cell motility seems to depend on mechanico-chemical system and cytoskeletal structures (subpellicular network of microtubules, intermediate filaments, actomyosin motor). They rather utilize several mechanisms of motility that correlate with various modifications of their cell cortex (epicyte), which differ between phylogenetical groups of gregarines. Present research was mostly focused on the motility of three lineages: blastogregarines, archigregarines and eugregarines parasitising marine invertebrates collected near to the White Sea Biological Station Moscow State University in Russia. Research was focused on principles of parasites' movement. To prove the need for dynamic turnover of actin polymerisation and stable network of subpellicular microtubules in gregarine motility, experiments with several commercial probes influencing the polymerisation of actin and tubulin were applied to suspensions of living parasites. Parasites were studied under light microscope and gradual changes in their motility were recorded in video. Incubated cells from each motility assay were fixed for electron (TEM, SEM) and confocal laser scanning (CLSM) microscopic analyses of induced cytoskeletal or other subcellular changes (such as potential membrane damage induced by drugs).

First of all, from blastogregarines, which take position in the basis of gregarines phylogenetic tree, *Siedleckia nematoides* was investigated (obtained by AV, cooperation by MK, publication in process). The elongated, flattened individuals of *S. nematoides*, parasite of polychaete *Scoloplos armiger*, perform wavy, pendular or undulating movement and are covered by a pellicle, consisting of the plasma membrane and IMC. Their surface appears smooth lacking any grooves or folds. Interestingly, subpellicular microtubules are organised in several layers. Subsequently *Selenidium* sp. from polychaete host was investigated. This parasite belongs to the ancestral group –archigregarines and exhibits pendular or rolling movement similar to that in *S. nematoides*. Nevertheless, the cell surface is formed by wide and low epicytic folds separated by grooves. The typical apicomplexan three-layered pellicle is underlined by a single layer of longitudinally oriented subpellicular microtubules and an additional layer of irregularly arranged microtubules. Experiments with probes influencing the polymerisation of actin and tubulin finalized in year 2015 confirmed that parasites are very resistant to drugs influence. In concentration under 10  $\mu$ M no effect on gregarine motility was detected and thus it was necessary to increase the drugs' concentration and prolong the incubation period. Parasites were incubated according to the type of probe from 90 minutes to 9 hours [2]. Second part of experimental work included the research of various ions concentrations in artificial sea water, and evaluation of their influence on gregarines' motility. This study was analysed and video-documented. Finally septate eugregarine *Cephaloidophora* cf. *communis* parasitising the crustacean *Balanus balanus* was collected and fixed for EM and (immuno)fluorescent analyses. Surface of this gregarine is covered with numerous high and narrow, undulated longitudinal epicytic folds and exhibits the typical unidirectional gliding on substrate.

Other outcomes from 2015 include cooperation with AV in study focused on the attachment strategy, cell structure and host-parasite interactions of the protococcidian *Eleutheroschizon duboscqi* [3].

## Future plans:

Further microscopic analyses of model marine apicomplexans. Continue with research using molecular-biological techniques. Performing (immuno)fluorescent labelling of eugregarine (aseptatorina) *Difficilina* sp., species which is rigid and capable of gliding motility. In addition, the method of freeze-etching will be tested on samples of marine apicomplexans, to investigate the supramolecular organisation of membrane structures.

### Acknowledgement

The studies have been supported from the project no. GBP505/12/G112 from the Grant Agency of the Academy of Sciences of the Czech Republic.

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Figure 1 *Selenidium* sp. parasitising marine polychaete (LM)

# Influence of life traits and hybridization on parasite community structure in roach (*Rutilus rutilus*) and common bream (*Abramis brama*) hybrids

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Existence of the fish hybrids is well documented and the hybrids generally represent the object of recently published ecological, immunological and evolutionary studies. Creation of the hybrid fish with new features inherited from parental species is representative new habitat for the parasite. The hybridization may disrupt the system of co-adapted host-parasite genes and affect parasite resistance in fish. However, the temporal variability and density dependent processes of the host populations may influence the parasite infection.

Metazoan parasites were collected during spring and autumn 2011, 2012 and 2013 from the common bream (*Abramis brama*), roach (*Rutilus rutilus*) and their respective hybrids. Parental and hybrid fish were identified following the molecular (mitochondrial cyt b gene and 9 microsatellite loci) and morphological characteristics. This study was focused to compare the parasite infection in parental and hybrid fish and to detect the effect of hybridization and influence of hybrid maternal origin on parasite infection.

On the basis of cytochrome b sequence analysis a total of 6 mitochondrial haplotypes in roach and 12 mitochondrial haplotypes in common bream were identified. The hybrids of both maternal origins were identified (5 haplotypes of roach and 8 haplotypes of common bream). Our analyses revealed the significant effect of hybridization on the parasite abundance. Parasite diversity was higher but parasite abundance was lower in hybrids when compared to each of the parental species. Both specialist parasites of two parental species and generalist parasites were presented in parasite communities of hybrids. However, concerning the highly specific parasites of *Dactylogyrus* genus, the hybrids harbor the specialists of roach but the occurrence of common bream specific *Dactylogyrus* in hybrids was restricted. Significant differences in parasite abundance and parasite species richness were observed between hybrids of different maternal origins. The significant effects of season fish group (including the categories common bream, roach and hybrids) and year of collection on the composition of the metazoan parasite communities were found. The effects of the fish group and sex on the fish condition were found. The analyzed measures of non-specific immunity were not affected by fish group.

## Future plans

The variability of the MHC genes and associations between MHC and parasite load in the studied hybrid system will be analyzed.

## Acknowledgement

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# Molecular identification of metacercariae of *Diplostomum* spp. in fishes from the River Danube

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Species of *Diplostomum* are important parasites of fish, but reliable data on their diversity in natural populations are virtually lacking due to the simple morphology of the metacercariae and the lack of reliable differentiating features. This study is the first to apply a barcoding approach to the diversity of *Diplostomum* in fishes from a large river in Europe.

A total of 27 species of fish (173 specimens) was sampled opportunistically in the River Danube near Nyergesújfalu in Hungary and Štúrovo in Slovakia. Specimens of *Diplostomum* spp. were recovered only from the lenses of 15 species of fish, predominantly cyprinids. Overall parasite intensity was low (1–15 metacercariae per fish) with few exceptions for *Abramis brama* (25–43 metacercariae per fish; 4 cases) and *Blicca bjoerkna* (27 metacercariae per fish). Twelve species of fish, for which fewer specimens were examined, were not infected: *Anguilla anguilla*, *Barbus barbus*, *Esox lucius*, *Gobio gobio*, *Lepomis gibbosus*, *Neogobius melanostomus*, *Perca fluviatilis*, *Ponticola kessleri*, *Sander lucioperca*, *S. volgensis*, *Zingel zingel* and *Z. streber*. We used the molecular framework and the recently generated genetic datasets for species of the genus *Diplostomum* to investigate species diversity of collected material. The two mitochondrial genes, *cox1* and *nad3*, were applied. The *nad3* primers were newly-designed for this study.

Barcoding of representative isolates (*cox1*; n = 75) provided molecular identification of five species: *D. spathaceum*, *D. pseudospathaceum*, 'D. mergi Lineage 2' and *Diplostomum* sp. We also generated 30 novel *nad3* sequences. Neighbour-joining analysis of this dataset yielded a tree with strongly supported clades that correspond to three species: *D. spathaceum*, *D. pseudospathaceum* and 'D. mergi Lineage 2' (Fig. 1). The comparison of the level of genetic differentiation between *cox1* and *nad3* has shown that the mitochondrial *nad3* gene is distinctly more divergent (Table 1) and thus more promising molecular diagnostic marker than *cox1*.

This study is also the first to establish a link between cercariae from *Radix auricularia* and metacercariae of the 'D. mergi Lineage 2' in fish hosts thus partially elucidating the life-cycle of this putative new species [1].

## Future plans

- (i) Morphological and morphometric assessment of the sequenced metacercariae and publication of the results.
- (ii) Large-scale sequencing of *cox1* for *D. spathaceum* and *D. pseudospathaceum*.
- (iii) Publication of a molecular phylogeny of the superfamily Echinostomatoidea.

## Acknowledgement

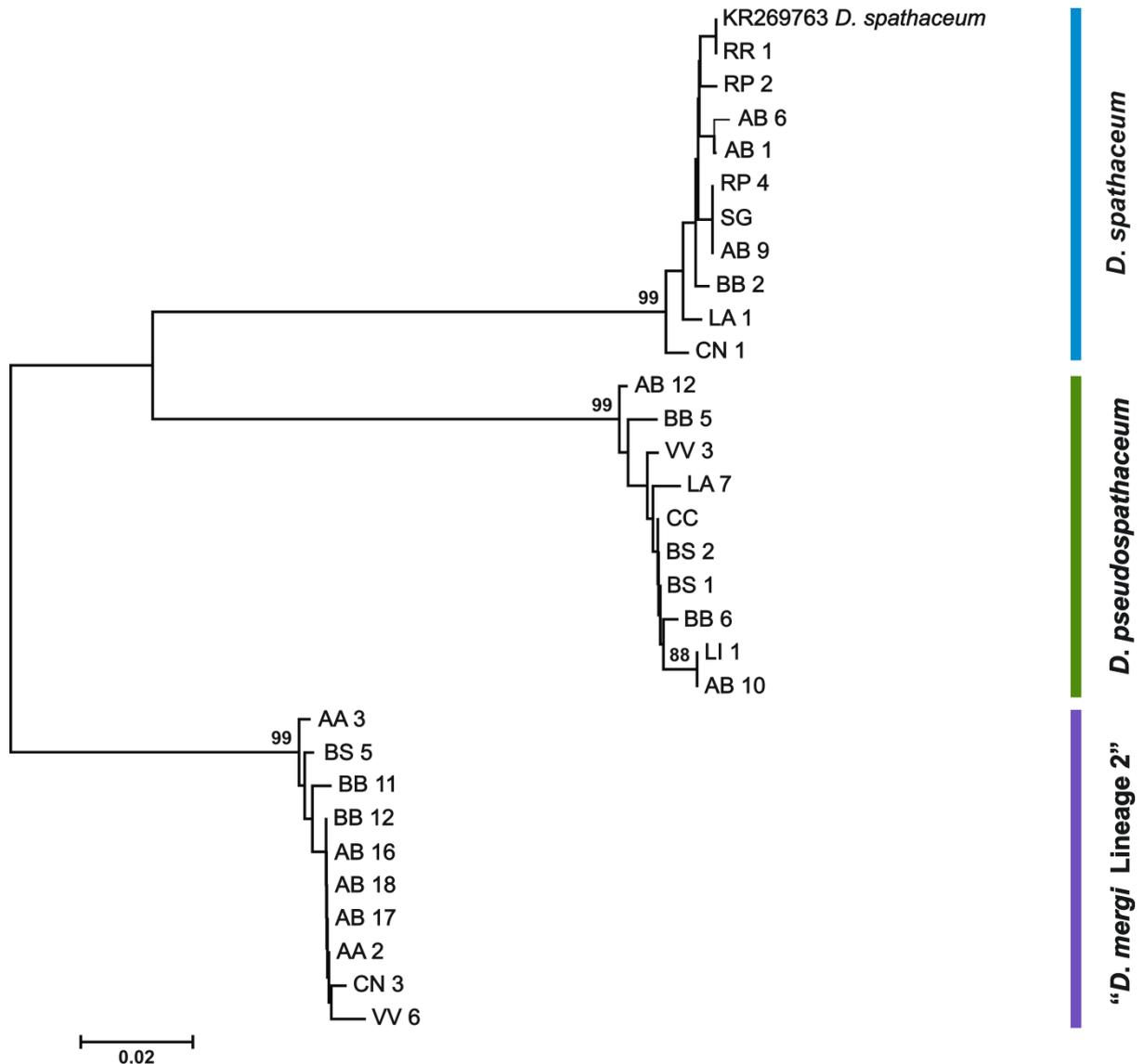
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**Table 1** Levels of divergence (p-distance in %) for *cox1* and *nad3* gene sequences in interspecific comparison of *Diplostomum* spp.

Comparison	<i>cox1</i>	<i>nad3</i>
<i>D. pseudospathaceum</i> vs <i>D. spathaceum</i>	9.0–10.7	15.7–17.4
<i>D. pseudospathaceum</i> vs ‘ <i>D. mergi</i> Lineage 2’	11.2–12.9	14.6–16.2
<i>D. spathaceum</i> vs ‘ <i>D. mergi</i> Lineage 2’	10.0–11.7	15.4–16.8



**Figure 1** Neighbour-joining phylogram reconstructed using newly-generated *nad3* sequences for isolates of *Diplostomum* spp. from fishes sampled in the River Danube and a sequence of *D. spathaceum* from GenBank (KR269763). The scale bar indicates the expected number of substitutions per site.

# Molecular phylogeny of the Bothriocephalidea (Cestoda): molecular data challenge morphological classification

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In this study, the relationships of the cestode order Bothriocephalidea, parasites of marine and freshwater bony fish, were assessed using multi-gene molecular phylogenetic analyses. The dataset included 59 species, covering approximately 70% of currently recognised genera, a sample of bothriocephalidean biodiversity gathered through an intense 15 year effort. The order as currently circumscribed, while monophyletic, includes three non-monophyletic and one monophyletic families. Bothriocephalidae is monophyletic and forms the most derived lineage of the order, comprised of a single freshwater and several marine clades. Biogeographic patterns within the freshwater clade are indicative of past radiations having occurred in Africa and North America. The earliest diverging lineages of the order comprise a paraphyletic Triaenophoridae. The Echinophallidae, consisting nearly exclusively of parasites of pelagic fish, was also resolved as paraphyletic with respect to the Bothriocephalidae. *Philobythoides* sp., the only representative included from the Philobythiidae, a unique family of parasites of bathypelagic fish, was sister to the genus *Eubothrium*, the latter constituting one of the lineages of the paraphyletic Triaenophoridae. Due to the weak statistical support for most of the basal nodes of the Triaenophoridae and Echinophallidae, as well as the lack of obvious morphological synapomorphies shared by taxa belonging to the statistically well-supported lineages, the current family-level classification, although mostly non-monophyletic, is provisionally retained, with the exception of the family Philobythiidae, which is recognised as a synonym of the Triaenophoridae. In addition, a new species, *Bothriocephalus timii*, was described from *Cottoperca gobio* collected on the Patagonian shelf off Argentina. It is the first tapeworm species reported from a bovicthiid fish. Moreover, *Schyzocotyle* was resurrected to accommodate the invasive Asian fish tapeworm, *Schyzocotyle acheilognathi* (syn. *Bothriocephalus acheilognathi*), which is of veterinary importance, and *Schyzocotyle nayarensis* (syn. *Ptychobothrium nayarensis*). The genus is morphologically characterised by a wide, heart-shaped scolex with narrow, deep bothria.

## Future plans

Several undescribed species of bothriocephalidean cestodes from marine and freshwater teleosts will be described and a new genus will be proposed to accommodate a new species from freshwater fishes in the Neotropical region.

## Acknowledgement

This study has been supported by the Czech Science Foundation (project no. P505/12/G112).

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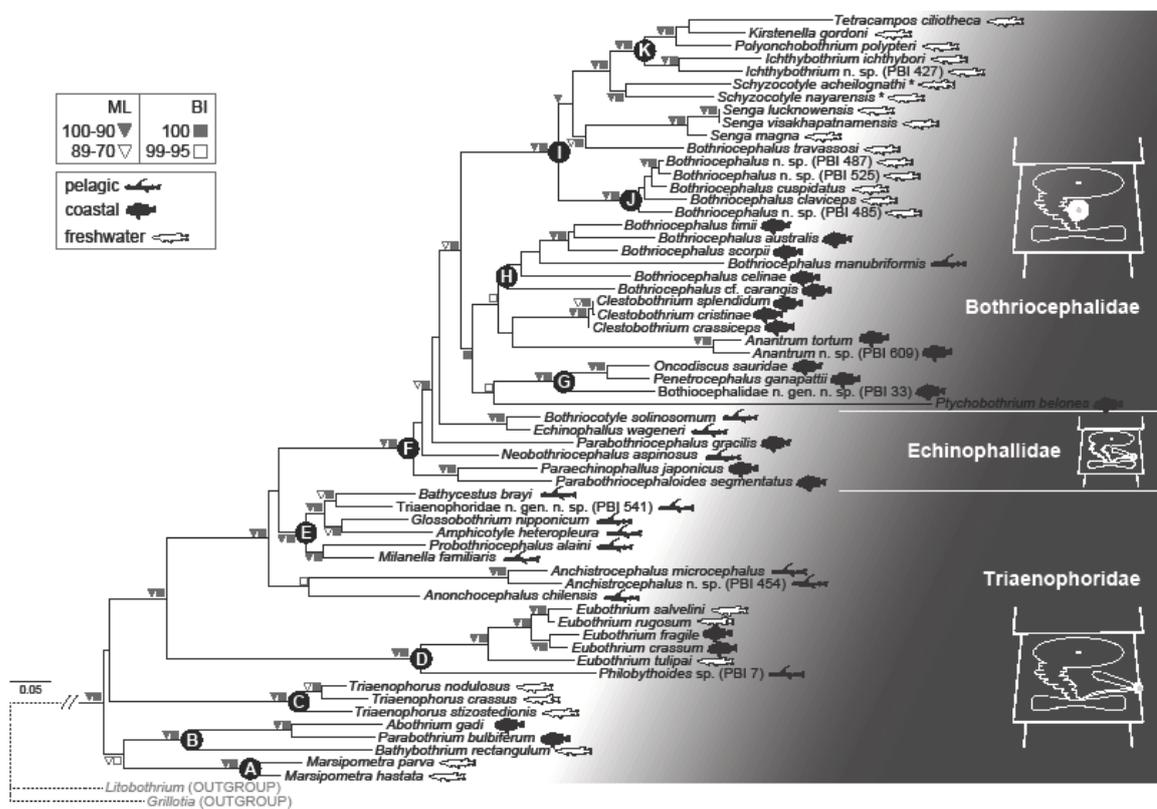
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# Methodological issues affecting the study of fish parasites

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Methods presently used in the study of fish parasites may have a significant influence on the results obtained. Problem areas are likely to include the sampling method used to catch host fish, and especially the degree of physical contact (manipulation), and how live fish are maintained prior to dissection.

In this study, we sampled Prussian carp (*Carassius gibelio*) and held them in a 1 m<sup>3</sup> basin outside. Twenty fish were dissected each day over the following six days. In addition, we compared the parasite community of the common roach (*Rutilus rutilus*) sampled repeatedly from the same site using three common lentic sampling methods: electrofishing, beach seine and gill-nets.

Our results indicate changes in the ectoparasite community over time in the holding tanks, with the number of *Gyrodactylus* increasing after three days in cold season and the number of *Dactylogyrus* increasing in hot period. Level of physical manipulation by sampling gear (e.g. gill nets) was associated with absence of some common ectoparasite species, while host fish 'personality' (e.g. inquisitiveness) may not only increase the risk of exposure to higher numbers of endoparasites but also increase the fish's likelihood of capture. In order to obtain a representative assessment of a fish species' parasite community, therefore, these two important factors need to be taken into account; hence, we suggest using sampling methods that involve less physical contact (e.g. electrofishing) in future parasite community studies and that fish are dissected no later than three days after sampling.

## Future plans for 2016

1. To confirm a species identification of introduced *Posthodiplostomum* cf. *minimum*, a parasite of invasive pumpkinseed (*Lepomis gibbosus*), by molecular methods.
2. The comparative study of parasite communities of three gudgeon species, *Gobio gobio*, *Romanogobio belingi*, and *R. vladykovi*.
3. The study of the invasive Chinese sleeper (*Perccottus glenii*) in the Danube basin as the vector of introduction of parasite species.

## Acknowledgement

This study was supported by ECIP (European Centre of Ichthyoparasitology); centre of excellence program of the Czech Science Foundation; project No. P505/12/G112)

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## Comparative study of the eggs morphology of human diphylobothriids (Cestoda)

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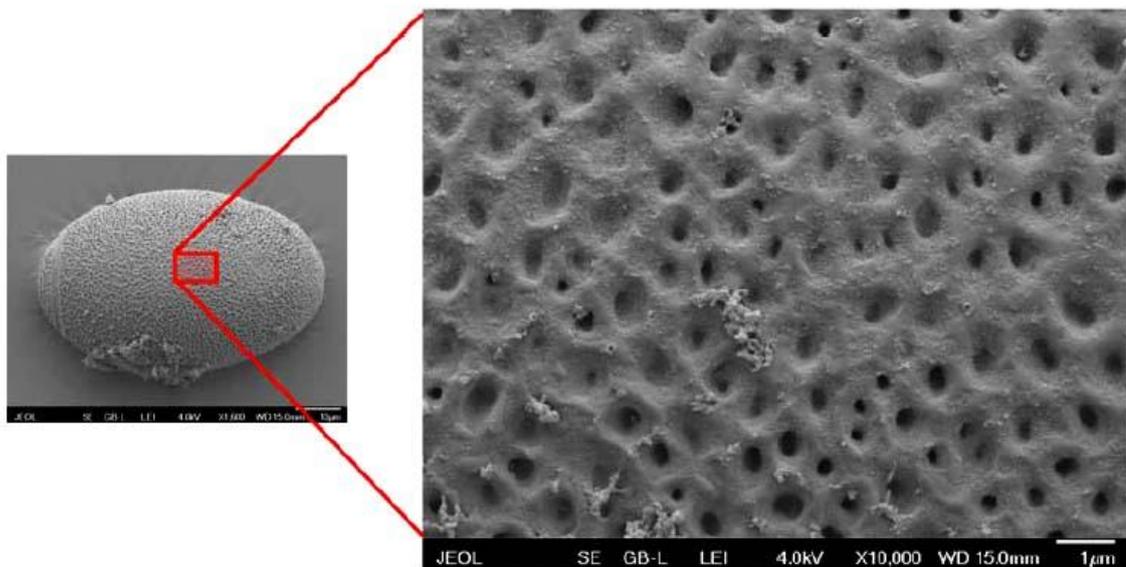
Tapeworms of the order Diphylobothriidea are well-known parasites of tetrapodes, including man. They use fish as second intermediate hosts. Identification of the members of the genus *Diphylobothrium* Cobbold, 1858 is difficult because of their morphological uniformity. The eggs of 58 samples of 8 species (*Adenocephalus pacificus*, *Diphylobothrium dendriticum*, *D. cf. cameroni*, *D. cordatum*, *D. hians*, *D. latum*, *D. nihonkaiense* and *D. stemmacephalum*) from human as well as natural and experimentally infected hosts were studied. Their size (length and width) and the surface were studied using light and scanning electron microscopy (SEM). Combining morphological and morphometrical data made it possible to distinguish all of the species. The surface of all marine species is covered by numerous deep pits with species-specific distribution, whereas the surface of freshwater species is smoother or with isolated wrinkles only. From the four most common causative agents of the human disease, diphylobothriosis, the eggs of *A. pacificus* are the smallest with the surface covered by numerous pits. In contrast, the eggs of *D. dendriticum*, *D. latum* and *D. nihonkaiense* are morphologically similar and their differentiation based on their size is impossible; therefore, molecular markers should be used for correct identification of these species.

### Future plans

The manuscript based on these data will be prepared.

### Acknowledgement

This study has been partly supported by the Czech Science foundation (project No. P505/12/G112 – European Centre of IchthyoParasitology – ECIP).



**Figure.** Photomicrograph of the egg and egg surface of *Diphyllobothrium lanceolatum* from *Erignathus barbarus*.

# Morphological and Molecular Studies on Anisakid Parasitic Nematodes from Antarctic Fish-Eating Bird

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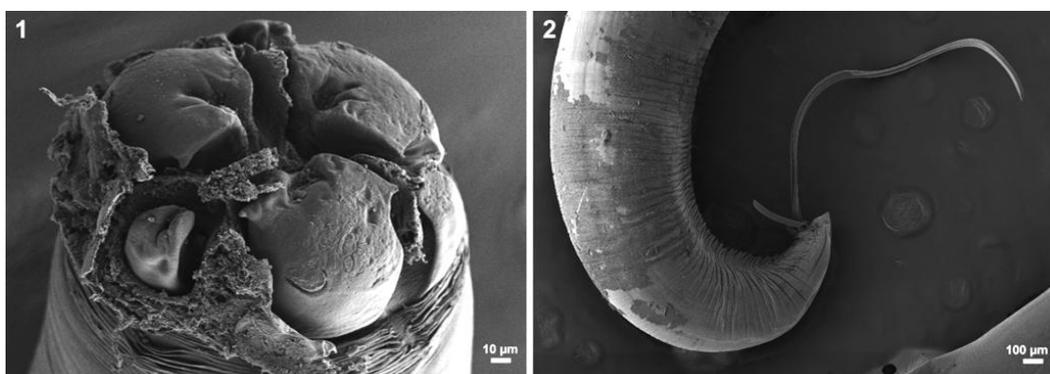
A survey on anisakid nematode parasites collected from the skua's regurgitated pellet [South Polar Skua - *Catharacta maccormicki* (Saunders, 1893), Stercorariidae] in James Ross Island in February 2014 was conducted. We examined one pellet and among others parasites, 53 adults and larvae of genus *Contraecaecum* Railliet et Henry, 1912 were found. These nematodes from family Anisakidae are cosmopolitan parasites of fish-eating avifauna which could be infected by ingestion of intermediate or paratenic host (aquatic invertebrates, fishes). Our material was washed in physiological saline and preserved in 80% ethanol. For light microscopical (LM) examination, nematodes were cleared with glycerine and examined using an Olympus BX 50 microscope equipped with differential interference contrast optics and a digital image analysis system. Scanning electron microscopy (SEM) and LM revealed the detailed structure of the head region and tail, the number of caudal papillae and size and shape of spicules in males, presence of the ventricular appendix and intestinal caecum. Also measurements of morphological structures were obtained. Further molecular analyses were performed to elucidate the exact species determination. Genomic DNA was extracted from parasites' tissues and applied as template for PCR reactions. To amplify specific region of exploitable rDNA markers (ITS2, 18S), specific primer combinations (initially designed for ascarids) were used. The PCR amplicons of our samples were subsequently purified and analyzed by Sanger sequencing (ABI 3130 Genetic Analyzer). Obtained sequences confirm identification of nematode as *Contraecaecum osculatum* (Rudolphi, 1802). It probably represents accidental infection, because it is common parasite of pinnipeds.

## Future plans

Since *Contraecaecum osculatum* forms species complexes, we have to differentiate which sibling species is presented in our material. Internal transcribed spacer (ITS) sequence analysis cannot be applied to distinguish between complexes dwelling in Antarctic region – *C. osculatum* D and *C. osculatum* E because of their identical composition in the ITS1 and ITS2 fragments. After finishing analyses, the obtained sequences will be compared and potential variability between samples will be inferred. In the case of informative and conclusive results for the exact species identification in final data set, the evolutionary distances between relative nematode species will be computed and phylogeny reconstruction may be performed by common statistical methods.

## Acknowledgement

The studies have been supported from the project no. GBP505/12/G112 from the Grant Agency of the Academy of Sciences of the Czech Republic. We acknowledge the Czech Antarctic Station "J. G. Mendel" and its crew for their support and we thank to Marcel Kosina and Ivo Sedláček for providing us with skua's regurgitated pellet.



**Figure 1, 2** Scanning electron micrographs of *Contraecaecum osculatum* from pellet of *Catharacta maccormicki*. (1) Head region (2) Caudal part of male with spiculae.

# Sea parasites biodiversity and their basic epidemiology

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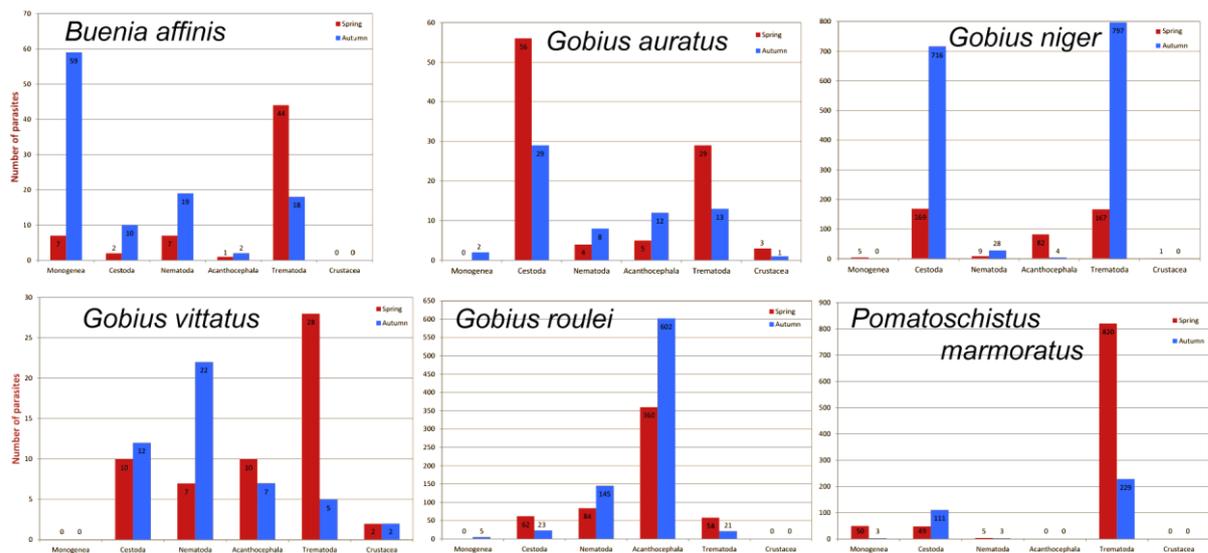
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Two surveys on different metazoan parasites is provided. First is concerning with composition and structure of metazoan parasites in selected gobiid fish (Gobiidae) in Northern Adriatic (Croatia). Basic epidemiological data are known for 364 specimens of the following six gobiid species from the two seasons in 2014: *Buenia affinis*, *Gobius auratus*, *G. niger*, *G. roulei*, *G. vittatus* and *Pomatoschistus marmoratus*. Figure 1 shows comparison of parasite numbers in two seasons (spring and autumn). Second survey is concerning with composition and structure of metazoan parasites in nototheniid fish (Nototheniidae) in Prince Gustav Channel (Weddell Sea, Antarctica). Basic epidemiological data are stated for 102 specimens of the following six nototheniid fish species from the Czech Antarctic expedition in 2014: *Trematomus hansonii*, *T. bernacchii*, *T. newnesi*, *Notothenia coriiceps*, *Parachaenichthys charcoti*, *Pagothenia borchgrevinki* (Fig. 2). All host individuals were infected with at least one parasite taxa. Mean total parasite abundance across the hosts was 76. The most parasitized host was *Notothenia coriiceps* with the mean parasite abundance of 117. Preliminary epidemiological data were published [1]. Now we are working on determination of parasites from Antarctica and Croatia.



**Figure 1** Croatia fish species and total numbers of their parasites in spring and autumn season.

## Future plans

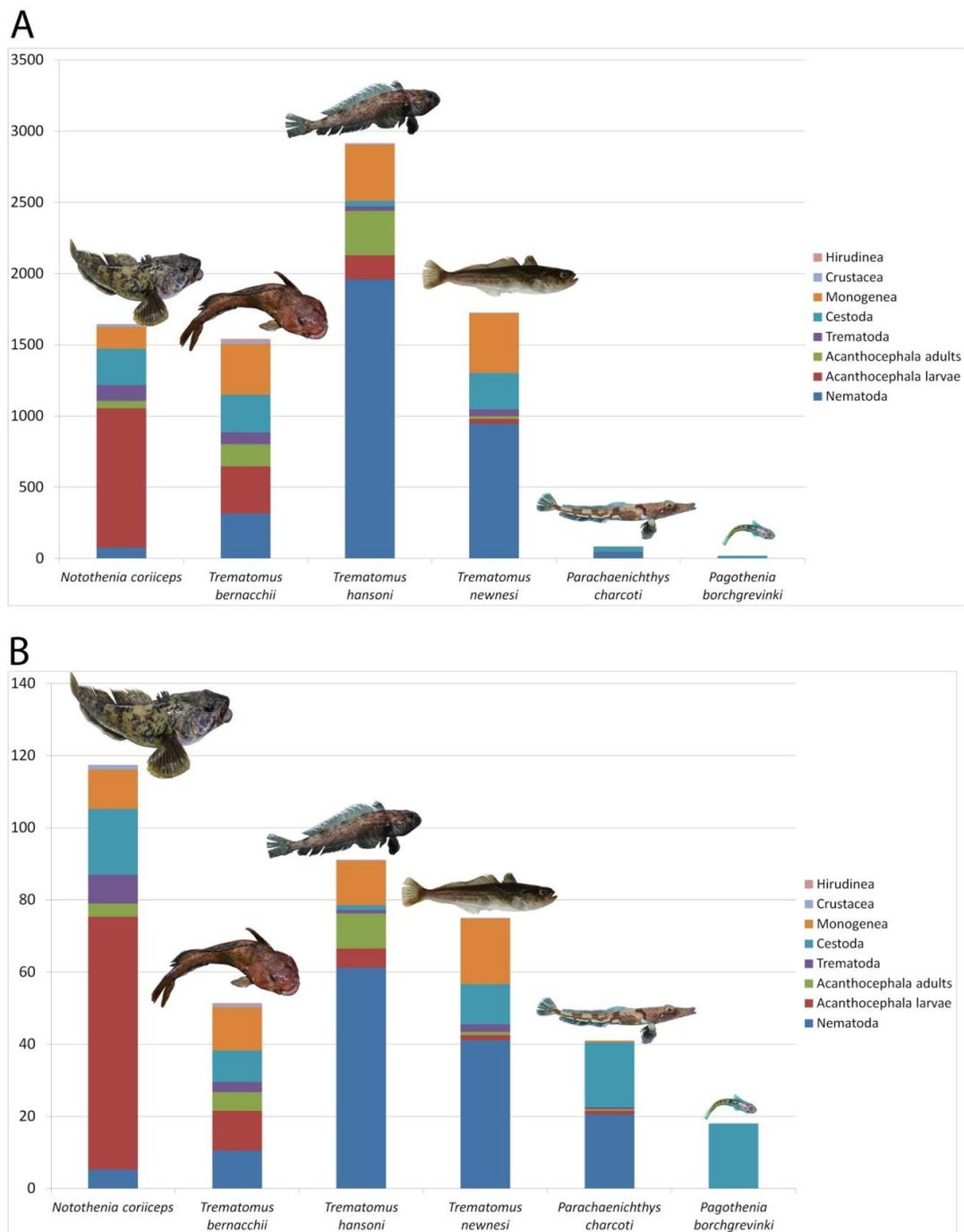
After determination of all parasitic specimens to the species level, analyses of the parasite community structure will be conducted for both surveys.

## Acknowledgement

The studies have been supported from the project no. GBP505/12/G112 from the Grant Agency of the Academy of Sciences of the Czech Republic. We acknowledge the Czech Antarctic Station “J. G. Mendel” and its crew for their support and We would like to thank to our colleagues Petra Zahradníčková, Nikol Kmentová, Markéta Pravdová, and Marcelo Kovačić for the cooperation during the fieldwork.

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**Figure 2** Antarctic fish species and their groups of parasites. (A) Total numbers of parasites at single fish host (B) Mean parasite abundance per one fish. (Reprinted with permission of Masaryk University Press from Czech Polar Reports, Fig. 3 by Nezhybová et Mašová 2015.)

# Systematics of freshwater monogeneans of the Amazon River basin

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Monogeneans (Platyhelminthes: Monogenea) are mainly ectoparasites of marine and freshwater fishes. These parasites exhibit the highest host specificity among platyhelminths, which make a suitable model of study to assess questions on their evolution with respect to their fish hosts. As a result of extensive sampling efforts focused on freshwater catfishes (Siluriformes) from the Peruvian Amazonia carried out from 2004 to 2011, a remarkable total of 99 species of the family Dactylogyridae were found infecting 42 species of catfishes of the families Auchenipteridae, Callichthyidae, Doradidae, Heptapteridae, Loricariidae and Pimelodidae.

Of these 99 species found, 6 were described as new for science: *Aphanoblastella aurorae* Mendoza-Palmero, Scholz, Mendoza-Franco & Kuchta, 2012 from *Goeldiella eques* (Heptapteridae), *Demidospermus brevicirrus* Mendoza-Palmero, Scholz, Mendoza-Franco & Kuchta, 2012 from *Pimelodus* sp., *D. curvovaginus* Mendoza-Palmero & Scholz, 2011 from *Pimelodus* sp. and Pimelodidae gen. sp., *D. mortenthaleri* Mendoza-Palmero, Scholz, Mendoza-Franco & Kuchta, 2012 from *Brachyplatystoma juruense* (type host), *D. peruvianus* Mendoza-Palmero & Scholz, 2011 from *Pimelodus ornatus*, *Pimelodus* sp., *P. blochii* and Pimelodidae gen. sp. and *D. striatus* Mendoza-Palmero & Scholz, 2011 from *Pimelodus* sp. and *Pimelodus blochii* [1,2]. Additionally, the status of 3 species was reviewed and new morphological data and geographical records for six known species were provided [2].

For the first time, the phylogenetic relationships of monogenean parasites on Neotropical catfishes of the subfamily Ancyrocephalinae Bychowsky, 1937, along with African and Asian representatives of the Ancylo-discoidinae Gussev, 1961, were assessed using partial sequences of the 28S rRNA gene. Bayesian inference (BI) and maximum likelihood (ML) analyses reveal that dactylogyrids of Neotropical catfishes do not represent a monophyletic group [3]. The phylogenetically diverse group of Neotropical ancyrocephalines should be considered in future attempts to propose a new classification of the subfamilies within the Dactylogyridae based on the phylogenetic relationships among its members.

The status of all monogenean parasites of freshwater fishes from the Neotropical region was updated and all available records of these parasites, along with new data resulted from this study were summarized in a checklist [4].

## Future plans:

Description of additional species found in catfishes from the Peruvian Amazonia will be achieved combining morphology and molecular approaches. Additionally, the checklist of monogenean species recorded from the Neotropical region will be completed and submitted to Zookeys (IF = 0.933).

## Acknowledgement:

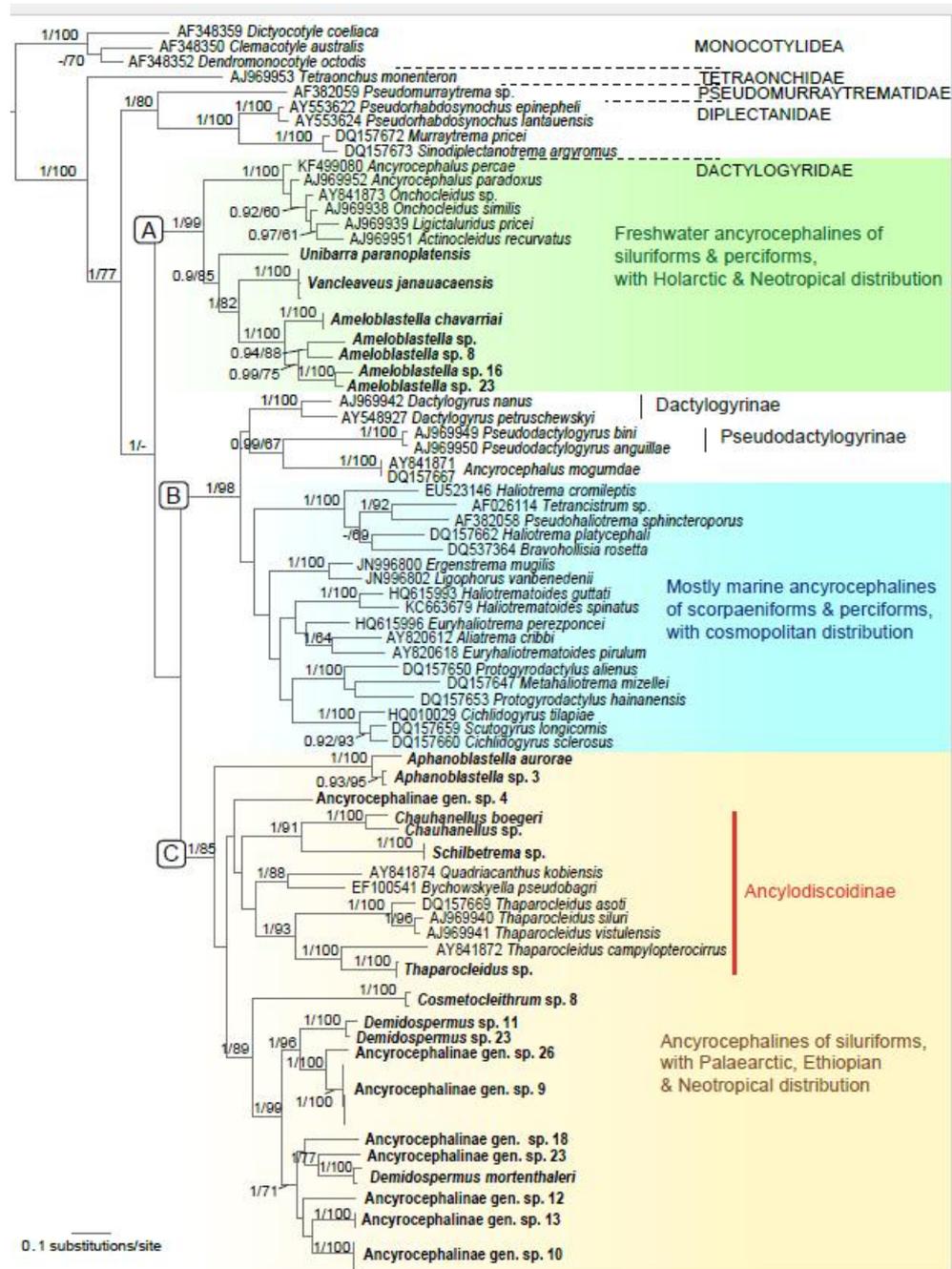
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**Figure 1** Molecular phylogeny of the Dactylogyridae estimated by Bayesian inference using partial sequences of the 28S rRNA gene (695 nt long) (from Mendoza-Palmero et al., 2015).

# Myxozoan diversity as revealed by eDNA

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Myxozoa are microscopic cnidarian endoparasites with a two-host life cycle. Geographic sampling is still patchy and new species are being described frequently indicating that myxozoan biodiversity is still largely undiscovered yet. This is especially true for the marine lineage of Myxozoa and the earliest lineage, Malacosporea. In order to cast light on the unknown Myxozoan biodiversity we are currently testing three different strategies: i) environmental sampling with subsequent amplicon Next Generation Sequencing (NGS), ii) systematic screening of different taxa of the marine meiofauna for myxozoan infection, and iii) mining of existing datasets of amplicons from environmental samples for myxozoan sequences.

i) For the environmental screening we collected three different environmental samples two from different ponds near České Budějovice and one from Bryozoans in shallow waters in Florida. DNA was extracted using the MP FastDNA SPIN kit for soil samples or the QIAamp DNA Stool Mini Kit. All three samples were tested using standard PCR techniques with positive results. We designed different primer pairs with short product lengths suitable for NGS, targeting variable regions of the small ribosomal subunit gene of Myxozoa to the exclusion of other eukaryotes. We tested these versus standard myxozoan specific primers using PCR. Two primer pairs amplified and sequenced Myxozoa and were chosen for NGS on the three environmental samples.

Preliminary analyses of this data revealed that one primer pair performing well in standard PCR failed to retrieve Myxozoa in the NGS setting but sequenced a broad range of other metazoa and some other groups of eukaryotes. The other newly designed primer pair revealed several different myxozoan genotypes, but in low percentages of the overall number of sequences obtained. A higher number of sequences could be assigned to *Xenopus tropicalis*, the majority of sequences, however, could not be assigned to any taxon. The universal primer set used for the characterization of eukaryote communities revealed a similar pattern than the first primer pair, with only four myxozoan sequences in the raw data.

ii) Several different taxa of marine meiofauna, including proseriates, acoels, nemertodermatids oligochaetes, polychaetes and ostracods were screened for myxozoan infections using standard myxozoan specific protocols and Sanger sequencing. Preliminary data show that Otoplanidae (Proseriata, Platyhelminthes) may be serving as host for Malacosporea, a group formerly known only from freshwater habitats and bryozoans.

iii) The TARA expedition (<http://taraoceans.sb-roscoff.fr/EukDiv/>) collected marine environmental samples around the world. About 350 NGS amplicon datasets have been created and made publicly available. In the original taxonomic analyses published by de Vargas et al. (2015) [1] two sequences were assigned to Myxozoa, both belonging to the Malacosporea, formerly known only from freshwater habitats. We are mining these datasets for Myxozoan sequences by blasting the individual sample datasets against custom build Myxozoan queries. This resulted in several more sequences identified as possibly belonging to Myxozoa.

## Future plans:

We intend to finish analyzing the NGS data comparing the performance of the different primer pairs on the three different samples. Protocols for the handling of environmental data will be adapted accordingly. Preliminary data also hints to the presence of a species of Myxozoa causing anal cysts in carp not previously recorded in Czech Republic. This will be verified by species-specific PCR assays.

Otoplanidae as first platyhelminth host for Myxozoa will be verified by screening other specimens for myxozoan infection.

Mining of the TARA datasets is on-going. Sequences found so far appear to belong to a new lineage, requiring detailed analyses.

## Acknowledgement:

This study was supported by ECIP (European Centre of IchthyoParasitology); centre of excellence program of the Czech Science Foundation; project No. P505/12/G112.

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# Metazoan parasites of African annual killifish (Nothobranchiidae)

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Annual killifish of the genus *Nothobranchius* inhabit annually desiccating pools across the African savannah and survive the dry season as developmentally arrested embryos. Their discontinuous non-overlapping generations make for unique predictions regarding their parasite fauna.

We investigated the relationship between local (vegetation, pool size, host density and diversity, diversity of potential intermediate hosts) and global (climate, altitude) environmental factors and fish parasite community structure. 21 populations of 4 *Nothobranchius* species (*Nothobranchius furzeri*, *N. orthonotus*, *N. kadleci* and *N. piensari*) were examined for metazoan parasites to test the role of environmental factors on the structure of the parasite communities.

Seventeen parasite taxa were recorded. Larval trematodes (metacercariae) were most common (prevalence 75%), followed by larval cestodes and nematodes. The parasites recorded were predominantly allogenic, apparently linked to the life cycle of killifish hosts. Host body size was not linked to parasite load and diversity. Sympatric host populations shared similar parasite communities and most of the parasites infected several killifish species. Parasite abundance was highest in climatic region with intermediate aridity and parasite diversity was associated with local environmental characteristics and positively correlated with the taxonomic diversity of fish hosts.

Our results suggest that parasite communities of sympatric *Nothobranchius* species are similar and dominated by larval parasite stages. *Nothobranchius* therefore serve as important intermediate or paratenic hosts of parasites, with piscivorous birds and, in some cases piscivorous fish, being their most likely definitive hosts.

## Future plans:

As a part of my dissertation thesis, metabolic parameters of *Diplostomum*-infected and control European bitterling will be investigated during the next year using intermittent flow respirometry in order to study the physiological consequences of parasitic infection.

## Acknowledgement:

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# Non-native fishes as a source of introduced parasites and/or reservoir for local parasites

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After the introduction into new environments an introduced host species may lose some of its parasites or acquire parasites from the new environment. In relation to the local ecosystem, non-native species can also serve as a reservoir for one or several pathogen species already present in the area and, importantly, non-native species may also transport new parasite species that can invade native host populations. We used a host-parasite system including parasites and their non-native fish host species, *Lepomis gibbosus* (Centrarchidae), introduced from North America over a hundred years ago to Europe.

Thirteen populations of *L. gibbosus* were investigated for metazoan parasites in four drainages in Europe: Black Sea, Aegean Sea, Mediterranean and North Sea drainage. Co-introduction of seven monogenean species (*Gyrodactylus macrochiri*, *Onchocleidus acer*, *O. dispar*, *O. similis*, *Actinocleidus oculatus*, *A. recurvatus*, *Cleidodiscus robustus*) and one digenean species (*Posthodiplostomum* cf. *minimum*) was documented. Abundance and species richness of monogenean parasites decreased along the latitudinal gradient from the south to the north. Metacercariae of *P. cf. minimum* were observed at four different sites in the Danube River basin in Bulgaria and the Czech Republic. Whilst transmission of monogenean parasites to local fauna is not expected due to their high specificity level, using local fish species as suitable host for *P. cf. minimum* remains a question. Molecular analyses, performed previously for this species complex in its native range, indicated that also *P. cf. minimum* shows high level of specificity. However, the affiliation of the parasites collected in Europe to particular species within the species complex has to be confirmed by further genetic analyses.

The susceptibility of non-native *L. gibbosus* to the local fauna resulted in relatively high abundance of metacercariae of *Diplostomum* spp. and *Tylodelphys clavata* at few sites in the Danube and Elbe basins. Ergasilid copepods were observed to parasitise this fish species at almost all sites, mainly with medium abundances. However, due to relatively low fish density at particular sites, their importance as reservoir for either diplostomid trematodes or ergasilid copepods is not expected to be high. Other parasite species (e.g. larval cestode *Valipora campylancristrota*, larval nematodes *Contraoecum* sp., *Spiroxys contortus* or *Raphidascaaris acus*) occurred rather rarely in parasite communities of *L. gibbosus*.

## Future plans for 2016:

1. To confirm a species identification of introduced *Posthodiplostomum* cf. *minimum* by molecular methods.
2. To identify the first intermediate host of this introduced parasite.
3. To evaluate the susceptibility of local fish hosts to *Posthodiplostomum* metacercariae infection.
4. To analyse the parasite community data of *L. gibbosus* with respect to the geographical and ecological parameters.

## Acknowledgement:

This study was supported by ECIP (European Centre of Ichthyoparasitology); centre of excellence program of the Czech Science Foundation; project No. P505/12/G112)

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# Fish tapeworms (Cestoda) in North America: filling the gaps in the knowledge of evolutionary important parasites

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Fish parasitology has a long tradition in North America, but it became a rather neglected research area during last decades (Scholz and Choudhury, 2014). As a result, there still remain numerous gaps in the knowledge of the diversity, biology and genetic structure of all parasite groups, including monozoic tapeworms (Caryophyllidea) that represent a typical component of the parasite fauna of North America. Systematic research on caryophyllideans in the Nearctic zoogeographical region started by Hunter's (1930) extraordinary monograph and was the most intensive in the 1960's and 1970's, when several authors described a number of caryophyllidean species. Surprisingly, almost no attention has been paid to these cestodes since the late 1980's (Scholz and Choudhury, 2014), with a single species described just recently (Haley and Barger, 2014).

In this study, the monozoic cestodes of the recently amended genus *Promonobothrium* Mackiewicz, 1968, parasites of suckers (Catostomidae) in North America, was surveyed, with the information on host specificity and geographical distribution of individual species, together with new data on the scolex morphology of seven species studied using scanning electron microscopy (SEM) for the first time. On the basis of the evaluation of type and voucher specimens from museum collections and newly collected material of most species, the following nominal species, most having been placed originally in *Monobothrium* Diesing, 1863 and *Rogersus* Williams, 1980, are considered to be valid: *Promonobothrium minytremi* Mackiewicz, 1968 (type species); *P. ingens* (Hunter, 1927); *P. hunteri* (Mackiewicz, 1963); *P. ulmeri* (Calentine and Mackiewicz, 1966); *P. fossae* Williams, 1974 and *P. mackiewiczii* Williams, 1974. In addition, *Rogersus* Williams, 1980 with its type species *R. rogersi* is transferred to *Promonobothrium* based on morphological characters typical of *Promonobothrium* and molecular data, and two new species are described from buffalos (*Ictiobus bubalus* – type host, and *I. niger*) and from eastern creek chubsucker (*Erimyzon oblongus*) in North America. Molecular phylogenetic analyses of six species based on sequences of the small and large subunits of nuclear ribosomal RNA genes (ssrDNA, lsrDNA) confirmed the monophyletic status of the genus and supported the validity of the species analysed. A key to identification of all species of *Promonobothrium* based on morphological characteristics is also provided.

## Future plans:

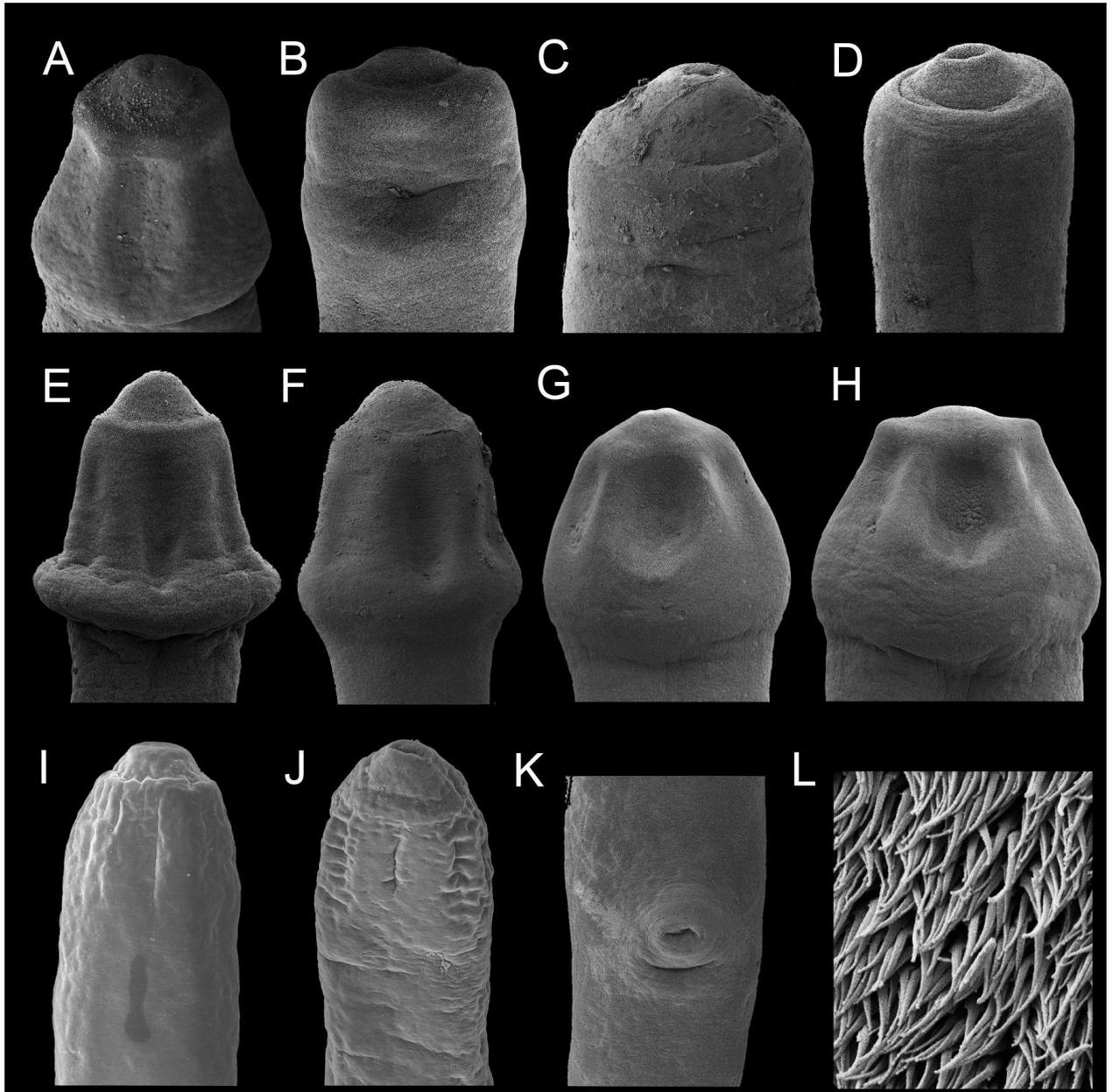
1. Based on a survey of freshwater fish from the areas with limited knowledge of fish parasites, to obtain material of tapeworms suitable for a complex morphological and molecular study.
2. Using combined approaches of integrative taxonomy, to provide reliable data on the species diversity, interrelationships and host-parasite-associations of selected groups of fish tapeworms.
3. To revise Nearctic species of monozoic tapeworms of the order Caryophyllidea and assess their phylogenetic relationships to those from other zoogeographical region, thus testing putative monophyly of the former group.

## Acknowledgement:

This study was supported by the ECIP (European Centre of Ichthyoparasitology); Centre of excellence program of the Czech Science Foundation (project No. P505/12/G112)

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**Figure 1** Scanning electron micrographs of species of *Promonobothrium* (A–J – anterior part with scolex, K – gonopores, L – acicular filitriches) A – *P. minytremi*, B – *P. ingens*, C – *P. hunteri*, D – *P. ulmeri*, E, F, K, L – *Promonobothrium* sp. 1., G, H – *P. rogersi* n. comb., I, J – *Promonobothrium* sp. 2.

## Evolution of diploid-polyploid *Carassius auratus* complex

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The presence of diploid and triploid forms of *Carassius auratus* complex in the Czech Republic is known for over 40 years. This complex consists of gynogenetic triploid females (triploid males are also recorded in very small frequency) and sexually reproducing form including males and females with similar frequencies. In this study, the *C. auratus* complex was studied from the selected locality in the connecting channels Lednice-South Moravia situated on the Dyje river. Using molecular analysis of mitochondrial DNA (D-loop region) the collected specimens of the complex were determined as "subspecies" of *Carassius auratus*, *C. gibelio*, *C. landsdorfi* and M-line. The majority of specimen belong to the *C. gibelio* haplotype.

We focused on selected measured of physiology and immunity including the specific immunity (IgM antibodies) and non-specific immunity (i.e. respiratory burst, lysozyme activity and complement activity). We also compared the haematological parameters and the level of parasite infection between two life strategies (sexual diploids versus gynogenetic polyploids) of *Carassius auratus* complex. Samples were collected in August during three consecutive years.

Our analyses revealed no difference in non-specific immunity between two reproductive forms but the difference in IgM production between gynogens and sexuals was found. Concerning the haematological parameters, only erythrocyte count and hematocrit was clearly affected by ploidy status. High investment in the reproduction was found for both gynogenetic and sexual females when compared to sexual males. No obvious difference was found in the abundance of different parasite group between diploid sexuals and triploid gynogens.

### Future plans:

1. Analyses of immune investment in diploid and triploid forms, the contribution of immunocompetence to the evolution of *C. auratus* complex.
2. Analyses of MHC variability and the selection pressure acting on MHC genes in sexual diploids and gynogenetic triploids.

### Acknowledgement:

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# Temperature-dependent proliferation of blood stages of *Sphaerospora molnari* in common carp (*Cyprinus carpio*) determined by real-time PCR

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Due to climate change, many fish species are affected by increased temperatures, rainfall and extreme weather conditions. Fish and their parasites respond to these changes and the latter show variations in their transmission, reproduction rates and disease effects. In myxozoans, moderate to medium high summer temperatures in Central Europe are often linked to faster parasite replication rates and higher host susceptibility caused by altered host immune function. In this study, we aimed at determining the temperature-dependent proliferation of *Sphaerospora molnari*, an emerging myxozoan pathogen in common carp [1], which proliferates in the blood prior to spore formation in the gills (Fig. 1).

We tested the effect of temperature on the prevalence of these blood stages over time. Specific pathogen free (SPF) carp fingerlings were acclimatized to temperatures mimicking winter to peak summer temperatures in Czech ponds (5°C to 30°C). Fish were infected with *S. molnari* by intraperitoneal and intramuscular injection of a defined number of blood stages and thereafter were bled repeatedly (10 times) over 100 days. DNA and RNA were extracted at each sampling date. An *S. molnari*-specific real-time PCR assay was designed and parasitemia levels were measured with two different parasite markers (SSU rDNA and EF2) [2]. Quantification was performed relative to host actin copies (Fig. 2).

The initial results demonstrate strong temperature-related differences in the onset of parasite proliferation and changes in proliferation rates, revealing the complexity and interplay between the host immune system, myxozoan parasitemia and water temperature. The results of this study will allow for predictions regarding *S. molnari* outbreaks in relation to the continuously rising pond temperatures in Central Europe.

## Future plans:

For a better understanding of the host immune response in relation to the number of parasites and to different temperatures, we are currently determining the expression of a variety of host immune markers (mostly cytokines) in these samples.

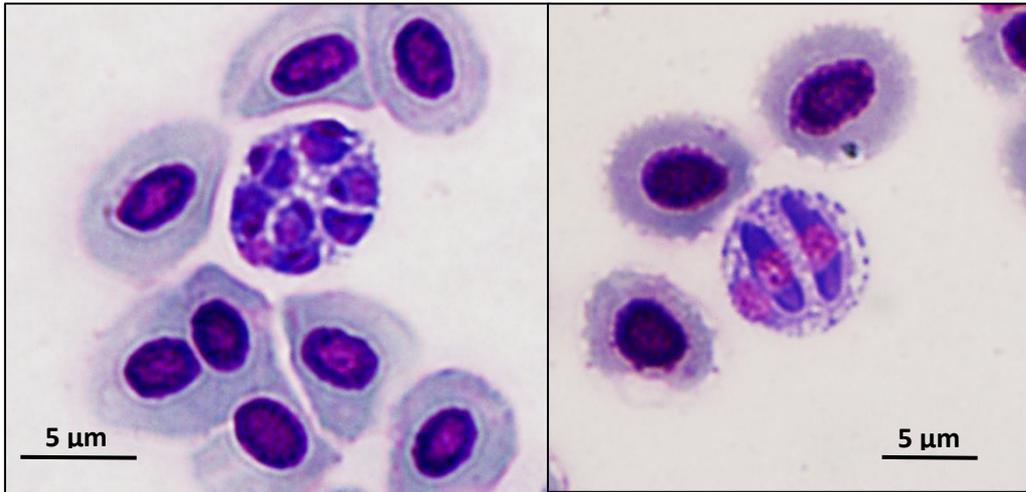
## Acknowledgement:

This study was supported by ECIP (European Centre of IchthyoParasitology); centre of excellence program of the Czech Science Foundation; project no. P505/12/G112.

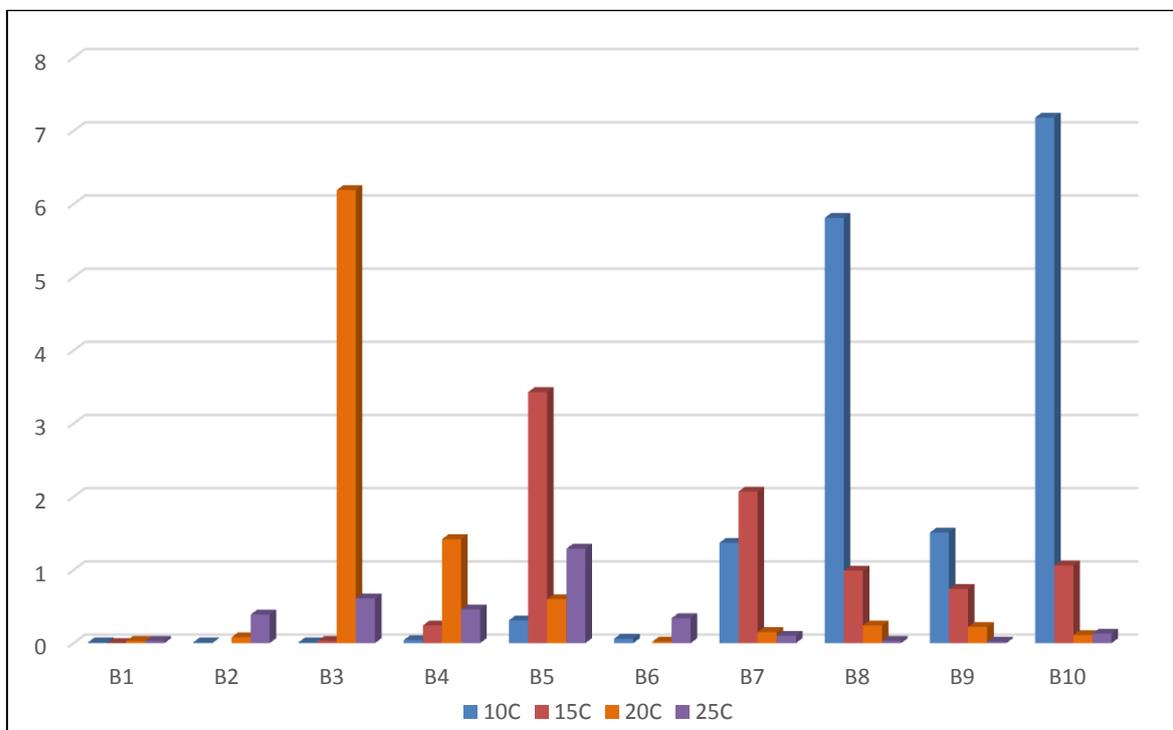
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**Fig 1:** Giemsa stained blood smears showing *S. molnari* proliferative parasite stages.



**Fig 2:** Relative quantification of parasitemia levels of *S. molnari* (SSU rDNA) in comparison to host (carp) actin, during 100 days post infection. X-axis indicates the bleeding times (for e.g. B1= bleeding 1) at 10 day intervals, Y-axis indicates parasitemia levels and colors of bars relate to different temperatures as indicated in the diagram.

# The attractiveness of the continents beginning from “A” for studies of various monogenean parasites

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Gyrodactylidea Bychowsky, 1937 (Monogenea) includes species that parasitize predominantly on the gills or the fins of fish. To date, only nine species of the genus *Gyrodactylus* Nordmann, 1832 (Gyrodactylidae Cobbold, 1864) and five species of the three genera of Tetraonchoididae (*Allotetraonchoides* Dillon & Hargis, 1968, *Neopavlovskioides* Dillon & Hargis, 1968 and *Pavlovskioides* Bychowsky, Gusev & Nagibina, 1965) have been described from fishes living in Antarctic waters. Parasites collected during the Antarctic summer expedition 2013 and 2014 from various Nothothenid fishes were studied. Species identification based on morphometric analysis of hard parts of attachment organ shown the presence of seven species from genera *Gyrodactylus* and *Pavloskoides*. From *Nothothenia coriiceps* two *Gyrodactylus* species were identified, *Gyrodactylus coriicepsi* and *Gyrodactylus* sp. 1. From the fish of *Trematomus* (*T. newnesi*, *T. bernacchi*, *T. eulepidotus* and *T. hansonii*) four *Gyrodactylus* species and two *Pavlovskoides* species were recognized, namely *G. antarcticus*, *Gyrodactylus* sp. 1, *Gyrodactylus* sp. 2, *Gyrodactylus* sp. 3 and *Pavlovskoides* sp. 1 and *Pavlovskoides* sp. 2. The molecular characterization of these specimens is currently in the process.

The genus *Afrodiplozoon* was proposed by Khotenovsky in 1981 when *Afrodiplozoon polycotyleus* (Paperna, 1963) has been excluded from the genus *Neodiplozoon* Tripathi, 1959 based on the number of clamps on the attachment apparatus. Specimens of *A. polycotyleus* were collected during a fish survey carried out in April and July 2014 in the Venda region, Limpopo Province, South Africa, from *Labeobarbus maraquensis* (n=36; mean total length=7.9 cm) and *Barbus paludinosus* (n=1; total length=6.6 cm). A prevalences of 64% and 59% were recorded for *A. polycotyleus* during April and July, respectively. Morphological analysis of the composition of the internal organs and attachment clamps using different microscopic methods (light microscopy of stained/unstained specimens and scanning electron microscopy, SEM) provided details for the redescription of the genus. The type material was also studied. Molecular characterization based on the variability of the second internal transcribed spacer (ITS2) rDNA showed the taxonomic relationship to other representatives of Diplozoidae. The obtained data provided sufficient results that the revision of the genus is needed [1].

The largemouth bass *Micropterus salmoides* (Lacepède, 1802) is an alien invasive species in South Africa (SA) that was introduced into the country in 1928 from England. Following introduction it was stocked for angling throughout SA. In North America, its native range, *M. salmoides* host several monogenean species. During this year, we determined whether any of these monogeneans were introduced via its hosts into SA. *Micropterus salmoides* were collected during October 2014 and March 2015 from Boskop Dam and Mooi River, respectively, North-West Province, SA and screened for monogeneans. Based on the morphometrics of the hard structures of the attachment organs, two monogeneans were identified, e.i. *Clavunculus bursatus* (Mueller, 1936) and *Onchocleides* sp. The latter species was compared with specimens of *Onchocleides dispar* (Mueller, 1936) previously found on *Lepomis gibbosus* L., 1758 from the Czech Republic, Slovakia, Bulgaria and Croatia, because of the very close morphological similarities of male copulatory organs. Differences in the 18S and 28S regions of rRNA sequences revealed that South African *Onchocleides* sp. represents new species. This is the first time that these parasites have been confirmed from Africa.

## Plans for next year:

To work on the description of new alien monogenean parasite of the *M. salmoides* from South Africa.  
To finish manuscript on the revision of the genus *Afrodiplozoon*.

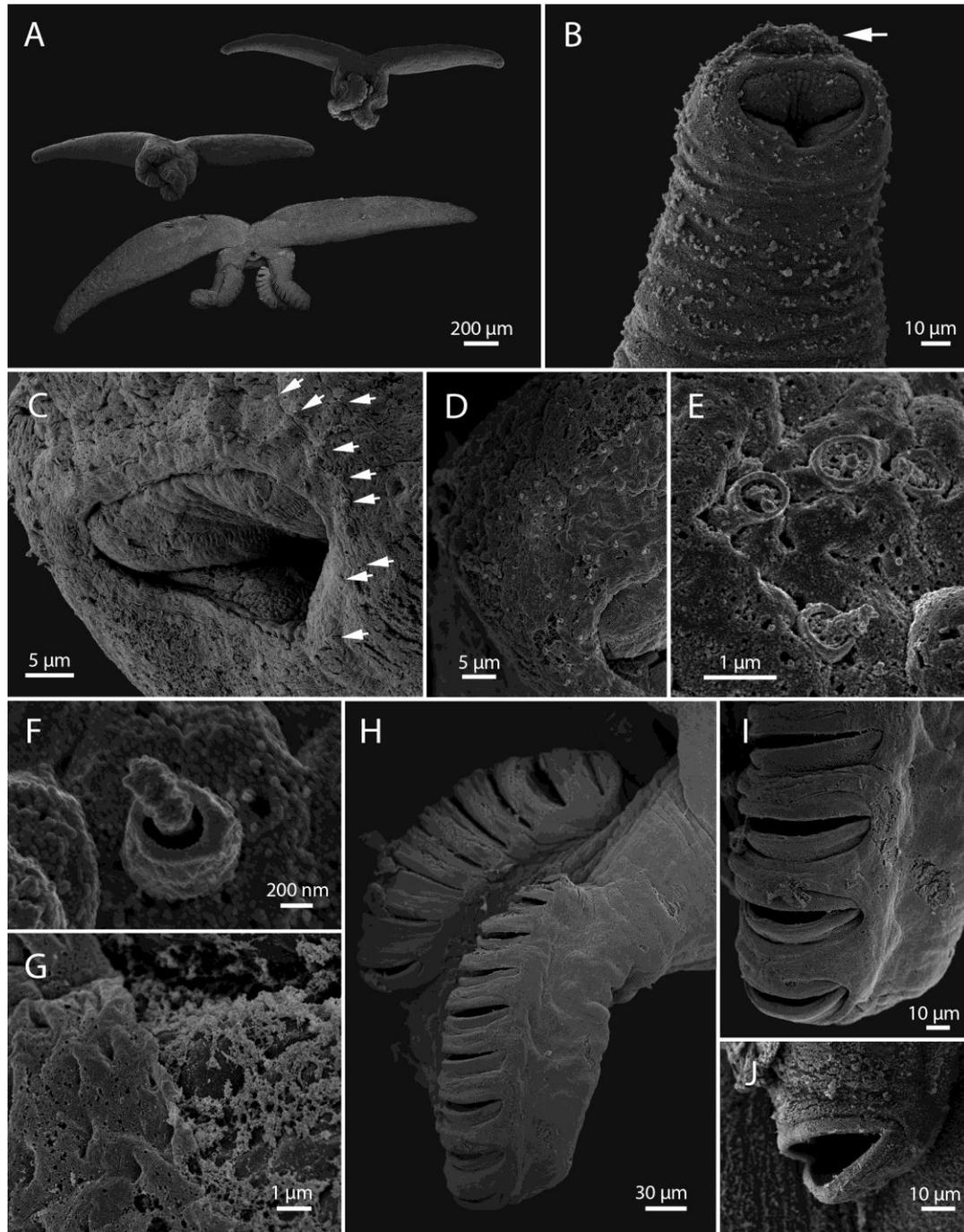
To finalize molecular characterization of Antarctic monogeneans of Gyrodactylidea and to start with new species descriptions of *Gyrodactylus* spp.

## Acknowledgement

The studies have been supported from the project no. **GBP505/12/G112** from the Czech Sciences Foundation, the fieldwork in South Africa was funded by the Biodiversity Research Chair (University of Limpopo).

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**Figure 1** Scanning electron micrographs of *Afrodiplozoon polycotyleus*: —A. Adult pairs in different views, —B. Forebody, —C. Detail of mouth —D., E. Detailed views on sensory organs, —F. Detail of ciliated organ, —G. Detail view on surface of integument showing surface secrets and micropores, —H. Attachments apparatus - opisthohaptor with the clamps organized in two rows, —I. Detail of one row of haptor, —J. Detail of terminal clamp. (Author of photos Š. Mašová)

# A phylogenetic perspective on species diversity: *Cichlidogyrus* (Dactylogyridae) parasitizing Lake Tanganyika cichlid tribes

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Lake Tanganyika, the deepest and oldest lake in Africa, harbors the most genetically, morphologically and ecologically diverse cichlid assemblages of the African Great Lakes. Its mostly endemic cichlids are considered as model to study adaptive radiation and rapid diversification. Because of the apparently high host specificity of gill monogeneans, there is an increasing interest to use phylogenetic analysis based on the molecular data of these parasites in order to study their host's diversity, evolution and host-parasite interactions. Based on our knowledge on freshwater fish monogeneans, we can confirm that the Lake Tanganyika cichlid fish harbor more parasites than cichlid species as suggested in the past. Currently, African cichlids can host five different genera of dactylogyrideans, *Cichlidogyrus* being the most diverse with more than 95 species recorded from more than 70 cichlid hosts. Members of *Cichlidogyrus* are gill ectoparasites. Species of *Cichlidogyrus* are identified morphologically by studying the sclerotized structures of haptor and male copulatory organ in addition with molecular data. The goal of this study is to investigate the phylogenetic affinities between the *Cichlidogyrus* fauna of different Tanganyika cichlid tribes employing different molecular markers (28S rDNA, 18S rDNA, ITS-1 and COX1). Monogenean species are described from *Cyprichromis microlepidotus* and *Eretmodus marksmithi*, the first representatives of cyprichromine and eretmodine hosts to be studied for monogeneans. Based on preliminary results, we suggest that phylogenetic relationships among *Cichlidogyrus* parasitizing the different Lake Tanganyika cichlid tribes may help us to elucidate the historic and ecological associations between cichlid tribes and to determine the origin of these Lake Tanganyika cichlid monogeneans.

## Acknowledgments

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# Sexual selection and male reproductive parasitism

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Sexual selection is a powerful mechanism of evolutionary change that results in differential reproductive success among individuals of the same sex. It explains both the emergence and the evolution of sexually dimorphic traits, including traits related to health and parasite load status. The extravagant male ornaments in many species are good indicators of the action of sexual selection. These ornaments are usually disadvantageous to a male's viability or survival, but they are advantageous in that they increase a male's mating success. An example of striking sexual ornamentation are males of Neotropical and especially African annual fishes. In the book chapter [1], we reviewed our current understanding of the extent, significance, role and constraints of sexual selection in annual fishes.

In a separate study [2], we focused on a neglected aspect of sexual selection – cognitive ability.

The ability to attract mates, acquire resources for reproduction, and successfully outcompete rivals for fertilisations may make demands on cognitive traits - the mechanisms by which an animal acquires, processes, stores, and acts upon information from its environment. Consequently, cognitive traits may undergo sexual selection. We investigated the role of cognitive traits on the reproductive performance of male rose bitterling (*Rhodeus ocellatus*), a freshwater fish with a complex mating system and alternative mating tactics. We quantified the learning accuracy of males and females in a spatial learning task and scored them for learning accuracy. Males were subsequently allowed to play the roles of a guarder (territorial tactic) and a sneaker (parasitic tactic) in competitive mating trials, with reproductive success measured using paternity analysis. By significant interaction between male mating role and learning accuracy on reproductive success, we found that the best performing males in maze trials showing greater reproductive success in a sneaker role (as reproductive parasites) than as a guarder. Using a cross-classified breeding design, learning accuracy was demonstrated to be heritable, with significant additive maternal and paternal effects. Our results imply that male cognitive traits undergo intra-sexual selection and demonstrate that parasitic male tactic is associated with enhanced spatial cognitive abilities. This is adaptive as the parasitic males suffer high sperm competition and need to distribute their ejaculates carefully.

## Future plans

In 2016, I plan to focus on data analysis and manuscripts preparation using the data from *Nothobranchius* field expeditions and laboratory tests.

## Acknowledgement

The work has been supported from the project no. P505/12/G112 from the Grant Agency of the Academy of Sciences of the Czech Republic.

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# Research on diversity of monogeneans from tropical and subtropical fishes

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Our current research concerning to monogeneans can be divided into the following five project lines:

**(1)** Structural and molecular diversity of *Haliotrema*-like dactylogyrids parasitizing goatfishes (Mullidae) off New Caledonia. Eleven of 13 species of goatfishes examined during our study were parasitized by 15 new and three previously described species of *Haliotrema*-like dactylogyrids. Morphological analysis of sclerotized structures suggests that there are at least six groups/morphotypes within the dactylogyrids parasitizing these fishes, with regard to the basic structure of the male copulatory organ. The division into five groups based on morphological criteria was also supported by results of a phylogenetic analysis using 28S rDNA sequences. Phylogenetic analysis also indicated that *Haliotrema* species parasitizing goatfishes off New Caledonia form a monophyletic clade within other representatives of *Haliotrema*-like dactylogyrids [1].

**(2)** Structural and molecular diversity of dactylogyrids parasitizing African tetras (Alestidae). Our survey of monogeneans found on the gills of 4 species of African tetras from Lake Turkana, Kenya, revealed the presence of four new and four previously described species of *Annulotrema*: *A. alestesnursi* Paperna, 1973 (from *Brycinus nurse*); *A. ansatum* n. sp., *A. besalis* Řehulková, Musilová and Gelnar, 2014, *A. bipatens* n. sp., *A. cucullatum* n. sp., *A. nili* Paperna, 1973, and *A. pontile* n. sp. (from *Hydrocynus forskahlii*); and *A. elongata* Paperna and Thurston, 1969 (from *Alestes baremoze* and *Alestes dentex*) [2].

**(3)** Structural and molecular diversity of monogeneans parasitizing African catfishes. Five species (one new) of *Quadriacanthus* were described and/or reported from the gills of 4 species of catfishes (Siluriformes) from Lake Turkana (Kenya): *Q. bagrae* Paperna, 1979 from *Bagrus bajad* and *B. docmac* (Bagridae); *Q. aegypticus* El-Naggar and Serag, 1986, *Q. clariadis* Paperna, 1961, and *Q. numidus* Kritsky and Kulo, 1988 from *Clarias gariepinus* (Clariidae); and *Q. mandibularis* n. sp. from *Heterobranchus bidorsalis* (Clariidae) [3].

**(4)** Species composition and structural diversity of monogeneans parasitizing the main African cichlid lineages.

Taxonomical evaluation of monogeneans found on two species of the lamprologine cichlids, *Lamprologus callipterus* (Burundi) and *Neolamprologus fasciatus* (Zambia), revealed the presence of 8 species of *Cichlidogyrus*, which will be described as new to science. One of the new species found possesses haptoral features that are unique among species of *Cichlidogyrus*. However, a phylogenetic analysis showed that this “unique” species clusters with *Cichlidogyrus* species possessing the “typical” haptoral configuration. Thus, although future discoveries of monogeneans from lamprologine cichlids may justify or require proposal of a new genus for this “unique” species, proposing a subgenus distinguishing it from all other species of *Cichlidogyrus* is presently preferable.

**(5)** Recently we examined five specimens of the African snake fish, *Erpetoichthys calabaricus*, with severe infestation of *Macroglyrodactylus polypteri* on the skin and fins. In the prepared paper, the original description of this species will be supplemented by morphological data on intraspecific variability of *M. polypteri* from different fish hosts, and scanning electron microscopy was used to outline the morphology, surface topography and attachment attitude (Figs. 1, 2)

## Future plans

In 2016, we intend to complete data on monogeneans presented above and make them publishable as soon as possible. The following tentatively entitled papers are in preparation: Dactylogyrids (Monogenea: Polyonchoinea) parasitizing goatfishes (Perciformes: Mullidae) off New Caledonia: what is the real *Haliotrema*?; Ancyrocephalidae (Monogenea) of Lake Tanganyika: V: *Cichlidogyrus* parasitizing species of Lamprologini (Teleostei, Cichlidae), with descriptions of eight new species and a proposal of *Neocichlidogyrus* as a subgenus within *Cichlidogyrus*; Morphological aspects of *Macroglyrodactylus polypteri* Malmberg, 1957 (Monogenea: Gyrodactylidae) from *Erpetoichthys calabaricus* Smith, 1865 (Polypteriformes: Polypteridae) by light and scanning electron microscopy.

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3. Francová K., Seifertová M., Blažek R., Gelnar M., Řehulková E.: *Quadriacanthus* (Monogenea: Dactylogyridae) from the gills of four species of African catfishes, with description of *Q. mandibularis* n. sp. parasitizing *Heterobranchus bidorsalis* (Siluriformes: Clariidae) [Submitted in *Parasitology Research*].

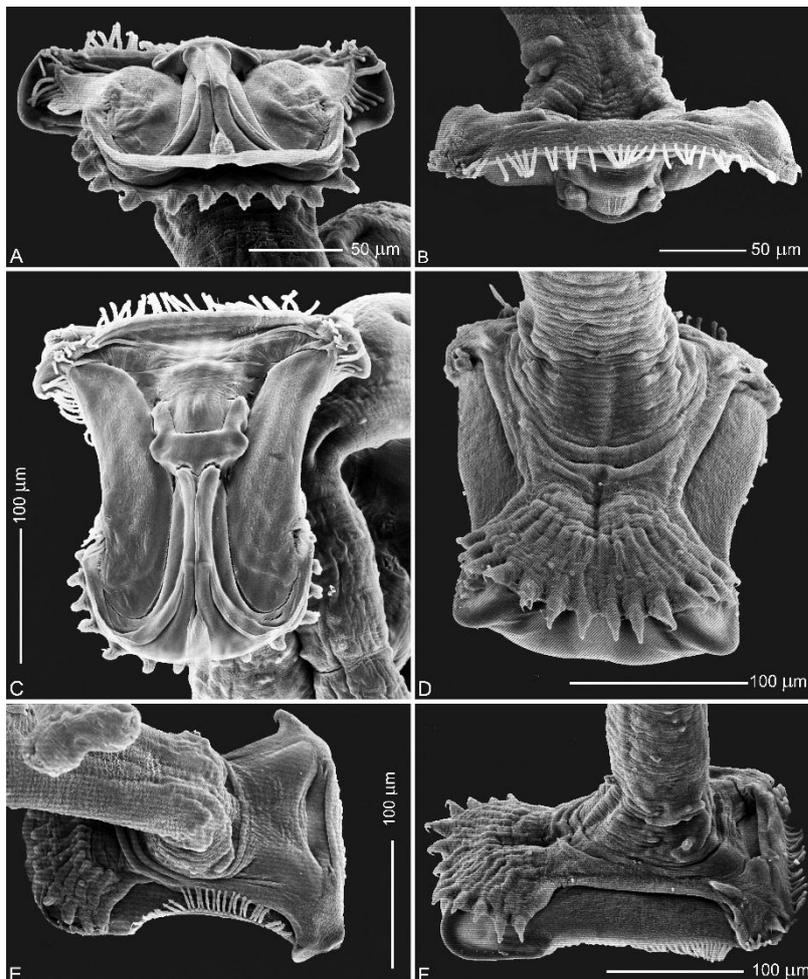


Fig. 1 *Macrogyrodactylus polypteri*, different views on haptor. SEM

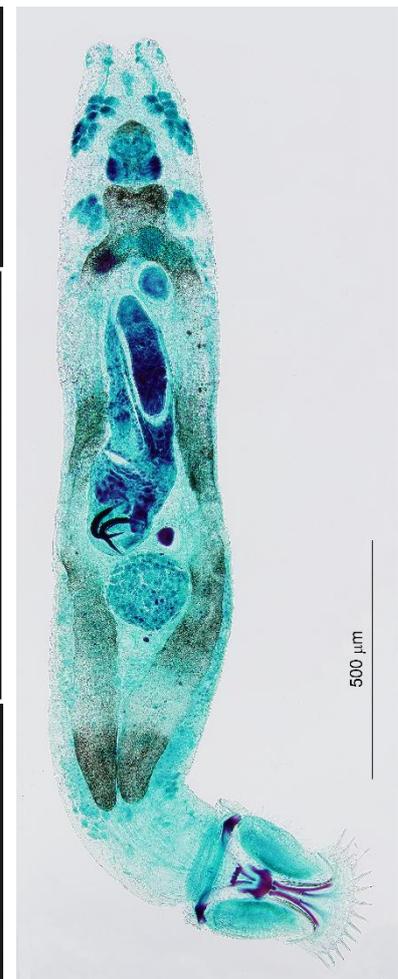


Fig. 2 *M. polypteri*, whole worm. Gomori's trichrome staining.

# Incorporating molecular data in systematics of selected African fish parasites

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The application of molecular methods into systematic studies may expand our understanding of African fish parasite diversity. Therefore, molecular characterization and investigation phylogenetic relationships of selected dactylogyrids and parasitic copepodes of African freshwater fishes were performed using different nuclear and mitochondrial DNA markers.

The evolutionary history of dactylogyrids infecting catfishes from the White Nile and Blue Nile (Sudan) was investigated based on partial sequences of large subunit nuclear ribosomal RNA (28S rDNA). Seventeen newly generated 28S rDNA sequences were used for preliminary phylogenetic analysis, which confirmed their division into the five genera: *Quadriacanthus*, *Protoancylodiscoides*, *Schilbetrema*, *Synodontella* and *Bagrobdella* [1]. Investigating phylogenetic status of these genera within dactylogyrids of the siluriform fishes revealed that Dactylogyridae was split into two strongly supported clades. Clade I included the freshwater dactylogyrids of Neotropical region. Clade II contained parasites of siluriform fishes from three distinct geographical regions (Ethiopian, Oriental and Neotropical). Monogeneans of African catfishes (*Bagrobdella*, *Schilbetrema*, *Synodontella*, and *Protoancylodiscoides*) clustered as a strongly supported monophyletic group together with Asiatic species. *Quadriacanthus* was found to form clade with Asiatic species *Q. kobiensis* and *Bychowskiella pseudobagri*.

Molecular characterization and phylogenetic analyses of *Annulotrema* species (Monogenea: Dactylogyridae) of African tetras (Characiformes: Alestidae) from Lake Turkana (Kenya) were performed based on 28S rDNA [2]. The first molecular data for *A. ansatum*, *A. cucullatum*, *A. besalis*, *A. nili* and *A. pontile* were obtained. The phylogenetic analyses including the newly obtained 28S rDNA data and molecular data for other species of Dactylogyridae parasitizing African freshwater fishes revealed that *Annulotrema* species parasitizing African tetra *Hydrocynus forskahlii* formed a strongly supported monophyletic group.

The phylogenetic relationships among the parasitic copepodes (Ergasilidae and Lernaeidae) of African freshwater fishes collected in Senegal and Kenya were examined based on partial sequences of 18S and 28S rDNA. Preliminary phylogenetic analyses confirmed division of the examined specimens into five genera within Lernaeidae (*Lernaea*, *Opistholernaea*, *Dysphorus*, *Lernaeogiraffa* and *Lamproglena*) and one genus in the Ergasilidae (*Ergasilus*). All the analyses suggested that all six genera are monophyletic.

## Future plans:

Processing of DNA samples of dactylogyrids of African catfishes will be completed and molecular analyses clarifying phylogenetic relationships with representatives from Ethiopian, Oriental and Neotropical regions using 28S and 18S rDNA sequences will be performed. Co-phylogenetic analyses of dactylogyrids of African catfishes and their hosts (based on mitochondrial *cyt.b*) will be performed. The phylogenetic relationships among parasitic copepodes based on the sequences of the mitochondrial cytochrome c oxidase subunit I gene (*COI*) will be clarified.

## Acknowledgement

The studies have been supported from the project no. GBP505/12/G112 from the Grant Agency of the Academy of Sciences of the Czech Republic.

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# Diversity and interrelations of tapeworms (Cestoda: Proteocephalidea), parasites of teleost fishes

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Proteocephalidean tapeworms form a diverse group of parasites with most of 315 valid species known from freshwater fishes (predominantly catfishes). The largest molecular dataset of these cestodes comprising over 100 species (30 new), including representatives of 54 genera (80%) and all subfamilies, was analysed using partial sequences (D1–D3 region) of the 28S rRNA gene sequences. The Old World origin of proteocephalideans and their more recent expansion in South America were confirmed. The earliest diverging lineages are composed of Acanthotaeniinae and Gangesiinae but most of the presently recognized subfamilies (and genera) appear not to be monophyletic. It is thus obvious that a reorganisation of the order is needed and the present subfamilial system should be abandoned. Some new characters, such as the pattern of uterus development, relative ovary size, and egg structure were defined. Although several improvements over previous works regarding phylogenetic resolution and taxon coverage were achieved in this study, the major polytomy in our tree, composed largely of siluriform parasites from the Neotropical Region, remained unresolved and possibly reflects a rapid radiation. The genus *Spasskyellina* Freze, 1965 is resurrected for 3 species of *Monticellia* bearing spinitriches on the margins of their suckers (de Chambrier, Waeschenbach et al., 2015).

An update is provided of the previous list of adult proteocephalidean tapeworms (Cestoda) parasitizing freshwater teleosts from the Peruvian Amazon, which was presented by de Chambrier et al. (2006a). Four new samplings made it possible to almost double the number of species found, all of them representing new geographical records from Peru. With 35 newly added species, a total of 63 proteocephalidean cestodes (46 named species of 27 genera) are now reported from Amazonia in Peru (compared to 54 named species of 28 genera from its Brazilian part). The highest number of proteocephalideans is reported from *Pseudoplatystoma fasciatum* (a total of 10 cestode species), *Zungaro zungaro* (previously named *Paulicea luetkeni*; 9 species) and *Phractocephalus hemioliopertus* (8 species). A high number of unnamed species found in Peru (17), which most probably represent taxa new to science including at least two new genera, demonstrates that the species richness of proteocephalidean cestodes in Amazonia is still poorly known (de Chambrier et al., 2015).

A new genus, *Frezella* gen. n., was proposed to accommodate *Frezella vaucheri* sp. n. from poorly known auchenipterid fish, *Tocantinsia piresi* (Miranda Ribeiro), from the Xingú River, one of the principal tributaries of the lower Amazon River in Brazil. The new genus belongs to the Proteocephalinae because of the medullary position of the testes, ovary (yet some follicles penetrate to the cortex on the dorsal side), vitelline follicles and uterus. It differs from other proteocephaline genera in the morphology of the scolex, which includes a metascolex composed of two distinct zones: anterior, strongly wrinkled part posterior to the suckers, and posterior, sparsely folded zone. *Frezella vaucheri* is the first helminth parasite reported from *T. piresi*, which occurs in the lower reaches of the Amazon and Tocantins River basins in Brazil (Alves et al., 2015).

## Future plans

Other molecular markers including mitochondrial gene sequences will be used to resolve major polytomies in the phylogenetic tree of the order. In addition, recent sampling in South America (Brazil) will provide samples of new taxa for future molecular phylogenetic analyses, but also for formal description of putative new taxa including new genera that form monophyletic lineages in the present analyses inferred from the 28S rRNA gene sequences. Three long stays of the first author in Brazil (in total 9 months and 8 collecting trips in the Amazon and Paraná River basins) in 2013–2015 and his co-supervising of a Brazilian graduate student, Philippe Vieira Alves, will enable a deeper study on the Neotropical taxa based on morphological and molecular data.

## Acknowledgement

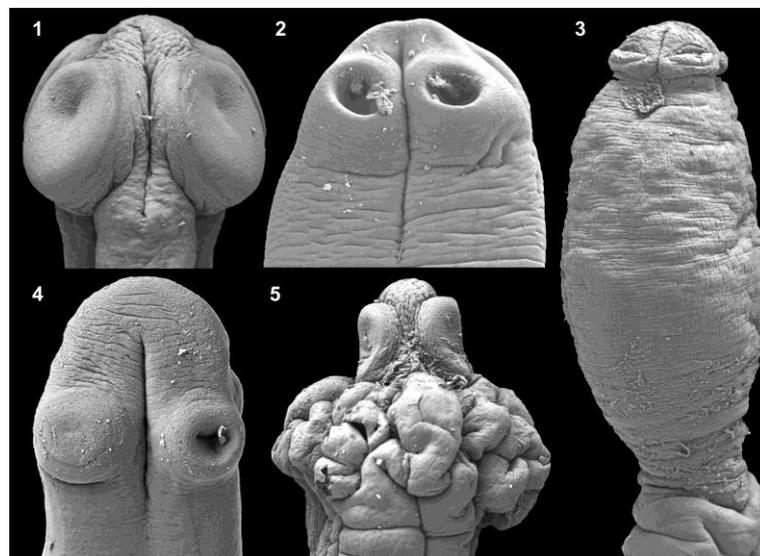
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**Figures 1–5** Scanning electron micrographs of scoleces of proteocephalidean cestodes found in the Peruvian Amazonia. 1 – *Nomimoscolex lopesi* from *Pseudoplatystoma punctifer* (PI 708); 2 – *Proteocephalus* sp. 2 from *Pterodoras granulosus* (PI 635); 3 – *Jauella glandicephalus* from *Zungaro zungaro*; 4 – *Proteocephalus kuyukuyu* from *Megalodoras uranoscopus* (PI 324); 5 – *Spatulifer rugosa* from *P. punctifer* (PI 708). 1, 3, 5 – lateral view; 2, 4 – dorsoventral view (compiled from de Chambrier et al., 2015).

# Molecular phylogeny of *Dactylogyrus* species: a potential tool to investigate the historical biogeography of cyprinids

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Over evolutionary time periods the diversity of parasite assemblages is shaped by coevolutionary and historical biogeographical processes. Host specificity of fish parasites seems to be the most important parasite characteristic for understanding of freshwater fish biogeography.

*Dactylogyrus* are gill parasites highly specific to freshwater fish of Cyprinidae. This highly diversified parasite genus includes many strictly host specific species but also the species specific to congeneric or phylogenetically closely related host species. Many *Dactylogyrus* coexist on the same host species. *Dactylogyrus* evolve mainly by intrahost speciation and the species coexisting in the same host differ in their niche position. Concerning their hosts, cyprinids originate from Asia and classically are divided into several subfamilies: Cyprininae, Rasborinae, Acheilognathinae, Xenocyprinae, Gobioninae, Tincinae, Leuciscinae and Alburninae. The cyprinids show the specific pattern of biogeographical distribution. Whilst the members of all subfamilies are widely distributed in Asia, some cyprinid groups are absent in Europe. Only the representatives of Leuciscinae in North America are present. African cyprinids consist of Cyprininae (barbins and labeonins) and Rasborinae. Following the dispersal's view, the cyprinids dispersed to Africa via connection in the north-east (18-16 mya). Five independent dispersal events from Eurasia to Africa have been proposed.

The aims of his study were to investigate the phylogenetic position of African *Dactylogyrus* parasites in relation to European and Asian *Dactylogyrus* lineages and (2) to evaluate whether *Dactylogyrus* is a suitable biological marker to infer cyprinid biogeography.

The phylogenetic position of West African *Dactylogyrus* and *Dactylogyrus*-related parasites and the phylogenetic position of North African *Dactylogyrus* parasites within *Dactylogyrus* phylogeny including the representatives from Europe and Asia were investigated. Our analyses revealed that *Dactylogyrus* is not monophyletic. *Dogielius* and *Dactylogyroides* were nested in *Dactylogyrus* of Cyprininae which suggests the need of taxonomical revision for *Dactylogyrus*-closely related genera. We showed that *Dactylogyrus* of West Africa originated from Asian Cyprininae and their diversity likely result from multiple dispersal of Asian cyprinids to Africa. The phylogenetic analyses focussed on North African *Dactylogyrus* suggest three independent origins (European, African and Asian) of *Dactylogyrus* diversity in North African cyprinids which is in line with their historical biogeography, and especially supports the different origins and historical biogeography of large sized barbs and small sized *Luciobarbus* species in North Africa.

## Future plans

The different scenarios of historical biogeography of cichlid fish species across different continents will be analyzed using the phylogenetic reconstruction of cichlid specific monogenean parasites.

## Acknowledgement

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# Motility and host-parasite interactions in early emerging apicomplexans

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Phylum Apicomplexa comprises exclusively parasitic protists that infect a wide spectrum of vertebrates and invertebrates. In contrast to intensively studied apicomplexan etiologic agents of human and animal diseases (e.g. malaria, toxoplasmosis, cryptosporidiosis), only little attention is paid to the basal lineages restricted to the invertebrate hosts remain. To fulfil the gaps in our understand of evolutionary pathways and biodiversity of Apicomplexa, our research focuses on early emerging groups with emphasis on lower coccidia, blastogregarines, archigregarines and eugregarines restricted to marine invertebrates.

The detailed study on host-parasite interactions of protococcidian *Eleutheroschizon duboscqi*, inhabiting the intestine of the marine polychaete *Scoloplos armiger*, was finished and published [1]. Study showed that *E. duboscqi* develops attached to the host intestinal epithelium in an epicellular position. Attached parasites share features of cryptosporidia and gregarines, i.e. they conspicuously resemble a maturing trophozoite of epicellular eugregarines with morphologically pronounced attachment apparatus, but are contained within a host-derived parasitophorous sac similar to that in cryptosporidia. In contrast to these organisms, however, *E. duboscqi* parasites have no intimate contact with the enterocyte membrane.

Second study, focusing on the motility of blastogregarine *Siedleckia nematoides* parasitising *Scoloplos armiger*, was finalised and summarised in a manuscript [2] (under English proof-reading, will be submitted in November 2015). Using a combined microscopic approach, for the first time we present an experimental study on motility of marine apicomplexan restricted to the invertebrate host. Study revealed that despite the presence of key glideosome components such as actin including its filamentous form, myosin associated with cortex, subpellicular microtubules, numerous micronemes and prominent glycocalyx layer, we cannot conclude that motility of *S. nematoides* is based on above described glideosome machinery known from zoite stages of another apicomplexans [3]. It is especially because *S. nematoides* individuals move independently on a solid substrate (combination of pendular, twisting, undulation, and spasmodic movements) and show no signs of gliding motility. We succeed to prove the essential role of polymerised form of actin and tubulin in *S. nematoides* motility, and our observations suggest that the subpellicular microtubules organised in several layers are the real leading motor structures. Furthermore, the majority of detected actin was stabilised in a polymerised form and it rather appeared to be located deeper (i.e. beneath the inner membrane complex) than generally described in glideosome. We speculate that this putative actin cytoskeleton associates lengthwise with subpellicular microtubules to force their synchronised bending in some cell regions and this might generate the typical undulating movements of *S. nematoides*.

Next model organism used for our research on gregarine motility is the aseptate eugregarine *Polyrhabdina* sp. parasitising the polychaete host. Interesting new data on drug-induced depolymerisation of cytoskeletal elements, as well as their re-polymerisation after parasites' recovering, were obtained this year. Parasites were also collected for additional approaches, such as freeze etching and molecular-biological analyses.

## Other outcomes

New samples of *S. armiger* intestine parasitised with *E. duboscqi* epicellular stages were collected for freeze etching analysis to investigate the supramolecular organisation of host-parasite interactions. While preliminary evaluation proved the protocols used for material fixation and subsequent preparation for freeze etching to be suitable for our research purposes, further sampling will be needed to obtain publishable data.

Putatively new species of marine apicomplexans from polychaete and nemertean hosts were collected and processed for preliminary analyses.

Other outcomes partially funded from this project include two recently prepared manuscripts dealing with the phylogeny (AV is co-author) and ultrastructure (AV is first author) of *Nematopsis temporariae* - the first gregarine reported from a vertebrate host. Research was performed in collaboration with Miloslav Jirků (Institute of Parasitology BC ASCR, České Budějovice).

Obtained data were also used for educational purposes in the form of 2 invited lectures at the seminars for MSc. and PhD. students at the Department of Zoology, Faculty of Natural Sciences of Comenius University in Bratislava (October 2015) and Faculty of Science Charles University in Prague (November 2015).

### Future plans

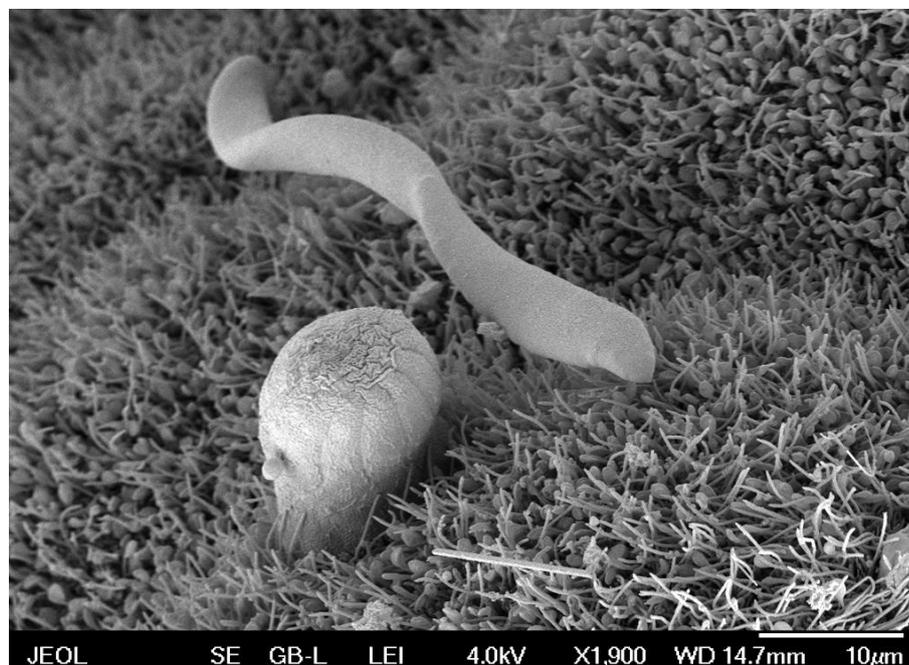
Ongoing material collection, fulfilling the missing data/methods, searching for new model marine apicomplexans, work on organisms found or even partially processed during sampling in previous years but not listed in this abstract, preparation or finalisation of planned manuscripts, supervising the PhD students involved in this research.

### Acknowledgement

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**Figure 1:** *Eleutheroschizon duboscqi* (left) and *Siedleckia nematoides* (right) attached to the *Scoloplos armiger* intestinal epithelium (SEM).

# (African) monogeneans in the context of speciation, biological indicators and invasive species

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We continued to study the phylogeny and biodiversity of Monogenea of teleost radiations, especially belonging to Cichlidae from Lake Tanganyika and the rest of the Congo Basin.

Together with PhD student N. Kmentová (supervised with M. Gelnar), we focused on the monogenean parasites of deepwater cichlids from Lake Tanganyika, showing that *Cichlidogyrus casuarinus* has a wide host range, probably as an adaptation to low host availability in its deepwater habitat, while also noticing that other lineages of *Cichlidogyrus* were able to infect deepwater hosts. A study on scanning electron microscopy of *Cichlidogyrus* (with W. Fannes and G. Paladini) also used *C. casuarinus* as a model. A number of studies linking the phylogeny, systematics and ecology of monogeneans and their trophic cichlid hosts were published (from the PhD theses of myself, M. Van Steenberghe and P. Hablützel, the MSc thesis of A. Grégoir and J. Bamps, and the post-doc of J. Raeymaekers). The description of morphologically very divergent monogeneans of lamprologine Tanganyika cichlids is being finalized together with E. Řehulková. Our understanding of the diversity and phylogeny of dactylogyridean monogeneans parasitizing other Tanganyika cichlid tribes is continuously improving thanks to the PhD research of C. Rahmouni (supervised with A. Šimková), who was the first to look at the gill parasites of eretmodine and cyprichromine cichlids.

Another, more applied, aspect of our research deals with anthropogenic impacts in the Congo Basin. MSc student G. Kapepula Kasembele (Université de Lubumbashi) (co-supervised with A. Chocha Manda) is looking at the diversity monogeneans of catfishes and “tilapiine” cichlids in the Lufira Basin (D.R.Congo), aiming to assess the influence of chemical pollution as a result of mining activities. PhD student M. Jorissen (Hasselt University) (supervised with T. Huysse and T. Artois) is looking, from a parasitological perspective, at the effects of the introduction of Nile tilapia into various areas of the Congo Basin. He is currently focusing on the characterization of the monogenean fauna of native “tilapiine” hosts in the Upper Congo. These PhD students both successfully finished their MSc under my (co-)supervision in 2015, being awarded the dean’s prize of the Faculty of Science (N. Kmentová) and shortlisted for the Jacques Kets Prize for Biology of the Royal Zoological Society of Antwerp (M. Jorissen).

We also reported on the monogeneans of some invasive alien gobies in Belgium.

We collaborate with numerous African ichthyologists and parasitologists regarding the parasitology of a wide range of other hosts. For example, I co-organised with S. Wamuini Lunkayilakio a workshop in the Lower Congo region, taught with A. Pariselle and F. Muterezi Bukinga. In agreement with the interest of the Brno parasitology team in dactylogyridean parasites of African cyprinids, PhD students M. Beletew (Addis Ababa University, Ethiopia) (supervised with A. Getahun) and I. Rahmouni (Université Mohammed V, Morocco) (supervised by O. Berrada-Rkhami, A. Pariselle and A. Šimková) carried out a research visit to Belgium. E. Netherlands (University of Leuven/North-West University) (supervised with L. Brendonck, N. Smit and L. Du Preez) is starting a PhD on the blood parasites of South African amphibians.

These projects require a profound understanding of the fish hosts, which is supplemented by colleagues such as S. Koblmüller, C. Sturmbauer, K. Sefc (University of Graz), J. Snoeks, E. Vreven, E. Decru (Royal Museum for Central Africa), H. Verreycken (Research Institute for Nature and Forest) and F. Volckaert (University of Leuven).

## Perspectives for 2016

Finalising and submitting several manuscripts of the abovementioned PhD students and from my own PhD research (parasites of Balkan sand gobies and Middle Congo Basin cichlids). Sampling and field training in Madagascar and the D.R.Congo. Improving our skills to work with biodiversity indicators.

## Acknowledgement

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## “Omics” and population genetic tools applied on selected species from class Monogenea.

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Our study is focused on three model organisms *Eudiplozoon nipponicum*, *Paradiplozoon homoion* and *Paradiplozoon gracile* all of them are representing blood sucking fish ectoparasites from group Monogenea: Diplozoidae. Our project consists of two parts according to the used methods.

The first part combining the modern pyro-sequencing methods and computer databases for the faster and more accurately identification of numerous sets of protein molecules, which are essential for life of monogeneans.

The second part of study is more focused on population genetics and description of each sample at the individual level. In order to obtain this type of data, we decided to use amplified fragment length polymorphism technique (AFLP).

Our four aims are realized; (i) compare the genetic diversity in populations of monogenean species, (ii) determine the level of genetic variability of the two permanently fused worms isolated from the same fish (“sex factor”), (iii) reveal the intra- and inter-population patterns and (iv) evaluate the effect of different host species on genetic plasticity of monogenea with generalist life strategy.

We started the preliminary analyses leading to generation of *E. nipponicum* genome, transcriptome and proteome databases. We would like to use data platforms for identification and further characterization of protein molecules involved into the interaction between host and parasite.

In the order to check the volume of obtained genomic data vs. reality we used the DNA fluorescent double staining method, which represents simple and easy checking point for obtained bioinformatics data. This method was designed for single-celled organisms and therefore we had to optimise the dying protocol and we are currently working on analysis settings for multi-celled organisms.

The mRNA *E. nipponicum* adult in the form of sort reads/transcripts was analyzed using specific software tools. e.g. Trimmomatic, SPAdes, TopHat, Bowtie2, Trinity, SOAPdenovo and Velvet-Oases. Whole data set contains approximately 20 000 unique sequences.

The preliminary experiments related to the population genetics have been initiated; 8 *Eudiplozoon nipponicum* worms were separated (“head part of body”, “leg part of body”, “fused part of body”), DNA was isolated and primers for AFLP are designed.

### Future plans

- Finish the first experimental estimation of *E. nipponicum* genome size.
- Perform the complete annotation of *E. nipponicum* transcriptomic data.
- Finish the “sex” factor analyses of *E. nipponicum*.
- Collect appropriate number of *P. homoion* and *P. gracile* adults and resolve the taxonomic relevancy of these species. start working on population genetics and phylogenetic analyses

### Acknowledgement

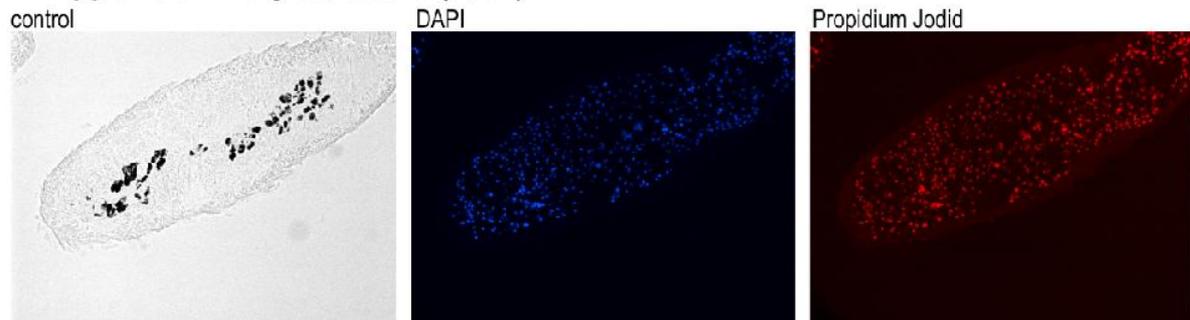
The study was supported from the projects: Czech Science Foundation (GBP505/12/G112, P506/12/1258), grants of the Masaryk University (MUNI/A/1484/2014) and grants of the Charles University in Prague (UNCE, PRVOUK P41, SVV, GAUK 502313).

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### ***E. nipponicum* - cryosections (100x)**



# An overview of new data about genus *Gyrodactylus* parasitizing African cichlids: patterns in the occurrence and diversity

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Parasites of the genus *Gyrodactylus* von Nordman, 1832 are worldwide spread viviparous parasite, predominantly parasitising gills, fins and skin of bony fishes. In Africa, the knowledge about diversity of these viviparous monogeneans is still poorly known. To date, only 33 species of *Gyrodactylus* have been described from host of various families. Present survey identified and prepared for publication evidence of 13 species of *Gyrodactylus* from 12 host species belonging to the family Cichlidae (1, 2).

Specimens of host fishes were collected from 10 localities located in four drainage basins, Limpopo River, Zambezi River, Congo River and Nile River. All studied hosts belong to 15 tribes but only fishes from six tribes have been found to be infected by *Gyrodactylus* spp.: Coptodini, Ectodini, Eretmodini, Haplochromini, Oreochromini and Tilapiini. In total 63 of 425 host individuals from 13 species were infected by species of *Gyrodactylus* species, these are *Astotatilapia burtoni*, *Coptodon rendalli*, *Ctenochromis horei*, *Eretmodomus marksmithi*, *Hemichromis bimaculatus*, *Ophthmotilapia nasuta*, *Oreochromis niloticus*, *Pharyngochromis acuticeps*, *Pseudocrenilabrus philander*, *Simochromis diagramma*, *Tilapia sparmanii* and *Tilapia zilli*. Diversity of *Gyrodactylus* species were relatively high, 16 species were identified including *G. thysi*, *G. thlapi*, *G. ulinganisus* and *G. yacatli*. Based on similar morphological features of opisthaptoral hard parts, these were divided into six morphologic groups. Subsequently molecular and phylogenetic analyses were performed based on rDNA ITS sequences. Both approaches reveal same results, i.e. relative species share the similar morphologic type of hard parts (see Fig. 1). Differences in the species composition of *Gyrodactylus* and intensities of infection on its hosts between drainage basins were observed. The highest proportion of examined hosts infected 33% and the highest species as diversity of *Gyrodactylus*, 11 species, were observed in Zambezi River drainage basin. The majority of individuals of *Gyrodactylus* were collected from fins and skin, only *G. nyanzae* was found on the gills and three species, including *G. sturmbaueri*, *G. yacatli* and *Gyrodactylus* sp. infect both sites either gills and fins.

In addition, the suitability of two morphometric approaches (geometric morphometry and elliptic Fourier analyses) evaluating the shape of the opisthaptoral hard parts for discrimination of cryptic species of *Gyrodactylus* were evaluated. Species determination was based on the shape of two main morphologic determination features hamuli and marginal hooks. Both analyses show high sensitivity to discrepancies of hooks orientation. Results also showed that for the purpose of more objective assessment and subsequently potential practical application geometric morphometry approach based on landmark and semi-landmarks is more appropriate.

## Future plans

Work on the manuscript dealing with discrimination of eight species with same morphologic features of opisthaptoral hard parts, including description of three new species, new records for *G. thysi*, *G. thlapi* and evidence for other three *Gyrodactylus* spp. Further analysis based on the molecular and distribution data available.

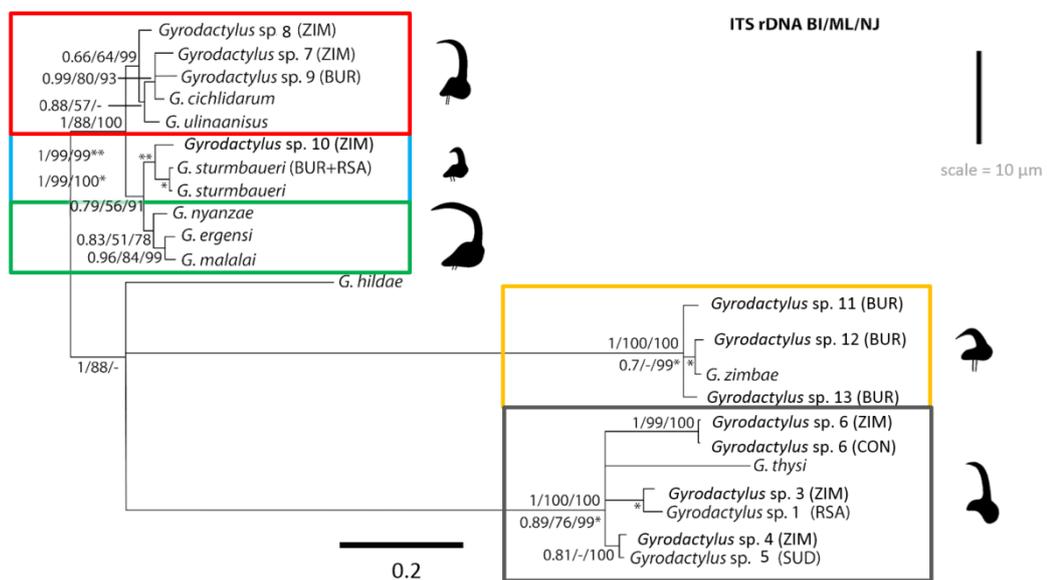
## Acknowledgement

The studies have been founded from the project no. GBP505/12/G112 from the Grant Agency of the Academy of Sciences of the Czech Republic and in collaboration with Biodiversity Research Chair, University of Limpopo.

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2. Petra Zahradníčková, Maxwell Barson, Wilmien J. Luus-Powell, Milan Gelnar, Iva Přikrylová. Identification of eight cryptic species of *Gyrodactylus* von Nordmann, 1832 parasitizing African cichlids. (in preparation)



**Figure 1** Phylogram of studied *Gyrodactylus* spp. based on ITS rDNA gene sequences. Tree topology was identical across Neighbour Joining (NJ), Maximum Likelihood (ML) and Bayesian inference (BI). Frames represents species division into morphologic groups according to opishaptoral hard parts appearance. Marginal hook of one species from each morpho-group is presented in scale.

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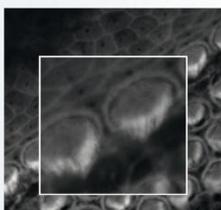
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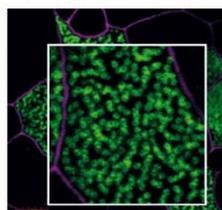


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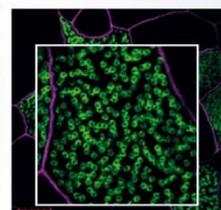


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# Schoeller

**dodávky a servis laboratorní techniky a spotřebního materiálu**

- Hlubokomrazicí boxy
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SCHOELLER INSTRUMENTS s. r. o., Vídeňská 124, 148 00 Praha 4

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## KULTIVACE ROSTLIN, ROSTLINNÝCH TK A HMYZU

### Komory pro pěstování rostlin a rostlinných TK

Panasonic



#### MLR 352

Boční osvětlení ze 3 stran  
Celková kultivační plocha 0,98 m<sup>2</sup>  
Teplotní rozsah: 0 až +50 °C ±0,3 °C  
Osvětlení 0 - 20 000 luxů (nastavitelná intenzita)  
5 nastavitelných polic  
Verze **352 H** s regulací vlhkosti 55 - 90%

### Univerzální box pro pěstování rostlin a rostlinných TK

Conviron

#### ADAPTIS A1000

Objem 1000 l  
Osvětlovací kity pro rostliny, TK, hmyz  
Teplotní rozsah +4°C až +40°C při zhasnutých světlech  
Teplotní rozsah +10°C až +45°C při zapnutých světlech  
Regulace vlhkosti a intenzity osvětlení



DODÁVKY-SERVIS-MONTÁŽ

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