

**6th Workshop  
of the European Centre of IchthyoParasitology**

**Programme and Abstracts**



**Michal Benovics, Zuzana Kobíková, Milan Gelnar (Eds.)**

**Hotel Skanzen, Velehrad  
27–29 November 2017**



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Scanning electron micrograph

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## Workshop sponsors

The organizers gratefully acknowledge the generous support.

# OLYMPUS

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INSTRUMENTS



# ECIP Scientific Board

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## List of attendants

### Research group of Milan Gelnar – Department of Botany and Zoology, Faculty of Science, Masaryk University, Brno

Michal Benovics	Martin Kašný	Simona Strouhalová
Andrei Diakin	Vadym Krasnovyd	Andrea V. Šimková
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Daniel Barčák	Roman Kuchta	Bjoern C. Schaeffner
Gabrielle van Beest	Jiří Kyslík	Tomáš Scholz
Viviane Carvalho	Sneha Patra	

### Research group of Pavel Jurajda – Institute of Vertebrate Biology, Czech Academy of Sciences, Brno

Radim Blažek	Pavel Jurajda	Martin Reichard
Jitka Fojtů	Caroline Methling	Kevin Roche
Michal Janáč	Libor Mikl	Ludek Šlapanský

# Programme

## Monday, November 27th

16:00 – 22:00 Arrival and accommodation

19:00 – 22:00 Dinner

## Tuesday, November 28th

7:00 – 9:00 Breakfast

### Workshop opening

9:00 – 9:10 **Gelnar:** Introduction and general programme information

### Diversity of parasites and molecular phylogenetics I – Protista (chairman S. Patra)

9:10 – 9:30 **Diakin A.:** Actin and tubulin visualisation in two species of urosporid gregarines from marine polychaete *Travisia forbesii*

9:30 – 9:50 **Patra S.:** Phylogenetic congruence of myxozoans belonging to *Sphaerospora sensu stricto* and their vertebrate hosts

9:50–10:00 **Kyslík J.:** Identification of novel minicollagens in the myxozoan polar capsule gene repertoire

10:00 – 10:10 **Van Beest Gabrielle:** Use of *in vivo* fluorescent dyes to determine the infectivity and penetration pattern of *Cardiocephaloides longicollis* (Rudolphi, 1819) Dubois, 1982 (Strigeidae) into the gilt head seabream *Sparus aurata* L.

10:10 – 10:40 **Coffee Break**

### Diversity of parasites and molecular phylogenetics II – Ectoparasites (chairman M. Vanhove)

10:40 – 10:50 **Gelnar M.:** Brno brief summary 2017

10:50 – 11:10 **Vanhove M.:** Exploring mitogenomics for phylogenetic reconstruction of African monogeneans

11:10 – 11:20 **Potrok A.:** Monogenean parasites and their effect on the expression of Toll-like receptor genes in *Abramis brama* x *Rutilus rutilus* hybrid system

11:20 – 11:30 **Vyčítalová K.:** Parasites of endemic cyprinids from the Balkan Peninsula – host specificity, endemism and diversity of metazoan parasite communities

11:30 – 11:40 **Benovics M.:** Coevolutionary patterns between host specific monogeneans of genus *Dactylogyrus* and Peri-mediterranean cyprinids

11:40 – 11:50 **Rahmouni C.:** *Cichlidogyrus* Paperna, 1960 (Monogenea: Dactylogyridae) gill monogenean parasites of cichlid fish species (Perciformes: Cichlidae) from Lake Tanganyika: overview and perspectives

11:50 – 13:30 **Lunch**

### Diversity of parasites and molecular phylogenetics III – Endoparasites (chairman B. Schaeffner)

13:30 – 13:50 **Scholz T.:** Endoparasitic helminths: results in 2017 and future plans

13:50 – 14:00 **Barčák D.:** Taxonomic revision of the subfamily Corallobothriinae (Cestoda: Onchoproteocephalidea) from North American ictalurid catfishes

14:00 – 14:10 **Schaeffner B.:** Novel insights on life histories of trypanorhynch cestodes from elasmobranchs and crustaceans of Moreton Bay (Queensland, Australia)

14:10 – 14:40 **Break**

14:40 – 15:00 **Sponsor presentation – Olympus, Dr. Tomáš Jendrulek**



### Host-parasite relationships and interactions (chairman A. Vetešníková-Šimnková)

15:00 – 15:10	<b>Krasnovyd V.:</b> Interruption of the host-parasite co-adaptation by hybridization tested using the monogenean infection in cyprinids
15:10 – 15:20	<b>Ilgová J.:</b> Immunomodulatory function of cysteine peptidase inhibitor from <i>Eudiplozoon nipponicum</i>
15:20 – 15:30	<b>Roudnický P.:</b> Functions of novel serpin from <i>Eudiplozoon nipponicum</i> (Monogenea)
15:30 – 15:40	<b>Vorel J.:</b> Could nanopore sequencing help us improve genome assembly of <i>Eudiplozoon nipponicum</i> (Polyopisthocotylea, Diplozoidae)?
15:40 – 16:00	<b>Coffe Break</b>

### Evolutionary epidemiology and behavioral ecology of parasites (chairman P. Jurajda)

16:00 – 16:20	<b>Jurajda P.:</b> Institute of Vertebrate Biology – annual report 2017
16:20 – 16:35	<b>Janáč M.:</b> Invasive gobiids show little impact on native fish assemblages in the Austrian Danube
16:35 – 16:50	<b>Reichard M.:</b> Coevolutionary dynamics in host Parasite association modulates invasional meltdown

16:50 – 17:00 **General Discussion**

17:00 – 18:00 **ECIP Board Meeting**

### Social evening

19:00 – 24:00 **Buffet dinner**  
**Wine and plum brandy research trip (21.00–23.00)**

### Wednesday, November 29<sup>th</sup>

8:00 – 10:00 Breakfast and departure

## Abstracts

Abstracts in the following section are sorted alphabetically according main author. Actual or past members of ECIP are presented **in bold**.

# Amended diagnoses of two old monotypic genera of proteocephalidean tapeworms (Cestoda: Proteocephalidae), parasites of the relictual pimelodid catfish *Phractocephalus hemioliopus*

Philippe Vieira Alves<sup>1,2\*</sup>, Alain de Chambrier<sup>3</sup>, José Luis Luque<sup>4</sup>, Tomáš Scholz<sup>2</sup>

<sup>1</sup>Programa de Pós-Graduação em Biologia Animal, Universidade Federal Rural do Rio de Janeiro, Seropédica, RJ, Brazil;

<sup>2</sup>Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, České Budějovice, Czech Republic;

<sup>3</sup>Department of Invertebrates, Natural History Museum, Geneva, Switzerland;

<sup>4</sup>Departamento de Parasitologia Animal, Universidade Federal Rural do Rio de Janeiro, Seropédica, RJ, Brazil

\*corresponding author, e-mail: philippe-vieira@hotmail.com

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Dating back to the 19th century, Diesing [1] erected two monotypic genera, *Ephedrocephalus* Diesing, 1850 and *Zygobothrium* Diesing, 1850 (Cestoda: Proteocephalidae), based on peculiar morphology of their species, both parasites of the early-diverged pimelodid catfish *Phractocephalus hemioliopus* (redtail catfish) in the Neotropical Region. Although relatively extensive morphological data on these taxa have been accumulated over more than one and half century, there are still substantial inconsistencies and gaps in the diagnoses of both genera and morphological descriptions of their type and only species, *Ephedrocephalus microcephalus* Diesing, 1850 and *Zygobothrium megacephalum* Diesing, 1850.

In this study, both generic diagnoses are amended and the above-mentioned species are redescribed to provide a robust baseline for the future re-arrangement of the classification of proteocephalid cestodes, which has been revealed as artificial, not corresponding to phylogenetic relationships of these parasites [2].

*Ephedrocephalus* is typified by the medullary position of the ovary and uterus, the cortical distribution of vitelline follicles (dispersed throughout almost the entire ventral cortex) and the testes in one dorsal field. *Zygobothrium* is primarily characterised by its possession of a robust scolex bearing four uniloculate suckers with two openings each and by the tetralobed velum (lacinations) on every proglottid (two on the ventral and two on the dorsal sides). *Phractocephalus hemioliopus* is the definitive host of seven species of proteocephalid cestodes, which apparently colonised this host several times through its long evolutionary history. Some species including *Z. megacephalum* belong to the earliest diverged parasites of Neotropical catfishes, being closely related to African and North American proteocephalids from catfishes, whereas others such as *E. microcephalus* belong to more recently diverged taxa with uncertain interrelations. Unlike most of the proteocephalids of the redtail catfish, which infect almost always the anterior parts of the host intestine, *E. microcephalus* and *Z. megacephalum* occur exclusively in its posterior third.

## Future plans

Application of the Next Generation Sequencing (NGS) approach to characterise complete mitochondrial genomes of Neotropical proteocephalids to better unravel their phylogenetic relationships.

## Acknowledgement

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

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## References

1. Diesing K.M. (1850) *Systema Helminthum*. Vol. I, Braumüller, Vienna, 679 pp.
2. de Chambrier A., Waeschenbach A., Fisseha M., Scholz T. & Mariaux, J. (2015) A large 28S rDNA-based phylogeny confirms the limitations of established morphological characters for classification of proteocephalidean tapeworms (Platyhelminthes, Cestoda). *ZooKeys* 500: 25–59.

# Taxonomic revision of the subfamily Corallobothriinae (Cestoda: Onchoproteocephalidea) from North American ictalurid catfishes.

Daniel Barčák<sup>1,2\*</sup>, Tomáš Scholz<sup>2</sup>

<sup>1</sup>Institute of Parasitology of the Slovak Academy of Sciences, Košice, Slovakia

<sup>2</sup>Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, České Budějovice, Czech Republic

\*corresponding author, e-mail: barcak@saske.sk

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The subfamily Corallobothriinae was erected to accommodate proteocephalidean tapeworms that possess a metascolex (mass of tissue surrounding the scolex from its posterior side) and medullary position of internal organs [1]. In fact, six genera, namely *Corallobothrium* from Africa and North America, *Choanoscolex*, *Megathylacus* and *Sciadocephalus* from South America, and *Corallotaenia* and *Megathylacooides* from North America, were placed in this family. However, recent phylogenetic studies have revealed that the presence or absence of a metascolex is homoplastic character and that the subfamily is not monophyletic. In contrast, three genera parasitising ictalurid catfishes in the Nearctic region, i.e. *Corallotaenia*, *Megathylacooides*, and recently erected genus *Essexiella*, form a well-supported monophyletic group [2, 3]. However, species composition of these genera, their actual host spectrum and phylogenetic relationships are insufficiently known.

Preliminary results suggest that the genus *Corallotaenia* comprises minute tapeworms (body length up to 26 mm) with a spherical scolex that has a flat or convex apical part. An apical organ is absent, the metascolex is weakly developed and may slightly overlap four circular suckers, which lack muscular sphincters. The vitellarium is formed by two compact fields of vitelline follicles alongside lateral margins of proglottids and their posterior parts may be bent medially and partly overlap ovary. The uterus is well developed in gravid proglottids and often fulfils almost the whole parenchyma.

Tapeworms of the monotypic *Essexiella* have a large strobila and a massive scolex without an apical organ. The metascolex is well-developed and its folds are protruding anteriorly and often cover the suckers. Sphincters in suckers are missing. The vitellarium is compact, formed by two lateral fields which bent medially near the posterior margin of proglottids, but do not overlap a follicular ovary. The uterus is well-developed and possesses several conspicuous, mostly lateral branches reaching laterally to vitelline follicles.

The most conspicuous discriminative trait of *Megathylacooides* is the presence of a semicircular sphincter in each sucker. This genus includes large tapeworms (body length of 100 mm and more) that possess a spherical scolex with a convex apical part; an apical organ may be present. The metascolex is weakly developed. Two lateral fields of vitelline follicles never bent medially. Pre-gravid proglottids (i.e. those containing the eggs without formed oncosphere) are conspicuously longer than wide, the ovary is H-shaped or butterfly-shaped, and branches of the uterus do not reach vitelline follicles laterally.

## Future plans

Within these three genera, ten species are considered as valid. In order to resolve unclear taxonomy and systematics of this group, available material from museum collection as well as newly collected specimens will be analysed using integrated morphological and phylogenetic approach.

## 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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1. Freze V.I. (1965) Proteocephalata in fish, amphibians and reptiles. In: K. I. Skryabin (ed.). *Essentials of cestodology*. Vol. V., Nauka, Moscow, 538 p.
2. Rosas-Valdez R., Choudhury A., & Pérez-Ponce de León G. (2004) Phylogenetic analysis on genera of Corallobothriinae (Cestoda: Proteocephalidea) from North American ictalurid fishes, using partial sequences of the 28S ribosomal gene. *J. Parasitol.* 90: 1123–1127.
3. Scholz T., de Chambrier A., Mariaux J., & Kuchta R. (2011) Redescription of *Corallobothrium solidum* (Cestoda: Proteocephalidea) and erection of a new genus, *Essexiella*, for tapeworms from channel catfishes (Ictaluridae). *J. Parasitol.* 97: 1142–1151.

# Structure and evolution of cysteine protease inhibitors in parasitic cnidarians: Additional unique feature of the Myxozoa

Pavla Bartošová-Sojková<sup>1\*</sup>, Ashlie Hartigan<sup>1</sup>, Gema Alama-Bermejo<sup>1,2</sup>, Ivan Fiala<sup>1</sup>, Jerri L. Bartholomew<sup>2</sup>, Astrid S. Holzer<sup>1</sup>

<sup>1</sup>Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, České Budějovice, Czech Republic

<sup>2</sup>The Natural History Museum, London, U.K.

<sup>3</sup>Department of Microbiology, Oregon State University, Corvallis, Oregon, USA

<sup>4</sup>National Scientific and Technical Research Council, National University of Comahue – ESCiMar, San Antonio Oeste, Argentina

\*corresponding author, e-mail: bartosova@paru.cas.cz

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The Myxozoa is a major group of cnidarian parasites with some species significantly impacting aquaculture and fisheries. However, parasitism among the Cnidaria takes several forms. While myxozoan development typically occurs within definitive and final hosts, and free-living life stages are spores, other parasitic cnidarians such as *Polypodium hydriforme* and *Edwardsiella lineata* have parasitic larvae and can continue their development in the absence of a second host. Our research seeks to understand the origin, evolution and diversity of cysteine protease inhibitors (cystatins and stefins) in cnidarians with a special interest in parasitic taxa. These proteins are involved in protection of cells from unwanted proteolysis and in control of intra- and extracellular protein degradation [1] but are also important pathogenicity factors of parasites as they act as key elements involved in host-parasite interactions [2]. Therefore, cysteine protease inhibitors of parasites have been exploited as vaccine and chemotherapeutic targets as well as serodiagnostic markers [3]. So far, both true cystatins and stefins have been identified in free-living cnidarian species [1] whereas only a stefin homolog with an acquired signal peptide was reported from the myxozoan parasite *Thelohanellus kitauei*, the causative agent of intestinal giant cystic disease in common carp [4].

Cystatin superfamily homologs were identified from newly produced and published cnidarian data, including genomes, transcriptomes and ESTs of twelve myxozoan species, by a combined motif- and sequence similarity-based blast search strategy. We performed a comprehensive phylogenetic analysis based on the amino acid sequences of cnidarians and other metazoans. The gene structure evolution was traced by a more detailed phylogenetic analysis restricted to cnidarians. The gene organization was studied by a comparison of sequences obtained from cnidarian genomes and transcriptomes.

Cystatins were lost in all myxozoans included in the search, while they are present in free-living cnidarians as well as in *P. hydriforme* and *E. lineata*. Stefins were identified in both free-living and parasitic cnidarians, including myxosporeans (Myxozoa). Surprisingly, some myxosporean species additionally evolved stefins with acquired signal peptide indicating secretion and transport outside of the parasite (typical feature of true cystatins) possibly as a novel defense or host immune system interference adaptation. Such atypical stefins are unique in eukaryote stefins and have been only reported from some trematode species [5]. No cysteine protease inhibitors have been found in malacosporeans (Myxozoa), most likely due to incompleteness of the EST data. Cystatins and stefins of metazoans form two separate phylogenetic groups. Parasitic taxa are spread throughout the whole phylogeny in both groups. Interestingly, a separation reflecting the different structure of myxosporean stefins with or without signal peptide is evident in their evolution. The gene organization of stefins is very similar among cnidarian species with two introns delimited by GT and AG pairs, as is also found in trematodes and mammals. Our findings suggest functional diversification and lineage-specific adaptations of cysteine protease inhibitors, potentially related to the parasitic lifestyle of this primitive metazoan group.

## Future plans

Future functional and biochemical characterization of myxozoan stefins will identify their roles in host-parasite interactions and aid their evaluation as candidates for the development of novel therapeutic targets to regulate myxozoan diseases in fish.

## 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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# Coevolutionary patterns between host specific monogeneans of genus *Dactylogyrus* and Peri-mediterranean cyprinids

Michal Benovics<sup>1\*</sup>, Yves Desdevises<sup>2</sup>, Andrea Šimková<sup>1</sup>

<sup>1</sup>Department of Botany and Zoology, Faculty of Science, Masaryk University, Brno, Czech Republic

<sup>2</sup>Observatoire Océanologique de Banyuls, Université Pierre et Marie Curie, Banyuls-sur-Mer, France

\*corresponding author, e-mail: michal.benovics@gmail.com

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Recently, the popularity of cophylogenetic studies has rapidly increased. Using parasites as the additional tool for solving phylogenetic relationships among their hosts, we can shed more light to some unresolved or unclear evolutionary processes and historical dispersion of many taxa from animal or plant kingdoms. The parasites, belonging to the highly diversified genus *Dactylogyrus* Diesing, 1850 (Monogenea) are known for their narrow host specificity. These strictly specific parasites of cyprinids appear to be the suitable model organism to study evolutionary history of cyprinids and to help to elucidate the phylogenetic ambiguities among cyprinids. During 2014-2017 we collected *Dactylogyrus* specimens from 78 endemic cyprinid species of Balkan and Apennine Peninsulas. In total 57 species were identified from which 10 were recognized as potentially new for science. We already described one new species from endemic *Aulopyge huegellii* (Bosnia and Herzegovina) which was phylogenetically closely related to *Dactylogyrus* of *Barbus* and *Luciobarbus* [1]. In general, endemic cyprinids are parasitized by endemic *Dactylogyrus* and widely distributed *Dactylogyrus* species as well. While majority of cyprinids are parasitized by one or more *Dactylogyrus* species, several congeneric species (e.g. representatives of *Pelagus*) appears to harbour no *Dactylogyrus*, or are parasitized by this genus very rarely. Comparing the phylogenetic trees based on partitioned data of partial 18S rDNA combined with partial 28S rDNA (for parasites) and cytochrome *b* (for hosts) we revealed several coevolutionary scenarios. Phylogenetic reconstruction of *Dactylogyrus* revealed 4 strongly or moderately supported groups including endemic *Dactylogyrus*, but also widely distributed species. Several endemic cyprinids were parasitized by *Dactylogyrus* of different origins, such as *Pachychilon pictum*, which harbours 5 different species from 3 distinct evolutionary lineages [2]. The similar results were observed also among Iberian and North-western African cyprinids [3]. Application of cophylogenetic methods revealed significant coevolutionary signal between cyprinids of genera *Aulopyge*, *Barbus* and *Luciobarbus* and their respective *Dactylogyrus*, such as *D. dyki*, *D. balkanicus*, *D. crivellius*, *D. petenyi*, *D. prespensis* and 3 new *Dactylogyrus* species of *Luciobarbus* and *Aulopyge*.

## Future plans

- (1) To identify and obtain molecular data of Iberian *Dactylogyrus* and their cyprinid hosts
- (2) To complete the dataset of *Dactylogyrus* and their hosts from whole Mediterranean Europe and Northern Africa
- (3) To describe new *Dactylogyrus* species collected in southern European Peninsulas

## Acknowledgement

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# Development of microsatellite markers for the studies of the Asian fish tapeworm *Schyzocotyle acheilognathi*

Jan Brabec<sup>1\*</sup>

<sup>1</sup>Institute of Parasitology, Biology Centre of the Academy of Sciences of the Czech Republic, České Budějovice, Czech Republic

\*corresponding author, e-mail: brabcak@paru.cas.cz

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The Asian fish tapeworm, *Schyzocotyle acheilognathi* (syn. *Bothriocephalus acheilognathi*) is globally distributed fish pathogen with extremely low host specificity that mirrors in the parasite's ability to invade a uniquely wide range of fish species across the globe to the exclusion of Antarctica only. The parasite has attracted attention of fish parasitologists' since its description in 1934 (for review see, e.g., Scholz et al., 2012), but only relatively recently has been the intraspecific diversity of the species addressed with molecular data, particularly with sequences of the nuclear ribosomal RNA gene loci. A further study by Brabec et al. (2016) comparing, among others, the whole mitochondrial genomes of eight representatives of the global diversity of the parasite than revealed that the individual isolates were surprisingly nearly uniform from the molecular perspective and that the former use of the nuclear ribosomal RNA loci data might have been problematic.

Here, we further exploit the Illumina high-throughput sequencing data gathered in the scope of the study of Brabec et al. (2016) in order to develop a panel of short tandem repeats (microsatellite) loci that could be used to study the phylogeography and diversification of populations of the Asian fish tapeworm. From the total number of 46 pure trimer microsatellite loci that has been bioinformatically selected and subsequently empirically tested in the laboratory using a suite of genomic DNA representing 12 populations and the global diversity of the parasite, 18 microsatellite loci were found to be polymorphic and universally amplifiable. The amplification of the 18 novel microsatellite markers has been optimized for multiplexing in three PCR reactions. We tested the robustness of the multiplexed genotyping on 10–20 individuals per each of five populations of *S. acheilognathi* from China, Hungary, Mexico, South Africa and Turkey. The observed fragment sizes ranged from 206 to 427 base pairs but most of the loci displayed low levels of heterozygotes. Further tests need to be undertaken to illuminate the possible source of the deficit of heterozygotes in our data.

## Future plans

Carry out tests to reveal the possible sources of the large heterozygous deficit within the genotyped populations of *S. acheilognathi* and assess its eventual impact on the estimations of the population genetic structure and phylogeographical history of these populations.

## Acknowledgement

This study was supported by the Czech Science Foundation (15-14198S) and ECIP (European Centre of Ichthyoparasitology); Centre of excellence program of the Czech Science Foundation; project No. P505/12/G112.

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# Actin and tubulin visualisation in two species of urosporid gregarines from marine polychaete *Travisia forbesii*

Andrei Diakin<sup>1\*</sup>, Andrea Valigurová<sup>1</sup>

<sup>1</sup> Department of Botany and Zoology, Faculty of Science, Masaryk University, Brno, Czech Republic

\*corresponding author, e-mail: ondredyakin@gmail.com

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The genus *Urospora* unites eugregarines of considerable morphological plasticity inhabiting body cavity and tissues of a wide range of marine and freshwater invertebrates. Only few members were investigated from ultrastructural and molecular phylogenetic viewpoint. Combining electron and confocal laser scanning microscopy, we present a morphological study of *Urospora travisiae* and *Urospora ovalis*.

The trophozoites of *U. travisiae* are of V-like shape with two narrowing branches converging at a point with attachment organelle. Their pellicle forms longitudinal epicytic folds (EF) along the axis of each branch. Detached trophozoites exhibit a gliding motility. In contrast, solitary ovoid trophozoites of *U. ovalis* float freely in the coelom and exhibit metabolic activity. Their surface is covered with EF and additional longitudinal superfolds, bearing a number of EF that form during metaboly in the region of contractions.

The phalloidin labelling showed that the distribution of filamentous actin fits to the pattern of EF. In *U. travisiae*, tiny filaments of actin lay superficially and parallel to each other along the longitudinal axis of the branch. In solitary *U. ovalis*, individual filaments are not clearly seen, however, general pattern of their distribution corresponds to that in *U. travisiae*. The specific immunolabeling of  $\alpha$ -tubulin showed that subpellicular microtubules in *U. travisiae* and *U. ovalis* distribute circumferentially along the cell axis, with abundant cytoplasmic clusters. Phalloidin staining of *U. ovalis* syzygies demonstrated that filamentous actin distributes in a bands along with superfolds, and individual longitudinal filaments pass along EF that run in-between superfolds. The microtubules in *U. ovalis* syzygies orient not circumferentially as in solitary trophozoites, but along longitudinal cell axis and repeat the pattern of the superfolds' organisation. Numerous clusters of  $\alpha$ -tubulin can be also seen in cytoplasm. Despite considerable differences in their morphology and motility mode, both species show similarities in cytoskeleton organisation.

## Acknowledgement

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# Amoeboid organisms associated with biofilms in rainbow trout production systems

Iva Dyková<sup>1\*</sup>, Tomáš Tým<sup>1,2</sup>

<sup>1</sup>Department of Botany and Zoology, Faculty of Science, Masaryk University, Brno, Czech Republic

<sup>2</sup>Centre for Polar Ecology, Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic

\*corresponding author, e-mail: dykova.iva@gmail.com

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Biofilms, defined as communities of microbes embedded in an organic matrix and attached to a physical surface, are studied in detail mainly as multicellular consortia of prokaryotes. This applies to both, freshwater networks used for human consumption and those in aquaculture production systems, where also pathogenic bacteria are expected to participate in biofilm formation. The major piscine bacteria of concern are *Photobacterium damsela*, *Vibrio* spp., *Aeromonas hydrophila*, *Flavobacterium columnare* and *Edwardsiella* spp. [1, 3].

Eukaryotic microorganisms that colonise freshwater biofilms and include also amoeboid protists have been studied intensely from a public health perspective [see e.g., review 4]. Less attention has been paid to amoeboid organisms that aggregate to biofilms in freshwater aquaculture systems despite the amoebic aetiology of nodular gill disease associated with direct losses in farmed rainbow trout *Oncorhynchus mykiss* [2]. Our research of biofilm-associated amoebae combines two objectives: (i) the search for potential sources of amphizoic amoebae known or suspected to be pathogenic for farmed rainbow trout and (ii) collection of new data on diversity of amoeboid protists. The study is currently based on the isolation of FLA from a defined amount of biofilm material scraped from plastic containers or concrete tanks at their water level. The collection of material takes place on farms that differ in water supply and the type of aquaculture system (indoor, outdoor, and recirculating). The initial phase of this study (i) revealed that generic assignment of most biofilm-isolated strains matches with that of gill-isolated strains known from our previous and current studies of potential pathogens of farmed *O. mykiss*; (ii) prepared conditions for molecular comparison of both sets of strains that is essential for the identification of species/strain identity; and (iii) improved our knowledge of amoeboid organisms thanks to the isolation of biofilm-associated strains that are new for our collection. The latter achievement is documented by the first results of our ultrastructural study of an imbricatean amoeboid organism (Cercozoa; Fig. 1 A – E).

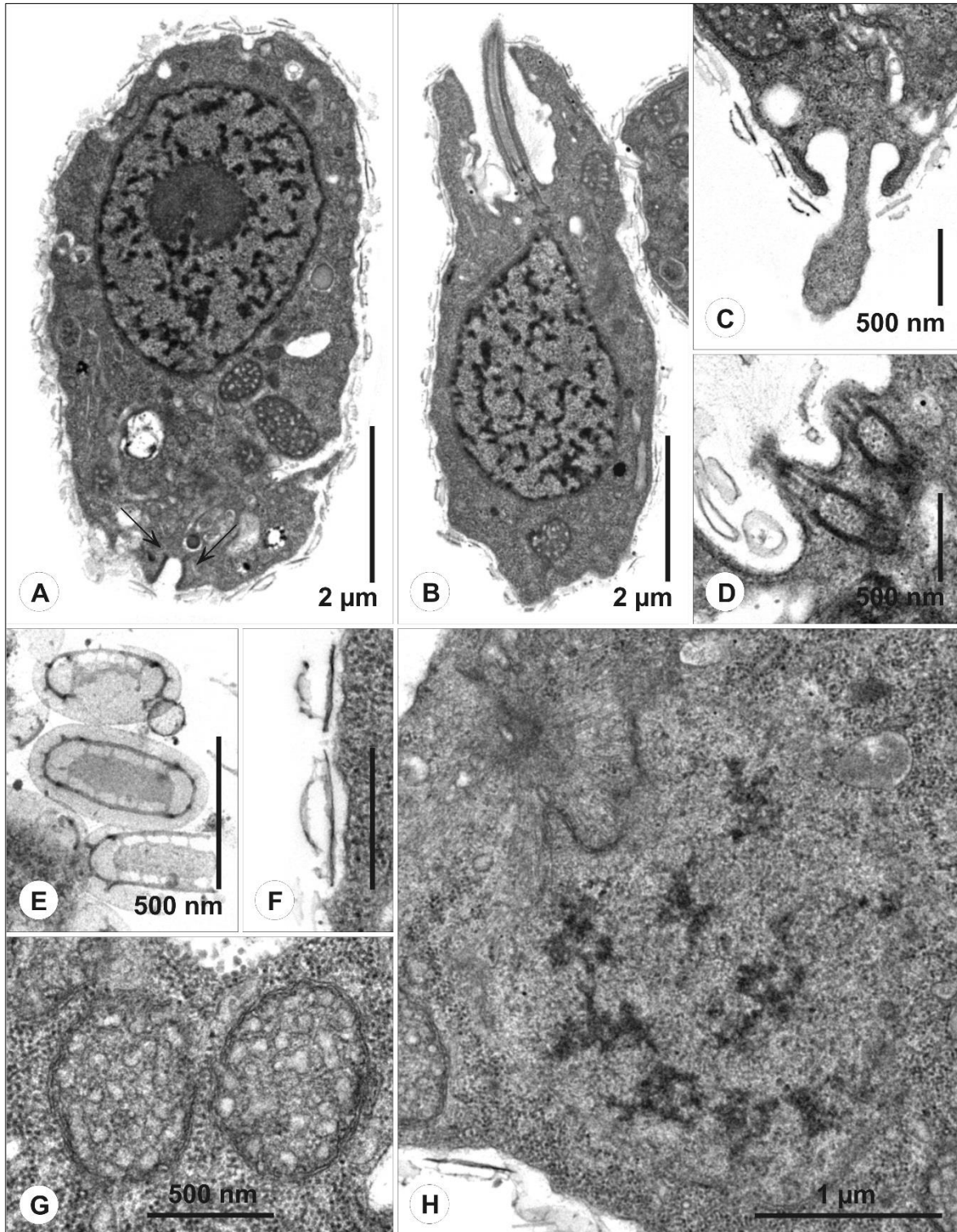
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**A.** New strain of thautomonad amoeboid organism (Imbricata). An overview of ultrastructure showing cell surface covered with imbricate scales, and cytoplasm with prominent nucleus and mitochondria. Arrows indicate ventral groove. **B.** Cell with one flagellum at the level of sectioning. **C.** Pseudopodium extending from ventral groove. **D.** Two kinetosomes at the level of sectioning prove the existence of biflagellated stage in the life cycle of this organism. **E.** Top view of two-tier scales resembling those of *Thautomonas/Allas* spp. **F.** Side view of scales (scale bar = 500 nm). **G.** Mitochondria with vesicular cristae. **H.** Mitotic division classified as open orthomitosis.

# How old are the Myxozoa? Evidence from molecular dating

Ivan Fiala<sup>1,2\*</sup>, Pavla Bartošová-Sojková<sup>1</sup>, Alena Lövy<sup>1</sup>, Ashlie Hartigan<sup>1,3</sup>, Astrid S. Holzer<sup>1,2</sup>

<sup>1</sup>Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, České Budějovice, Czech Republic

<sup>2</sup>Faculty of Sciences, University of South Bohemia, České Budějovice, Czech Republic

<sup>3</sup>The Natural History Museum, London, U.K.

\*corresponding author, e-mail: fiala@paru.cas.cz

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The evolutionary origin of Cnidaria is dated approximately 200 millions years before most of the stem bilaterian lineages arose. Hence, the Myxozoa, a large group of parasitic cnidarians with still enigmatic origin had to occur later in the cnidarian evolution when suitable hosts became available. To determine the date of origin of these parasites we performed molecular clock analysis.

The alignments used for our phylogenetic and molecular clock analyses contained 138 metazoans taxa including 10 myxozoans and four fungal outgroups. The alignment includes six nuclear housekeeping genes (2444 AA positions): aldolase, methionine adenosyltransferase, ATP synthase, elongation factor 1 alpha, triosephosphate isomerase and phosphofructokinase. We used available and newly obtained genome and transcriptome data to mine the protein coding myxozoan genes. Molecular clock analysis was performed using a Bayesian approach in BEAST2 program with Lognormal relaxed molecular clock model, Yule speciation prior and 14 calibration points.

The molecular clock dating analysis revealed that Cnidaria originated 725 million years ago (Ma) which is in agreement with most of published analyses [1]. According to our analysis the last common ancestor of *Polypodium hydriforme* and Myxozoa is dated to 651 Ma in the late Cryogenian era, approximately 75 million years after the origin of Cnidaria. The last common ancestor of the Myxozoa is dated to 588 Ma in the Ediacaran era. It correlates with the origin of Lophotrochozoa, group including both Annelida and Bryozoa, the definitive hosts of extant myxozoans. The origin of vertebrates, myxozoan intermediate hosts, is dated to 363 Ma in the late Devon correlating with the origin of the main myxozoan phylogenetic clades (e.g. the Freshwater hepatic biliary clade, the Kudoa clade etc.).

We suggest that myxozoan parasites first infected an invertebrate host, presumably the common ancestor of bryozoans and annelids, in a single host life cycle. Since cartilaginous fishes (chimaeras, sharks, skates, and rays) are hosts of most basal myxosporean lineages, we assume that acquisition of the second host happened before the cartilaginous fishes representing the oldest living group of jawed vertebrates diverged from a common ancestor of bony vertebrates. In accordance, we demonstrate that the origin of Malacosporea and Myxosporea as well as the three main myxosporean phylogenetic lineages happened before the radiation of most teleost orders. We revealed that parasitism in Cnidaria evolved early in the evolution and Myxozoa probably represent the first metazoan parasitic group.

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# Distribution and parasites of the pumpkinseed sunfish (*Lepomis gibbosus* L.) in the floodplain of the lower Dyje

Jitka Fojtů<sup>1,2</sup>, Markéta Ondračková<sup>1\*</sup>, Anna Bryjová<sup>1</sup>, Mária Seifertová<sup>2</sup>, Pavel Jurajda<sup>1</sup>

<sup>1</sup>Institute of Vertebrate Biology, Czech Academy of Sciences, Brno, Czech Republic

<sup>2</sup>Department of Botany and Zoology, Faculty of Science, Masaryk University, Brno, Czech Republic

\*corresponding author, e-mail: audrey@sci.muni.cz

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The pumpkinseed, *Lepomis gibbosus* (Linnaeus, 1758) is a centrarchid fish native to the eastern part of North America. Since its introduction to the Europe in 19th century, this species widely spread throughout the continent and now it is one of the most successfully introduced fish species [1]. In the Czech Republic, it was first recorded in 1929 and an eighty years later, pumpkinseed inhabited several water bodies in the basins of the Elbe and Morava Rivers. Recently, pumpkinseed spread into the number of water bodies in the floodplain of the lower Dyje where established several abundant populations. Preliminary results indicate that this recent spreading pumpkinseed population genetically differs from that ones established in the basins of the lower Morava and middle Danube twenty years ago, and, therefore, most likely results from a new introduction event rather than from natural dispersal.

In our study, we surveyed the pumpkinseed distribution in various habitats of the River Dyje floodplain and examined its parasites in selected localities, including both adult and juvenile fish. During the summer, presence of pumpkinseed was screened in the river Dyje and its tributary Kyjovka, in adjacent backwaters, oxbows and borrow pits. Adult fish were collected in spring before the reproduction to detect the fish reproductive status as well as parasite burden during this energy demanding period. Juvenile fish were collected and examined for parasites in late summer. For parasitological examination, fish were transported alive to the field station, measured and examined for metazoan parasites. In the laboratory, parasites were determined based on morphology. Pumpkinseed was regularly distributed along the small lentic water bodies in the Dyje floodplain and its occurrence was limited by flood-control dyke. Its density greatly varied between sites, but successful reproduction was recorded at all sites where adult fish were observed. Parasite fauna consisted of natural North-American species (monogenean *Onchocleidus dispar* and digenean *Posthodiplostomum cf. minimum centrarchi*), local acquired species (digenean *Diplostomum* spp., cestode *Triaenophorus nodulosus* and bivalve glochidia) and non-native acquired parasites (copepod *Neoergasilus japonicus*). Whilst parasite communities in adult fish significantly varied between the sites in both the species richness and abundance, juvenile fish exhibited more similar parasite fauna with dominance of co-introduced monogenean *O. dispar*. Our results confirmed introduction of two new parasite species into the region of the Dyje floodplain, the North-American monogenean *O. dispar* and digenean *P. cf. minimum centrarchi*. However, both parasites appear to be specific to centrarchid fishes [2,3] and then they possess low potential as a threat for local fish fauna.

## Future plans

Finish the parasite identification and data analysis and complete the Master's thesis.

## Acknowledgement

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

Kateřina Francov<sup>1\*</sup>

<sup>1</sup>Department of Botany and Zoology, Faculty of Science, Masaryk University, Brno, Czech Republic

\*corresponding author, e-mail: kfranc@sci.muni.cz

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In 2017, my work was focused on the following points, including dactylogyrids infecting African siluriforms and also other fish groups:

1) *Quadriacanthus* species parasitizing *Clarias gariepinus*, *Heterobranchus bidorsalis* (Clariidae) and *Bagrus docmak* (Bagridae) were studied in Kenya (Lake Turkana) and Sudan (Nile River Basin). Morphological and molecular approaches were used for the species identification. Seven species (including four new) of *Quadriacanthus* were identified: *Quadriacanthus aegypticus* El-Naggar & Serag, 1986, *Quadriacanthus clariadis* Paperna, 1961, *Quadriacanthus fornicatus* n. sp., *Quadriacanthus pravus* n. sp., and *Quadriacanthus zuheiri* n. sp. from *Clarias gariepinus*; *Quadriacanthus mandibulatus* n. sp. from *Heterobranchus bidorsalis*; and *Quadriacanthus bagrae* Paperna, 1979 from *Bagrus docmak*.

For both 18S-ITS1 and 28S rDNA regions, *Q. clariadis* from *Clarias* was found to be most closely related to *Q. bagrae* from *Bagrus*. *Quadriacanthus mandibulatus* n. sp. was observed to be the most distant species from the others which corresponds with the different morphology of its copulatory tube. The observed interspecific genetic relationships among *Quadriacanthus* species from clariids and *Q. bagrae* from *Bagrus* suggest a possible host-switching event in the evolutionary history of the genus [1,2].

Reconstruction of phylogenetic relationships were performed on dactylogyrids parasitizing African catfishes, including *Quadriacanthus* species and also representatives of other dactylogyridean genera [3].

2) Morphological identification of *Schilbetrema* spp. from Kenya, Sudan and Senegal, complemented with molecular analyses of nuclear ribosomal DNA sequence data (28S, 18S and ITS-rDNA), has been in process. Eleven *Schilbetrema* species have been identified. Two species have been considered as new for science.

3) "Guide to the Parasites of African Freshwater Fishes: Diversity, Ecology and Study Methods" publication (in process): participation on the catalogue of monogeneans and the drawings for identification key.

4) Collecting of parasitological material in Cameroon, Central Africa, and partly identification.

## Future plans

The plans for 2018 include completion of *Schilbetrema* spp. characterization and characterization of other dactylogyridean genera from African catfishes.

## Acknowledgement

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# Philometrid nematodes parasitising marine fishes in the Gulf of Hammamet, northeastern Tunisia

David González Solís<sup>1,2\*</sup>, Nessrine Ghanmi<sup>3</sup>

<sup>1</sup>El Colegio de la Frontera Sur, unidad Chetumal, Chetumal, Quintana Roo, Mexico;

<sup>2</sup>Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, České Budějovice, Czech Republic

<sup>3</sup>Unité de Recherche Bio-Écologie Animale et Systématique Évolutive, Faculté des Sciences, Université Tunis El Manar, Tunisia

\*corresponding author, e-mail: dgonzale@ecosur.mx

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Nematodes belonging to the family Philometridae represent a diverse group of parasites infecting freshwater, brackish water and marine teleost fishes around the world. They have a very particular morphology and biology, exhibit a high host and site specificity and a marked sexual dimorphism, where females are considerably larger than their conspecific males (Moravec and de Buron, 2013). Additionally, most philometrids have been described solely from the conspicuous females, whereas males are unknown in several species.

The Mediterranean Sea is one of the regions where these nematodes have been reported from fishes of various fish families (e.g., Serranidae, Uranoscopidae, Mullidae). To date, 12 species were found, of which in 9 both the males and females were described. Recent collections of nematodes resulted in finding three undescribed species, as well as conspecific unknown males of *Philometra serranellicabrillae* and specimens that allow to redescribe *P. filiformis*. The new species, one from *Trachinus draco*, one from *Trachinus radiatus* (Fig. 1; see Ghanmi et al. 2017) and one from *Mullus barbatus*, differ from their congeners in the different structures on the gubernaculum (e.g., smooth field between the two longitudinal lamellated parts, dorsal protuberance), as well as in the body, spicules and gubernaculum lengths. These findings increase the number of philometrids reported from the Mediterranean Sea to 15 species. Moreover, the male of *P. serranellicabrillae* is reported for the first time from the type host, but in a different locality. The morphology of its posterior end is different from those of other males in the shape of the caudal reniform mounds, which are clearly divided into dorsal and ventral lobes. *Philometra filiformis* is redescribed based on new material collected from the type host (*Pagellus erythrinus*) from the Tunisian coast and SEM examinations of male are reported for the first time. *Philometra filiformis* is for the first time reported from Tunisian coastal waters, being the seventh philometrid species so far recorded from marine fishes off Tunisia (see Moravec et al. 2017). Apparently, there is an important philometrid fauna in marine fish from the Mediterranean region.

## Future plans

Preparation and finalization of publications dealing with the new species and the first description of male of *P. serranellicabrillae*.

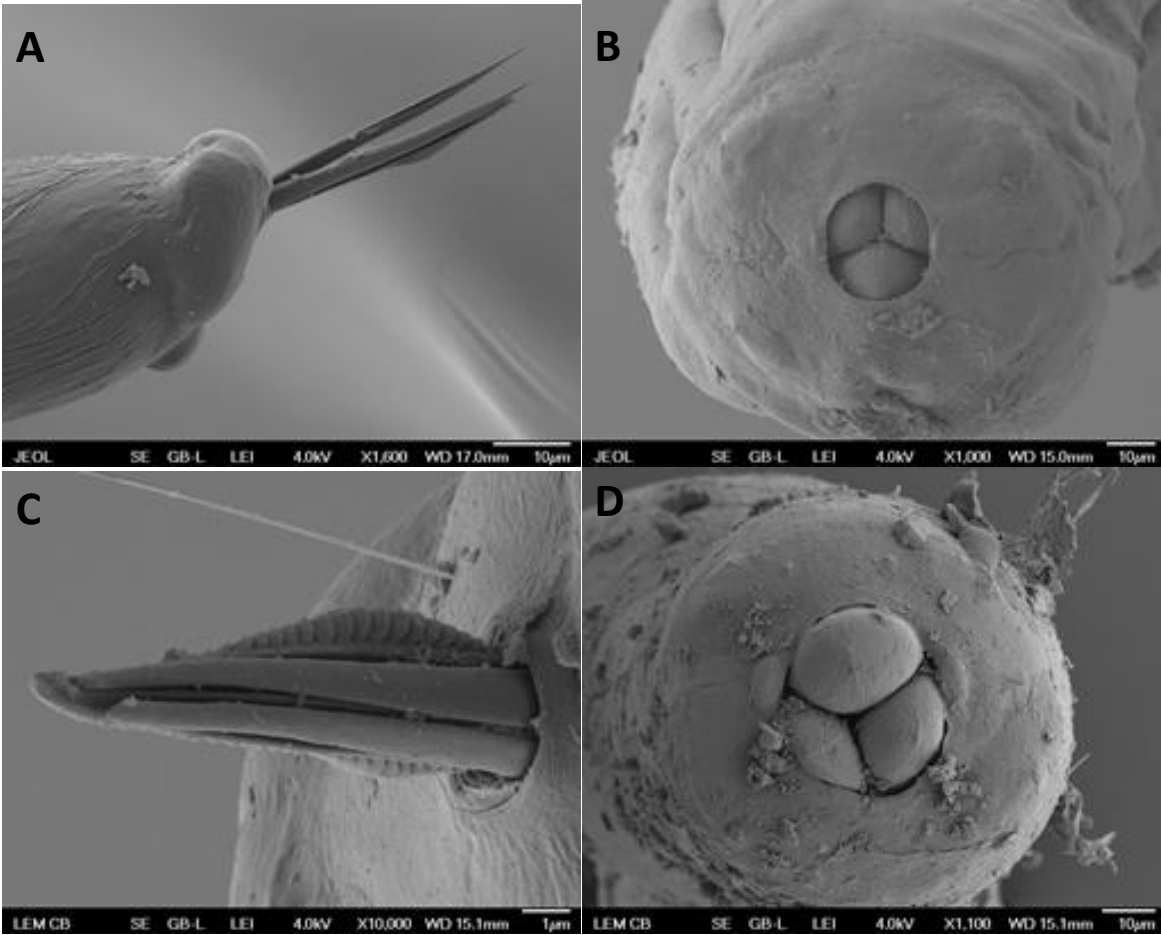
## Acknowledgement

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**A, B.** A new species of *Philometra* from *Trachinus draco*; posterior end of male and cephalic end of female, respectively. **C, D.** Another new species of *Philometra* from *Trachinus radiates*; gubernaculum/spicules and cephalic end of female, respectively.



# Host response of common carp to infection with the myxozoan *Sphaerospora molnari*

Astrid S. Holzer<sup>1\*</sup>, Ana Born-Torrijos<sup>1</sup>, Martina Jedličková<sup>1,2</sup>, Victor Sieranski<sup>1,3</sup>, Ivan Fiala<sup>1</sup>

<sup>1</sup>Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, České Budějovice, Czech Republic

<sup>2</sup>Faculty of Sciences, University of South Bohemia, České Budějovice, Czech Republic

<sup>3</sup>Faculty of Engineering and Natural Sciences, Johannes Kepler University, Linz, Austria

\*corresponding author, e-mail: astrid.holzer@paru.cas.cz

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*S. molnari* is the agent of skin and gill sphaerosporosis in common carp. We recently detected emerging numbers of presporogonic blood stages [1] in European carp cultures and aimed at determining the host reaction to this massive presporogonic development in controlled laboratory experiments.

Specific pathogen-free carp were IP injected with *S. molnari* blood stages and sampled on day 1, 2, 4, 7 and thereafter weekly until week 9 post infection (p.i.). Sampling included full hematological analyses and the collection of DNA/RNA samples from a number of organs to determine the whereabouts and concentration of parasite stages by qPCR as well as the expression of secreted IgM (IgMsec) and membrane-bound IgM (IgMmem) in different organs, as an indicator for acquired immunity.

*S. molnari* blood stages were first detected on day 14 p.i. and the highest blood stage concentration was measured 28 days p.i. Thereafter, the parasite was found to invade several organs, with highest parasite levels detected in the liver, on day 42 p.i. These were found to be six times higher than those present in the blood. A strong immunological response was raised by the host, characterized macroscopically by reno-, hepato- and splenomegaly, and microscopically by massive proliferation of lymphocytes (B cells) from day 21 p.i. onwards. Significant changes over time were also detected in the number of neutrophils, monocytes and thrombocytes. The sudden increase in secretory IgM expression from day 28 p.i. onwards strongly points to the development of a specific host immune response, which could provide some protection to reinfection.

The results of this study clearly demonstrate that *S. molnari* presporogonic stages are of high pathogenicity and they point to an important contribution of the parasite to the etiology of important diseases believed to be caused exclusively by other parasite taxa, e.g. swim bladder inflammation of common carp [2].

## Future plans

Ongoing in vitro experiments with sera from different fish as well as reinfection experiments after chemical parasite clearance are presently on the way to better explain and complement the results achieved. Furthermore, we are investigating the whereabouts of the parasite during the first 21 days of infection where it appears to hide in an immunoprotective, likely intracellular location.

## Acknowledgement

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# Immunomodulatory function of cysteine peptidase inhibitor from *Eudiplozoon nipponicum*

Jana Ilgová<sup>1\*</sup>, Pavel Roudnický<sup>1</sup>, Jiří Vorel<sup>1</sup>, Libor Vojtek<sup>2</sup>, Pavel Hyršl<sup>2</sup>, Jiří Salát<sup>3</sup>, Milan Gelnar<sup>1</sup>, Martin Kašný<sup>1,4</sup>

<sup>1</sup>Department of Botany and Zoology, Faculty of Science, Masaryk University, Brno, Czech Republic

<sup>2</sup>Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic

<sup>3</sup>Department of Immunology, Veterinary Research Institute, Brno, Czech Republic

<sup>4</sup>Department of Parasitology, Faculty of Science, Charles University, Prague, Czech Republic

\*corresponding author, e-mail: jana.ilgova@gmail.com

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*Eudiplozoon nipponicum* is a sanguivorous monogenean of East Asian origin which parasitizes the gills of the common carp, *Cyprinus carpio*. One of the mechanisms which enable successful parasitic lifestyle is the utilization of compounds contained in excretory-secretory products of the parasite for possible modulation of the host physiological processes. We identified cysteine peptidase inhibitor (type I cystatin, also known as stefin) among the molecules secreted by *E. nipponicum*. These inhibitory molecules are generally known as regulators of endogenous processes related to the protein degradation. Besides, they can affect the blood digestion, immunomodulation of the host and the repair of its tissue damaged by the parasite. Our study aims to reveal the immunomodulatory potential of the stefin of *E. nipponicum*.

During *in vitro* assays we used recombinant stefin which is capable of inhibiting cathepsin B and L as well as legumain. Using qPCR method we characterized the changes in the expression of cytokines by immune cells stimulated by LPS. Recombinant stefin of *E. nipponicum* caused significant downregulation of selected cytokines (IL-1 $\beta$ , IL-8, TNF- $\alpha$ , IL-6 and IL-10) by porcine alveolar macrophages and monocyte derived macrophages. Our finding indicates possible role of this inhibitor in immunomodulation of the host, i.e., the infiltration of neutrophils, suppression of inflammatory response and differentiation of T cells. Luminol-enhanced chemiluminescence assays did not prove any significant effect of stefin on the activation of complement in carp's plasma or oxidative burst in full blood.

## Future plans

We are optimizing protocols for *in vitro* cultivation of fish macrophages culture from head kidney and spleen. In order to test the effect of *E. nipponicum* stefin on the immune response of the fish host we plan to perform qPCR analysis of selected cytokines expressed by fish macrophages.

## Acknowledgement

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# Invasive gobiids show little impact on native fish assemblages in the Austrian Danube

Michal Janáč<sup>1\*</sup>, Kevin Roche<sup>1</sup>, Luděk Šlapanský<sup>1</sup>, Matej Polačik<sup>1</sup>, Pavel Jurajda<sup>1</sup>

<sup>1</sup>Institute of Vertebrate Biology, of the Czech Academy of Sciences, Brno, Czech Republic

\*corresponding author, e-mail: janac@ivb.cz

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As invaders of European and North American aquatic systems, Ponto-Caspian gobiids are believed to represent a significant negative threat to native fish assemblages. To date, relatively few studies have tried to document actual impacts, most being short-term and/or laboratory based.

When examining eight-years (2008-2015) of electrofishing data from a 1200 m stretch of rip-rap along the Austrian Danube, initiated following establishment of four non-native gobiids: tubenose goby *Proterorhinus semilunaris*, bighead goby *Ponticola kessleri*, racer goby *Babka gymnotrachelus* and round goby *Neogobius melanostomus*, only native bullhead *Cottus gobio* and the four non-native gobiids were caught regularly [1]. Other native species were caught only occasionally, probably due to the hostility of rip-rap banks to these species. The shorelines of navigable rivers are under considerable erosive pressure from vessel-induced wave action with detrimental impact on both eggs and specimens of these native fish (mainly cyprinids and percids, larvae and juveniles of which have positive or neutral buoyancy). Bullhead and non-native gobiids lack swim bladders and appear to be far more tolerant of such wave action.

River channelization thus appears to have negatively affected most of the native species in this area, thus creating a 'vacant' or underutilized niche, which has been effectively utilized by non-native gobiids but, for the same reason, preventing any further effect of gobiids on these native fish assemblages [2]. Though cottids are presently believed to be most vulnerable to gobiid invasion, we observed no negative trend in bullhead abundance over the eight-year dataset, the population remaining stable and at similar abundances to gobiids. We suggest that the lack of effect on cottids may be caused surplus resources (shelter, food, spawning grounds), limiting competition between gobiids and cottids in the Danube. Our data contradict previous reports of drastic impacts on cottid abundance by gobiids (mainly round goby), suggesting potential region-specific effects following invasion. To further determine source of this variability, it is essential to investigate hierarchy and/or synergy in mechanisms, through which gobiids affect native fish assemblages.

## Future plans

This part of the research has been finalised by the publication [1]. Further research will include meta-analysis of studies regarding impact of invasive gobiids on different recipient systems and pilot studies focused on investigation of hierarchy and/or synergy in mechanisms, through which gobiids affect native fish assemblages.

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# Diversity of dactylogyrids (Monogenea) parasitizing African tetras

Maria Lujza Červenka Kičinja<sup>1\*</sup>, Mária Seifertová<sup>1</sup>, Milan Gelnar<sup>1</sup>, Eva Řehulková<sup>1</sup>

<sup>1</sup>Department of Botany and Zoology, Faculty of Science, Masaryk University, Brno, Czech Republic

\*Corresponding author, e-mail: lujzika@mail.muni.cz

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African freshwaters harbour more than 200 characiform species currently arrayed in four families: Alestidae (118 spp.), Citharinidae (8 spp.), Distichodontidae (101 spp.), and Hepsetidae (5 spp.). Alestidae, often called African tetras, are known to be parasitized by monogeneans belonging to three genera: *Annulotrema* (41 spp.), *Characidotrema* (10 spp.), and *Afrocleidodiscus* (1 sp.). *Annulotrema* (7 spp.) and *Afrocleidodiscus* (3 spp.) have been recorded also as parasites of fishes from the Distichodontidae and Hepsetidae.

During fieldwork (2005 – 2017) carried out in eight African countries (i.e. Cameroon, Democratic Republic of the Congo, Kenya, Nigeria, Senegal, South Africa, Sudan, and Zimbabwe) twelve species of African tetras (*Alestes baremoze*, *A. dentex*, *Arnoldichthys spilopterus*, *Brycinus imberi*, *B. leuciscus*, *B. macrolepidotus*, *B. nurse*, *Hydrocynus forskahlii*, *H. brevis*, *H. vittatus*, *Micralestes acutidens*, and *M. elongatus*) were sampled. As a result, a total of 36 species of dactylogyrids were found: *Annulotrema* (28 spp.), *Characidotrema* (7 spp.), and *Afrocleidodiscus* (1 sp.).

In 2017, I continue to work towards on (i) species identification, which revealed the presence of two new species of *Annulotrema* from *Hydrocynus vittatus* in Zimbabwe: *A. bracteatum* and *A. pseudonili* [1], probably three new species of *Annulotrema* from *H. vittatus* and *B. imberi* in South Africa, and two undescribed species of *Characidotrema* from *B. imberi* and *B. macrolepidotus* in Cameroon, Democratic Republic of the Congo, South Africa and Zimbabwe [2]; (ii) evaluation of phylogenetic relationships within dactylogyrids parasitizing African tetras based on nuclear ribosomal DNA gene sequences: The preliminary phylogenetic analyses confirmed the monophyly of *Characidotrema* species [2]. Species of *Afrocleidodiscus hydrocynuous* and representatives of *Annulotrema* clustered together, while *Afrocleidodiscus* sp. from *Distichodus rostratus* (Characiformes: Distichodontidae) forms separate cluster close to the *Characidotrema* spp. Division of both *Afrocleidodiscus* species highlights the necessity of a revision of the latter genus; and (iii) investigation of the relative taxonomic importance of the characters of the reproductive organs and those of the haptor sclerites in accordance with molecular data. The most important feature for species determination within *Characidotrema* appears to be the morphology of the male copulatory organ and vagina [2].

## Future plans

Description of *Characidotrema* species found on African tetras from Cameroon, Democratic Republic of the Congo, Senegal, South Africa, Sudan and Zimbabwe, and *Annulotrema* species from Nigeria, Senegal, South Africa and Sudan, will be achieved by combining morphological and molecular approaches. Revision of *Afrocleidodiscus* will be prepared.

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# Monogenean population structure and demographic history in Lake Tanganyika's pelagic zone

Nikol Kmentová<sup>1\*</sup>, Maarten Van Steenberge<sup>2,3</sup>, Stephan Koblmüller<sup>4</sup>, Fidel Muterezi Bukinga<sup>5</sup>, Theophile Mulimbwa N'sibula<sup>5</sup>, Pascal Masilya Mulungula<sup>5</sup>, Milan Gelnar<sup>1</sup>, Maarten P.M. Vanhove<sup>1,2,3,6,7</sup>

<sup>1</sup>Department of Botany and Zoology, Faculty of Science, Masaryk University, Brno, Czech Republic

<sup>2</sup>Biology Department, Royal Museum for Central Africa, Tervuren, Belgium

<sup>3</sup>Operational Directorate Taxonomy and Phylogeny, Royal Belgian Institute of Natural Sciences, Brussels, Belgium

<sup>4</sup>Institute of Zoology, University of Graz, Graz, Austria

<sup>5</sup>Centre de Recherche en Hydrobiologie, Département de Biologie, Uvira, Democratic Republic of Congo

<sup>6</sup>Laboratory of Biodiversity and Evolutionary Genomics, Department of Biology, University of Leuven, Leuven, Belgium

<sup>7</sup>Centre for Environmental Sciences, Research Group Zoology, Hasselt University, Diepenbeek, Belgium

\*corresponding author, e-mail: kmentovan@mail.muni.cz

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Lake Tanganyika is a unique study area containing one of the biggest freshwater pelagic ecosystems worldwide. While the lake is famous for the rapid radiation processes of vertebrate as well as invertebrate taxa, its pelagic zone is characterised by lower species richness compared to the diverse littoral habitat. Unlike the strict or intermediate host specificity of monogenean parasites documented in the lake's littoral zone, a previous study has proved the pelagic environment to be challenging for specialisation of parasites [1]. The only reported intermediate generalist species of *Cichlidogyrus* in the lake, *Cichlidogyrus casuarinus*, is infecting six members of the cichlid tribe Bathybatini inhabiting the lake's pelagic zone. Although clupeids and latids dominate the lake's pelagic zone, there is almost nothing known about their parasite fauna. In our study, we asked the following questions: What is the monogenean species richness in the pelagic zone in non-cichlid tribes? Does the population structure and demographic history correspond with the situation documented in *C. casuarinus*?

Our samples originated from 25 localities throughout Lake Tanganyika. Morphological identification was based on measurements of sclerotized structures. Molecular characterisation was performed for four different markers from nuclear ribosomal (18S, 28S and ITS-1 rDNA) as well as mitochondrial (COI) markers. Intraspecific genetic structure and demographic history inferred from the COI region was analysed by haplotype networks, Bayesian skyline plot and mismatch distribution analysis.

According to morphological and genetic characterisation, three different monogenean species belonging to two different genera and families were recorded. Significant differences related to host species in haptor as well as genital hardparts were found in both dactylogyrid species and *Diplectanum lacustris*. Next, the previously suggested decrease of parasite host specificity in the pelagic and benthopelagic zone of Lake Tanganyika was confirmed in these other host taxa and monogenean genera. This result corresponds with other studies in marine systems. Haplotype networks show a core-satellite structure not related to geographic distance in any of the dactylogyrid species. The unimodal distribution of pairwise genetic differences indicates recent demographic expansion in both dactylogyrid species corresponding with the end of aridity connected with the last Ice age, a phenomenon previously documented in various cichlid species in the lake. While recent population expansion, with the start dated between 5,000 to 10,000 years ago, was documented for species of *Ancyrocephalus*, previous study reported the start of population expansion of *C. casuarinus* back to 90,000 years ago. No intraspecific variability in the COI region of *Diplectanum* sp. in Lake Tanganyika was documented.

## Future plans

To further investigate intraspecific genetic structure of *Ancyrocephalus*, more mitochondrial markers will be developed using mitochondrial genomes. Moreover, we will compare host and parasite population structure using genome-scale screening. Finally, we also plan to look at the metazoan parasite composition of latids and clupeids in Lake Tanganyika at different community levels and localities.

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# Selection of suitable housekeeping genes for gene expression studies in myxozoan parasites

Anush Kosakyan<sup>1</sup>, Gema Alama-Bermejo<sup>2</sup>, Pavla Bartošová-Sojtková<sup>1</sup>, Ana Born-Torrijos<sup>1</sup>, Radek Šíma<sup>1</sup>, Astrid S. Holzer<sup>1</sup>

<sup>1</sup>Institute of Parasitology, Czech Academy of Sciences, České Budějovice, Czech Republic.

<sup>2</sup>National Scientific and Technical Research Council Buenos Aires, Buenos Aires, Argentina

\*corresponding author, e-mail: anush.kosakyan@unine.ch

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Myxozoans are a group of microscopic cnidarian parasites some of which are known to cause fatal infections in wild fish and aquaculture. A range of new applications involving gene expression studies have been emerging rapidly over the last years in order to design efficient methods for prediction and mitigation of myxozoan diseases. Due to its high sensitivity and wide dynamic range, quantitative PCR (qPCR) is the most widely used approach for gene expression studies [1,2]. To obtain gene expression data, it is critical that qPCR results are normalized to a reference or housekeeping (HSK) gene, since the mRNA level itself is not only influenced by regulation of gene expression but by the number of parasite cells present. HSK genes are genes that are necessary for basic cell survival and the mRNA synthesis of these genes is considered to be stable and secure in various tissues, even under experimental treatments [3,4]. However, numerous studies have already shown that HSK gene expression may vary under experimental conditions from organism to organism [2]. That is why it is absolutely necessary to validate the HSK gene for each case before any gene expression studies.

For cnidarians, data on HSK genes is very scarce, for myxozoans in particular no solid data exist. The majority of papers dealing with gene expressions rely on HSK genes that have been “validated in previous or other publications” without testing their reliability [5,6]. For this purpose, we have conducted a study to identify the best HSK genes for myxozoans, which will become a valuable reference for the future numerous gene expression studies for myxozoans and other cnidarians.

We have chosen three myxozoan species *Sphaerospora molnari*, *Myxobolus cerebralis* and *Ceratomyxa shasta* belonging to three different myxozoan lineages. Our workflow consists of the following steps: 1) extensive literature survey and listing of the most common HSK genes, 2) candidate HSK genes mining in parasite transcriptomes of different life cycle stages, 3) RNA/cDNA isolation from different stages followed by gene specific primer design and quantification of target genes using qPCR. Based on obtained Ct values relative expression of target gene was calculated according to Pfaffl [7]. Since the parasite RNA is intermixed with that of fish and initial concentration of parasite RNA is not possible to determine by cell counts, the “similar ratio” approach was taken into account to predict that if we have 2 genes with the constant expression level in stage 1, the ratio between them should be the same in the stage 2.

Our preliminary calculations reveal that from the list of analyzed genes GDP (9 Glyceraldehyde-3-Phosphate Dehydrogenase), EF2 (Eukaryotic Translation Elongation Factor 2) and 28S (28S ribosomal RNA LSU) possibly have the most similar expression rate in different developmental stages of myxozoan parasites.

## Future plans

Comparative analyses using transcriptomic data from two life cycle stages of *S. molnari* which we recently produced will further aid the identification of homogeneously expressed genes in myxozoans that may serve as housekeeping genes for expression studies.

## Acknowledgement

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# Institute of Parasitology – Trematode team: research outlines for 2017

Aneta Kostadinova<sup>1\*</sup>

<sup>1</sup>Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, České Budějovice, Czech Republic

\*corresponding author, e-mail: aneta.kostadinova@uv.es

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During 2017 the research of the trematode team was focused on the integrative taxonomy approaches to the diversity of fish parasites. Our results include descriptions of two digenean species new to science and morphological and molecular characterisation of six species from two genera. Within *Macvicaria*, a new species was described, *M. crassigula* (sensu stricto) was delineated and reported for the first time in the Mediterranean, and four species were molecularly characterised: *M. gibsoni* and *M. crassigula* (s.s.) based on specimens ex *Diplodus vulgaris*, and *M. mormyri* and *M. maamouriae* based on specimens ex *Sparus aurata* from off the Algerian coast of the Mediterranean. Sequences associated with morphological description were also provided the type-species of Pseudopycnadena, *P. fischthali* based on material ex *D. vulgaris*. Phylogenetic analyses based on ITS and 28S rDNA data provided evidence for the distinct species status of eight Mediterranean species of *Macvicaria* [1]. Within *Skoulekia*, a new species, *S. erythrini*, was described from *Pagellus erythrinus* collected from several localities of the Western Mediterranean off Spain. A second species, *S. meningialis*, was described from a new sparid host, *Diplodus puntazzo*, from off Santa Pola, Spain. An amended generic diagnosis of *Skoulekia* was provided based on the novel morphological data for *S. erythrini* and *S. meningialis* and the re-examination of the type-material of *S. meningialis* [2]. Research on large-scale genetic characterisation of species of *Diplostomum* resulted in delineation of a number of species based on adults from North Europe and a significant enrichment of the cox1 database of the project based on metacercariae from a wide range of fishes in Europe and Asia.

## Acknowledgement

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# Bending (nematode-like) movement in ancestral apicomplexans represented by marine archigregarine *Selenidium* sp.

Magdaléna Kováčiková<sup>1\*</sup>, Andrei Diakin<sup>1</sup>, Gita G. Paskerova<sup>2</sup>, Andrea Valigurová<sup>1</sup>

<sup>1</sup> Department of Botany and Zoology, Faculty of Science, Masaryk University, Brno, Czech Republic

<sup>2</sup> Department of Invertebrate Zoology, Faculty of Biology, Saint-Petersburg State University, Petersburg, Russia

\*corresponding author, e-mail: kovacikova@sci.muni.cz

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Gregarines represent one of the ancestral lineages of apicomplexans widely dispersed in invertebrate hosts. Archigregarines are found exclusively in marine environment. Based on their pleomorphic characters, archigregarines are inferred to be the representatives of most ancestral gregarines and perhaps apicomplexans as whole [1]. The intestinal trophozoites of *Selenidium* species are morphologically similar to the infective apicomplexan zoites and hence are often termed as “hyperzoites” [2]. Vermiform trophozoites are covered with a cortex organised into shallow longitudinally running folds separated by grooves. Under the pellicle, one or more layers of subpellicular microtubules are localised, which are supposed to have important role on *Selenidium* spp. movement.

The bending (nematode-like) motility of archigregarine *Selenidium* sp. from marine polychaete *Pygospio elegans* Claparède, 1863, was studied throughout the experiments with drugs influencing depolymerisation/repolymerisation of the main cytoskeletal proteins: actin (jasplakinolide and cytochalasin D) and tubulin (colchicine and oryzalin). As was previously demonstrated by our light and confocal laser scanning microscopic (CLSM) observations [3, 4], the differences in presence and distribution of subpellicular microtubules were also monitored using the transmission electron microscopy (TEM). Drug-treated archigregarines displayed changes in their movement mode resulting in motility cessation in a relatively short time period after treatment with microtubule depolymerising probes. These changes in motility correlated with fading or complete loss of a number of subpellicular microtubules. In contrast, in control archigregarines incubated close to natural environment (seawater), the outermost microtubules were presented as continuous layer and the motility showed typical pattern.

## Future plans

Summarise data and submit the manuscript

## Acknowledgement

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# Interruption of the host-parasite co-adaptation by hybridization tested using the monogenean infection in cyprinids

Vadym Krasnovyd<sup>1\*</sup>, Lukáš Vetešník<sup>2</sup>, Andrea Šimková<sup>1</sup>

<sup>1</sup>Department of Botany and Zoology, Faculty of Science, Masaryk University, Brno, Czech Republic

<sup>2</sup>Institute of Vertebrate Biology, Czech Academy of Sciences, Brno, Czech Republic

\*corresponding author, e-mail: krasnovyd@gmail.com

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The host specificity is affinity of the parasite to the most suitable host species after the consequent process of host-parasite co-adaptation. Strict specificity of the monogenean parasites is linked to their specific adaptations to their fish host. Nevertheless, such host-parasite co-adaptation may be interrupted by host hybridization. Fish host hybridization and maternally inherited mitochondrial DNA are hypothesized to affect the level of parasite infection. Patterns of the parasite infection and distribution on hybridized fish have been recently investigated. However, different scenarios of the parasite infection in hybrids and their parental fish species have been observed. The aim of this study was to analyse the monogenean infection in the two phylogenetically related species, common bream (*Abramis brama*) and silver bream (*Blicca bjoerkna*), and their F1 hybrids. Breed lines of common bream, silver bream and their F1 hybrids were prepared for this study. All fish specimens resulting from experimental breeding were infected from the specimens of common bream and silver bream collected from the field. Fish were subjected to the dissection and all monogenean parasites were collected, fixed and identified. Similarity between monogenean infection of the parental species and hybrids with different maternal origins was evaluated using the Sørensen index.

Higher monogenean abundance was found in parental species (common bream and silver bream) when compared to their hybrids which supports the hybrid resistance hypothesis. However, parasite species richness of the F1 hybrids was higher when compared to the pure species as previously demonstrated also in other studies comparing the level of parasite infection between parental species and their hybrids. The effect of the maternal origin on the monogenean abundance in fish hybrids was found. Specialist and generalist parasites of the parental species were presented in the hybrids with different maternal origins. Higher abundance of the specialist parasites of *B. bjoerkna* in the hybrids with different maternal origins was found. Nevertheless, Sørensen index revealed high similarity between the parasite composition of common bream and the hybrids with different maternal origins. The presence of the parental species-specific monogenean parasites in the hybrids might indicate interruption of the host-parasite system of co-adapted genes. These results may be explained by the different degree of the host-parasite co-adaptation in *A. brama* and *B. blicca*.

## Acknowledgement

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# Knowledge of the trematodes in freshwater fishes of Africa

Olena Kudlai<sup>1,2\*</sup>, Nico Smit<sup>2</sup>, Tomáš Scholz<sup>1</sup>

<sup>1</sup> Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, České Budějovice, Czech Republic

<sup>2</sup> Unit for Environmental Sciences and Management, Potchefstroom Campus, North-West University, Potchefstroom, South Africa

\*corresponding author, e-mail: olena.kudlai@gmail.com

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Although the freshwater fish fauna in Africa is exceptionally diverse and comprise about 3,000 species, we have little understanding of the diversity of its parasites [1]. The knowledge of trematode fauna remains highly incomplete. Current estimates of trematode species revealed the presence of 66 species (based on adults) allocated in 33 genera of 20 families reported from 59 freshwater fish species [1–3]. The distribution of taxonomic diversity of trematodes is uneven, with thirteen families (Apocreadiidae, Aporocotylidae, Callodistomidae, Derogenidae, Deropristidae, Didymozoidae, Haploporidae, Hemiuridae, Gonocercidae, Macroderoididae, Opecoelidae, Opisthorchiidae, Orientocreadiidae) represented by one or two species and seven species-rich families, Cephalogonimidae (8 species), Gorgoderidae (8 species), Allocreadiidae (7 species), Cryptogonimidae (7 species), Plagiorchidae (6 species), Cladorchiidae (4 species) and Heterophyidae (4 species). Data on trematode larval stages from African freshwater fishes are rather questionable. Most of published reports do not contain morphological descriptions or illustrations. In addition, many of the earlier descriptions are not recognisable. However, the rough estimates of the diversity of trematode larvae demonstrated the presence of 40 species from 19 genera of six families. The species-richest families are Diplostomidae (16 species, 6 genera), Clinostomidae (11 species, 4 genera) and Heterophyidae (9 species, 7 genera).

Our current research focuses on trematode diversity of freshwater fishes in South Africa with the application of molecular tools in combination with morphological analysis. To date, a total of 127 fish of 20 species were sampled in the Phongolo River (Ndumo Game Reserve), Riet River (Mokala National Park) and Vaal River (North-West Province). Trematodes were recovered from eight fish species. Morphological and molecular analyses of collected material provided identification for 16 species of trematodes belonging to the families Clinostomidae, Diplostomidae, Gorgoderidae, Macroderoididae and Opisthorchiidae. The obtained results represent an important step to increase the knowledge on the diversity of trematodes from freshwater fishes in Africa.

## Future plans

- (1) Publication of data on trematodes reported from freshwater fishes in Africa.
- (2) Morphological assessment of the sequenced trematodes collected from fish in South Africa and publication of the results.

## Acknowledgement

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# Parasites of the invasive round goby, *Neogobius melanostomus* (Pallas, 1814) (Actinopterygii: Gobiidae), in the estuarine zone of the Elbe River, North Sea basin

Yuriy Kvach<sup>1,2\*</sup>, Markéta Ondračková<sup>1</sup>, Michal Janáč<sup>1</sup>, Vadym Krasnovyđ<sup>3</sup>, Eduard-Gabriel Ballon<sup>4</sup>, Pavel Jurajda<sup>1</sup>

<sup>1</sup>Institute of Vertebrate Biology, Czech Academy of Sciences, Brno, Czech Republic

<sup>2</sup>Institute of Marine Biology, National Academy of Sciences of Ukraine, Odessa, Ukraine

<sup>3</sup>Department of Botany and Zoology, Faculty of Science, Masaryk University, Brno, Czech Republic

<sup>4</sup>Branch office Elbe of the Institute of Applied Ecology, Marschacht, Germany

\*corresponding author, e-mail: yuriy.kvach@gmail.com

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The round goby, *Neogobius melanostomus* (Pallas, 1814) (Actinopterygii: Gobiidae), is Ponto-Caspian brackish water fish species, naturally ranged in the Black and Caspian seas and lower reaches of adjacent rivers. This species is one of the most successful fish invaders in many water bodies of Europe and North America [1]. Recently it was registered in the estuary of the Elbe River [2]. The North Sea is terminal point for two routs of aquatic invasions in Europe: Central and Southern corridors [3]. North Sea riverine estuaries and deltas have specific ecological conditions, with unstable salinity and water levels that change rapidly due to the presence of intensive tides [4]. Ponto-Caspian gobiids mainly inhabit brackish or high salinity waters in their natural range; however, some species, classed as neo-limnetic, have a brackish water origin but mainly inhabit freshwaters [5]. Taking into account the untypical environmental conditions for Ponto-Caspian gobiids, the aim of the present work was to study the colonization of the round goby by parasites in the estuarine Elbe.

The fish were sampled by angling in two localities in lower Elbe: 1. Süderelbe (53°28.266'N 9°59.133'E), 2. Geesthacht (53°25.643'N 10°21.905'E). The fish were transported alive in aerated cans with riverine water to the field laboratory, where dissected in accordance to recommendations. The data from the upper Elbe near Ústí nad Labem [6], were used for comparative study.

In total of 12 parasite taxa occurred in the estuarine zone of the Elbe river: 6 in the Süderelbe and 9 in Geesthacht. Fish parasite communities significantly differed among each of the three sites. Süderelbe fish were infected mostly by *Anguillicoloides crassus* larvae, which was almost absent in fish from the other two sites. While fish from Ústí nad Labem were infected mostly by *Pomporhynchus tereticollis* cysacaths and glochidia occurring sporadically, this pattern was reversed in Geesthacht fish and both these taxa were absent in the Süderelbe fish. Infracommunity species richness was not significantly different among the three sites, being generally poor with mean values reaching 0.75 in Süderelbe fish (range 0-3), 1.09 in Geesthacht fish (range 0-3) and 0.54 in fish from Ústí nad Labem (range 0-2). Total parasite abundance did not significantly differ between the Süderelbe and Geesthacht, but significantly fewer parasites were found at both German sites compared to fish from Ústí nad Labem.

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# Identification of novel minicollagens in the myxozoan polar capsule gene repertoire

Jiří Kyslík<sup>1,2\*</sup>, Ivan Fiala<sup>1,2</sup>

<sup>1</sup>Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, České Budějovice, Czech Republic

<sup>2</sup>Department of Parasitology, University of South Bohemia, Faculty of Science, České Budějovice, Czech Republic

\*corresponding author, e-mail: j.kyskikk@gmail.com

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Obligate parasitism is frequently associated with the considerable anatomic reduction and creation of specialized morphological structures. Myxozoa, a cnidarian parasitic group, is an illustrative example of this phenomenon. They changed their body plans into simple plasmodial forms, in which the myxozoan specific spore are developed. Spores are composed of spore valves, sporoplasm and polar capsules with coiled polar filament. Polar capsules are homologous structures to the cnidarian nematocysts and via extrusion of filaments they enable the spores to attach to the host.

Remarkable similarities between polar capsules and nematocysts suggested common evolutionary origin of Myxozoa and Cnidaria. The morphological affinities between these complex organelles were firstly confirmed by the identification of nematocyst specific gene in myxozoan EST data [2]. Recently this hypothesis was also supported by identification of nematocyst-specific cnidarian taxonomically restricted genes (TRGs) in the myxozoan genome [4,5]. Minicollagens, typical nematocyst-specific TRG, are short collagen proteins with specific sequence composition playing a critical role in the nematocyst structure. They contain small cysteine-rich domains (CRDs) joining together by disulfide bonds and short central collagen sequence surrounded by Poly-Proline repeats [1]. Based on their gene organization and domain architecture minicollagens were divided in Cnidaria into 17 types clustering into three groups. Diversity of myxozoan minicollagens was supposed to be reduced according to the low number of identified homologs (Ncol-1; Ncol-2; Ncol-3).

Here we report a discovery of four new minicollagen types in Myxozoa by mining of available myxozoan genomes and transcriptomes including our newly obtained transcriptome of *Myxidium lieberkuehni*. The minicollagen affiliation was proved by identification of specific Poly-Proline-CRD region together with phylogenetic analysis, which revealed that these novel myxozoan minicollagens types create four separated clades.

Hypothetical reduction of myxozoan minicollagen gene types due to the parasitic lifestyle turned out to be not true. Myxozoans have probably similar minicollagen gene repertoire as other cnidarians, which is in agreement with very similar morphology and ontogeny of myxozoan polar capsules and cnidarian nematocysts. However, two newly identified minicollagen groups seem to be distantly related from all other cnidarian homologs and thus may represent minicollagens specific for Myxozoa with unknown role in the polar capsule structure. Seven so far identified myxozoan minicollagens types may not be the final number since the identification of myxozoan minicollagens in the genomic data turned out to be very difficult.

Within the Cnidaria, the sequence complexity of minicollagens is associated with the different shape of nematocyst [1]. Whether minicollagens have similar function in Myxozoa was not proved as well as we do not know the localization of these cnidarian TRGs within the myxozoan polar capsule and their connections with other proteins involved in the polar capsule organisation (NOWA, spinalin etc.)

## Future plans

The following research will include a clarification of the function of these myxozoan minicollagens as well as to find out their localization within the polar capsule. We also plan to reveal their expression during the ontogeny of myxospore and to understand the key steps of myxozoan minicollagen evolution.

## Acknowledgement

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# Potential effectiveness of bio-manipulation on reservoir water quality

Mikl Libor<sup>1,2</sup>, Adámek Zdeněk<sup>1</sup>, Roche Kevin<sup>1</sup>, Jurajda Pavel<sup>1</sup>

<sup>1</sup>Institute of Vertebrate Biology, Czech Academy of Sciences of Czech Republic, Brno, Czech Republic

<sup>2</sup>Department of Botany and Zoology, Faculty of Science, Masaryk University, Brno, Czech Republic

\*corresponding author, e-mail: libor.mikl@seznam.cz

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During the summer of 2016, we undertook a pilot evaluation of the potential effectiveness of bio-manipulating fish populations on the water quality of five drinking water reservoirs in the River Morava drainage area. We first estimated the relative density of cyprinids and predators in each reservoir using gillnets and electrofishing. We then examined the digestive tracts (n = 480) from five native predator species: perch (*Perca fluviatilis*), northern pike (*Esox lucius*), European zander (*Sander lucioperca*), asp (*Leuciscus aspius*) and European catfish (*Silurus glanis*). At least 25% of the predators analysed were parasitised with nematodes, acanthocephalans and/or Cestoda. The five dominant species in the reservoirs were roach (*Rutilus rutilus*; 45%), perch (34%), bream (*Abramis brama*; 7%) and bleak (*Alburnus alburnus*; 4%). The relative density of predators was generally low, with all predators (except perch) in the five reservoirs having a density of < 3%. Dietary analysis indicated that the dominant fish species consumed by predators were roach (23%), perch (22%) and bleak (5%). As the majority of predatory species showed a high level of cannibalism on smaller individuals of their own species, there is a strong chance of a multiplying effect as parasites are concentrated within the species.

Our data suggest that current conditions of low predator and high cyprinid density are potentially having a negative effect on both water quality and level of parasitisation. Hence, bio-manipulation by increasing the predator density has a good chance of improving both water and fish quality in these reservoirs.

## Future plans

Preparation and finalisation of publications concerning the data collected and planning of a more comprehensive study including water quality parameters, zooplankton and zoobenthos levels and parasite community analysis.

## Acknowledgement

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# Some nematodes from freshwater fishes in Central Africa

František Moravec<sup>1\*</sup>, Miloslav Jirků<sup>1</sup>

<sup>1</sup>Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, České Budějovice, Czech Republic

\*corresponding author, e-mail: moravec@paru.cas.cz

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Freshwater bodies in Africa contain almost 25% of the world's 13,000 freshwater fish species [1], with a high degree of endemism at species and family levels. In contrast, the present knowledge of the parasite fauna of African fishes is fragmentary and incomplete, especially when compared with other continents, which makes it impossible to assess reliably their diversity, interrelations, distribution and potential effects on their fish hosts [2]. Twenty species (sixteen adult and four larval) of parasitic nematodes belonging to the Ascaridoidea, Camallanoidea, Cosmocercoidea, Dioctophymatoidea, Habronematoidea, Oxyuroidea, Seuratoidea, and Thelazioidea were collected from freshwater fishes of the Congo River basin in the Democratic Republic of the Congo and the Central African Republic in 2008 and 2012, respectively. Based on light and scanning electron microscopical examination, many species were redescribed in detail. This material also contained four previously unknown species, one new species of *Labeonema* Puylaert, 1970 from *Synodontis acanthomias* Boulenger, two new species of *Gendria* Baylis, 1930 from *Schilbe grenfelli* (Boulenger) and *Schilbe marmoratus* Boulenger, respectively, and one new species of *Cucullanus* Müller, 1777 from *Auchenoglanis occidentalis* (Valenciennes). The new species of *Labeonema* is mainly characterised by conspicuously long (210–228 µm) spicules, approximately twice as long as those in other congeners, the length (45–48 µm) of the gubernaculum and the host family (Mochokidae), whereas the new species of *Gendria* from *S. grenfelli* by very long (1.22 mm) spicules, the shape of the oesophagus and cephalic vesicle, distribution of postanal papillae and the host family (Schilbeidae). Main characteristic features of the new species of *Gendria* from *S. marmoratus* are the presence of a hexagonal oral aperture surrounded by lip-like structures, the posterior portion of the oesophagus moderately expanded, spicules and the gubernaculum 414–438 µm and 54–57 µm long, respectively, deirids located at the level of the posterior end of the oesophagus and the absence of cervical alae. The new species of *Cucullanus* differs from congeneric species parasitising African freshwater and brackish-water fishes mainly in the absence of a ventral preloacal sucker and lateral preanal papillae, the presence of a large median preloacal papilla-like formation, spicules 480–489 µm long and the location of the excretory pore in the region of the oesophago-intestinal junction. *Chabaudus* Inglis et Ogden, 1965 was considered a junior synonym of *Gendria* Baylis, 1930 and, consequently, species listed in the former genus were transferred to the latter as *G. alaini* (Alfonso-Roque, 1981) comb. n., *G. chabaudi* (Inglis et Ogden, 1965) comb. n., *G. dehradunensis* (Rizvi, Bursey et Maity, 2016) comb. n., *G. thysi* (Puylaert, 1970) comb. n. and *G. williamsi* (Puylaert, 1970) comb. n. The findings represent many new host and geographical records.

## Future plans

The above data will be published and then included into the intended monograph dealing with nematode parasites of freshwater fishes in Africa. Further studies on the diversity of philometrid nematodes (Philometridae) parasitic in marine fishes.

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# Ecological plasticity of invasive tubenose gobies (*Proterorhinus semilunaris*) in South Moravian water bodies

Markéta Ondračková<sup>1\*</sup>, Lucie Všetická<sup>1</sup>, Lukáš Kopeček<sup>1</sup>, Zdeněk Adámek<sup>1</sup>, Pavel Jurajda<sup>1</sup>

<sup>1</sup>Institute of Vertebrate Biology, Czech Academy of Sciences of Czech Republic, Brno, Czech Republic

\*corresponding author, e-mail: audrey@sci.muni.cz

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Over recent decades, the tubenose goby *Proterorhinus semilunaris* (Pallas, 1811) has spread throughout Europe from its original Ponto-Caspian range [1]. Tubenose gobies were first found in the Czech Republic in the Nové Mlýny reservoir system on the River Dyje in 1994, probably as a result of unintended introduction as bait fish [2]. Later, the species was found in the Rivers Dyje and Morava, downstream of their confluence [3]. In the Dyje/Morava basin, tubenose gobies have established populations in wide range of habitat types. In this study, we aimed to a) assess the present distribution of tubenose goby in South Moravia, b) evaluate its dietary preferences in relation to food availability and habitat type and c) its susceptibility to parasite infection. Fish sampling took place at 39 sites representing six major aquatic habitat types in the Dyje River basin, i.e. lowland rivers, backwaters, oxbow lakes, borrow pits, reservoirs and ponds. In each case, we recorded fish density and dominance, diet, macrozoobenthos density and parasite infection rate.

Tubenose gobies were recorded at 74% of sampling sites, with densities ranging from 3 (River Svatka) to 202 (Hvezda borrow pit) individuals per 100 m. There was no significant difference in tubenose goby density and/or dominance between habitats, though frequency of occurrence was lower in ponds and oxbows. Both fish length and condition differed significantly between sites, independent of habitat type. Diet composition ranged between 1 (Rohlik borrow pit and D2 oxbow) and 6 taxa (Udernik pond). Individual diet composition was positively correlated with fish length and condition. While diet did not differ significantly between habitats, there was a trend towards a more diverse diet in rivers when compared with oxbows. Index of fullness was not associated with either fish length or condition and did not differ between habitats. Food availability ranged from 2 (Melanbon borrow pit) to 19 taxa (Udernik pond), with a higher quantitative macrozoobenthos density in rivers and very poor availability in borrow pits and oxbows. In all habitats, there was negative selection for Gastropoda, Tubificidae, Hirudinea and Diptera (excluding Chironomidae). Positive selectivity varied considerably between sampling sites/habitats. Overall, the parasite community consisted of 18 metazoan taxa, including one monogenean, eight digeneans, one cestode, three nematodes, two acanthocephalans and three bivalve species. Maximum parasite species richness was found in rivers, with 6-7 species per site at the component community level and 5 species per fish at the infracommunity level across habitat types. Both component community and infracommunity species richness decreased significantly with tubenose goby dominance. The parasite community was dominated by larval trematodes, significantly affecting the overall parasite abundance at most sites. No association between abundance and fish condition was detected for any parasite species at any site.

Our results indicate that this small fish species appears to be tolerant of most habitats, from small lentic water bodies to large reservoirs and lotic waters. Its diet covers a wide range of macroinvertebrates, with diet varying in relation to local availability. The tubenose goby has acquired a diverse parasite fauna in its new range, though at relatively low intensities. None of the parasites appears to have had a negative effect on the condition of its host.

## Future plans

Preparation and finalisation of publications concerning the data in this abstract.

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# A common Eurasian fish tapeworm, *Caryophyllaeides fennica* (Cestoda) in western North America: further evidence of amphi-Pacific vicariance in freshwater fish parasites

Mikuláš Oros<sup>1\*</sup>, Anindo Choudhury<sup>2</sup>, Tomáš Scholz<sup>3</sup>

<sup>1</sup>Institute of Parasitology, Slovak Academy of Sciences, Košice, Slovakia

<sup>2</sup>Division of Natural Sciences, St. Norbert College, DePere, Wisconsin

<sup>3</sup>Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, České Budějovice, Czech Republic

\*corresponding author, e-mail: oros@saske.sk

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The freshwater fish fauna of the Palaearctic and Nearctic regions shows similarities but also notable differences, resulting in diverse distributions of their parasites. Relatively few parasite species occur in both regions and fewer still have been examined using molecular data. We report a rare example of ‘amphi-Pacific’ distribution, involving the common Palaearctic parasite, the caryophyllidean cestode *Caryophyllaeides fennica* (Schneider, 1902), in the chiselmouth *Acrocheilus alutaceus* Agassiz and Pickering, 1855, an endemic cyprinid in northwestern Nearctic, Oregon, USA. Available information on non-native fish species in Oregon suggests that the parasite is native to the area and not introduced. Molecular data (18SrDNA, 28SrDNA, ITS-2 and *cox1*) indicate very little genetic divergence between representatives from the Palaearctic and Nearctic, and possibly a relatively more recent colonisation of the Nearctic region by this cestode via the Beringian land bridge. This is remarkable considering that *Acrocheilus* has reportedly been in Oregon since the Miocene. We expect that further studies of northwestern cyprinids will provide insights into the zoogeography and evolution of western Nearctic parasites and the relationship of the western Nearctic fish fauna with that of the eastern Palaearctic.

## Acknowledgement

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# A new classification of *Glaridacris* Cooper, 1920 (Cestoda, Caryophyllidea), parasites of suckers (Catostomidae) in North America

Mikuláš Oros<sup>1\*</sup>, Dalibor Uhrovič<sup>1</sup>, Tomáš Scholz<sup>2</sup>

<sup>1</sup>Institute of Parasitology, Slovak Academy of Sciences, Košice, Slovakia

<sup>2</sup>Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, České Budějovice, Czech Republic

\*corresponding author, e-mail: oros@saske.sk

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A taxonomic study of monozoic cestodes of the genus *Glaridacris* Cooper, 1920 (Cestoda: Caryophyllidea), parasites of catostomid fishes in North America, confirmed artificial character of the genus which is split to two different, morphologically distinct and not closely related genera. The two morphologically distinct groups are called herein, in accordance with Mackiewicz (1976), as 'catostomi-group' and 'laruei-group'. *Glaridacris* is newly circumscribed to include only three species, *G. catostomi* Cooper, 1920 (type species), *G. terebrans* (Linton, 1893) and *G. vogeii* Mackiewicz, 1976, which are characterised by an elongate body, a cuneiloculate or wedge-shaped scolex with six shallow loculi, male and female gonopores at a distance from each other, follicular ovary and circummedullary vitelline follicles (lateral and median). The remaining three species ('laruei-group') differ from the species of *Glaridacris* listed above by the possession of a bothrioloculodiscate scolex with a terminal disc at the scolex apex, with a pair of deeper median and two shallower lateral loculi, male and female gonopores close together, a compact, non-follicular ovary, and vitelline follicles lateral, i.e. missing medially. For species of this group, a new genus will be erected. An annotated list of all species of both genera with data on their hosts and distribution, and keys to their identification are provided.

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# Red Queen dynamics in the diploid-polyploid complex of *Carassius auratus*

Tomáš Pakosta<sup>1\*</sup>, Lukáš Vetešník<sup>2</sup>, Andrea Šimková<sup>1</sup>

<sup>1</sup>Department of Botany and Zoology, Faculty of Science, Masaryk University, Brno, Czech Republic

<sup>2</sup>Institute of Vertebrate Biology, Czech Academy of Sciences of Czech Republic, Brno, Czech Republic

\*corresponding author, e-mail: pakostatomas@seznam.cz

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*Carassius gibelio* - member of *Carassius auratus* complex is an extraordinary cyprinid species exhibiting dual reproductive ways i.e. sexual way and asexual way termed gynogenesis. As four forms of *C. auratus* complex were described in the waters of Czech Republic, D-loop region of mtDNA was analysed to identify the specimens of *C. gibelio*, which represented the most abundant form.

On the basis of the Red Queen Hypothesis, asexual form (or the most common asexual genotype) is a target of parasite adaptation due to the limited genetic variability. In contrast, sexual reproduction by creating a wide range of genetically diverse forms is able to escape to parasites. As a result, we expected that genetically homogenous asexual form of *C. gibelio* is disadvantaged i.e. should be more parasitized when compared to the genetically variable sexual form. At the same time, we were interested in the response of the host immune system to the parasitic infection. The parameters of non-adaptive (i.e. respiratory burst, lysozyme activity and complement activity) and adaptive immunity (IgM antibodies) were compared between two forms in four consecutive years. A total of 7 species of *Dactylogyrus*, 3 species of *Gyrodactylus* and larval stage of *Paradiplozoon homoion*, 2 species of Nematoda, Trematoda and Crustacea, *Pisciola geometra*, larval stage of glochidium and *Ichthyophthirius multifiliis* were identified in *C. gibelio*. The maximum prevalence and the highest intensity of infection were found for the following monogenean species: *Dactylogyrus dulkeiti*, *D. anchoratus* and *Gyrodactylus sprostonae* in both forms of gibel carp. The similar level of parasite infection in both gynogenetic females and sexual individuals was found. However, our study revealed a temporal variation in parasite load in both forms which could potentially be explained by the dynamics of host-parasite interactions predicted by Red Queen hypothesis. Our study indicates significantly higher specific immunity expressed by IgM for 3n females when compared to diploid specimens. Concerning the haematological parameters, only erythrocyte count was clearly affected by ploidy status and haematocrit was influenced by sex. This suggests that asexual triploids dispose by lower cell count than diploid individuals; it can be compensated by larger cell size in triploid females.

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# Phylogenetic congruence of myxozoans belonging to *Sphaerospora sensu stricto* and their vertebrate hosts

Sneha Patra<sup>1\*</sup>, Pavla Bartošová-Sojková<sup>1</sup>, Hana Pecková<sup>1</sup>, Ivan Fiala<sup>1,2</sup>, Edit Eszterbauer<sup>3</sup>, Astrid S. Holzer<sup>1,2</sup>

<sup>1</sup>Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, České Budějovice, Czech Republic

<sup>2</sup>Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic

<sup>3</sup>Institute for Veterinary Medical Research, Centre for Agricultural Research, Budapest, Hungary

\*corresponding author, e-mail: snehampatra@gmail.com

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Myxozoa are a highly diverse and strongly morphologically group of Cnidarians that alternately infect invertebrates (bryozoans and annelids) and vertebrate hosts (predominantly fishes). Phylogenetically, myxozoans cluster into 4 well-defined clades. The *Sphaerospora sensu stricto* clade is the most basal myxosporean lineage in the phylogenetic tree of myxozoans. Sphaerosporids commonly parasitize the urinary system of marine and freshwater fishes but have also conquered amphibian hosts. Exclusive to this phylogenetic clade of myxozoans is the occurrence of highly proliferative and often motile presporogonic stages in the blood of their hosts [1]. Taxonomic sampling of sphaerosporids is restricted to 19 taxa sequenced to date, likely because they are difficult to detect (cryptic nature, seasonal infections) and even more difficult to sequence due to large variable inserts in the 18S and 28S rDNA regions.

In the present study, we characterised 17 new sphaerosporid 18S rDNA sequences from marine and freshwater fishes by intensive PCR screening of fish kidneys, applying a number of new, lineage-specific primers. Using 18S rDNA parasite sequences and full mitogenome host sequences of 26 taxa, we investigated the coevolutionary history of sphaerosporids and their vertebrate hosts using tree topology-based (CoRe-PA) and distance based (ParaFit) methods. We detected significant phylogenetic congruence of with a high number of cospeciation events. We detected a high diversity of sphaerosporids in cyprinid hosts and though this may be explained by the bias in host sampling, a strongly increased speciation rate in cyprinids can also be found in other myxozoan lineages, such as myxobolids. It is unclear if parasite diversity is a result of intense host specification but phylogenetic trees are highly congruent and our results indicate that host switching between closely related cyprinid hosts occurred several times. The present study provides proof for reciprocal influence of myxozoans parasites and their vertebrate hosts and allows for insights in the mechanisms that shape their common evolutionary history.

## Future plans

PCR screening of kidneys from evolutionary old fish groups (for e.g. cartilaginous fish) is currently performed as we expect additional sequence information confirming coevolution of sphaerosporids and their vertebrate hosts down to the most basal branches.

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# Local variation in embryo development rate in annual fish

Matej Poláčik<sup>1\*</sup>, Martin Reichard<sup>1</sup>, Milan Vrtílek<sup>1</sup>

<sup>1</sup>Institute of Vertebrate Biology, Academy of Sciences of the Czech Republic, Brno, Czech Republic

\*corresponding author, e-mail: polacik@ivb.cz

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Extreme asynchrony in embryo development, a typical feature of annual killifish living in temporary pools, represents a bet-hedging strategy to cope with unpredictable rainfall. African annual killifish are distributed across a large precipitation gradient, raising the potential for local adaptation in the degree of developmental asynchrony (e.g. higher in arid areas, lower in humid areas). Eight populations of two sister species, *Nothobranchius furzeri* and *N. kadleci*, from sites along the rainfall gradient were tested and compared for asynchrony and duration of embryo development. Degree of asynchrony and mean duration of embryo development did not differ across the examined range. Despite generally high developmental variability, fish from more arid regions (where rain is more erratic) produced a significantly higher proportion of short-developing embryos. Comparable developmental asynchrony, regardless of precipitation level, suggests that all populations tested need to cope with some level of rainfall stochasticity. By producing more short-developing embryos, however, fish in more arid areas with relatively more erratic rains are better adapted to very short pool durations and are more likely to produce multiple offspring generations within a single rainy season.

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# Monogenean parasites and their effect on the expression of Toll-like receptor genes in *Abramis brama* x *Rutilus rutilus* hybrid system

Adam Potrok<sup>1,2\*</sup>, Andrea Šimková<sup>1</sup>, Lenka Gettová<sup>1</sup>

<sup>1</sup>Department of Botany and Zoology, Faculty of Science, Masaryk University, Brno, Czech Republic

<sup>2</sup>Department of Animal Physiology and Immunology, Faculty of Science, Masaryk University, Brno, Czech Republic

\*corresponding author, e-mail: potrok.adamm@gmail.com

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Infection by monogeneans (the gill and skin helminths), can cause severe health problems in fish, especially those living in aquaculture. In response to parasite infection, multiple immunological processes are initiated. An important process associated with the immune response to infection is an expression of immune genes, which can adapt immune response against particular parasites. The understanding of immune genes expression and gene modulation by parasitic infection can bring additional information on the fish immune system. Subsequently, new methods to control parasitic infections might be established [1].

In our study, we aimed to determine the effect of infection by monogenean parasites on the expression of genes encoding Toll-like receptors (TLR2 and TLR21), a crucial component of fish innate immune system, in the fish lymphatic organs (spleen and head kidney) and gills (i.e. site of infection of the studied monogenean parasites). *Abramis brama*, *Rutilus rutilus*, and their hybrids collected from Hamry reservoir (N=10 for each group) were dissected and their parasites were determined. Monogenean parasites represented the most abundant group of parasites in studied fish. Differences in parasitic load between fish groups, as well as differences in expression of studied TLR genes in organs and between fish groups, were analyzed using Kruskal-Wallis test. The effect of monogenean abundance on the expression of TLRs was analyzed by general linear model (GLM) using the fish group as a covariate. The associations between the abundance of each monogenean species and expression of TLRs in the selected organ were analyzed using Pearson correlation.

The parasitic load differed between fish groups. *A. brama* had a significantly higher abundance of monogenean parasites in comparison with *R. rutilus* and hybrids. The analysis of gene expression showed a significantly higher TLR21 expression in the spleen of *A. brama* than in remaining groups. Additionally, a significant effect of monogenean abundance and fish group on TLR21 expression in the spleen was supported by GLM analysis. In case of TLR2, a significant difference in its expression in the head kidney was revealed between *R. rutilus* and hybrids. Expression of TLR2 and TLR21 in remaining tissues did not differ significantly between fish groups. However, in *A. brama* significant negative correlation was observed between the abundance of *Dactylogyrus wueneri* (the second most abundant monogenean parasite detected in our study) and expression of TLR21 in gills.

Our findings imply that infection by monogenean parasites has an effect on the expression of TLR genes in studied fish. However, the effect of other parasite groups besides Monogenea on the level of TLR expression cannot be excluded. Therefore, an experimental study involving infection by single monogenean parasite species is further needed in order to confirm these findings.

## Future plans

Study of the effect of single monogenean parasite species on fish immune genes expression is planned under experimental conditions. The selection of suitable monogenean parasite from genus *Dactylogyrus* is undertaken at the present time. Changes in expression of selected immune-related genes at various stages of infection, as well as changes in immunological parameters of fish blood during infection, will be examined.

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# Relationship between pharmaceuticals accumulated in hosts tissues and parasitic infection

Markéta Pravdová<sup>1\*</sup>, Markéta Ondračková<sup>1</sup>, Jitka Kolářová<sup>2</sup>, Kateřina Grabicová<sup>2</sup>, Tomáš Randák<sup>2</sup>, Pavel Jurajda<sup>1</sup>

<sup>1</sup> Institute of vertebrate Biology, Academy of Sciences of the Czech Republic, Brno, Czech Republic

<sup>2</sup> Faculty of Fisheries and Protection of Waters, University of South Bohemia in České Budějovice, Czech Republic

\*corresponding author, e-mail: pravdova8@e-mail.cz

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During the last two decades, close relationship between water and sediment contamination by various chemicals of natural and anthropogenic origin and parasitism has been documented, highlighting the potential use of parasites as indicators of environmental pollution. Parasites may serve as accumulation indicators and they may demonstrate biological availability of pollutants. Parasites may also play an important role as pollutant sinks. Via combined effect of parasitism and pollution, parasites may affect the health of their hosts. Finally, parasites may serve as indicators of contaminants and ecosystem health [1]. Environmental stressors, such as wastewater or industrial pollutants can result in an increase in fish parasites due to the decreased immunological defence and decreased resistance to infections. On the other hand, some works have reported that pollution may result in a decrease of abundance and prevalence of parasites through direct effects of pollutants on the viability and longevity of free-swimming stages. This has been mainly reported for parasites with complex life-cycle, and alternatively, local absence or a very low abundance of the intermediate host is presented as the most likely explanation [2].

Wastewater treatment plants represent one of the sources of pollution of the aquatic environment. Aquatic organisms living in sites affected by the effluent of sewage treatment plants are exposed throughout their lifetime to mixtures of various compounds such as pharmaceuticals, personal care products (PPCPs) and other chemicals. The treated effluent from sewage treatment plants is a major source of active pharmaceutical ingredients that enter the aquatic environment. Despite the relatively low concentrations of pharmaceuticals in the environment pharmaceuticals are of ecological concern due to their potential long-term adverse effects on humans and wildlife [3].

During our research we compared parasite infection, somatic condition and accumulation of selected pharmaceuticals in fish between Čežárka pond situated downstream the leaking waste water from sewage treatment plant and control site at Velká Outrata pond in Vodňany. In both sites the fish were of the same origin and were released to the ponds three years ago. Two species, common carp *Cyprinus carpio* and pikeperch *Sander lucioperca* were used in our study. The fish were examined for presence of metazoan parasites, immunological and condition parameters and concentration of pharmaceuticals in the host tissues during two samplings, in the spring and summer 2017. Currently, all the data are in the process of analysis.

## Future plans

Complete the data analysis and preparation of manuscript.

## Acknowledgement

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# Diversity of monogenean parasites in South Africa: the story continues

Iva Přikrylová<sup>1,2,3\*</sup>, Wilmien Luus-Powell<sup>3</sup>, Nico J. Smit<sup>2</sup>, Milan Gelnar<sup>1</sup>

<sup>1</sup> Department of Botany and Zoology, Faculty of Science, Masaryk University, Brno, Czech Republic

<sup>2</sup> Water Research Group (Ecology), Unit for Environmental Sciences and Management, North West University, Potchefstroom, South Africa

<sup>3</sup> Department of Biodiversity, School of Molecular and Life Sciences, University of Limpopo, Sovenga, South Africa

\*Corresponding author: e-mail: ivaprik@sci.muni.cz

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Two new species of *Enterogyrus* found coexisting in the stomach of the Mozambique tilapia, *Oreochromis mossambicus* (Peters) are reported. Newly identified species *Enterogyrus multispiralis* n. sp. and *Enterogyrus mashegoi* n. sp. were collected from the hosts sampled from the Luphephe-Nwanedi Dams of the Limpopo River System in South Africa. The two new species can be differentiated from other members of the genus based on the length and nature of spirality of the cirrus. *Enterogyrus multispiralis* n. sp. has a unique 8/9–2–4 cirrus spiral formula. At *E. mashegoi* n. sp. the length of the cirrus together with its spiral formula 5–2–3 make it morphometrically unique from other *Enterogyrus* spp. In both the species the two pairs of posterior uncinuli are smaller than the five anterior pairs. The sequences of the nuclear ribosomal DNA partial 18S and internal transcribed spacer (ITS1) and 28S rDNA were obtained and compared with available sequences entries of *Enterogyrus* in the GenBank database. The study brings the first data on 18S rDNA region of *Enterogyrus* species. The phylogenetic comparison including all and very limited 28S data of *Enterogyrus* spp. available were conducted. The closest species to the both newly identified species was an undescribed *Enterogyrus* sp. 2 from *Sarotherodon galilaeus* (Linnaeus) with origin in Senegal (i. e. 2.1 and 2.9% uncorrected pairwise genetic distance to *E. multispiralis* n. sp and *E. mashegoi* n. sp., respectively). The description of these two new species of the genus *Enterogyrus* will bring their total number to 14.

The Sharptooth catfish, *Clarias gariepinus* (Burchell), is one of the most widely distributed freshwater fishes in Africa. It has been introduced under either controlled or uncontrolled conditions to different regions of Africa, and are also found in Israel, Lebanon and Turkey. As such, it has also become a popular candidate for research, including parasitological work on Monogenea. From August 2011 to September 2016, field surveys were undertaken at eight localities in South Africa and two in Zimbabwe, and included the Zambezi, Limpopo and Vaal river basins. A total of 43 specimens of *C. gariepinus* were collected and studied for the presence of monogenean parasites. Morphometrical and molecular characterisation of parasites were performed to confirm their identity. Seventeen species from three genera were recorded as follows: four species of *Quadriacanthus* Paperna, 1961, three species of *Macrogyrodactylus* Malmberg, 1957 and 10 species of *Gyrodactylus* von Nordmann, 1832. The species composition varied among sampled sites, with the highest species diversity recorded from the locality inside of Kruger National Park. All *Quadriacanthus* and *Macrogyrodactylus* species collected are known, but an unexpected six new *Gyrodactylus* species were sampled.

## Plans for next year:

- (1) Maternity leave
- (2) Finalise MSs on new *Gyrodactylus* species descriptions of various origins.

## Acknowledgement

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# ***Cichlidogyrus* Paperna, 1960 (Monogenea: Dactylogyridae) gill monogenean parasites of cichlid fish species (Perciformes: Cichlidae) from Lake Tanganyika: overview and perspectives**

Chahrazed Rahmouni<sup>1\*</sup>, Maarten P. M. Vanhove<sup>1,2,3,4</sup>, Andrea Šimková<sup>1</sup>

<sup>1</sup>Department of Botany and Zoology, Faculty of Science, Masaryk University, Brno, Czech Republic

<sup>2</sup>Capacities for Biodiversity and Sustainable Development (CEBioS), Operational Directorate Natural Environment, Royal Belgian Institute of Natural Sciences, Brussels, Belgium

<sup>3</sup>Laboratory of Biodiversity and Evolutionary Genomics, Department of Biology, University of Leuven, Leuven, Belgium

<sup>4</sup>Hasselt University, Centre for Environmental Sciences, Research Group Zoology: Biodiversity & Toxicology, Diepenbeek, Belgium

\*corresponding author, e-mail: rahmouni.chahrazed@gmail.com

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Cichlids have grasped the attention of ichthyologists for many years and fascinated researchers in evolutionary biology and ecology. Among the 13 monogenean genera infecting cichlids, *Cichlidogyrus* Paperna, 1960 is the most speciose genus mostly restricted to African and Levantine hosts (few species were described from non-cichlid hosts). In fact, the knowledge on monogenean parasite diversity in African cichlid fish is still limited. Only 111 monogenean species were described from cichlids in the three largest African Lakes Victoria, Malawi and Tanganyika which are listed among the areas with the richest concentration of vertebrates on earth and harbour the most diverse cichlid assemblages with more than 1 500 valid species.

Lake Tanganyika, the deepest and oldest Lake in Africa, counts about 250 extraordinary endemic cichlids subdivided into 50 genera and 14 tribes. Research on the monogenean ectoparasites of Tanganyikan cichlids is limited, but has increased considerably during the last years enriching the taxonomic and molecular knowledge on African cichlid parasite fauna. Indeed, in 2013, nine endemic cichlid species were collected from the Burundese lakeshore and examined for their ectoparasite monogeneans. Gills of four of them i.e. *Simochromis diagramma* (Tropheini), "*Ctenochromis*" *horei* (Haplochromini), *Perissodus microlepis* (Perissodini) and *Boulengerochromis microlepidotus* (Boulengerochromini) were previously investigated for parasites and five *Cichlidogyrus* spp. were found on hosts sampled from non-Burundese localities [1–3; unpublished data for *P. microlepis*]. We found the same *Cichlidogyrus* species as reported in these studies, but intraspecific variability in the sclerotized structures of haptor and reproductive organs was observed in the same host species sampled in different localities in the lake. The morphometrical intraspecific variability in the Burundese *Cichlidogyrus* species will be analysed in near future using geomorphometry methods. Such approach may provide additional view to the classical morphometric study [4].

We recently described eight new *Cichlidogyrus* spp. on five endemic Tanganyikan hosts inhabiting the Burundese part of Lake Tanganyika i.e. *C. milangelnari* on *Cyprichromis microlepidotus* (Cyprichromini); *C. jeanloujustinei* on *Eretmodus marksmithi* (Eretmodini); *C. evikae* on *Tanganicodus irsacae* (Eretmodini); *C. aspiralis*, *C. glacicremoratus* and *C. rectangulus* on *Ophthalmotilapia nasuta* (Ectodini); and *C. pseudoaspiralis* and *C. discophonum* on *Aulonocranus dewindti* (Ectodini) (Rahmouni *et al.* 2017, in press). In addition, two *Cichlidogyrus* species were recorded last year on deep-water cichlid fish belonging to the tribes Benthochromini and Bathybatini sampled during the same expedition in Burundi [5]. Therefore, the list of *Cichlidogyrus* spp. infecting gills of Tanganyikan cichlids raised to 32 valid species when compared to 78 non-Tanganyikan species, the latter counts a single new member of *Cichlidogyrus* described recently on *Sargochromis mellandi* [6]. Our study represents the first parasitological examination of the cichlid tribes Cyprichromini and Eretmodini, while, previously investigated *Ophthalmotilapia* - representative of the Ectodini - inhabiting various localities along the lake showed to host four parasite species [7].

Moreover, during the last expedition in the R.D. Congo in 2016, we investigated the gills of various cichlid species i.e. *C. microlepidotus* (Cyprichromini), *Cardiopharynx schoutedeni*, *Callochromis melanostigma* and *Xenotilapia flavipinnis* (Ectodini), *Cyphotilapia frontosa* (Cyphotilapiini), *Petrochromis orthognathus*, *P. trewavasae*, *Interchromis loocki*, *Pseudosimochromis marginatus*, *P. curvifrons* and *P. babaulti* (Tropheini) and finally *Tylochromis polylepis* (Tylochromini). Ectodine hosts as well as the single cyphotilapiine have never been studied before this study, thus this investigation allowed us to record the first data on their gill parasite fauna. On the other hand, the remaining cichlid fish provide additional data on the high *Cichlidogyrus* spp. richness and the diversity of Tanganyikan cichlids, and on geographical variation in their gill ectoparasites throughout the lake [2]. More than 10 *Cichlidogyrus* spp. are considered new for science. Description of new species with the new host and locality records will be published in the near future.

*Cichlidogyrus* spp. are commonly studied based on the morphology of the sclerotized structures of their attachment organ (haptor) and reproductive organs (male copulatory organ, MCO, and vagina when visible). Haptoral structures in *Cichlidogyrus* spp. are characteristic for major phylogenetic lineages, while the MCO is important for species-level identification. Previous studies based on morphological and molecular approaches showed that *Cichlidogyrus* spp. (all non-Tanganyikan) clustered within four main haptoral groups [8]. Data from

Lake Tanganyika should stir this classification and “new” groups will probably appear. Indeed, our last study suggested that there are more than four haptoral groups. Due to the incomplete taxonomic coverage, it is still not possible to fully elucidate the evolution of the different haptoral configurations in *Cichlidogyrus* spp. Re-investigation of the structural diversity of the hook pairs in *Cichlidogyrus* spp. and identification of the exact “borders” between the haptoral groups are necessary. On the other hand, the heel (a small sclerotization attached to the basal bulb of copulatory organ) is a relevant feature in the MCO of *Cichlidogyrus* spp. However, when analysing Tanganyikan and non-Tanganyikan *Cichlidogyrus* species described so far, we found out that ten cichlid monogeneans are lacking this character. Moreover, when comparing *Cichlidogyrus* spp. based on their vagina and the length of their hook pairs, only two Tanganyikan representatives of the same haptoral group lack a sclerotized vagina. Our results shed light on the necessity to elucidate the evolutionary scenarios and the significance of the sclerotization in the vagina and in the heel in *Cichlidogyrus* spp.

In our research, molecular tools are combined with the basic taxonomical methods to successfully identify and differentiate between *Cichlidogyrus* species. Sequences data of 28S rDNA, 18S rDNA and ITS-1 are used for this purpose and phylogenetic analyses are carried out using the different methods of phylogenetic reconstruction. Our analyses showed that the clusters of *Cichlidogyrus* species follow the phylogenetic relationships of their Tanganyikan cichlids, and mirror the affinities revealed by morphological characters between *Cichlidogyrus* species. Combining our sequence data obtained for *Cichlidogyrus* spp. from cichlids of Lake Tanganyika and available on GenBank with morphological characters exhibited by *Cichlidogyrus* spp. seem to provide new information on the processes of the evolutionary history of the congeneric species. We suggest that this approach represents one of the potential tools allowing us to draw new conclusions on the evolutionary and biogeographic history of *Cichlidogyrus* and the cichlids-*Cichlidogyrus* evolutionary associations.

### Acknowledgement

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# Research on the diversity of (not only) African monogeneans

Eva Řehulková<sup>1\*</sup>

<sup>1</sup>Department of Botany and Zoology, Faculty of Science, Masaryk University, Brno, Czech Republic

\*corresponding author, e-mail: evar@sci.muni.cz

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In 2017, my research activities were a combination of continued and new projects examining the diversity of monogeneans, especially from African fishes. These activities can be divided into the following parts:

(i) Descriptions of four new species of *Quadriacanthus* [1] and two new species of *Annulotrema* [2] from three species of catfishes and one species of African tetras, respectively.

(ii) Contribution to forthcoming publication “Guide to the Parasites of African Freshwater Fishes: diversity, ecology and research Methods” as author of the chapter “Methods to study the principal groups of fish parasites - Monogenea” and co-author of two chapters “Parasitological examination of fish (dissection) – general rules” and “A systematic survey of parasites of freshwater fish in Africa – Monogenea”. Concerning the last chapter, the guide to the monogeneans was performed as keys to individual genera. The main outcomes obtained in an effort to compile the catalogue of the currently known monogenean species can be summarized as follows: Today, a total of 457 species of polyonchoineans (32 genera in three families) and seven species of oligonchoineans (three genera in two families) have been described. At the family level, the Dactylogyridae are represented currently by the greatest number of species (403 spp.) belonging to 26 genera: *Afrocleidodiscus* (3), *Ancyrocephalus* (s.l.) (4), *Annulotrema* (46), *Archidiplectanum* (1), *Bagrobdella* (3), *Birgiellus* (3), *Bouixella* (10), *Characidotrema* (10), *Cichlidogyrus* (104), *Dactylogyrus* (100), *Dogielius* (21), *Enterogyrus* (8), *Eutrianchoratus* (5), *Heteronchocleidus* (5), *Heterotesia* (1), *Insulacleidus* (3), *Nanotrema* (2), *Onchobdella* (8), *Paraquadriacanthus* (1), *Protoancylo-discoides* (6), *Quadriacanthus* (30), *Schilbetrema* (14), *Schilbetrematoides* (2), *Scutogyrus* (7), *Synodontella* (5), and *Urogyrus* (1). Only a single species of diplectanids, *Diplectanum lacustre*, has been reported from *Lates niloticus*. The viviparous gyrodactylids are the second largest family, with 53 species attributed to six genera, i.e.

*Afrogyrodactylus* (4), *Citharodactylus* (1), *Diplogyrodactylus* (1), *Gyrodactylus* (37), *Macrogyrodactylus* (9), and *Mormyrogyrodactylus* (1). The only representatives of the Oligonchoinea are six species belonging to *Paradiplozoon* (5), *Afrodiplozoon* (1) (Diplozoidae) and one species of *Heterobothrium* (Diclidophoridae) [3].

(iii) Organization of fieldwork in Cameroon (20 February – 19 March). A total of 50 species (353 specimens) of fishes (mostly belonging to Alestidae, Cyprinidae, and Mormyridae) were sampled in the Boumba River (near Lomié) and the Sanaga River Basin (near Batchenga) and examined for the presence of metazoan parasites. Monogeneans, with about 1500 specimens collected, were the group with the highest prevalence.

(iv) Processing of twenty specimens of *Haliotrema australe*, the type species of the genus, for morphological and molecular analyses. Sampled specimens were kindly collected by Scott Cutmore from *Parupeneus spilurus* in Moreton Bay (Australia).

## Future plans

(1) Morphometrical evaluation of monogeneans collected during fieldwork in Cameroon.

(2) Revision of *Haliotrema*-like monogeneans from goatfishes (Mullidae) off New Caledonia based on comparative structural morphology, host distribution, and molecular analyses.

## Acknowledgement

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# Functions of novel serpin from *Eudiplozoon nipponicum* (Monogenea)

Pavel Roudnický<sup>1\*</sup>, Jiří Vorel<sup>1</sup>, Jana Ilgová<sup>1</sup>, Libor Mikeš<sup>2</sup>, Lucie Jedličková<sup>2</sup>, John Dalton<sup>3</sup>, Jan Dvořák<sup>3</sup>, Lubomír Janda<sup>4</sup>, Adam Norek<sup>4</sup>, Milan Gelnar<sup>1</sup>, Martin Kašný<sup>1,2</sup>

<sup>1</sup>Department of Botany and Zoology, Faculty of Science, Masaryk University, Brno, Czech Republic

<sup>2</sup>Department of Parasitology, Faculty of Science, Charles University in Prague, Prague, Czech Republic

<sup>3</sup>School of Biological Sciences, Medical Biology Centre, Queen's University Belfast, Belfast, Northern Ireland

<sup>4</sup>Institute of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic

\*corresponding author, e-mail: p.roudnický@mail.muni.cz

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The properties of functional proteins of the members from Monogenea are still poorly investigated. We chose *Eudiplozoon nipponicum* as our experimental organism to address this issue. *E. nipponicum* (Diplozoidae, Polyopisthocotylea) is hematophagous ectoparasite which lives on the gills of common carp (*Cyprinus carpio*). The main aim of our current work is to understand the regulation of host/parasite peptidase activity related to numerous physiological processes. Among the key regulatory factors could be included the peptidase inhibitors, such as serpins - serine peptidase inhibitors. These functional proteins are generally known as important regulators of the coagulation cascade, complement, fibrinolysis, angiogenesis, inflammation etc. In the transcriptome of *E. nipponicum* we identified serpin gene (EnS), prepared it in recombinant form (rEnS) and investigated its properties.

We have been able to achieve approx. 70% of protein sample purity. Using western blot, the presence of purified rEnS in bacterial extracts and EnS in excretory-secretory products (ESP) was confirmed. These results were validated by mass spectrometry (MS). Fluorometric inhibition assays showed the rEnS ability to partially inhibit four serine peptidases (SP) playing a role in host-parasite interaction – digestion (trypsin), regulation of blood coagulation (factor Xa, plasmin) or tempering the inflammation (kallikrein). Due to properties mentioned above and presence of the serpin in ESP, we hypothesize that EnS might be one of the key factor of host-parasite interaction.

## Future plans

Publish article concerning the data presented here. Focus on the next topic related to the Ph.D. thesis – combination of laser microdissection and mass spectrometry for analysis of molecules in chosen tissue of *E. nipponicum*.

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# Diversity of monogenean gills parasites (Monogenea: Dactylogyridae) from African freshwater fishes in the light of molecular data

Mária Seifertová<sup>1\*</sup>

<sup>1</sup>Department of Botany and Zoology, Faculty of Science, Masaryk University, Brno, Czech Republic

\*corresponding author, e-mail: seifertovamaria@gmail.com

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Molecular characterization, study of intra- and interspecific genetic variability and reconstruction of phylogenetic relationships were performed on two different groups of monogenean parasites, namely dactylogyrids parasitizing African catfishes (Siluriformes) and dactylogyrids from African tetras (Characiformes: Alestidae).

1. Six species of *Quadriacanthus* (Monogenea: Dactylogyridae) parasitizing *Clarias gariepinus*, *Heterobranchus bidorsalis* and *Bagrus docmak* collected in Kenya and Sudan were described using the partial 18S rDNA, entire ITS1, and partial 28S rDNA sequences (1, 2). The sequences of the 18S-ITS1 region were 877-921 bp long, the sequences of the partial 28S region were 772-847 bp long. No intraspecific variability was found in 18S-ITS1 or 28S sequences for all observed species. Molecular characterisation of representatives of five dactylogyridean genera (*Bagrobdella*, *Protoancylodiscoides*, *Schilbetrema*, *Schilbetrematoides* and *Synodontella*) specific to African siluriform fishes was also performed. Results of phylogenetic analyses comprising all six genera from African catfishes confirmed their monophyletic nature. Species of *Schilbetrema*, *Schilbetrematoides*, *Synodontella*, *Bagrobdella* and *Protoancylodiscoides* clustered as a strongly supported monophyletic group together with Asiatic monogeneans of catfishes. *Quadriacanthus* species from Africa formed separate clade with Asiatic species *Q. kobinensis* and *Bychowskiella pseudobagri* (3).

2. Six species of *Characidotrema* collected from two alestid species (*Brycinus imberi* and *B. nurse*) in the Democratic Republic of the Congo, Senegal, Sudan and Zimbabwe were characterized using ribosomal (18S, ITS1, 28S) and mitochondrial DNA (COI) sequences (4). No intraspecific variation was found in the rDNA markers, but higher degree of variation was detected within the COI sequences. Phylogenetic analyses confirmed, that *Characidotrema* spp. represent monophyletic group sister to *Annulotrema* spp. The topology of phylogenetic tree was in agreement with the morphology of sclerotized structures.

## Future plans

Processing of DNA samples of dactylogyrids of African freshwater fishes will be completed and molecular analyses clarifying their phylogenetic relationships will be performed. The parasite phylogenies will be compared with phylogenetic relationships of their hosts in order to assess the existence of potential parallel speciation processes.

## Acknowledgement

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# Novel insights on life histories of trypanorhynch cestodes from elasmobranchs and crustaceans of Moreton Bay (Queensland, Australia)

Bjoern C. Schaeffner<sup>1\*</sup>, Ian Beveridge<sup>2</sup>

<sup>1</sup>Institute of Parasitology, Biology Centre, Czech Academy of Sciences, Česká Budějovice, Czech Republic

<sup>2</sup>Faculty of Veterinary and Agricultural Science, University of Melbourne, Veterinary Clinical Centre, Werribee, Australia

\*corresponding author, e-mail: b.schaeffner@unimelb.edu.au

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At present, information on the life cycle histories and transmission patterns of trypanorhynch cestodes are poorly known and infections of both definitive and intermediate host stages are hardly ever recorded.

During a recent study on trypanorhynchs, we examined the spiral valves of a total of 64 elasmobranchs of 13 species and the digestive glands of potential crustacean intermediate hosts from Moreton Bay (Queensland, Australia). Thirteen fully identifiable species of trypanorhynchs were recovered, including two new species of *Dollfusiella*; the first from *Maculabatis toshi* (Whitley), *Himantura cf. astra* and *Glaucostegus typus* [Anonymous, in Bennett, 1830], and the second species from *Aetobatus ocellatus* (Kuhl). Metacestodes were recovered from the digestive glands of 'yabbies', *Trypaea australiensis* (Dana) (Crustacea) and were identified as an undescribed species of *Dollfusiella*. Adults were found in the spiral valves of *Neotrygon trigonoides* (Castelnau) and *Ae. ocellatus*. Six additional species of trypanorhynch metacestodes were recovered from the digestive glands of pistol shrimps - *Alpheus richardsoni* Yaldwyn and *Al. papillosus* Banner & Banner (Crustacea). Of these, three were identified as *Parachristianella monomegacantha* Kruse, 1959, *Prochristianella aciculata* Beveridge & Justine, 2010 and *Zygorhynchus robertsoni* Beveridge & Campbell, 1988; the adults of all three species were also recovered from batoid hosts. Two undescribed species of *Dollfusiella* and an undescribed species of *Parachristianella* were also obtained from crustaceans and batoids. However, the material available was insufficient to provide formal species descriptions. This study also revealed the presence of *Prochristianella omunae* Beveridge & Justine, 2010, which is reported from Australia for the first time; there it has been obtained from its type host, *Neo. trigonoides*. In addition, *Prochristianella odonoghuei* Beveridge, 1990 is synonymized with *Prochristianella butlerae* Beveridge, 1990, based on an identical body morphology and features of the tentacular armature. Overall, the identification of second intermediate hosts provides a significant advance in understanding the life cycles of these poorly studied cestodes, although the initial copepod intermediate host stage necessary to commence the multi-host life cycle is not yet known.

## Future plans

Identification of the first intermediate host and large-scale collecting efforts of copepods from Moreton Bay. Presentation and publication of the first life cycle of trypanorhynchs from Australia.

## Acknowledgement

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# Digest of fish tapeworms

Tomáš Scholz<sup>1\*</sup>, Roman Kuchta<sup>1</sup>

<sup>1</sup>Institute of Parasitology, Biology Centre of Czech Academy of Sciences, České Budějovice, Czech Republic

\*corresponding author, e-mail: tscholz@paru.cas.cz

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Members of the following seven of a total of 19 cestode orders mature in ray-finned fishes (Actinopterygii): Amphilinidea (mainly in acipenseriforms and osteoglossiforms; 8 species in 6 genera), Bothriocephalidea (in freshwater and marine fishes; 129/47), Caryophyllidea (in cyprinids, suckers and catfishes; 117/42), Haplobothriidea (in bowfin; 2/1), Nippotaeniidea (in osmeriforms and perciforms; 6/1), freshwater Onchoproteocephalidea (mainly in catfishes 194/55), and Spathebothriidea (in freshwater and marine fishes; 6/5). Updated information on species diversity, host associations, interrelations and geographical distribution is provided for every group. The existing phylogenetic hypotheses suggest that tapeworms colonised ray-finned fishes several times and form several independent lineages. From a total of 461 fish tapeworms only 92 species are exclusively marine. No general patterns in host use can be observed at the level of fish definitive hosts because cestodes of fishes occur in not closely related host groups. Nevertheless, only three fish orders host almost three fourth of all tapeworms of fishes, namely Siluriformes (36% of all fish cestode species), Cypriniformes (22%) and Perciformes (16%). Nearly two thirds (61%) of fish tapeworms have a strict (oioxenous) specificity and one third (33%) is stenoxenous. The highest proportion (8%) of euryxenous species is among the bothriocephalideans, including one of the most opportunistic fish helminth, the invasive Asian fish tapeworm (*Schyzocotyle acheilognathi*). Tapeworms of fishes are more diverse in the temperate zones of the northern hemisphere than in the tropics, with the exception of onchoproteocephalideans in South America. However, this may reflect a lower sampling effort in tropical regions and the southern hemisphere. Caryophyllideans dominate in the Australasian (58% of all species, but only 12 species found) and Nearctic (56%; total number = 102) realms, and together with onchoproteocephalideans in the Ethiopian (both 41%; n = 39), Palaearctic (both 37%; n = 90) realms, and with bothriocephalideans in the Oriental realm (34%; n = 36). The Neotropical region is dominated by onchoproteocephalideans (n = 112), which represent as many as 95% of all species reported. The major part of the undescribed diversity of fish tapeworms regarding the total number of species can be expected in the latter region, but this concerns only a single cestode family, Proteocephalidae.

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# First freshwater bothriocephalidean (Cestoda) from tropical South America, closely related to African taxa

Tomáš Scholz<sup>1\*</sup>, Ricardo Massato Takemoto<sup>2</sup>, Roman Kuchta<sup>1</sup>

<sup>1</sup>Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, České Budějovice, Czech Republic

<sup>2</sup>Universidade Estadual de Parana, Maringa, Brazil

\*corresponding author, e-mail: tscholz@paru.cas.cz

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A new genus is proposed to accommodate a new bothriocephalid cestode (family Bothriocephalidae) from flatfish *Catathyridium jenynsii* (Chabanaud, 1922) (Pleuronectiformes: Acharidae; type host) and another three freshwater fishes of the orders Characiformes, Cyprinodontiformes and Siluriformes in the Neotropical Region. The new genus is placed in the Bothriocephalidae because it possesses medioventral uterine and mediodorsal genital pores, and a follicular vitellarium. It is characterised by possessing a tiny, slightly subovate scolex narrower than the strobila, with an apical disc armed with two semicircles of 15–17 tiny hooks in each, and an acraspedote strobila. New genus differs from all bothriocephalid cestodes that have a scolex armed with hooks by their small size (maximum length less than 20 µm only), triangular shape with the basal part (handle or basal plate) shorter than the distal coniform part (blade). In the other hooked bothriocephalids, hooks have a longer handle than a blade. This new species is the third bothriocephalidean cestode described from freshwater teleosts in South America, but first out of Patagonia. Molecular phylogenetics considers the new species as a member of a lineage consisting of freshwater bothriocephalids from the Ethiopian biogeographic region, thus indicating Gondwanan relationship.

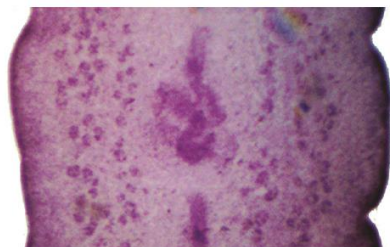
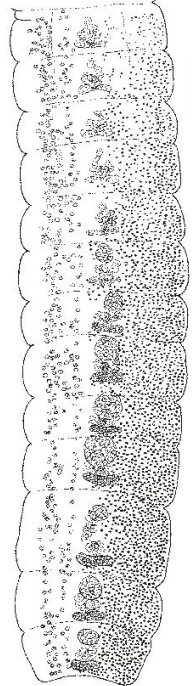
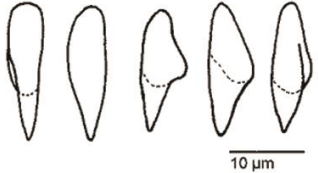
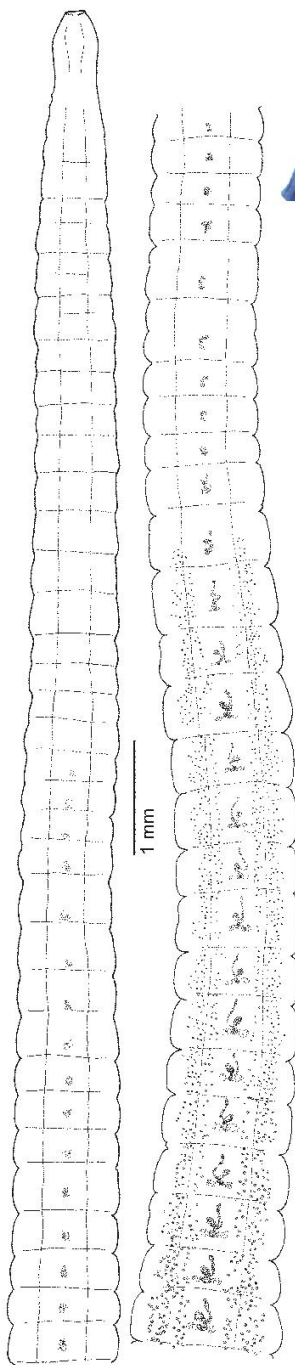
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# Parasites of freshwater fish: evolutionary and ecological studies

Andrea Šimková<sup>1\*</sup>

<sup>1</sup>Department of Botany and Zoology, Masaryk University, Brno, Czech Republic

\*corresponding author, e-mail: simkova@sci.muni.cz

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Several ecological and evolutionary aspects of parasite communities of freshwater fish were studied. First, the potential risk of parasite transmission associated with cichlid introduction from Africa to Madagascar was studied. The communities of metazoan parasites in seven following cichlid species endemic to Madagascar were investigated in the selected localities of North and central parts of Madagascar: *Paratilapia lamena*, *Paratilapia* sp. 1, *Paratilapia* sp. 2, *Ptychochromis grandidieri*, *Ptychochromis* sp. 1, *Ptychochromis* sp. 2 and *Paretroplus polyactis*. In the same localities four cichlid species introduced from Africa to Madagascar were also identified and examined for parasites: *Oreochromis niloticus*, *O. mossambicus*, *Coptodon rendalli*. Our study revealed rare presence of endemic Malagasy cichlids. Introduction of African cichlids was linked with introduction of their metazoan parasites indicating parasite spillover. Our study revealed the introduction of some highly invasive parasite species which represents a potential risk for endemic cichlids. Malagasy cichlids were found as competent hosts for specific monogeneans of African cichlids (*Cichlidogyrus* and *Scutogyrus*) but monogeneans of Malagasy cichlids (*Insulacleidus*) were strictly specific to their original hosts. We suggest that the source and time of introduction seems to represent the factor determining the local cichlid fauna in Malagasy cichlids.

Second, the metazoan parasite communities of cyprinid fish species were studied in the Mediterranean area. High level of endemism was documented for host specific monogeneans. *Dactylogyrus* parasites were applied as the potential tool to investigate historical biogeographical contacts among cyprinid fish species of South Mediterranean Europe (Iberian Peninsula) and Northwest Africa. We showed that *Dactylogyrus* of Iberian *Luciobarbus* (Barbini, Cyprininae) were included in two phylogenetic lineages of *Dactylogyrus*. The similar pattern was observed for Cyprininae distributed in Northwest Africa. Our results indicated two historical contacts between Iberian and Northwest African Cyprininae associated with host switches of *Dactylogyrus*. We suggest that there are two historical ways of *Dactylogyrus* dispersion – north way from European Leuciscinae that likely originated in Siberia and dispersed from North to South Europe and south way from Asian Cyprininae to Africa and then to the Iberian Peninsula.

Next, the extensive review of current knowledge on the major histocompatibility complex (MHC) in fish, specifically focusing on the MHC IIB class in cyprinid species was prepared. The general hypotheses on the origin of MHC genes and the mechanisms maintaining the polymorphism of these genes were presented and exemplified by studies performed on fish. Parasite-mediated selection (balancing selection) and reproductive mechanisms based on mating preferences were the most often cited mechanisms maintaining the extensive MHC polymorphism in fish. MHC polymorphism in fish populations is driven by parasites through overdominant selection or frequency-dependent selection. However, the intermediate number of MHC alleles was documented at the individual level associated with the lowest parasite load. A trade-off between investing in MHC and other components of life maintenance has been hypothesized and documented by comparative analyses and within-species studies. Sexual selection is the main actor affecting the evolution of MHC polymorphism. Good or compatible genes of the males are generally selected by the females by mating choice. A mating preference for MHC genes has been documented in several fish species. It was proposed that the MHC diversity of the potential mating partner is evaluated by the olfactory system.

## Acknowledgement

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# Site fidelity and individual movement of round gobies in a lowland river

Luděk Šlapanský<sup>1,2\*</sup>, Michal Janáč<sup>1</sup>, Kevin Roche<sup>1</sup>, Libor Míkl<sup>1,2</sup>, Pavel Jurajda<sup>1</sup>

<sup>1</sup> Institute of Vertebrate Biology, Academy of Sciences of the Czech Republic, Brno, Czech Republic

<sup>2</sup> Department of Botany and Zoology, Faculty of Science, Masaryk University, Brno, Czech Republic

\*corresponding author, e-mail: 270489@mail.muni.cz

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A number of Ponto-Caspian Gobiid species have greatly increased their geographical ranges over recent decades. Although they are generally considered to be less mobile than larger bodied species, relatively little is, in fact, known about their movement patterns and home ranges [1]. In this study, we have observed the round goby *Neogobius melanostomus* site fidelity and individual movement along non-navigable river. Monitoring took place in the lower part of the Dyje River within several sampling campaigns and round goby movement was monitored by individual marking, visible implant elastomer (VIE) tagging and recolonization experiment.

Round goby demonstrated high site fidelity with 58.9 % being recaptured within the same 10-m stretch in which they were originally tagged one month after tagging, with 23.5 % of fish resting within the same 10-m stretch for more than year. The results, however, also indicated that some individuals were capable of moving at least 580 m within 30 days. In an experiment simulating release of large numbers of propagule from a point source, round gobies showed a preference for upstream spreading, with relatively large distances swum (mean upstream distance reached within 30 days = 199.17 m, mean downstream distance = 65.65 m). While the distance was independent on size in females (GLM, df= 1,66, P = 0.193), smaller males moved significantly more than the larger ones (GLM, df=1, 37, P = 0.038). Another experiment, simulating invasion front conditions, have demonstrated no effect of fish mean size or sex on the distance reached when round gobies were allowed to recolonise stretches cleaned them.

We can confirm that round goby in the established area show a great fidelity to a particular place in the river. However, a small percentage of individuals exhibit relatively high level of movement activity over longer distances. These individuals can significantly contribute to the expansion of this species. It has also been shown that higher level of movement activity was performed by smaller individuals and they can carry the migration potential of round gobies. The movement activity of round gobies is mostly upstream oriented and they are able to quickly fill the vacant habitat at the site of an accidental introduction or on the invasion front.

## Future plans

Preparation and finalization of publication concerning the data in presented abstract.

## Acknowledgement

This study has 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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# ***Rosculus* sp. (HGR lineage of Cercozoa) as an opportunist in a mixed culture with *Sappinia* sp. (Amoebozoa, Thecamoebidae)**

Tomáš Tým<sup>1,2\*</sup>, Iva Dyková<sup>1</sup>

<sup>1</sup>Department of Botany and Zoology, Faculty of Science, Masaryk University, Brno, Czech Republic

<sup>2</sup>Centre for Polar Ecology, Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic

\*corresponding author, e-mail: tomastym@gmail.com

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The genus *Rosculus* Hawes, 1963 established for amoeboid organism isolated from the rectum of a European grass snake (*Natrix natrix*) has a complicated taxonomic history in which it has been declared a member of various superior taxa [2, 3]. The lack of clear distinctive morphological features was probably the cause why this genus comprised *R. ithacus* as the type and only species until recently. In 2016, when Bass et al. generated the 18S rRNA gene sequence of the strain deposited in CCAP as a neotype and analysed it phylogenetically, this genus found its place within Cercozoa as a member of HGR lineage [1].

In the course of our free-living amoeba studies, *Rosculus*-like organisms have been frequently observed as tiny contaminants in cultures of amoebozoan species, however, only a recent co-isolation of *Rosculus* and *Sappinia* amoebae from mineral soil mixed with king penguin guano collected in the Subantarctic zone and their safe morphological and molecular determination offered the opportunity of observing interesting interactions of these organisms in culture.

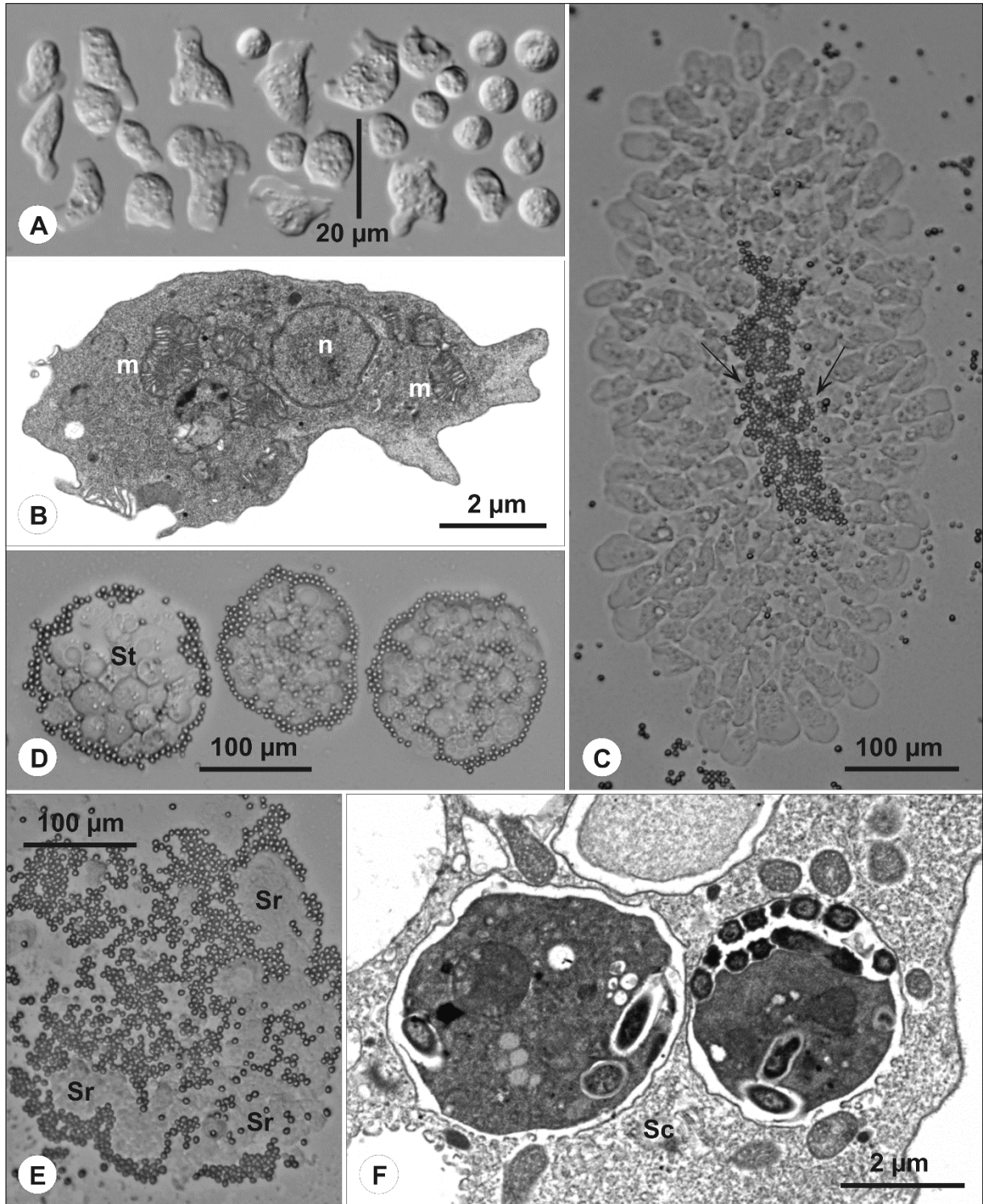
*Rosculus* and *Sappinia* cells were subcultured for 6+ months and studied in over 30 subsequent passages. The establishment of the *Rosculus* strain was accomplished thanks to its tolerance to the temperature above 30°C which was not tolerated by *Sappinia* cells. At the subculturing temperature well tolerated by *Sappinia* (20°C) its trophozoites remained intermixed with those of *Rosculus* despite continuous efforts to obtain a clean *Sappinia* culture. Light microscopical and ultrastructural study convinced us that this situation was due to an unusual nature of mutual relationships of the two organisms: *Rosculus* cells, very small in comparison with *Sappinia*, were not the main source of food for the latter; trophozoites of *Rosculus* were able to encyst inside the cytoplasm of *Sappinia*; *Rosculus* trophozoites aggregated around aging trophozoites of *Sappinia* which disintegrated within their mass. This allows us to speculate that the presence of *Rosculus* was responsible for preventing *Sappinia* trophozoites from cyst formation. These findings together with a remarkable growing potential of *Rosculus* let us think about an opportunistic role of *Rosculus* in a mixed culture with *Sappinia*. Whether this newly observed behaviour is typical of *Rosculus* also in natural populations or just occurred under culturing conditions is hard to tell at the moment.

## **Acknowledgement**

This research was supported by the Czech Science Foundation (505/12/G112), the Centre for Polar Ecology, Faculty of Science, University of South Bohemia and the Laboratory of EM, Biology Centre of CAS. Both the latter institutions were funded by the MEYS CR (grants LM2015078 and LM2015062, respectively). We are grateful to Josef Elster, head of the Centre for Polar Ecology, who kindly shared with us samples collected during expeditions to subantarctic islands.

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Novel strain of *Rosculus* sp. (Cercozoa) co-isolated with *Sappinia* sp. from mineral soil mixed with king penguin guano collected in the Subantarctic (South Georgia, the Salisbury Plain). **A.** Trophozoites and spherical cysts (right) seen in hanging drop preparation (Nomarski DIC). **B.** Overview of trophozoite ultrastructure (n - nucleus, m - mitochondria). **C.** Small electron dense *Rosculus* cells (arrows) within multiplying *Sappinia* trophozoites. **D.** Ageing *Sappinia* trophozoites (St) surrounded by *Rosculus* cells. **E.** Remnants of disintegrated *Sappinia* trophozoites (Sr) accompanied by *Rosculus* cells. **F.** The ability of *Rosculus* trophozoites to encyst in *Sappinia* cytoplasm (Sc) is instrumental in opportunistic coexistence of these two organisms.

# *Sappinia* Dangeard, 1896 (Thecamoebidae, Amoebozoa): contribution to the knowledge of subgeneric diversity

Tomáš Tým<sup>1,2\*</sup>, Iva Dyková<sup>1</sup>

<sup>1</sup>Department of Botany and Zoology, Faculty of Science, Masaryk University, Brno, Czech Republic

<sup>2</sup>Centre for Polar Ecology, Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic

\*corresponding author, e-mail: tomastym@gmail.com

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The genus *Sappinia*, listed among coprophilic amoebae, belongs to those long ago established taxa for which no original type material for molecular analyses is available. Based on careful early descriptions and line drawings from the pre-molecular era of amoeba studies, neotype strains were defined for two *Sappinia* species [1; 3]. Currently, *Sappinia* comprises three nominal species, *S. pedata* [1], *S. diploidea* [3], and *S. platani* [5] described from material of the same type (bark of sycamore tree) as the neotype of *S. diploidea*. Of these *Sappinia* species, known to inhabit rather curious substrates, one has also been diagnosed as agent of encephalitis of man [2; 4], which has directed attention to gaps in the knowledge of taxonomy and morphological and molecular diversity of the genus.

To expand the knowledge on *Sappinia*, we reported on a novel strain (SG10G) which we isolated from a mixture of mineral soil and guano of the king penguin, *Aptenodytes patagonicus* (collected in the Salisbury Plain, South Georgia, the Subantarctic) and kept in culture for 6+ months. The origin of the strain is a remarkable addition to the list of habitats of coprophilic amoebae. The unusual morphology of trophozoites, (binucleate condition with nuclei closely apposed to one another) indicated generic assignment of the isolated strain to *Sappinia* Dangeard, 1896. Our phylogenetic analyses, built on a significantly larger dataset of the 18S rRNA gene sequences than those of previous authors, clearly show that the lineage containing SG10G consists of strains that are distinct from the described *Sappinia* species. The diversity of 18S rRNA gene sequence, however, is not as high as in the other thecamoebid genera (i.e., *Stenamoeba*, *Thecamoeba*).

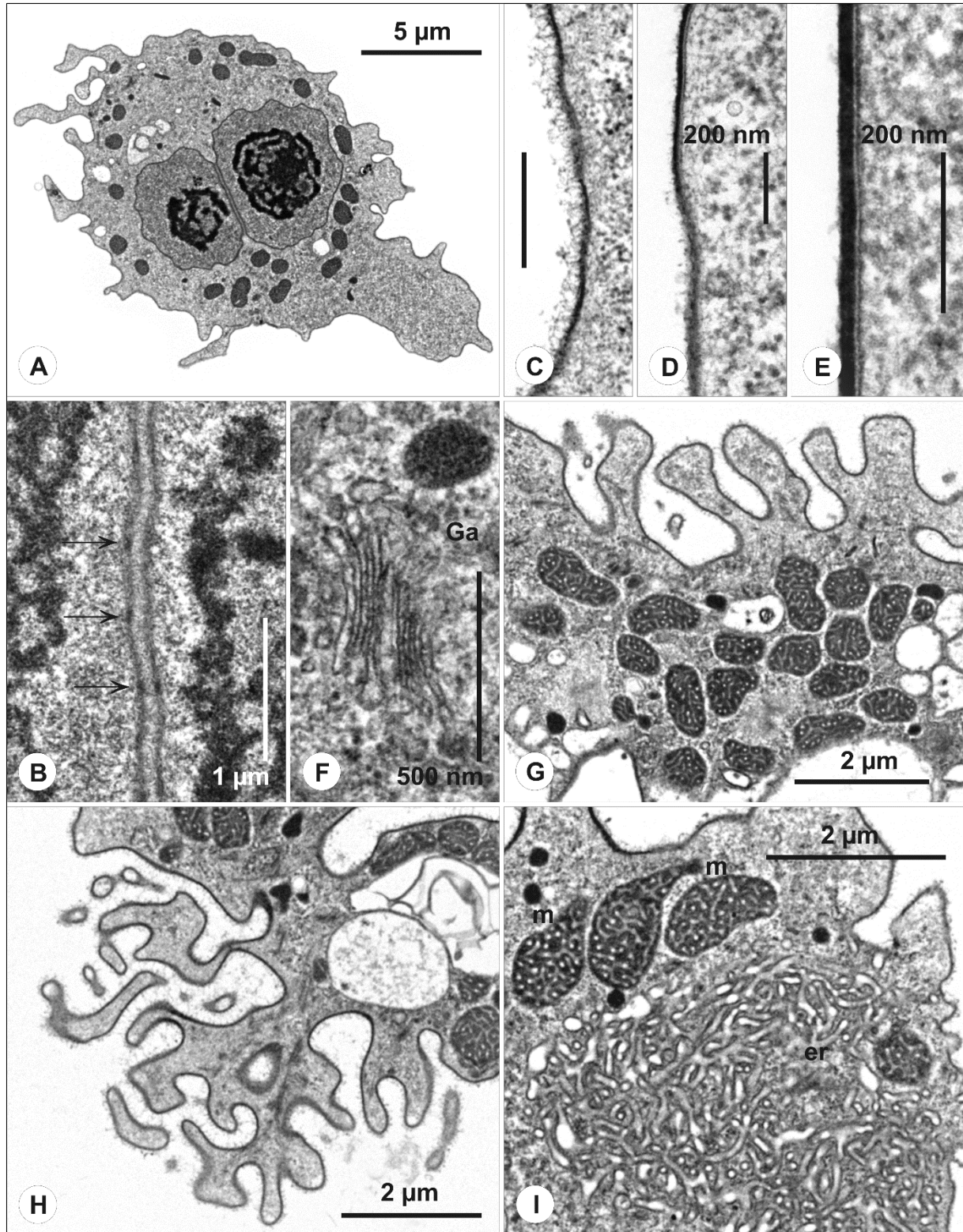
The results of ultrastructure study of the new *Sappinia* strain combined with conclusions of phylogenetic analyses demonstrate that most features used for differential diagnoses of the described species require a thorough critical re-evaluation. Descriptions of new strains should include data on fine structure of the cell surface, the type of nucleus, the character of posterior uroid-like structure, the arrangement of endoplasmic reticulum as well as a reliable information about presence/absence of the dictyosomal type of Golgi apparatus.

## Acknowledgement

This research was supported by the Czech Science Foundation (505/12/G112), the Centre for Polar Ecology, Faculty of Science, University of South Bohemia and the Laboratory of EM, Biology Centre of CAS. The two latter institutions were funded by the MEYS CR (grants LM2015078 and LM2015062, respectively). We are grateful to Josef Elster, head of the Centre for Polar Ecology, who kindly shared with us samples collected during expeditions to the Subantarctic islands.

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Ultrastructure of novel *Sappinia* strain isolated from mineral soil mixed with king penguin guano collected in the Subantarctic (South Georgia). **A.** Overview of trophozoite showing a pair of identical closely apposed nuclei, the hallmark feature of the genus. **B.** Contact of two nuclei in detail (arrows indicate nucleopores). **C–E.** Due to different fixatives applied, trophozoites differ in electron density and cell surface organisation. **F.** Dictyosome type of Golgi apparatus (Ga). **G.** Mitochondria accumulated in posterior part of trophozoite. **H.** Plicate type of uroid. **I.** Mitochondria with tubular branching cristae (m) and dense network of cisternal profiles of endoplasmic reticulum (er).



# Use of *in vivo* fluorescent dyes to determine the infectivity and penetration pattern of *Cardiocephaloides longicollis* (Rudolphi, 1819) Dubois, 1982 (Strigeidae) into the gilt-head seabream *Sparus aurata* L.

Gabrielle S. van Beest<sup>1\*</sup>, Mar Villar-Torres<sup>1</sup>, Francisco E. Montero<sup>1</sup>, Juan A. Raga<sup>1</sup>, Ana Born-Torrijos<sup>1,2</sup>

<sup>1</sup>Cavanilles Institute for Biodiversity and Evolutionary Biology, University of Valencia, Valencia, Spain.

<sup>2</sup>Institute of Parasitology, Biology Centre of the Academy of Sciences of the Czech Republic, České Budějovice, Czech Republic.

\*corresponding author, e-mail: gavanbe@alumni.uv.es

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The trematode parasite *Cardiocephaloides longicollis* (Rudolphi, 1819) Dubois, 1982 (Digenea, Strigeidae) highly parasitizes the gilt-head seabream (*Sparus aurata* L.), one of the most important marine fish in Mediterranean aquaculture (up to 53.9 % prevalence) [1]. The cercariae penetrate the skin and migrate to the brain, where they encyst as metacercariae. The cysts could cause significant vision impairment leading to an increase in "conspicuous" behaviour favouring parasite transmission to the final host, as discussed in other sparids [2]. For the study of the infectivity and penetration pattern of *C. longicollis* into its second intermediate host, different experimental assays were performed. First, the effect of different *in vivo* fluorescent dyes on the activity and survival of labelled cercariae was tested. Thereafter, a designated dose of one of the dyes helped to determine the penetration points of *C. longicollis* into *S. aurata*. And finally, the effect of both dyes on the infectivity capacity of labelled cercariae was also tested.

Two different fluorescent dyes were tested: (1) 5(6)-Carboxyfluorescein N-hydroxysuccinimidyl ester (CFSE), which stains intracellular amines in life cells and concentrates in the acetabular gland of the cercariae, and (2) Hoechst 33342 (NucBlue), which specifically stains DNA, i.e. the nuclei of life or fixed cells. Three different ascending concentrations were tested for each dye (CFSE as 20 µM, 50 µM and 100 µM, and NucBlue as 1 drop/mL, 2 drops/mL and 3 drops/mL), and their effect on cercarial activity was recorded during 24 hours, showing no differences between concentrations and unlabeled cercariae. Additionally, intermediate doses of both dyes were selected based on labeling efficacy over time, to test the effect of dyes on cercarial activity and survival during the first five hours post labeling (hpl), i.e. the time during which cercariae should be highly efficient during penetration. Intermediate doses of CFSE enabled a quick and accurate location of the cercariae on the host body surface without affecting cercarial survival, suggesting a centralization of the larvae around the facial areas of the fish.

In order to evaluate the post-labeling infectivity of the larval trematodes, labelled and control cercariae were used to infect *S. aurata*. The water where the fish were infected was analyzed to count the tails as cercariae lose them when they penetrate the host. Additionally, 20 days post infection, fish brains were analyzed for metacercariae, showing no significant differences between the number of metacercariae developed from NB-labelled and control cercariae. On the contrary, the number of metacercariae developed from CFSE-labelled cercariae was significantly lower compared to the control treatment.

In conclusion, the *in vivo* fluorescent dye CFSE is the most adequate labeling treatment in experimental assays studying the infectivity capacity and penetration strategy of cercariae, such as *C. longicollis*.

## Future plans

Experimental assays are currently being performed in order to increase our understanding of the life cycle of *C. longicollis*. Currently, the migratory route that cercariae follow within the fish to reach the target organ, i.e. the brain, and encyst as metacercariae, is being studied.

## Acknowledgement

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# Exploring mitogenomics for phylogenetic reconstruction of African monogeneans

Maarten P.M. Vanhove<sup>1,2,3,4,5\*</sup>, Andrew Briscoe<sup>6</sup>, Wouter Fannes<sup>2</sup>, Michiel W.P. Jorissen<sup>2,4</sup>, D. Tim J. Littlewood<sup>6</sup> & Tine Huyse<sup>2,3</sup>

<sup>1</sup>Department of Botany and Zoology, Faculty of Science, Masaryk University, Brno, Czech Republic

<sup>2</sup>Invertebrate Section, Biology Department, Royal Museum for Central Africa, Tervuren, Belgium

<sup>3</sup>Laboratory of Biodiversity and Evolutionary Genomics, Department of Biology, University of Leuven, Leuven, Belgium

<sup>4</sup>Hasselt University, Centre for Environmental Sciences, Research Group Zoology: Biodiversity & Toxicology, Diepenbeek, Belgium

<sup>5</sup>Capacities for Biodiversity and Sustainable Development, Operational Directorate Natural Environment, Royal Belgian Institute of Natural Sciences, Brussels, Belgium

<sup>6</sup>Division of Parasites & Vectors, Department of Life Sciences, Natural History Museum, London, United Kingdom

\*corresponding author, e-mail: maarten.vanhove@kuleuven.be

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The Gyrodactylidea and the Dactylogyridea are probably the most species-rich monogenean taxa, with representatives that can be harmful fish pathogens, especially in intensive aquaculture or after anthropogenic co-introduction. In Africa, several cichlids and clariids are relatively well-studied for monogeneans, among which the most important aquaculture fishes on the continent, many of which have also been widely introduced outside Africa [1]. A limitation, however, is the small number of molecular markers available for these worms. The need for additional and higher-resolution markers is especially apparent when studying processes on a recent timescale, such as population genetics, phylogeography and barcoding. A small set of established mitochondrial gene fragments currently constitutes the most variable markers available, with advantages such as a lack of recombination, a high mutation rate... These mitochondrial sequences also served in flatworm systematics on a higher, macro-evolutionary scale. The high (amino acid) sequence variability within the mitogenomes of monogeneans (and other flatworms) has however hampered the availability of universal barcoding primers [2]. With this in mind, we intend to apply a mitogenomic approach for primer development (by capturing the most variable mitochondrial regions), starting with a number of monogenean species collected during our ongoing work on the monogenean fish parasites of various parts of the Congo Basin in the D.R.Congo [3]. We here present the first mitogenomic data on African monogeneans (from cichlid and clariid hosts), based on Illumina technology, and explore questions on phylogenetic position and gene order. Our results confirm the basal position of the Neotropical oviparous *Aglaiogyrodactylus* (and not of the African *Macrogryrodactylus*) with respect to other gyrodactylids, and confirm the paraphyly of *Gyrodactylus*. Within the Dactylogyridae, additional taxon sampling seems necessary to increase phylogenetic resolution. Between the studied gyrodactylid and dactylogyrid species, within-family gene order changes appear limited and simple, but phylogenetically informative.

## Future plans

Finalisation of a manuscript on mitochondrial phylogenomics of selected African monogeneans. Further exploration of mitogenomics for phylogenetics, phylogeography and population genetics within various PhDs on either the monogenean fish parasites of Lake Tanganyika and other parts of the Congo Basin, or on symbiotic rhabdocoels in marine invertebrates.

## Acknowledgement

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# Could nanopore sequencing help us improve genome assembly of *Eudiplozoon nipponicum* (Polyopisthocotylea, Diplozoidae)?

Jiří Vorel<sup>1\*</sup>, Marie Jankůvová<sup>2</sup>, Jan Oppelt<sup>2</sup>, Filip Pardy<sup>3</sup>, Pavel Roudnický<sup>1</sup>, Jana Ilgová<sup>1</sup>, Hana Dvořáková<sup>4</sup>, Lucie Jedličková<sup>4</sup>, Libor Mikeš<sup>4</sup>, Dagmar Jirsová<sup>1,5</sup>, Božena Koubková<sup>1</sup>, Milan Gelnar<sup>1</sup>, Martin Kašný<sup>1,4</sup>

<sup>1</sup>Department of Botany and Zoology, Faculty of Science, Masaryk University Brno, Czech Republic

<sup>2</sup>National Centre for Biomolecular Research, Faculty of Science, Masaryk University, Brno, Czech Republic

<sup>3</sup>Central European Institute of Technology, Masaryk University, Brno, Czech Republic

<sup>4</sup>Department of Parasitology, Faculty of Science, Charles University, Prague, Czech Republic

<sup>5</sup>Department of Forest Botany, Dendrology and Geobiocoenology, Faculty of Forestry and Wood Technology, Mendel University, Brno, Czech Republic.

\*corresponding author, e-mail: vorel@mail.muni.cz

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Ectoparasitic flatworms from the family Diplozoidae (Platyhelminthes, Monogenea) represent a serious bloodfeeding fish pathogens. Until now, the running research has been focused mainly on morphological and phylogenetical characteristics of these worms and the information related to the biochemical and molecular nature of the physiological processes is rather sporadic. To this date only two monogenean genomes are deposited in public sequence databases. Genomes of *Gyrodactylus salaris* (Monopisthocotylea, Gyrodactylidae) [1] and *Protopolystoma xenopodis* (Polyopisthocotylea, Polystomatidae) [2]. Therefore, we designed whole-genomic sequencing of selected monogenean representative - *Eudiplozoon nipponicum* Goto 1891, which was performed by three sequencing techniques. 454/Roche (Junior sequencing platform), Illumina (MiSeq and HiSeq sequencing platforms) and modern Oxford Nanopore technique (MinION sequencing platform).

Using 454/Roche Junior and both Illumina sequencing platforms, 164,773,962 short raw reads were originally generated. After trimming of low quality reads (Trimmomatic v. 0.36) and removing contamination - reads given by fish host (*Cyprinus carpio*), 130,741,241 reads were used for the draft of genome assembly (assemblers SPAdes v. 3.6.0, SOAPdenovo v. 2.0 and MaSuRCA v. 3.2.2 were tested). Finally, 766,162 scaffolds were generated (by MaSuRCA, 9,451 scaffolds were longer than 10,000 base pairs) in total length 1,322,576,402 base pairs (1.3 Gb). But most of assembled scaffolds were identified as a *Spiroplasma* sp. or *Emticicia oligotrophica* (bacterial contaminations), which could mean that significant number of *E. nipponicum* reads could be unintentionally filtered out and final scaffold is incomplete.

For purpose to improve *E. nipponicum* genome draft, we have additionally included reads generated by modern third generation sequencing method – nanopore sequencing. The MinION platform (Oxford Nanopore Technologies, UK) is a small and portable, real time working, low-cost USB device, producing long-read (theoretically up to 250 Kb). 500 working channels (nanopores) produced 1,097,634 reads with length up to 198,101 base pairs, which are currently further bioinformatically processed.

## Future plans

Use newly generated long reads from MinION sequencing platform for merge previously assembled scaffolds (derived from Illumina and 454/Roche reads) into longer constructs of whole genome.

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# Parasites of endemic cyprinids from the Balkan Peninsula – host specificity, endemism and diversity of metazoan parasite communities

Kateřina Vyčítalová<sup>1\*</sup>, Michal Benovics<sup>1</sup>, Bořena Koubková<sup>1</sup>, Anna Faltýnková<sup>2</sup>, Tomáš Scholz<sup>2</sup>, Simona Georgieva<sup>2</sup>, Yuriy Kvach<sup>3</sup>, Andrea Šimková<sup>1</sup>

<sup>1</sup>Department of Botany and Zoology, Masaryk University, Brno, Czech Republic

<sup>2</sup>Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, České Budějovice, Czech Republic

<sup>3</sup>Institute of Vertebrate Biology, Academy of Science of Czech Republic, Brno, Czech Republic

\*corresponding author, e-mail: vycitalova@mail.muni.cz

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The Mediterranean area is distinguished from most of the other parts of Europe in occurrence of many unique endemic fish species, especially cyprinids. However, studies focussing on the investigation of parasitofauna of endemic cyprinids are rather exceptional and were performed just in a few localities of Mediterranean area. In the present study, we analysed the metazoan parasite communities of endemic cyprinid species in a large part of the Balkan Peninsula. A total of 577 specimens belonging to 57 cyprinid species were sampled during 2014 – 2015 in selected localities in Albania, Bosnia and Herzegovina and Greece and examined for the presence of metazoan parasites. This study revealed over 100 species of metazoan parasites. We analysed the infection level of parasites and the diversity of parasite communities. In addition, we focussed on host specificity and endemism of parasites. The highest species diversity was reported within Monogenea – a total of 103 species were recorded, from which 36 are likely new for science. Number of parasite species per endemic cyprinid species ranged from 1 to 16. Diversity of parasite communities was analysed at the levels of metacommunity and infracommunity using indices of diversity. Using multidimensional scaling on the similarity of parasite communities expressed by the indices of similarity, the parasite communities of cyprinids from Greece and Albania were similar, whilst parasite communities of cyprinids from Bosnia and Herzegovina were different from both of them. The similarity of parasite metacommunities of congeneric hosts belonging to genera *Squalius* and *Barbus* were found. While the majority of species of Monogenea found in endemic cyprinids are characterised by a high degree of host specificity and include taxa endemic to the Mediterranean area, some monogeneans reported in endemic cyprinid species are commonly distributed in Europe. The remaining ectoparasites and all endoparasites are common parasites of cyprinids widely distributed in Palaearctic Region. We concluded that species diversity of parasites and their distribution in endemic cyprinid hosts are influenced by cyprinid biogeographical history (especially concerning the monogeneans), range of distribution of fish, coevolution processes and other abiotic and biotic factors. The study of parasite diversity in endemic cyprinids of the Balkan Peninsula (especially parasites exhibiting high host specificity) may represent a suitable model to investigate the potential historical contacts among cyprinid hosts as the evolution of these parasite species.

## Future plans

- (1) to perform complex analysis of diversity of metazoan parasites of endemic cyprinids from the Mediterranean area
- (2) to reconstruct the molecular phylogeny of *Gyrodactylus* parasites
- (3) to analyse parasite-host associations using cophylogenetic analyses

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# Comparative morphology of surface ultrastructure of diphylobothriidean tapeworms (Cestoda: Diphylobothriidea)

Aneta Yoneva<sup>1,2,\*</sup>, Tomáš Scholz<sup>2</sup>, Roman Kuchta<sup>2</sup>

<sup>1</sup>Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 2 Gagarin Street, 1113 Sofia, Bulgaria

<sup>2</sup>Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, Branišovská 31, 370 05, České Budějovice, Czech Republic

\*corresponding author, e-mail: anetayoneva@gmail.com

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The tegument of tapeworms (Platyhelminthes: Cestoda) is covered with unique surface structures referred to as microtriches, associated with a diversity of functions, such as nutrient absorption, attachment, digestion, protection, excretion and movement [1]. Microtriches represent one of the synapomorphies of all tapeworms and they are absent in closely related groups of parasitic flatworms (Neodermata, i.e., Trematoda and Monogenea) [2]. Numerous studies have shown that microtrich patterns may be of taxonomic importance and may help in phylogenetic studies. In the present study, the ultrastructure of microtriches of species of all three families of diphylobothriideans (i.e., Cephalochlamydiidae from frogs, Solenophoridae from snakes and lizards, and Diphylobothriidae from fish-eating birds and mammals) is compared.

The basic structure of the tegument of the seven species studied does not differ markedly from that found in other cestodes. The main characteristic is the presence of electron-dense bodies and vesicles in the distal cytoplasm. However, the present study has shown differences in the morphology of microtriches even among species of the same family. Two different types of microtrich patterns were found: filitriches and spinitriches, with the latter represented by two forms. Our study reveals that capilliform filitriches are most commonly found in Diphylobothriidea. They were observed mainly on the strobila and the scolices of all but one studied species; *L. intestinalis* bears only coniform spinitriches on its surface. The same type of microtriches was found on the cirrus of *D. latum*. Gladiate spinitriches cover the scolex of both species of *Duthiersia* and gladiate spinitriches interspersed with capilliform filitriches were observed on the anterior part of the strobila of *D. fimbriata* and the posterior part of the scolex of *B. pithonis*. *C. namaquensis* was covered only by small acicular filitriches. Even though we studied representatives of different genera and families of diphylobothriideans that mature in markedly distinct hosts (frogs, snakes, monitors, fish-eating birds, mammals) from three zoogeographical realms (Ethiopian, Oriental and Palaearctic) [3], we did not find any phylogenetic pattern in the type and distribution of microtriches on the surface of these cestodes. It thus seems that surface ultrastructure may not provide a sufficiently robust phylogenetic signal as to the evolutionary history of diphylobothriidean cestodes, but may help in species identification in some cases.

## Future plans

Future research will focus on the ultrastructure of the tegument and associated structures of the plerocercoid of *Diphylobothrium latum* and thus a detailed comparison between larval and adult stages will be made.

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# Preliminary list of publications dedicated to ECIP – 2017

Actual or past members of ECIP are presented in bold.

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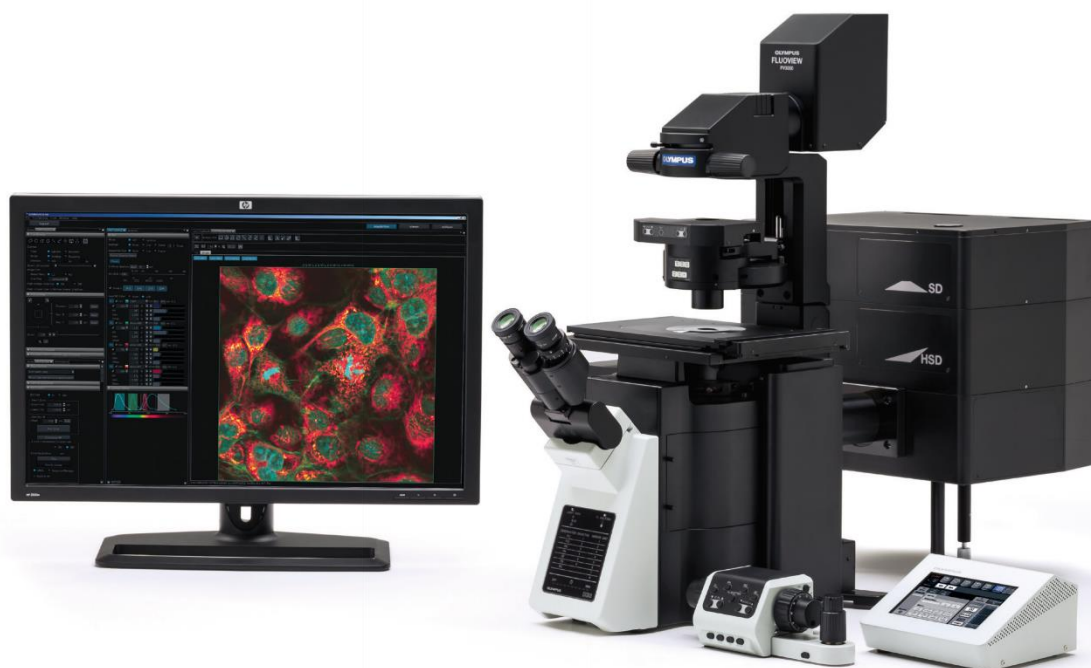
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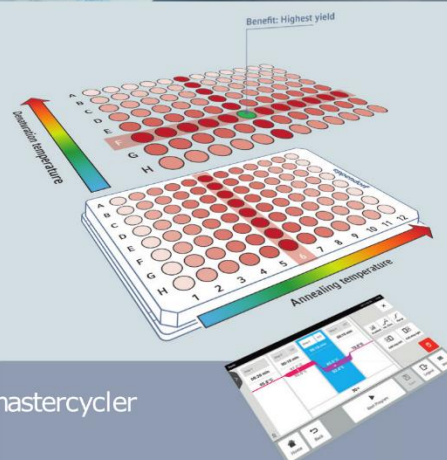
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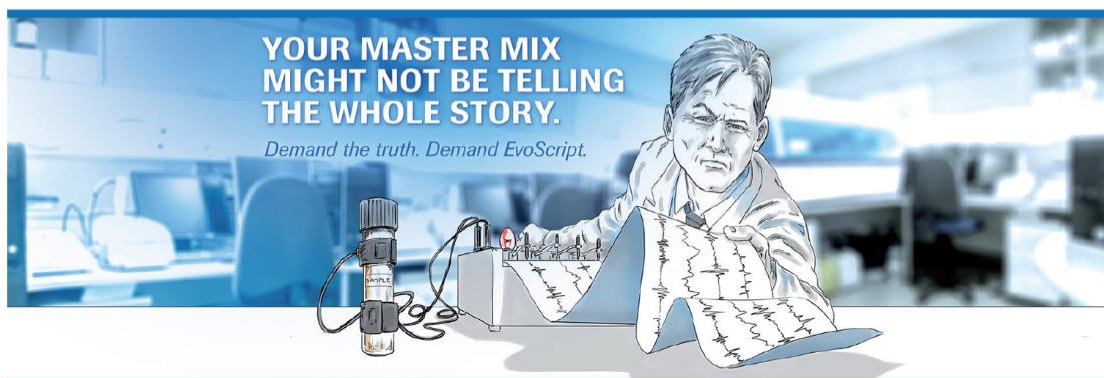


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