

# 5th Workshop of the European Centre of Ichthyoparasitology

## Programme and Abstracts



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



Hotel Beatrice, Prušánky  
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**Organizer:** Department of Botany and Zoology, Faculty of Science,  
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## List of attendants

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Michal Benovics	Maria Lujza Kičinjaová	Pavel Roudnický
Jaroslav Červenka	Nikol Kmentová	Eva Řehulková
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Michal Janáč	Martin Reichard	Luděk Šlapanský
Karel Janko	Kevin Roche	Teodora Trichova
Pavel Jurajda	Jan Röslein	Andrea Slaninová



# Programme

## Monday, November 28th

16:00	Arrival and accommodation
19:00	Dinner

## Tuesday, November 29th

7:00 – 8:30	Breakfast
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### Workshop opening

8:30	<b>Gelnar:</b> Introduction and general programme information
8:45	<b>Gelnar, Scholz &amp; Jurajda:</b> ECIP 2016 in numbers
9:15	<b>Kobíková, Přikrylová &amp; Gelnar</b> – Summer School of Fish Parasitology 2016 – a brief summary

### Diversity of parasites and molecular phylogenetics I – Protista

9:30 – 9:50	<b>Fiala:</b> No regrets, suppression of a traditional genus is needed: <i>Henneguya</i> is “just” a <i>Myxobolus</i> with long spore projections
9:50 – 10:00	<b>Patra:</b> Molecular diversity of new members of the <i>Sphaerospora</i> sensu stricto clade (Cnidaria: Myxozoa)
10:00–10:10	<b>Bartošová-Sojková:</b> Radiation of <i>Ceratomyxa</i> species in the clinid fish in the rock pools of South Africa
10:10 – 10:20	<b>Diakin:</b> Ultrastructure of oocysts and sporozoites of urosporid gregarine from marine polychaete
10:20 – 10:30	<b>Kováčiková:</b> Different motility modes in marine gregarines representing the early emerging group of Apicomplexa
10:30 – 11:00	Coffee Break

### Diversity of parasites and molecular phylogenetics II – Ectoparasites

11:00 – 11:20	<b>Vanhove:</b> Diversity and invasion biology of cichlid parasites, with focus on a case study in Madagascar
11:20 – 11:30	<b>Rahmouni:</b> Morphological diversity of <i>Cichlidogyrus</i> species (Dactylogyridae) in Tanganyikan and non-Tanganyikan cichlids
11:30 – 11:40	<b>Kmentová:</b> Different parasite origin of freshwater sardines and latids in Lake Tanganyika
11:40 – 11:50	<b>Kičinjaová:</b> A preliminary phylogenetic analysis of dactylogyrids (Monogenea) parasitizing African tetras
11:50 – 12:00	<b>Benovics:</b> Phylogenetic relationships between two cyprinid genera – <i>Telestes</i> and former <i>Rutilus</i> from the perspective of host-specific <i>Dactylogyrus</i> (Monogenea) parasites
12:00 – 12:10	<b>Jirounková:</b> Leeches of notothenioid fishes in Antarctica: new species discovery and phylogenetic position
12:10 – 12:25	<b>Sponsor presentations No I – Olympus, Jendrulek:</b> News in confocal microscopy
12:30 – 13:30	Lunch

### Diversity of parasites and molecular phylogenetics III – Endoparasites

13:30 – 13:50	<b>Kuchta:</b> Endohelminths – summary 2016
13:50 – 14:00	<b>Barčák:</b> Taxonomic revision of the genus <i>Caryophyllaeus</i> Gmelin, 1790, (Caryophyllidea), monozoic tapeworms of cyprinid fishes

14:00 – 14:10	<b>Georgieva:</b> A North American alien species of <i>Posthodiplostomum</i> Dubois, 1936 Digenea: Diplostomidae) widespread in European freshwaters
14:10 – 14:20	<b>Faltýnková:</b> Strigeid trematodes (Digenea) from Iceland – an integrative taxonomic approach
14:20 – 14:50	<b>Sponsor presentations No II – SEQme, Vácha:</b> Next-Generation Sequencing at SEQme – parasite like projects examples
14:50 – 15:50	<b>Sponsor panel – exhibition stands with Coffee Break</b>

### Host-parasite relationships and interactions

15:50 – 16:10	<b>Kašný:</b> Monogenea: parasite-host interactions at molecular level
16:10 – 16:20	<b>Vorel:</b> <i>Eudiplozoon nipponicum</i> (Monogenea): the molecules transcribed by hematophagous parasite of fish
16:20 – 16:30	<b>Roudnický:</b> <i>Eudiplozoon nipponicum</i> (Monogenea): analysis of secretome and characterization of selected proteins – serpins
16:30 – 16:40	<b>Ilgová:</b> Molecular and biochemical characterization of cysteine peptidase inhibitor from <i>Eudiplozoon nipponicum</i> (Monogenea)
16:40 – 16:50	<b>Jirsová:</b> <i>Eudiplozoon nipponicum</i> : "Together – chance or destiny?"
16:50 – 17:00	<b>Krasnovyd:</b> Immunocompetence and parasite infection in hybrids of roach ( <i>Rutilus rutilus</i> ) and common bream ( <i>Aramis brama</i> )
17:00 – 17:20	Coffee Break

### Evolutionary epidemiology and behavioral ecology of parasites

17:20 – 17:40	<b>Reichard:</b> Evolutionary ecology of annual fishes
17:40 – 17:50	<b>Blažek:</b> Costly defence in a fluctuating environment – sensitivity of annual <i>Nothobranchius</i> fishes to predator kairomones
17:50 – 18:00	<b>Kvach:</b> First record of metacercariae of North American digenean, <i>Posthodiplostomum cf. minimum</i> (Strigeiformes: Diplostomidae) in Europe
18:00 – 18:10	<b>Janáč:</b> Effect of exotic species on host-parasite interactions

### Social evening

19:00 – 24:00	Buffet dinner
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### Wednesday, November 30<sup>th</sup>

8:00 – 10:00	Breakfast and departure
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## Abstracts

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

# Untangling convoluted taxonomy of *Chambriella* Rego, Chubb and Pavanelli, 1999 (Cestoda: Proteocephalidae), with erection of a new genus and description of a new species from pimelodid catfishes in the Neotropical Region

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Neotropical freshwater fishes host an extraordinary diversity of helminth parasites, but our knowledge of individual groups is still fragmentary. Siluriforms (catfishes) represent one of the dominant groups in this fish fauna and some of them serve as definitive hosts of proteocephalid tapeworms (Eucestoda: Onchoproteocephalidea) [1,2]. As part of a complex revision of proteocephalid cestodes parasitic in freshwater bony fishes of the Neotropical Region, the genus *Chambriella* Rego, Chubb & Pavanelli, 1999 is redefined based on detailed examination of type specimens and newly collected material of both nominal species of the genus. This examination has revealed that the type species, *C. agostinhoi* (Pavanelli & Santos, 1992), from *Zungaro jahu* (Ihering) (type host) and *Z. zungaro* (Humboldt) is indistinguishable from *Lenhataenia megacephala* (Woodland, 1934) from *Sorubimichthys planiceps* (Spix & Agassiz) (all hosts Siluriformes: Pimelodidae), type and only species of the genus. New molecular data (partial sequences of lsrDNA) support conspecificity of these taxa. The second species of *Chambriella*, *C. paranaensis* (Pavanelli & Rego, 1989), from *Hemisorubim platyrhynchos* (Valenciennes) should be transferred to a new genus. Additional specimens from *S. planiceps*, *Phractocephalus hemiolopterus* (Bloch & Schneider) and *Z. zungaro* are to be described as a new species within this new genus, which is placed in the subfamily Monticelliinae because of the cortical position of the testes, ovary, vitelline follicles and uterus, and is mainly characterized by the possession of a sigmoid cirrus-sac with voluminous, chambered internal seminal vesicle, and a bilobate sucker.

## Future plans

A phylogenetic analysis of the nuclear ribosomal RNA and mitochondrial genes is currently being performed on additional representatives from the Neotropics, some of them being hitherto undescribed taxa, in order to shed more light on the evolutionary history of this group of fish cestodes.

## 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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## References

1. Rego A.A. (1994) Order Proteocephalida Mola, 1928. In: L.F. Khalil, A. Jones and R.A. Bray (Eds.), *Keys to the Cestode Parasites of Vertebrates*. CAB International, Wallingford, pp. 257–293.
2. Rego A.A., Chubb J.C., Pavanelli G.C. (1999) Cestodes in South American freshwater teleost fishes: keys to genera and brief description of species. *Rev. Bras. Zool.* 16: 299–367.

# Taxonomic revision of the genus *Caryophyllaeus* Gmelin, 1790, (Caryophyllidea), monozoic tapeworms of cyprinid fishes

Daniel Barčák<sup>1,2\*</sup>, Mikuláš Oros<sup>1,2</sup>, Vladimíra Hanzelová<sup>1</sup>, Tomáš Scholz<sup>2</sup>

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Tapeworms of the genus *Caryophyllaeus* Gmelin, 1790 (Caryophyllidea: Caryophyllaeidae) are common parasites of cyprinid fishes in the Palaearctic Region including *C. laticeps* (Pallas, 1781), which served as a model for pioneer ecological and epidemiological studies in the late 1960's. So far, 42 nominal taxa have been placed in the genus during its long history. However, detailed morphological studies on specimens in museum collections and newly-collected material supported by molecular data has shown that only several species could be considered as valid. Moreover, phylogenetic analysis of the partial mitochondrial *cox1* gene and large subunit of ribosomal gene (*lsu* rDNA) in relation to morphological data revealed unexpected phenotypic plasticity within two most widespread species of the genus.

Within the type species, *Caryophyllaeus laticeps*, five possibly host-specific morphotypes were recognised and morphologically characterised. In fact, some of them were initially considered as new species based on the morphology of the anterior part of the body, until molecular analyses showed their genetic similarity. Besides the morphological differences, they are sharing characters, i.e. form of the cirrus-sac, vas deferens and surrounding structures located on the posterior part, which were established as novel species-specific morphological traits.

Since its original description, *Caryophyllaeus brachycollis* Janiszewska, 1953 was considered as a parasite of barbels and chubs. Recent studies, however, revealed another morphotype, which morphologically partly resembles *C. laticeps* and also parasitizes same fishes, freshwater breams. However, second morphotype of *C. brachycollis* can be easily distinguished based on a massive, elongated cirrus-sac and vas deferens surrounded by vitelline follicles, in contrast to a subspherical, thin-walled cirrus-sac with internal seminal vesicle and conspicuous vas deferens of *C. laticeps* morphotype 1.

Taxonomic status of rare species of the genus *Caryophyllaeus*, such as *C. auriculatus*, *C. fimbriiceps* and *C. syrdarjensis*, was assessed based on whole-mounts deposited in museum collections and records in the literature. Until new material suitable for genetic analyses will be collected, their validity remains rather unclear.

## Future plans

The life cycle of *C. laticeps* will be studied in laboratory conditions. It is an initial step in the effort to elucidate probably host-driven phenotypic plasticity of this species. Following previous genetic analyses, population study by using microsatellite markers of *C. laticeps* from different geographical localities and fish hosts has been initiated.

## Acknowledgement

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

## References

1. Bazsalovicsová E., Králová-Hromadová I., Brabec J., Hanzelová V., Oros M., Scholz T. (2014) Conflict between morphology and molecular data: a case of the genus *Caryophyllaeus* (Cestoda, Caryophyllidea), monozoic tapeworm of cyprinid fishes. *Folia Parasitol* 61: 346–352.
2. Barčák D., Oros M., Hanzelová V., Scholz T. (2014) Phenotypic plasticity in *Caryophyllaeus brachycollis* Janiszewska, 1953 (Cestoda: Caryophyllidea): does fish host play a role? *Syst Parasitol* 88: 153–166.
3. Hanzelová V., Oros M., Barčák D., Miklisová D., Kirin D., Scholz T. (2015) Morphological polymorphism in tapeworms: redescription of *Caryophyllaeus laticeps* (Pallas, 1781) (Cestoda: Caryophyllidea) and characterisation of its morphotypes from different fish hosts. *Syst Parasitol* 90: 177–190.

# Radiation of *Ceratomyxa* species in the clinid fish in the rock pools of South Africa

Pavla Bartošová-Sojková<sup>1\*</sup>, Alena Kodádková<sup>1</sup>, Tereza Tomková<sup>2</sup>, Cecile C. Reed<sup>3</sup>, Ivan Fiala<sup>1,2</sup>

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Intertidal rock pools harbour large numbers of fishes and invertebrates, which are well adapted to life in harsh conditions with a strong fluctuation of water temperature and salinity. These rock pools with close contact of fish and invertebrates represent ideal conditions for parasite life cycles. We focused on different host species - clinid fish (*Clinus acuminatus*, *C. brevicristatus*, *C. cottoides*, *C. superciliosus* and *Muraenoclinus dorsalis*) collected from the intertidal pools from different localities along the temperature gradient of the South African coast (Fig. 1). *Clinus cottoides* has a clear population structuring in the tip of South Africa given by the ocean currents [1]. Representatives of three myxosporean genera *Henneguya*, *Ceratomyxa* and *Sphaeromyxa* (Cnidaria: Myxozoa) have so far been described from South African clinids [2, 3]. *Ceratomyxa* is a species-rich genus characteristic by morphologically uniform spores with crescent shape and two polar capsules. Individual species commonly differ by host preference and in sequences. Two *Ceratomyxa* species have so far been described from clinid fishes, *Ceratomyxa cottoidi* from *Clinus cottoides* and *C. dehoopi* from *Clinus superciliosus* both from South Africa [2].

We aimed to 1) examine the species diversity of the genus *Ceratomyxa* in the South African clinids and to 2) examine if these parasites show a similar trend in population structuring as reported for *Clinus cottoides*.

*Ceratomyxa* spores and plasmodia were found both in host gall bladders and livers. We morphologically and molecularly characterized seven *Ceratomyxa* species found in a variety of clinid fish species and localities (Fig. 1). Our comprehensive phylogenetic analyses based on the newly obtained SSU rDNA, LSU rDNA, ITS sequences revealed that ceratomyxids from South African clinid fishes create a widely diversified clade of closely related species forming a sister branch to *Ceratomyxa aegyptiaca* from the soleid fish from Tunisian lagoon. Within this clade, seven main lineages – species were identified, two of which match with the previously described *Ceratomyxa cottoidi* and *C. dehoopi*. Three *Ceratomyxa* species including *C. dehoopi* were strictly host specific, whereas four ceratomyxids were found in more than one fish host. In this respect, the most diversified species was *C. cottoidii* which parasitized four fish species. Sequences within the *Ceratomyxa cottoidi* lineage did not cluster according to the fish host species and geographic locality so the population structuring of this parasite did not reflect the trend observed in its fish host. Co-infections of one fish host by different *Ceratomyxa* species were commonly found in our samples. *Clinus superciliosus* was found to be the most frequently parasitized host as it hosted all but one *Ceratomyxa* species.

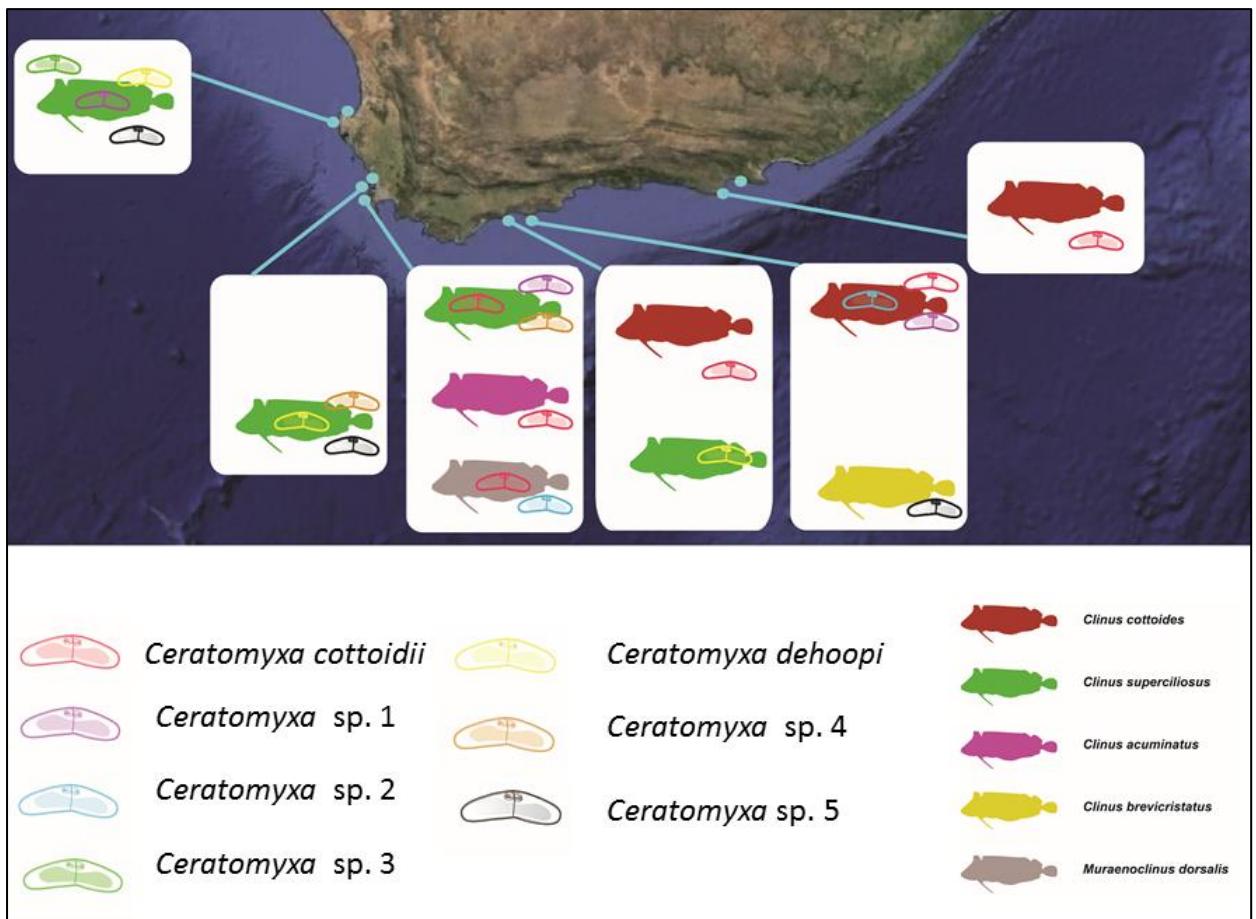
We have revealed a high degree of species diversity and close relationships of *Ceratomyxa* spp. from the intertidal coastal fishes, the Clinidae, in the coast of South Africa. Such parasite radiation was most probably affected by the limited dispersal of the fish hosts within this endemic area. Differences in population structuring of *Ceratomyxa cottoidi* and its fish intermediate host are probably affected by the possible co-evolution of the parasite with its definitive host, which is an unknown polychaete.

## 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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**Figure 1.** The diversity and distribution of *Ceratomyxa* spp. in the clinid fishes from the rock pools of South Africa.

# Phylogenetic relationships between two cyprinid genera – *Telestes* and former *Rutilus* from the perspective of host-specific *Dactylogyrus* (Monogenea) parasites

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Distribution of cyprinid genera over Southern European peninsulas is unequal. Of multitude of European cyprinid genera, only *Squalius* represents the genus distributed over all three southern peninsulas. Contrariwise, phylogenetically related *Telestes* and *Rutilus* are inhabiting only Balkan and Apennine Peninsulas.

While the genus *Telestes* emerged on basis of morphological and molecular diagnostics from the former genus *Leuciscus*, genus *Rutilus* was just recently revised. According to morphology, ecology, genetics and biogeography, several former *Rutilus* species were moved to two new genera. The first one is *Leucon* comprising northern Apennine and western Balkan species previously included in *Rutilus* genus. The other one is monotypic genus *Sarmarutilus* including only Italian *Sarmarutilus rubilio*.

Over years 2014-2016 several sampling trips were conducted on Balkan and Apennine Peninsulas with the aim to analyze recent diversity of *Dactylogyrus* parasitizing *Telestes* and *Rutilus* and to confirm the scenario proposed for the diversification of these cyprinids. A total of 7 species of former *Rutilus* and 9 species of *Telestes* were investigated, and parasitological data, especially ectoparasites of genus *Dactylogyrus*, was collected.

*Dactylogyrus* species richness from *Rutilus* hosts endemic for southern peninsulas was lower in comparison to widely distributed *Rutilus rutilus* which harbours up to 9 different *Dactylogyrus* species [1]. Collected *Rutilus* species were parasitized by 1 to 5 *Dactylogyrus* species with the highest prevalence of *D. erhadovae* and shared three *Dactylogyrus* species i.e. *D. sphyra*, *D. caballeroi* and *D. crucifer* with common *R. rutilus*. Four species of *Dactylogyrus* were collected from examined *Telestes* host species. The most common *Dactylogyrus* parasite species was *D. vistulae* which is considered as generalist parasitizing a wide range of cyprinid host species including several genera of Cyprinidae. *Telestes* and *Rutilus* shared the complex of *Dactylogyrus* species morphologically close to the *D. rarissimus* which is common parasite of *R. rutilus*. Phylogenetic reconstruction of *Dactylogyrus* species parasitizing *Rutilus* and *Telestes* species (based on concatenated sequences of partial 18S, ITS1 region, 5.8S and partial 28S rDNA) revealed that *D. rarissimus* collected from *R. heckelii* and *R. rutilus* forms monophyletic group nested within the clade of morphologically related species parasitizing *Telestes* and *Rutilus*.

## Future plans

1. to analyze the recent diversity of *Dactylogyrus* in endemic cyprinid fish species of the Mediterranean area,
2. to describe new *Dactylogyrus* species from Mediterranean region,
3. to use the *Dactylogyrus* of highly diversified cyprinid genera as a tool for testing patterns and processes linked to the evolution and biogeography of endemic cyprinid hosts.

## Acknowledgement

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# Structural adaptation to external embryonic development and active host finding strategy in *Cardiocephalooides longicollis* miracidia (Digenea, Strigeidae)

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Egg and free-living larvae that hatch from eggs, i.e. miracidia, play critical roles in the transmission of trematodes. Once eggs are released via final host faeces, these life stages have to be capable to detect a suitable molluscan host, ensuring larval viability and infectivity to establish in such a host. The study of the development of the egg and the miracidial structures inside can lead to a better understanding of the parasite's biology and transmission, as well as of the infection process.

In view of the, to our knowledge, nonexistent research on strigeid embryonic stages (only one study on miracidial surface and argentophilic structures of 3 strigeids, [1]), we decided to investigate the embryonic development and functional ultrastructure of *C. longicollis* miracidia in naturally released eggs of the strigeid *Cardiocephalooides longicollis* (Rudolphi, 1819) Dubois, 1982 by visualization of live eggs with light and transmission electron microscopy.

Eggs are released into the marine environment, where they typically develop and mature, hatch and penetrate the first intermediate host, benthic nassariid snails [2]. Our results showed that this species has operculated eggs and embryogenesis occurs in the marine environment before an actively infecting ciliated miracidium hatches. Six different developmental stages were identified. The lack of pores in the eggshell indicates its impermeability and the miracidium's dependency on glycogen nutritive reserves, contained in numerous vitellocytes in early embryos. As the development advances, these merge into larger vitelline vacuoles that encircle the miracidium and may aid its hatching once completely mature. Tissue and primary organ differentiation were observed in advanced stages, i.e. terebratorium, cerebral ganglion, glands and eyespots, which are key structures in free swimming miracidia that use photoreception for host detection. The terebratorium contains a single apical and paired lateral glands for the secretion of histolytic substances, as well as three types of sensory endings, which permit location, adhesion and penetration of the host.

The eggs of *C. longicollis* demonstrate the structures required for an active-host-finding strategy following external embryogenesis of immature eggs to mature miracidia. It shares some features with other, well described species whose life cycle is unfortunately unknown, thus lacking an understanding of deviant special ultrastructural details (e.g. the pleurogenid *Brandisia turgida* and the prosthogonimid *Mediogonimus jourdanei*, [3,4]. This study highlights that ultrastructural data have to be analyzed in relation to parasite biology to understand the adaptations to specific parasite strategies.

## Ongoing studies on *C. longicollis*

Experimental assays are currently being performed in order to assess the effect of temperature on the hatching and development of *C. longicollis* eggs.

## Acknowledgement

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# Ultrastructure of oocysts and sporozoites of urosporid gregarine from marine polychaete

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Apicomplexans represent a very successful group of unicellular parasites that consists entirely of parasitic species infecting a wide range of vertebrates and invertebrates. Gregarines represent a basal group of the phylum Apicomplexa. The life cycle of eugregarines is in general characterised by a lack of merogony (asexual reproduction for autoinfection), but a presence of syzygy (pregamic paired association of gamonts) and gametocyst stage. Within gametocyst, gametogony (sexual reproduction), fusion of gametes and sporogony (formation of invasive stages) take place. The crucial step for parasite dissemination and effective infection of new hosts is formation of sporozoites within a protective envelope during the sporogony. The knowledge of the morphology of these stages can provide a possible evolutionary pathways particularly within gregarines and apicomplexans in general.

In the body cavity of polychaete *Travisia forbesii* brown bodies comprising white spheres were found. Most of these spheres were identified as cysts of gregarines, that inhabit the host's body cavity (*Urospora* spp.). Each cyst was surrounded by several closely adjacent layers of flattened host coelomocytes, forming a brownish capsule around them. The wall of the cyst consisted from three distinct layers: (i) multi-lamellar innermost, (ii) hyaline middle and (iii) outermost layer consisting of short fibrils. Under this wall numerous spindle-shaped and heteropolar oocysts, typical of the urosporids [1], were observed under this wall. At the one pole of oocyst, a transparent funnel was situated, while the opposite end was elongated into a narrowing, transparent tail. Inside the oocysts, a refringent residuum and eight sporozoites were observed. The latter were localised by fours at the each oocyst pole. The envelope consisted of two-layers: (a) outermost layer (= exospore), consisted of one membranous lamellae, (b) innermost (= endospore), was made of two membranous lamellae. The distance between exo-, endospore and sporozoite pellicle varied considerably, however, in most cases endospore was closely appressed to the sporozoites. The banana-shaped sporozoites were covered with a three-layered apicomplexan pellicle. The pellicle was underlain by 20 longitudinal subpellicular microtubules, a large nucleus was often localised in the cell middle region along long axis of sporozoite. The structures resembling conoid and polar ring placed at apical end of sporozoites, the anterior third of the cell was packed with rhoptries and numerous micronemes. Among the oocysts, zygotes with envelope were found. They were round, covered by pellicle and contained different inclusions and vacuoles. We also observed dead cells and solitary "naked" sporozoites.

Our observations are in agreement to the previous ones performed on spores of *Urospora* spp. and other urosporids [1]. General morphology of cysts and oocyst walls, as well as sporozoite morphology are comparable to species investigated to date [2]. The presence of dead cells and function of solitary sporozoites remain undiscovered.

## Future plans

Preparation and finalisation of publications concerning the data in presented abstract and morphology and molecular phylogeny of agamococcidia from the same host. Further morphological and molecular analyses of gregarines from marine environment.

## Acknowledgement

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# No regrets, suppression of a traditional genus is needed: *Henneguya* is “just” a *Myxobolus* with long spore projections

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Genus *Henneguya* is one of the most traditional genera of the Myxozoa described by Thelohan in 1892. It comprises currently about 210 species and thus is the third largest genus within the Myxozoa. Species of the genus *Henneguya* are characterised by myxospores with ellipsoidal or rounded spores with two polar capsules lying in the sutural plane and with two caudal projections of the spore valves. The latter character is the one that distinguishes them from species of the genus *Myxobolus*. Both of these genera are taxonomically classified to the family Myxobolidae that harbours half of the total number of described myxosporeans. Most of them are histozoic in freshwater fishes producing large polysporic plasmodia in the tissues.

Phylogenies based on the SSU rDNA did not support a monophyly of *Myxobolus* and *Henneguya* (e.g. Fiala 2006). Recent study of Carriero et al. (2013) including more than one hundred species supported several independent evolutionary origin of *Henneguya* morphotype within *Myxobolus* radiation.

We performed broad phylogenetic analysis of all myxobolid species available in GenBank including more than 250 taxa (Fig. 1). We supported the hypothesis that during myxosporean evolution the character of spore projections appeared several times independently. We traced the character evolution (using Mesquite program) and found at least 15 independent evolutionary events of the origin of spore projections and 6 apparent secondary losses of this typical spore characteristic of the genus *Henneguya*.

Furthermore, we performed transmission electron microscopy (TEM) of *M. wulii* and *M. oralis* spores. Latter species was characterised to show the myxospores with aberrant tailed forms (Liu et al. 2014). Ultrastructure of the aberrant spores of *M. oralis* revealed caudal appendages originated from the sutural ridge at the posterior end of the spore indicating that caudal appendages of *Henneguya* and *Myxobolus* have the same ontogeny. The relatively small caudal appendages were observed on *M. wulii* spores under TEM but cannot be seen under light microscopy. This could mean that more *Myxobolus* species have the genetic capacity to express the caudal appendages on the myxospore. This character may be neglected in former descriptive works using only light microscopy and this may be more common trait.

Beside the clear polyphyletic phylogenetic pattern of *Henneguya* species, the similar morphology and origin of caudal appendages of *Henneguya* and some *Myxobolus* myxospores proved that caudal appendages are not a valid character for distinction of the genera *Myxobolus* and *Henneguya*. Moreover, several *Myxobolus* species including the type species – *M. muelleri* were found to produce aberrant forms of spore with caudal projections. Suppression of the genus *Henneguya* is proposed in the manuscript under preparation.

## Future plans

Continue in the phylogenetic studies that aim to gradually change the myxozoan taxonomy to mirror the phylogenetic relationships of myxozoan parasites.

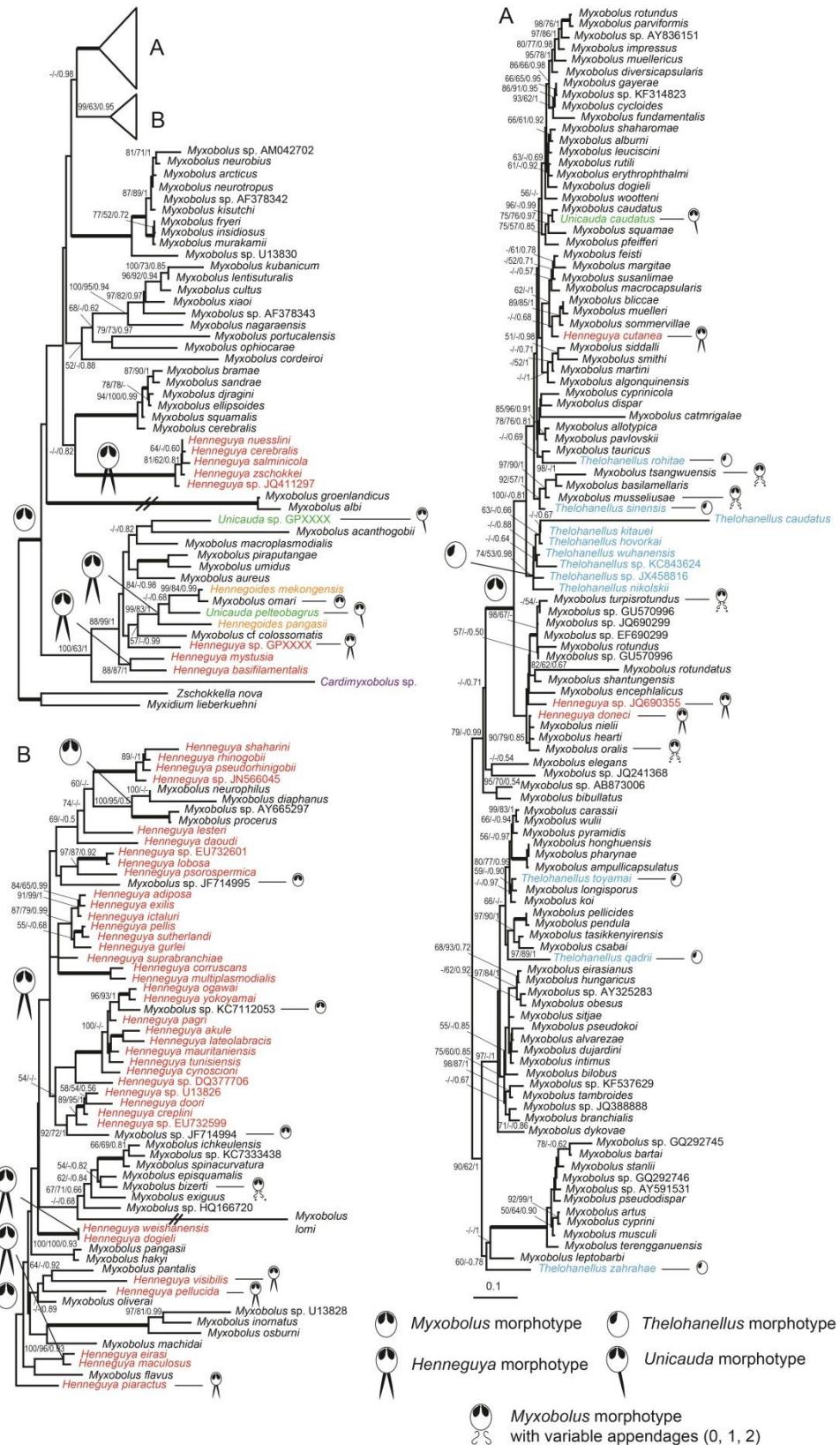
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**Figure 1.** The phylogenetic relationships of the *Myxobolus* clade containing mostly *Myxobolus* and *Henneguya* species (in red colour).

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

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Seven species (four new) of *Quadriacanthus* were collected from the gills of three species of catfishes from Lake Turkana (Kenya) and the Nile River Basin (Sudan). These were as follows: *Quadriacanthus aegypticus* El-Naggar and Serag, 1986, *Quadriacanthus clariadis* Paperna, 1961, *Quadriacanthus fornicatus* n. sp., *Quadriacanthus pravus* n. sp., and *Quadriacanthus zuheiri* n. sp. from *Clarias gariepinus* (Clariidae); *Quadriacanthus mandibulatus* n. sp. from *Heterobranchus bidorsalis* (Clariidae); and *Quadriacanthus bagrae* Paperna, 1979 from *Bagrus docmak* (Bagridae). Taxonomic evaluation of the monogeneans found was made on the basis of both morphometrical observation and molecular methods. The interspecific relationships among *Quadriacanthus* spp. parasitizing catfishes (Siluriformes) in the African region inferred from ribosomal DNA sequences were investigated for the first time (also see abstract of MS). *Quadriacanthus mandibulatus* n. sp. was observed to be the most distant species from others. The separation of *Q. mandibulatus* n. sp. from the other species corresponds with the different morphology of its copulatory tube [1].

The morphological evaluation of *Schilbetrema* spp. from Kenya and Sudan, complemented with molecular analyses of nuclear ribosomal DNA sequence data (28S, 18S and ITS-rDNA), has been in process. Eleven *Schilbetrema* species have been distinguished. Phylogenetic analysis shows division of *Schilbetrema* species into two main clades corresponding to host species (A – *Schilbe intermedius* and *S. uranoscopus*; B – *S. mystus*).

Participation on “Summer School of Fish Parasitology 2016: Parasites of Fish in Africa” course was realized through preparing the study material and providing practical lessons.

## Future plans

The plans for 2017 include completion of *Schilbetrema* spp. characterization (including also material from other localities, if possible) and characterization of other dactylogyridean genera from African catfishes.

## Acknowledgement

This research was supported by the Czech Science Foundation, project no. P505/12/G112 (ECIP).

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# A North American alien species of *Posthodiplostomum* Dubois, 1936 (Digenea: Diplostomidae) widespread in European freshwaters

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Species of *Posthodiplostomum* Dubois, 1936 represent a group of diplostomid trematodes with wide distribution; they are pathogenic to their fish hosts and may exert serious impacts on freshwater fish communities. Currently, the genus includes 14 nominal species; of these, two, i.e. *Posthodiplostomum cuticola* (von Nordmann, 1832) Dubois, 1936 (type-species) and *P. brevicaudatum* (von Nordmann, 1832) Dubois, 1936, have been reported from a wide range of fish hosts and localities in Europe.

We carried out the first study addressing integrated molecular and morphological approaches to the species diversity of *Posthodiplostomum* in Europe using isolates of larval and adult digenetic stages. Most of the studied material originated from fish hosts in Lake Atanasovsko (Bulgaria) where 134 *Gasterosteus aculeatus* (L.) (Gasterosteidae) and 107 *Lepomis gibbosus* (L.) (Centrarchidae) were sampled. Comparative materials included metacercariae recovered from *Perca fluviatilis* L. in Lake Mácha (Czech Republic) and *Lepomis gibbosus* in a blind river arm of the River Danube near Šturovo (Slovakia), an adult isolate from *Ardea cinerea* L. from a bird recovery centre in Catalonia (Spain) and a cercarial isolate from *Planorbis planorbis* (L.) in the Curonian Bay near Juodkrantė (Lithuania). The newly-obtained isolates were characterised both morphologically and molecularly. Partial sequences for the mitochondrial *cox1* barcode region, the complete ITS1-5.8S-ITS2 gene cluster and 28S rRNA gene have been generated for most of the isolates. Species boundaries were assessed in phylogenies via maximum likelihood and Bayesian inference analyses. We also provided the first sequences for a representative of *Ornithodiplostomum* Dubois, 1936 from the Palaearctic, *O. scardinii* (Schulman in Dubinin, 1952) ex *Scardinius erythrophthalmus* (L.), used as the outgroup in phylogenetic analyses.

Phylogenies inferred from *cox1* and ITS1-5.8S-ITS2 sequence datasets confirmed the distinct status of three species in Europe: i.e. *Posthodiplostomum cuticola* (ex *Planorbis planorbis*), *P. brevicaudatum* (ex *Gasterosteus aculeatus* and *Perca fluviatilis*) and "Posthodiplostomum sp. 3" sensu Moszczyńska et al. (2009) and Locke et al. (2010) (ex *Lepomis gibbosus*) in contrast to the generally accepted opinion that only *P. brevicaudatum* and *P. cuticola* occur in the Palaearctic. The unidentified "Posthodiplostomum sp. 3" from centrarchid fishes in Canada had identical sequences with the European isolates recovered by us from the alien pumpkinseed sunfish *L. gibbosus*. Further, we obtained a *cox1* sequence from an adult isolate recovered from *Ardea cinerea* L. Thus, our study provides the first report of this alien North American digenetic species in a definitive host in Europe. The morphological study of metacercariae from pumpkinseed sunfish in Europe and the comparison with the previously described metacercariae from centrarchid hosts from North America allowed assigning our specimens to a widespread species-group taxon believed to be endemic for the Nearctic Region. The latter parasite, occurring in the alien host *L. gibbosus* and a Palaearctic definitive host, is also supposed to be alien for Europe. Our results suggest that this species of *Posthodiplostomum* is widespread in Europe and it could be expected that its distribution probably coincides with that of its centrarchid second intermediate host. Further studies are needed to gain more insights into the diversity and distribution of this invasive species as well as its impact on the native biodiversity.

## Future plans

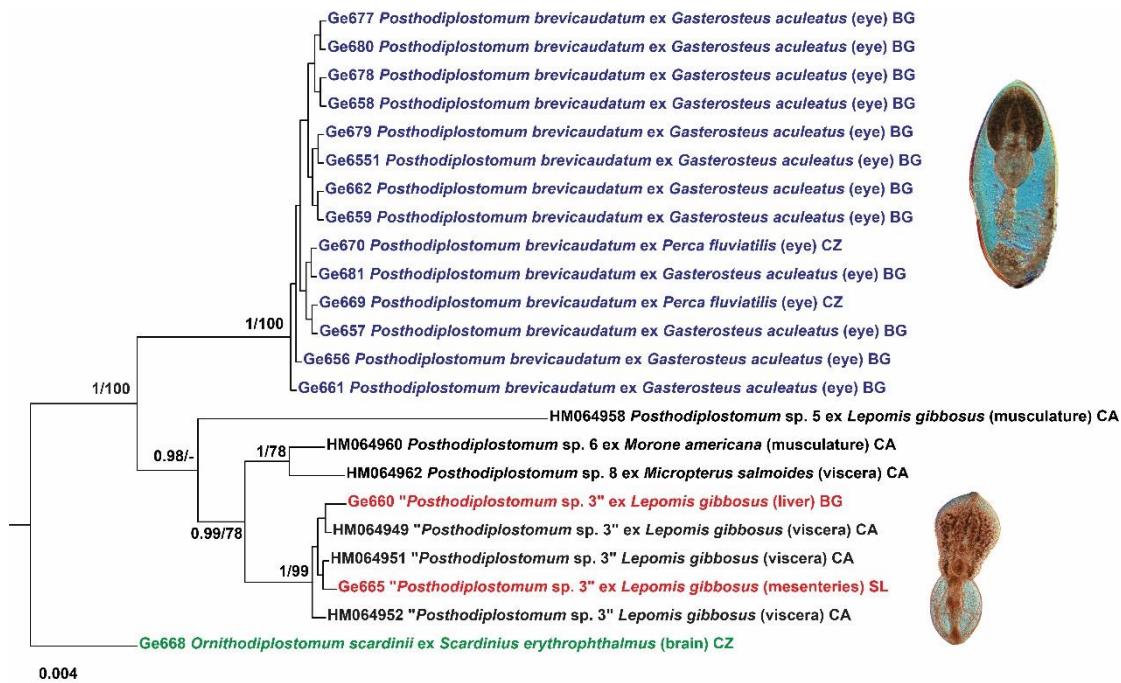
Morphological and molecular characterisation of newly-obtained diplostomids and other digenetics from various fish-eating bird and snail hosts from Iceland. Morphological and molecular characterisation of digenetics of sparid and scombrid fishes from the Western Mediterranean coasts.

## Acknowledgements

This research was partially supported by ECIP (European Centre of IchthyoParasitology); Centre of Excellence programme of the Czech Science Foundation (Project No. P505/12/G112), WETLANET (FP7 CAPACITIES Grant 229802), CEBDER (National Science Fund, Ministry of Education and Science of the Republic of Bulgaria, Grants DO 02-15/2009) and YSDO 02-271/18 (NSF, Bulgaria). SG profits from a postdoctoral fellowship of the Czech Academy of Sciences.

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**Figure 1.** Bayesian inference (BI) tree for *Posthodiplostomum* spp. inferred from ITS-5.8S-ITS sequence dataset. Nodal support values from BI and maximum likelihood (ML) analyses are indicated as BI/ML; only values > 0.95 (BI) and > 70 (ML) are shown. The scale-bar indicates the expected number of substitutions per site. Sequence annotations are as in the GenBank database; host, site and country of origin are also indicated. Abbreviations: BG, Bulgaria; CA, Canada; CZ, Czech Republic; SL, Slovakia.

# ***De-novo development of microsatellite markers for Dactylogyrus vistulae***

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In the last few years, the microsatellite markers have become one of the most popular tools for a wide spectrum of applications in population genetics, e.g. individual identification, parentage or population structure analyses. For non-model species where microsatellite loci cannot be sufficiently discovered through cross-species amplification, development of next-generation sequencing technologies such as Illumina sequencing technology provide fast, efficient and still cost-effective methods for microsatellite isolation. In Monogenea, a heterogeneous group of mainly fish ectoparasites, microsatellite markers are only known for the three representatives of the *Gyrodactylus* genus [1]. The most of the genetic population studies of monogenean parasites are based preferentially on the mitochondrial DNA.

In our study, we aimed to characterize *de-novo* microsatellite markers for *Dactylogyrus* parasites. *Dactylogyrus vistulae* was selected as a model organism owing to its relatively large body size in comparison to the other members of this genus. Approximately 300 specimens of *D. vistulae* were collected from European chub *Squalius cephalus* of the Dyje River. Genomic DNA was isolated and subsequently prepared genomic libraries were subjected to Illumina MiSeq (provided by CEITEC, MU, Brno). QDD program [2] was used for selecting microsatellite markers and primer design. Following criteria used in Gettová et al. (2013) 70 sequences with target microsatellites were selected.

Monogenean parasites, especially dactylogyrids, display a high level of host specificity. As a result genetic structure of monogenean parasites might be host-dependent. Microsatellite markers developed for *Dactylogyrus* parasites might, therefore, enable us to reconstruct the evolution history of their fish hosts. In addition, in the case of rare generalist *Dactylogyrus* parasites (such is *D. vistulae*), we expect that the microsatellites will represent a useful tool to analyse the structure of parasite populations parasitizing different host species and/or geographically isolated parasite populations across Europe.

## **Future plans**

Selection of polymorphic microsatellites is currently performed and cross-species amplification of different *Dactylogyrus* parasites is planned.

## **Acknowledgement**

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# ***Orientattractis brycini* sp. nov. (Nematoda: Atractidae) from characiform freshwater fishes in Gabon, Africa**

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During a short visit to the Museum of Natural History of Geneva in 2012, the examination of parasitic nematodes collected from freshwater fishes in Gabon, Africa, revealed the presence of very peculiar atractid specimens. These parasites were found in two characiform fishes, *Brycinus macrolepidotus* Valenciennes (Alestidae) and *Xenocharax spilurus* Günther (Distichodontidae) which are freshwater fishes that inhabit rivers and lakes in Lower Guinea, from Cameroon to the Chiloango River Basin, the Nile system and the Zaire (= Congo). They feed on insects, crustaceans and vegetation (Froese & Pauly, 2016). The nematodes belong to an unknown species of *Orientattractis* Petter, 1966.

Fishes were collected by angling in 2 localities from the Franceville area, Southeast Gabon, in November 2010. All nematodes were washed in physiological saline, fixed in hot 4% formaldehyde solution and cleared a glycerine-water mixture. For scanning electron microscopy (SEM), specimens were postfixed in 1% osmium tetroxide (in phosphate buffer), dehydrated through a graded acetone series, critical-point-dried and sputter-coated with gold; they were examined using a JEOL JSM-7401F scanning electron microscope at an accelerating voltage of 4 kV (GB low mode). All collections were made in the frame of a Scientific Research Convention between the Centre International de Recherches Médicales de Franceville (CIRMF) and the Muséum d'Histoire naturelle de Genève (MHNG) with the research permit AR0017/09/MESRSDT/CENAREST/CG/CST/CSAR.

*Orientattractis brycini* sp. nov. was described from the intestine of *B. macrolepidotus* (type host) (body length 21.4 cm) and *X. spilurus* (body length 15.5–20.2 cm) in Bridge on Ogooué River, Haut-Ogooué, Gabon (01°38'24"S; 13°31'.48"E; elev. 300 m) (type locality). Prevalence and intensity in each host were *B. macrolepidotus*: prevalence 25% (4 fish examined/1 infected), intensity 24 nematodes; *X. spilurus*: 43% (7/3), 4.3. The generic diagnosis was emended, based on the already described species and present data, since several important features were not included in the original description (see Petter, 1966). Morphological features as the structure of the oral opening, presence of deirids, among others, were incorporated to the diagnosis for making it easier to distinguish *Orientattractis* from closely related genera (e.g., *Klossinemella* and *Paraorientattractis*) within the Atractidae. Thus, *Orientattractis* and *Paraorientattractis* have four bicornate submedian structures surrounding mouth, whereas *Klossinemella* shows eight pairs; the two first genera differ in the number of lips (6 vs. 4) and presence of ornateations on the dorsal surface of body in *Paraorientattractis*. These changes do not modify the systematic position of the genus. The new species is similar to several of its congeners, but differs from all in the size of spicules (except in *O. hamabatrachos*), gubernaculum and number and distribution of caudal papillae.

*Orientattractis brycini* was found in two fish species of the order Characiformes, but from different families (Alestidae and Distichodontidae) and sampling localities (Ogooué and Mpassa), but no differences in the morphology and biometrical values among the nematodes from both hosts were found. The present finding represents the eighth species in the genus *Orientattractis* and the fourth being reported from fish hosts, since other members were reported in tortoises (*O. levanhoai*, *O. leiperi*), frog (*O. hamabatrachos*), and turtle (*O. asymmetrica*). This is also the first record of a species of *Orientattractis* in Africa.

## Acknowledgement

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# Diversity of monogeneans of deep-sea sharks and fish from the western Mediterranean deep-sea

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In recent years, there have been an increasing number of studies on the helminth communities of deep-sea fish from the Western Mediterranean deep-sea [1,3]. However, despite the considerable progress in deep-sea fish parasitology, major gaps still exist especially in the knowledge of the systematics, diversity and biology of most helminth parasites from this part in the ocean. In this study, we report the diversity of the monogeneans found on deep-sea elasmobranchs and teleosts from off the western Mediterranean coast of Valencia, Spain. Three species of sharks (i.e. bluntnose sixgill shark *Hexanchus griseus* (Bonnaterre) [n = 2], kitefin shark *Dalatias licha* (Bonnaterre) [n = 4] and velvet belly *Etmopterus spinax* (L.) [n = 56]), and two species of teleosts (i.e., dragonfish *Stomias boa boa* (Risso) [n = 9] and Mediterranean slimehead *Hoplostethus mediterraneus* Cuvier, 1829 [n = 2]) were examined. Freshly fishes were obtained the Valencian Gulf (Balearic Sea) (38°04'–40°09'N, 0°04'–0°25'E) from a depth of 400 m. Three species of monogeneans were collected: the hexabothriid *Protocotyle grisea* (Cerfontaine, 1899) Euzet & Millard, 1974 (Fig. 1) from the gills of the bluntnose sixgill shark (both sharks infected), *Protocotyle* sp. from the kitefin shark (1 out of 4 sharks) and the plectanocotylid *Euzetplectanocotyle hoplosteti* Mamaev & Tkachuk, 1979 from the gills of the Mediterranean slime head (1 out of 2 fish). Morphological data, including illustrations and scanning electron microscopy photomicrographs of all the newly-collected specimens are provided. This is the first report of *P. grisea* from the bluntnose sixgill shark from the Spanish Mediterranean coast, and a new geographical record of *E. hoplosteti* in the Mediterranean Sea. The kitefin shark is reported as a host of hexabothriids for the first time.

## Future plans

Detailed morphological descriptions of these specimens will be published including the molecular characterization of partial sequences of the 28S rRNA gene for our specimens of *Protocotyle*. This study will provide the first sequences for this genus of hexabothriid monogeneans, and will clarify if specimens from both species of sharks are conspecific.

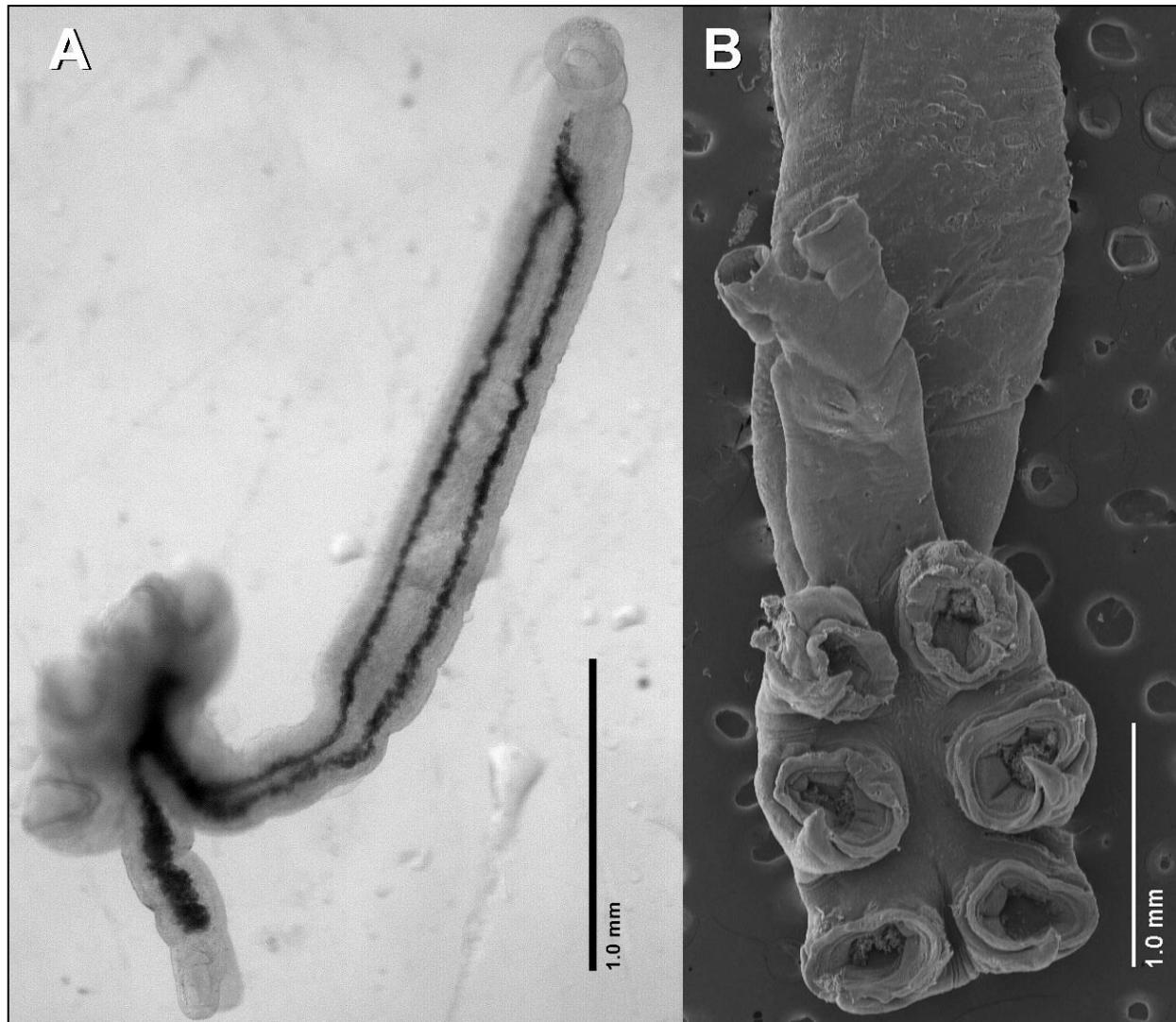
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**Figure 1.** *Protocotyle grisea* (Cerfontaine, 1899) Euzet & Millard, 1974 (Monogenea: Hexabothriidae) from the gills of the bluntnose sixgill shark *Hexanchus griseus* (Bonnaterre). **A**, whole worm, ventrolateral view; **B**, scanning electron micrograph of the haptor and appendix, ventral view.

# Molecular and biochemical characterization of cysteine peptidase inhibitor from *Eudiplozoon nipponicum* (Monogenea)

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Inhibitors of cysteine peptidases (cystatins) are proteins produced by a wide range of organisms, including parasites. Besides regulation of the basic physiological functions of parasites they may act either as modulators of the host immune system or regulators of blood-digestion.

We identified that cystatin genes are expressed and their protein form probably secreted by *Eudiplozoon nipponicum*. In following experiments we focused on molecular and functional characterization of cystatin from *E. nipponicum* (Monogenea). We performed bacterial expression of *E. nipponicum* cystatin gene (pET19b plasmid vector, *E. coli* BL21DE3 RIPL cells) and purified the recombinant protein [1]. Phylogenetic trees inferred using Bayesian inference, maximum likelihood, neighbour-joining methods revealed close phylogenetic relationship with cystatins of cestodes. The inhibitory properties and stability of cystatin was fluorometrically measured by adoption of fluorogenic peptide substrate (FR-AMC) and recombinant cysteine peptidases (mouse cathepsin L and cathepsin L3 from *E. nipponicum*). Successful inhibition of cathepsin L3 from *E. nipponicum*, probably major molecule responsible for host blood degradation by this monogenean parasite [2], lead us to presumption of cystatin possible role in regulation of this process. Succeeding assays showed that cystatin inhibits the digestion of haemoglobin caused by soluble crude extract from *E. nipponicum*, its E/S products and recombinant cathepsin L3.

Presence of cystatin was successfully detected in both soluble crude extract and excretory-secretory products from *E. nipponicum* by using anti-cystatin antisera generated in mice and rabbits. Sera were used also as primary antibodies in the immunohistochemical detection of cystatin on cryosections and paraformaldehyde-fixed paraffin sections of *E. nipponicum*.

In order to test the immunomodulatory potential of cystatin we also carried out a preliminary study by using *in vitro* cultures of porcine alveolar macrophages (PAMs) stimulated by pathogenic bacteria *Haemophilus parasuis* which triggers the expression of proinflammatory cytokines by infected PAMs. qPCR analysis showed moderate downregulation of cytokines IL-1  $\beta$  and TNF-  $\alpha$  caused by cystatin of *E. nipponicum*.

## Future plans

The attention will be focused on a detailed study of the immunomodulatory potential of *E. nipponicum* cystatin, e.g. the influence on the expression and production of anti- and pro-inflammatory cytokines by macrophages stimulated *in vitro* using qPCR and ELISA.

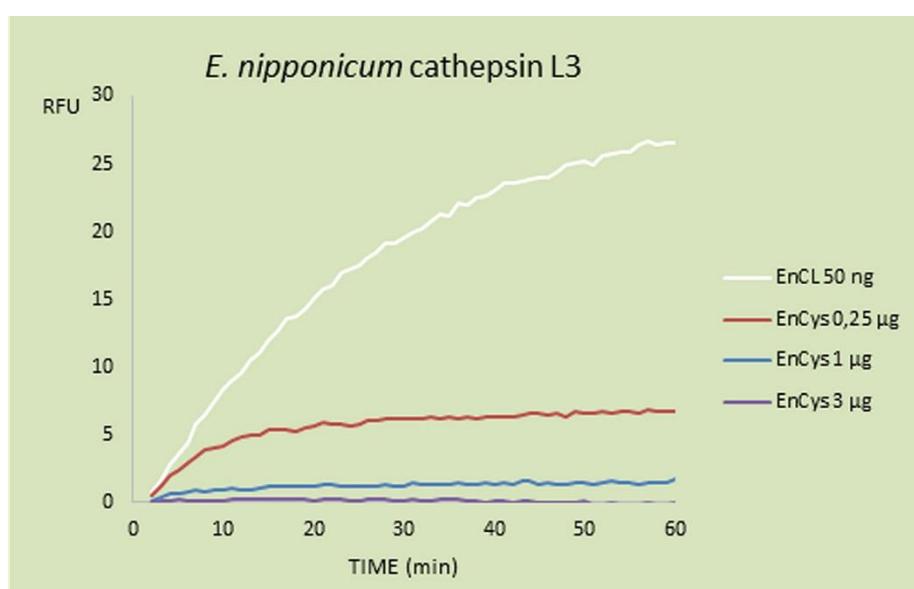
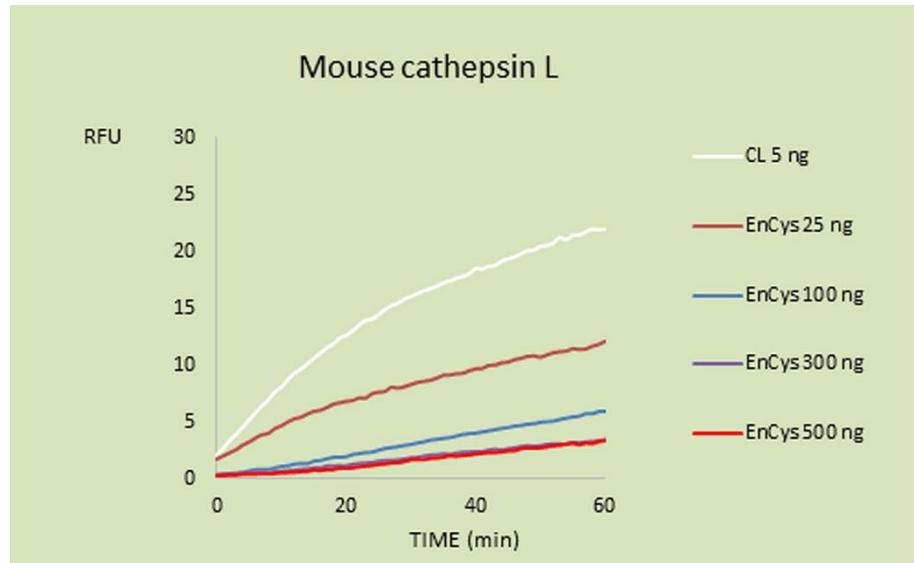
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**Figure 1.** Inhibition of enzymatic activity of mouse and *E. nipponicum* cathepsins by recombinant *E. nipponicum* cystatin (EnCys).

# Leeches of notothenioid fishes in Antarctica: new species discovery and phylogenetic position

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Leeches (Hirudinea) in Antarctica occur in marine habitats. The family Piscicolidae is predominantly a marine group of subclass Hirudinea and its members feed on a variety of fish species. Some members of this family are restricted to the Southern Ocean [1]. Notothenioid fishes (Perciformes; Notothenioidei) is the most dominant Antarctic fish taxon distributed throughout the Southern Ocean and is adapted to low and stable temperatures. Most of the species prefer benthic habitats and some of them including representatives of the genus *Trematomus*, *Pagothenia* and *Notothenia*.

In our study, we focused on leeches from notothenioid fishes in Prince Gustav Channel (James Ross Island, Weddell Sea) collected during the Czech Antarctic expedition 2014. We report their species diversity and phylogenetic position in polar areas. In total, 100 specimens of five notothenioid fish (*Trematomus bernacchii*, *T. hansonii*, *T. eulepidotus*, *Pagothenia borchgrevinki* and *Notothenia coriiceps*) were examined. Collected leeches were measured and documented by light microscopy as well as scanning electron microscopy. Phylogenetic analyses were performed combining a portion of the nuclear large ribosomal subunit (28S rRNA) gene with mitochondrial nicotinamide adenine dinucleotide dehydrogenase subunit one (ND1) and cytochrome c oxidase subunit one (COI) fragments.

The leeches were identified as representatives of four different genera (Fig. 1): *Nototheniobdella* (Piscicolinae), *Trulliobdella* (Piscicolinae), *Moorebdellina* (Pontobdellinae), and *Crangonobdella* (Platybdellinae). Three of collected species have been already described (*T. capitata*, *N. sawyeri* and *M. cf. uschakovi*). Details of infection parameters and site of infection are presented in Table 1. Since previous studies documented species of *Moorebdellina* mostly in their free-living stage and only one specimen was collected from Pycnogonida, our study reported the first observation of the genus feeding on fish host in Antarctica. Specimens considered as the new species also provided the first record and molecular data of the genus *Crangonobdella* in Antarctica. Phylogenetic reconstruction of piscicolids in polar areas placed the collected species into both major clades and confirmed previous suggestions about the polyphyly of the subfamily Pontobelinae as well as the genus *Calliobdella*. Moreover, the results support historical connections between Arctic and Antarctic leeches probably promoted by host migration in the past. Phylogenetic reconstruction shows low support in most deeper nodes indicating the still high portion of undescribed species richness and missing molecular data.

## Future plans

Our main purpose for the near future is analysing the global phylogenetic relationships of piscicolids in order to reveal the origins of selected leech species in Antarctica.

## Acknowledgement

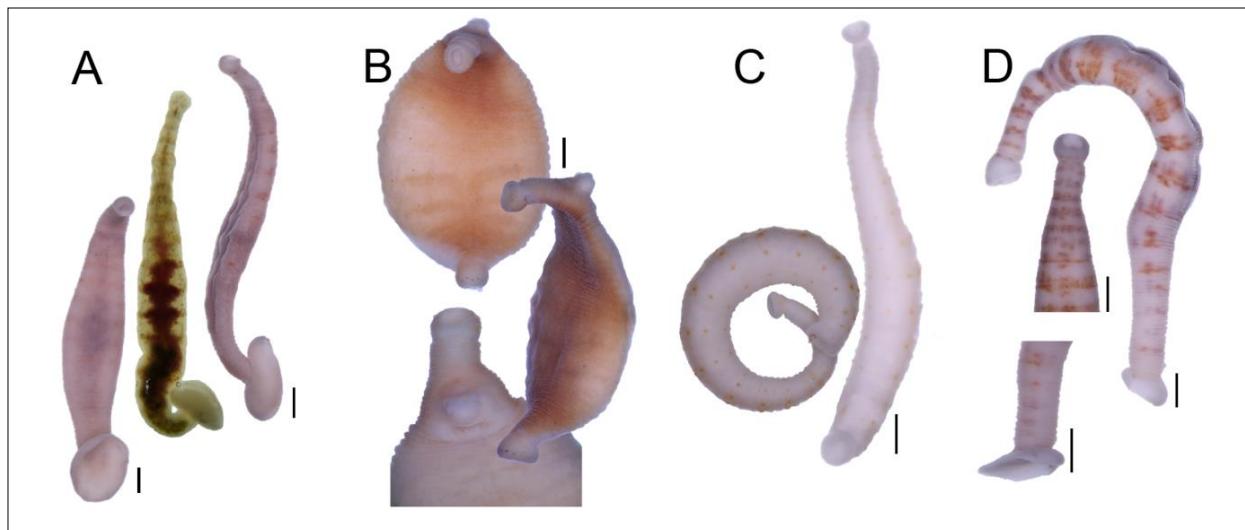
This study was supported by the Czech Science Foundation (project P505/12/G112). We acknowledge the Czech Antarctic Station "J. G. Mendel" and its crew for their support.

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**Table 1.** Overview of infection parameters of piscicolid leeches on the different host species.

Host species	Piscicolid species (subfamily)	Infected/ Dissected	Prevalence	Intensity of infection	Site of infection
<i>Trematomus hansonii</i>	<i>Crangonobdella</i> sp. (Platybdelinae)	3/33	9%	1–2	behind operculum
<i>Trematomus bernacchii</i>	<i>Nototheniobdella sawyeri</i> (Piscicolinae)	10/30	33%	1–2	body surface (skin)
	<i>Truliobdella capitis</i> (Piscicolinae)	1/30	3%	1	fin
	<i>Crangonobdella</i> sp. (Platybdelinae)	1/30	3%	3	gills
<i>Notothenia coriiceps</i>	<i>Moorebdellina</i> cf. <i>uschakovi</i> (Pontobdellinae)	1/17	7%	1	gills



**Figure 1.** Overview of recorded leeches in Antarctic fishes. A - *Nototheniobdella sawyeri* from *Trematomus bernacchii* and *T. hansonii*; B - *Truliobdella capitis* from *T. bernacchii*; C - *Moorebdellina* cf. *uschakovi* from *Notothenia coriiceps*; D - *Crangonobdella* sp. from *T. bernacchii*. Scale bar = 1 mm.

# ***Paradiplozoon hemiculteri*, real one!**

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The members of family Diplozoidae are bloodsucking ectoparasites which live on the gills of freshwater fish. Our study is focused on Asian diplozoons, because the morphological and molecular knowledge of this group of organisms is unclear. Due to the lack of publications in English, complex taxonomic and phylogenetic studies using together the morphological and molecular characteristics, it is difficult to distinguish between closely related Asian diplozoid species.

In order to prove the existing information, we collected the samples of diplozoons from freshwater fish originated from southern part of China (province Guandong). We dissected 29 freshwater fish species from Hanjiang, Wuijang and Zhenshui River. Three of the fish species were infected by diplozoons, including fish host *Hemiculter leucisculus* parasitized by *Paradiplozoon hemiculteri*. The taxonomic position of this parasite is not clear and type material is missing.

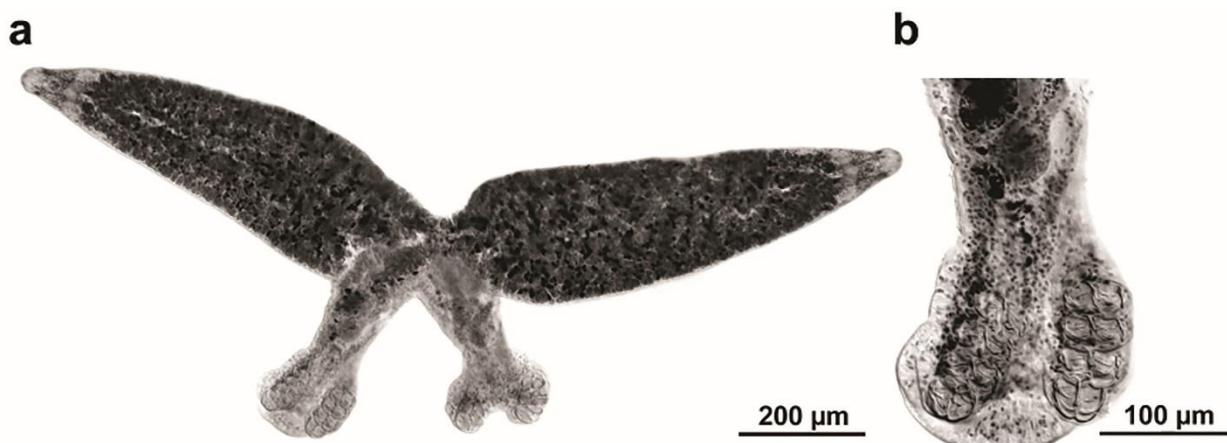
Therefore collected 12 individuals of *P. hemiculteri*, which were morphologically and molecularly analysed. The length and width of the body, buccal suckers, pharynx, attachment clamps and sickle and handle of central hooks were measured. Shape of anterior and posterior part of median plate, anterior and posterior joining sclerites were accurately documented. The phylogenetic analyses based on the sequence of second internal transcribed spacer (ITS2) of ribosomal DNA were performed. Based on our data, we designated the neotype of *Paradiplozoon hemiculteri* (Ling 1973).

## **Future plans**

Finish the phylogenetic analysis and submit a paper concerning the redescription of *P. hemiculteri*.

## **Acknowledgement**

This research was supported by Czech Science Foundation (GBP505/12/G112).



**Figure 1.** *Paradiplozoon hemiculteri*. A; total view. B; two rows of clamps on opisthaptor.

# ***Eudiplozoon nipponicum*: "Together – chance or destiny?"**

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The family Diplozoidae Tripathi, 1959 involves a specific obligatory blood sucking ectoparasites localized on the gills of freshwater fish. In our study we used experimental model *Eudiplozoon nipponicum*, isolated from common carp (*Cyprinus carpio*) originated from two different South Moravia localities (ponds). The life cycle of this parasite represents a unique biological phenomenon – two immature individuals (diporpa) meet on the gills of fish, subsequently permanently fuse together and perform X-shape body. The fusion of two individual larvae evokes many fundamental biological questions, which can be addressed. Thus far, this topic was rather overlooked and there is no satisfactory explanation how the two partners find, recognize and stimulate each other for fusion.

We applied whole genome scanning method – amplified fragment length polymorphism (AFLP) to find, if the permanently fused worms share genetic pattern specific for the “couple” or if they pair up randomly.

All the adult worms were placed buccal suckers facing upwards and split into three parts individual A, individual B and center section with material from both individuals – C.

Our preliminary results showed that individuals from same locality share the similar genetic pattern. This locality-specific pattern might be crucial for selection and further fusion of diporpa into the adult worm. Moreover, we also discovered that only one of both parts - A or B corresponds more to C part of the worm. This phenomenon might indicate presence of dominating genome that can possibly affect the genetic gear in following generation.

## **Future plans**

Finish the biochemical analyses of expressed recombinant protein molecules (*E. nipponicum* Kunitz proteins, serpins, cystatins)

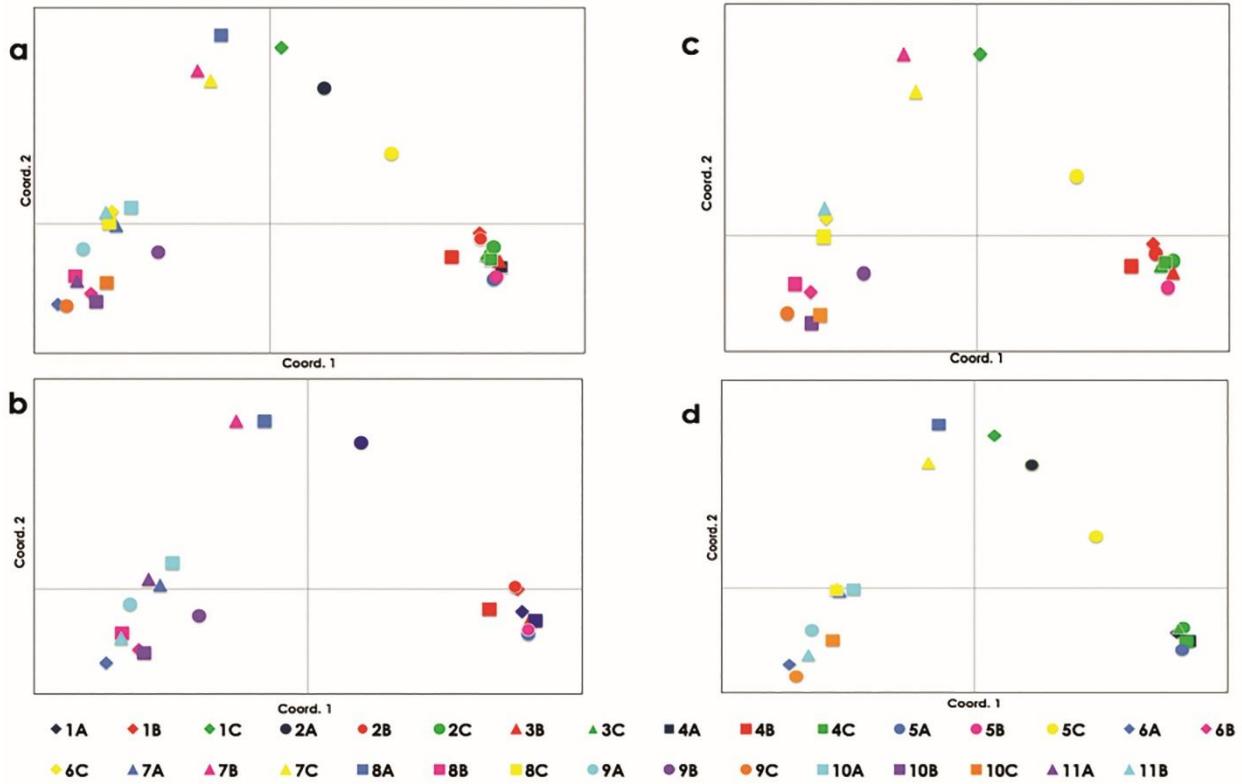
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**Figure 1.** Results of PCoA based on genetic distances of samples from a) all parts of adult worm A, B, C; b) A and B; c) B and C; d) C and A part.

# Monogenea: parasite-host interactions at molecular level

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The majority of known monogenean species are ectoparasites of fish with monoxenous life cycle in freshwater or marine ecosystems. Several species are of high economic importance, being highly pathogenic to fish and causing significant mortality, which can result in losses in aquaculture. In contrast to these facts almost nothing is known about their interactions with fish hosts at molecular level and functions of particular monogeneans molecules. Up to now, only the results of number of studies focused on taxonomy, phylogeny, zoogeography, morphology/anatomy, basic biology, ecology, ecotoxicology and pathogenicity were predominantly published.

Therefore, in the frame of the project ECIP, we developed an effort leading to change this situation and adopted the experimental organisms *Eudiplozoon nipponicum* (Diplozoidae, Heteronchoinea); a blood-feeding monogenean inhabiting gills of common carp (*Cyprinus carpio*). This worm originates in East Asia and became an invasive species – in Europe it was for a first time recorded in 1983 (farmed carps in France) (Denis et al. 1983). Currently it is spread over the Europe, including Czech Republic. Its pathogenic effect consists mainly of the induction of hypochromic microcytic anemia as a result of continuous intake of blood by the worms attached to the gills.

Hematophagy is a successful trophic strategy employed by many multicellular parasites; it is evident that it has evolved repeatedly, independently within several taxa, including members of, e.g., Platyhelminthes, Nematoda, Annelida, Arthropoda. There are some crucial issues for every blood-feeder which must be effectively solved on molecular basis: inhibition of hemostasis by anticoagulant factors, avoiding/blocking host immunity by inhibition or modulation of immune mechanisms like complement cascade, inflammation etc., and effective digestion of blood proteins by proteolytic enzymes.

Our research approach deals with molecular mechanisms underlying these aspects and three groups of serine and cysteine peptidase inhibitors (Kunitz proteins, serpins and cystatins) together with proteolytic enzyme counterparts of *E. nipponicum* are studied. All of these types of molecules play crucial roles in living organisms by regulation of endogenous proteolytic activities involved in almost all aspects of physiology.

## Future plans

Finish the biochemical analyses of expressed recombinant protein molecules (*E. nipponicum* Kunitz proteins, serpins, cystatins)

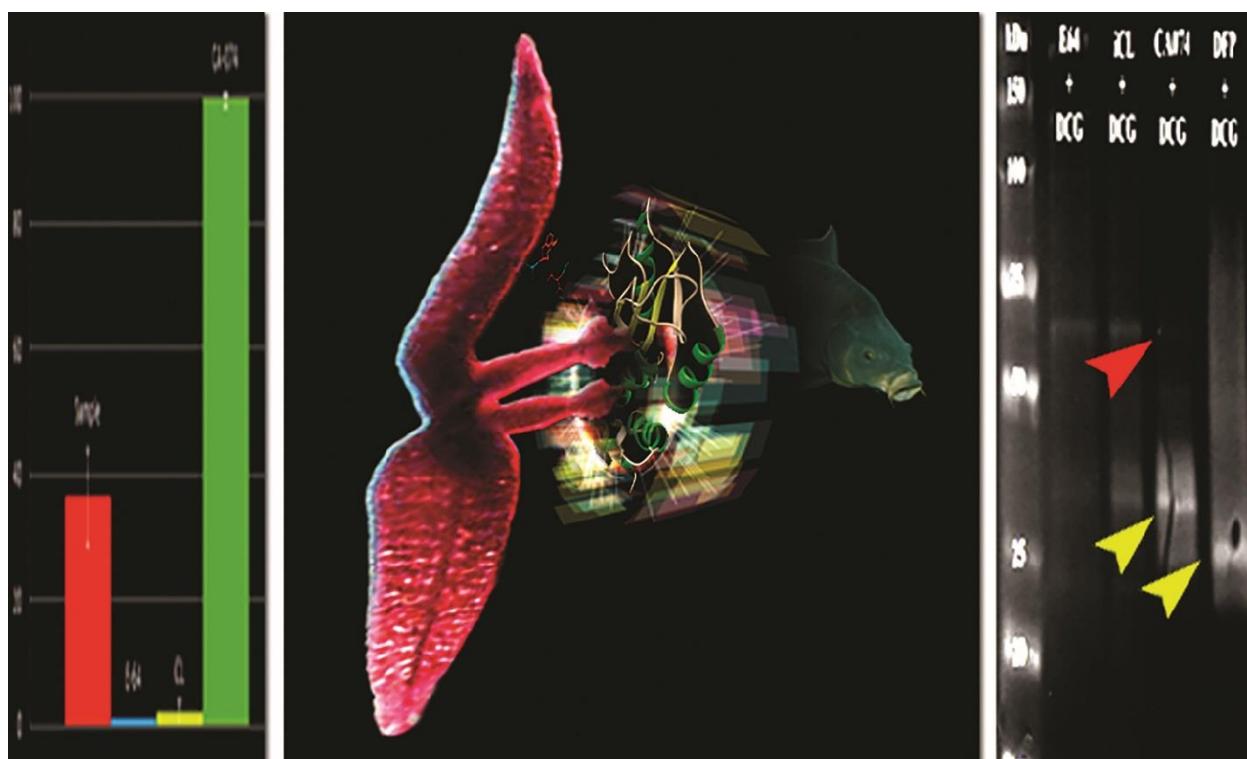
## Acknowledgement

This research was supported by Czech Science Foundation (GBP505/12/G112, P506/12/1258) and grants of the Masaryk University (MUNI/A/1325/2015).

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**Figure. 1.** Example of molecular nature of parasite-host interaction. On the left side inhibitory effect of cystatin molecules; on the right side the identification of cysteine peptidases by adoption of fluorescent probe DCG-04.

# A preliminary phylogenetic analysis of dactylogyrids (Monogenea) parasitizing African tetras

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The order Characiformes is one of the largest and most diverse components of the Neotropical and African ichthyofaunas. African freshwaters harbour more than 200 characiform species currently arrayed in four families: Alestidae (118 species), Citharinidae (8 species), Distichodontidae (101 species), and Hepsetidae (5 species). Alestidae, often called African tetras, are known to be parasitized by monogenean platyhelminths representing three genera: *Annulotrema* (39 species), *Characidotrema* (10 species), and *Afrocleidodiscus* (1 species).

During field trips (2007–2015) we sampled 11 species of African tetras (*Alestes baremoze*, *A. dentex*, *Arnoldichthys spilopterus*, *Brycinus imberi*, *B. leuciscus*, *B. nurse*, *Hydrocynus forskahlii*, *H. brevis*, *H. vittatus*, *Micralestes accutidens*, *M. elongatus*), and revealed the presence of monogeneans belonging to three genera within the Dactylogyridae: *Annulotrema* (19 species), *Characidotrema* (5 species), and *Afrocleidodiscus* (1 species).

In 2016, I continue to work towards my Ph.D. goals that are as follows: (A) to investigate species diversity of dactylogyrids of African tetras from seven African countries (Democratic Republic of the Congo, Kenya, Nigeria, Senegal, South Africa, Sudan, and Zimbabwe [1]); (B) to evaluate the relative taxonomic importance of the characters of the reproductive organs and those of the haptoral sclerites in accordance with molecular data [2]; and (C) to investigate the relationships between dactylogyrid species from African tetras based on nuclear ribosomal DNA gene sequences. Preliminary phylogenetic analyses confirmed the monophyly of *Characidotrema* species [3]. Species of *Afrocleidodiscus hydrocynous* and representatives of *Annulotrema* clustered together, while *Afrocleidodiscus* sp. 1 from *Distichodus rostratus* (Characiformes: Distichodontidae) forms separate cluster close to the *Characidotrema* spp. Division of both *Afrocleidodiscus* species highlights the necessity of revision this genus.

## Future plans

Description of species new to science found on African tetras from the Democratic Republic of the Congo, Nigeria, and Sudan will be achieved combining morphological and molecular approaches. Phylogenetic manuscript dealing with position of dactylogyrids from African tetras and selected representatives of Dactylogyridae of the Old World and Neotropics based on nuclear gene sequences will be prepared.

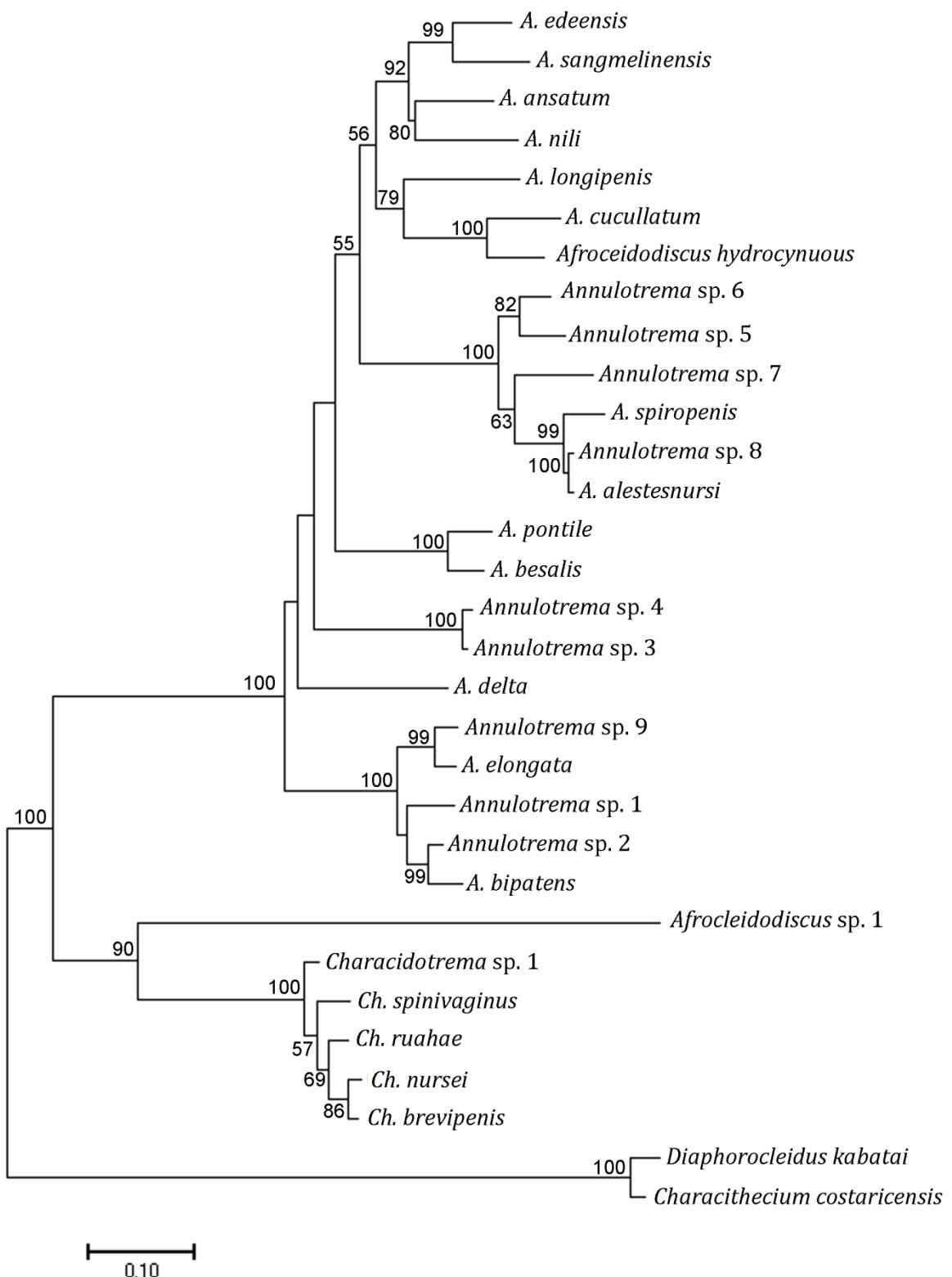
## Acknowledgement

This research was supported by the Czech Science Foundation No. P505/12/G112 (ECIP). A special thank is due to Iva Přikrylová, Maarten Vanhove and Carlos A. Mendoza Palmero for collecting monogeneans and/or their hosts.

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**Figure 1.** Molecular phylogeny of Dactylogyridae parasitizing African tetras estimated by Maximum Likelihood using partial sequences of the 28S rRNA gene. *Diaphorocleidus kabatai* and *Characithecium costaricensis* from *Astyanax aeneus* (Characidae) were used as outgroup taxa.

# Different motility modes in marine gregarines representing the early emerging group of Apicomplexa

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Apicomplexa, comprising exclusively parasites of invertebrates and vertebrates, represents a highly monitored group of protists. The motility of apicomplexan invasive stages (zoites), facilitated by a mechanism of substrate-dependent gliding, was investigated in important human pathogens as an essential mechanism for parasites' migration and host cell invasion [1]. Gregarines represent a highly diversified group of ancestral apicomplexans, with diverse host-parasite interactions and modes of locomotion, mechanism of which differs from apicomplexans zoites.

In gregarines, the principles of motility are discussed in phylogenetically distinct groups (e.g. archigregarines, eugregarines). In our research, the so called pendular or rolling movement (Fig. 1) in archigregarine *Selenidium* sp. from marine polychaete was investigated after the application of drugs inducing assembly or disassembly of actin filaments (jasplakinolide, cytochalasin D) and depolymerisation of microtubules (oryzalin, colchicine). For archigregarines belonging to the family Selenidiidae, it was generally suggested that the regular sets of subpellicular microtubules lying under low and broad bulges separated by grooves formed by the parasite three-layered pellicle represent the main motility motor [2, 3]. Our study confirms the influence of microtubules depolymerising drugs on *Selenidium* sp. motility. Despite the evidence that movement was blocked, experiments also revealed the resistance of gregarines to cytoskeletal drugs' concentrations routinely used in other apicomplexans and their prolonged survival (90 min - 9 h) in extremely high doses (10 µM - 100 mM) of these probes; after transfer to fresh sea water their motility was restored. Treated parasites were fixed for electron (SEM, TEM) and confocal laser scanning (CLSM) microscopic analyses. In SEM observation no superficial changes of *Selenidium* sp. surface were observed, while the evident changes in distribution of cytoskeletal proteins after CLSM analysis were documented [4].

Moreover, the influence of changed ions concentration in artificial sea water (prepared according to the salinity of 25 ppt and ions composition of the White Sea) on archigregarine (*Selenidium* sp.) and eugregarine (*Polyrhabdina* sp.) motility was investigated. Both species parasitise the same host, but represent phylogenetically distinct groups and their motility modes significantly differ (pendular vs. gliding motility). The composition of artificial sea water was evaluated as suitable for gregarines' survival, and even after prolonged incubation their movement did not show any evidence of modification. Further experiments were performed with changed concentration of ions: Na<sup>+</sup>, K<sup>+</sup>, Mg<sup>2+</sup> and Ca<sup>2+</sup>. Gregarines were incubated in these conditions long time period and the influence of sea water composition on gregarines' movement was monitored. Motility index was calculated in set time intervals for both species. Although no significant changes in gregarines' survival and motility were observed under the light microscope, gregarines were fixed for ultrastructural analysis (TEM).

Furthermore, the gliding motility was studied in marine eugregarine *Cephaloidophora* cf. *communis* parasitising the crustacean host *Balanus balanus*. The structures related to attachment and motility in trophozoites and gamonts covered with a three-layered pellicle organised in longitudinal epicytic folds were investigated using SEM, TEM and CLSM. The results of the study showed, that the unusually active and variable modes of motility (e.g. jumping and rotational movements with rapid changes in the gliding direction and cell flexions) are facilitated by a unique architecture of parasite epicytic folds.

## Future plans

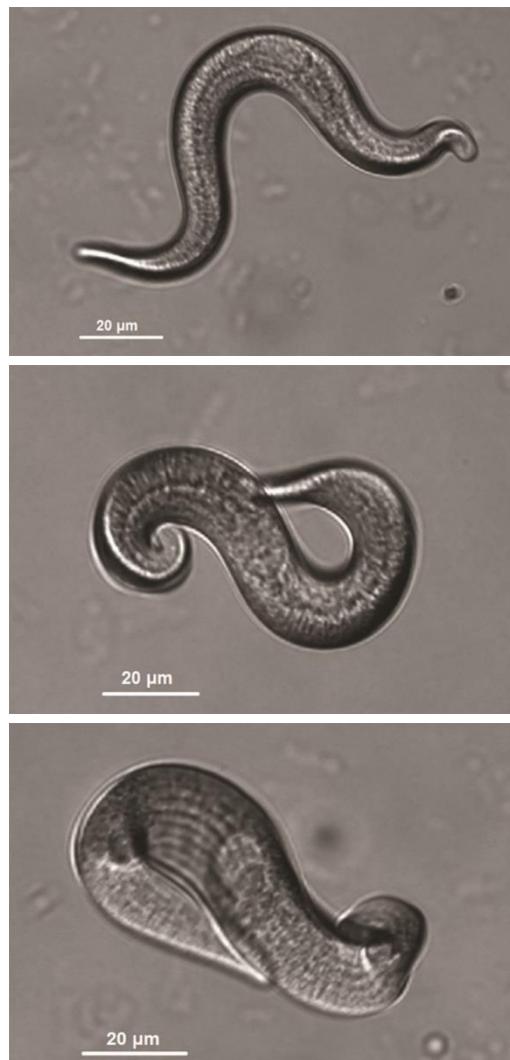
Further microscopic analyses of model marine apicomplexans, to define the motility mechanisms and to summarise obtained data for publication.

## Acknowledgement

This study was supported by ECIP (European Centre of Ichthyoparasitology); centre of excellence program of the Czech Science Foundation; project No. GBP505/12/G112.

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**Figure 1.** Sequences of movement showing the pendular/rolling motility in *Selenidium* sp.

# Immunocompetence and parasite infection in hybrids of roach (*Rutilus rutilus*) and common bream (*Abramis brama*)

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Hybrid heterosis may underlie the success of the hybrid vigour (measured for example as hybrid resistance against parasites). Nevertheless, hybridization may also disrupt the system of host-parasite gene co-adaptation. As the result, different parasite load in parental species and hybrids is expected. Hybrids with features inherited from both parental species may express different physiology and immunity when compared to those of their parental species. Consequently, it also affects parasite infection and the composition of parasite communities in the hybrid fish hosts.

For this study, all metazoan parasites were collected from common bream (*Abramis brama*), roach (*Rutilus rutilus*) and their respective hybrids during spring and autumn 2011, 2012 and 2013. All fish specimens were identified using morphological traits and molecular markers (partial mitochondrial cyt *b* gene and 12 microsatellite loci). Basic physiological indexes were calculated to measure fish condition, vigour and immunocompetence. The aim of this study was to compare the physiological indexes, hematological parameters and selected measures of immunity between parental species and their hybrids and to search for the potential associations between host physiology (and immunity) and parasite load.

Spleen-somatic (SSI) and gonado-somatic (GSI) indexes were significantly affected by hybridization. We found that total parasite infection correlated negatively with the SSI in hybrid females and males of common bream. The positive relationship between SSI and the hepato-somatic index (HSI) was found for common bream. The positive relationship between HSI and total parasite infection in males of roach was found, but this relationship was not evidenced in common bream and hybrids. GSI and lysozyme activity were positively correlated to total parasite infection in males of common bream, nevertheless, both HSI and SSI were negatively correlated to total parasite load in this fish group. In addition, lysozyme activity was also positively correlated to parasite infection in females of common bream. The positive correlation between lysozyme activity and condition factor, and negative correlation between SSI and total parasite infection were also found in hybrid females. Interestingly, oxidative burst activity correlates negatively with endoparasites in hybrid fish males.

## Acknowledgement:

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# Molecular diversity of *Tylodelphys* spp. in fishes from South Africa

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Diplostomid metacercariae are important fish pathogens that have become in recent years a focus of intensive studies on most continents, especially in Europe and North America. Africa remains the continent where this group has not been studied intensively using modern molecular tools in combination with quality morphological analysis, an approach that proved to be extremely successful in similar studies elsewhere. The diversity of diplostomids in South Africa is poorly understood, with just two forms from fish and amphibians identified to the species level, i.e. *Tylodelphys xenopi* and *T. mashonensis*.

To extend the knowledge of diplostomid diversity in fish from South Africa, a pilot study was performed at the Lower Phongolo River in Ndumo Game Reserve in May 2016. A total of 10 species of fish belonging to six families was sampled opportunistically. Three species of fish were found to be infected with metacercariae of *Tylodelphys*. Metacercariae were recovered from the cranial cavity and vitreous humour of *Clarias gariepinus* (Clariidae) and *Nothobranchius orthonotus* (Aplocheilidae), and only from the vitreous humour of *Oreochromis mossambicus* (Cichlidae). Overall parasite intensity in the cranial cavity was remarkably high (7–249 metacercariae per fish for *N. orthonotus*; 151–1,343 metacercariae per fish for *C. gariepinus*). Seven species of fish examined during this study, *Acanthopagrus berda* (Sparidae), *Glossogobius giuris* (Gobiidae), *Labeo cylindricus*, *Labeobarbus marequensis* (Cyprinidae), *Tilapia rendalli* and *T. sparrmanii* (Cichlidae), were not infected with *Tylodelphys* spp.

Molecular identification was carried out using novel and recent data for the mitochondrial (cox1) and nuclear (28S and ITS1-5.8S-ITS2) loci for *Tylodelphys* spp. Bayesian inference and maximum likelihood analyses of the cox 1 gene dataset yielded a tree with strongly supported clades that correspond to three species: one named species, *Tylodelphys mashonensis* and two unidentified species of *Tylodelphys* (Fig. 1); this was corroborated by the 28S rDNA and ITS sequences. This study is the first to apply a barcoding approach to diplostomid diversity in South Africa and to report metacercariae of *Tylodelphys* from *N. orthonotus* [1].

## Future plans

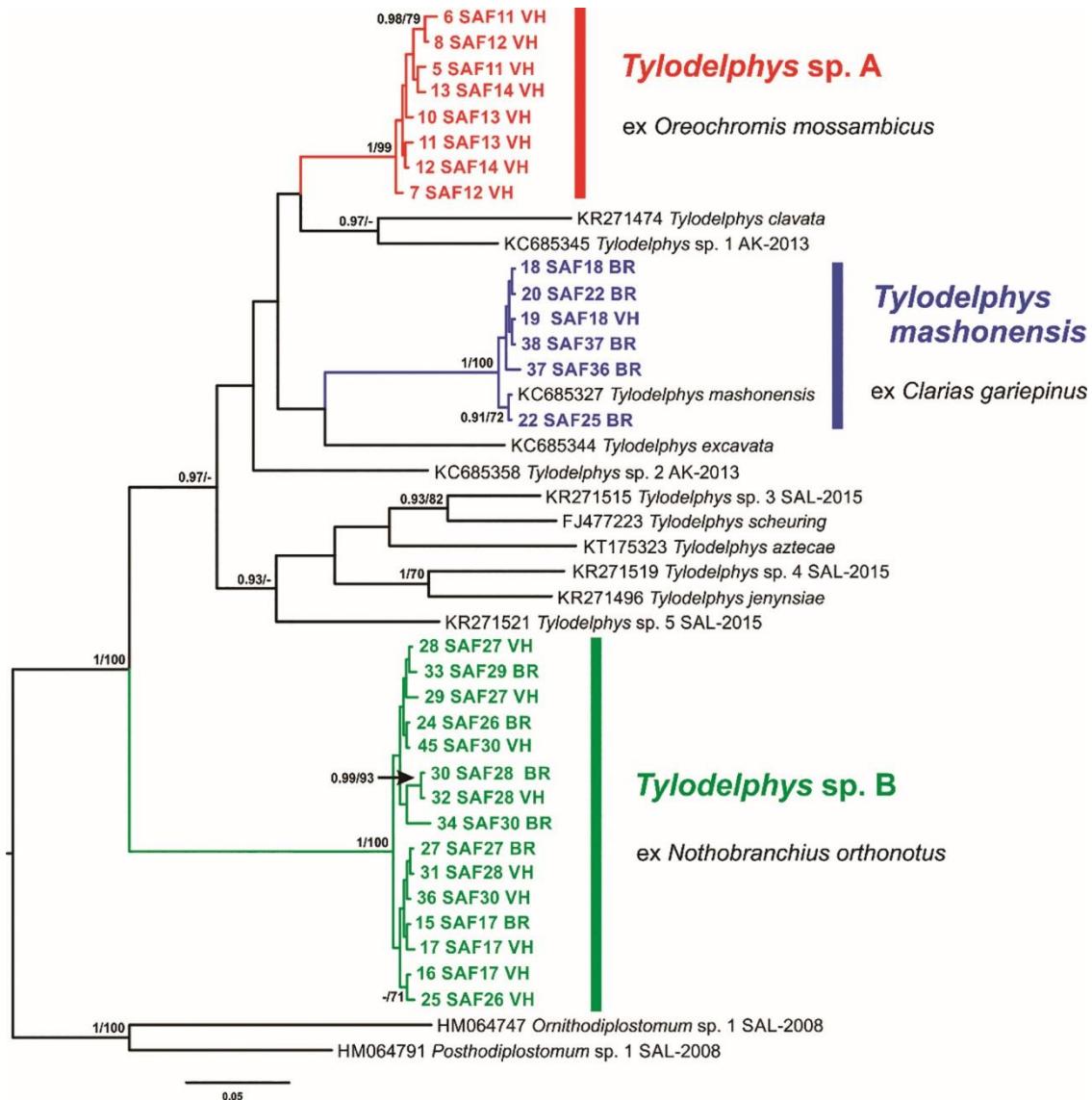
Morphological and morphometric assessment of the sequenced metacercariae and publication of the results. Morphological and molecular characterisation and phylogenetic relationships of metacercariae from different groups of diplostomids in fishes and amphibians from South Africa.

## Acknowledgement

This study was partially supported by ECIP (European Centre of IchthyoParasitology); centre of excellence program of the Czech Science Foundation; project No. P505/12/G112) and North-West University, Potchefstroom Campus, South Africa.

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**Figure 1.** Bayesian inference tree based on the mitochondrial gene *cox1* sequences for isolates of *Tylodelphys* spp. The newly-sequenced African isolates are shown in different colours. Bayesian inference posterior probabilities /maximum likelihood bootstrap support values are shown at the nodes. Support values < 0.90 (BI) and 70 (ML) not shown. The scale bar indicates the expected number of substitutions per site.

# **Ultrastructure of embryonated eggs of the cestode *Gyrocotyle urna* (Gyrocotylidea) using cryo-methods**

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Ultrastructure of eggs of the cestode *Gyrocotyle urna* Grube and Wagener 1852 with fully formed shells and early embryonic stages is described for the first time. Multiple techniques used included cryo-methods of high-pressure freezing with freeze substitution combined with field emission scanning electron microscopy and transmission electron microscopy (TEM). After 20 days of in vitro maturation, eggs are composed, from the exterior to the interior, of a thick egg shell of vitelline origin, an early embryonic envelope, a ring of numerous large vitellocytes, and a group of differentiating blastomeres situated in the center, forming the early presumptive larva. The thick resistant egg shell is similar to that reported from other polylecithal neodermatan flatworms. The embryonic envelope is poorly formed at this stage and not yet divided into inner and outer envelopes as occurs in all neodermatans described to date. Vitellocytes are separate, not forming a syncytium, and are filled with diverse vitelline materials as well as lipid droplets and associated endomembrane components having ultrastructure consistent with macroautophagy systems seen among many animals in apoptotic or autophagic cells. These results largely corroborate the basic observations and interpretations of previous TEM studies on other neodermatans and provide insight into a powerful suite of cryo-techniques that can enhance our work with these challenging subjects.

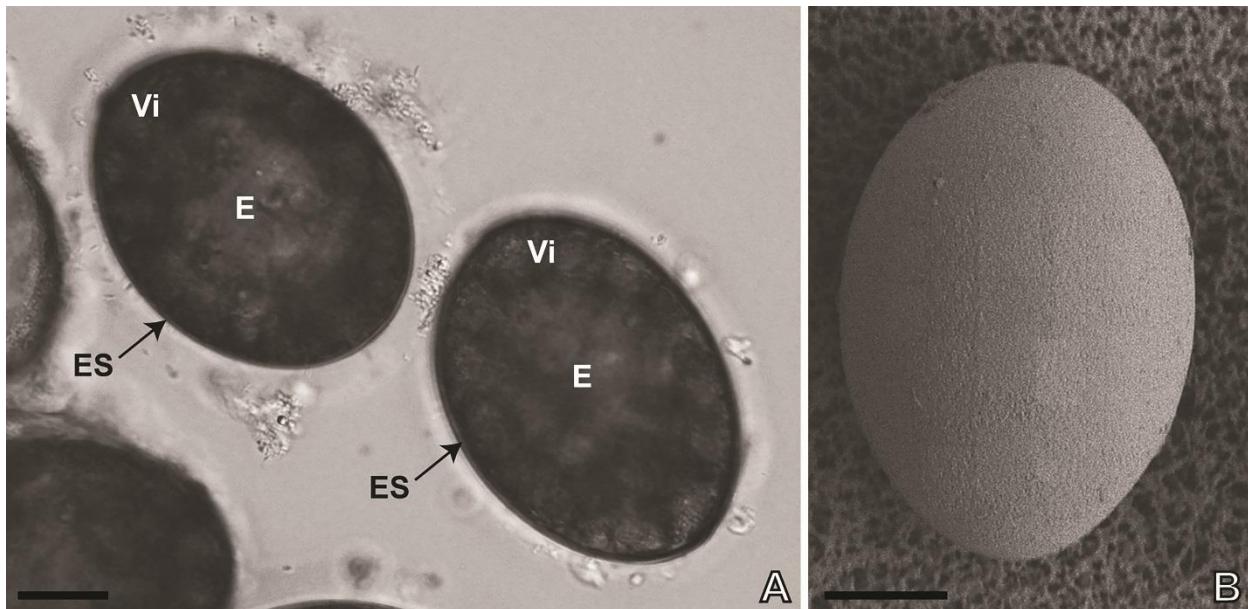
## **Acknowledgement**

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

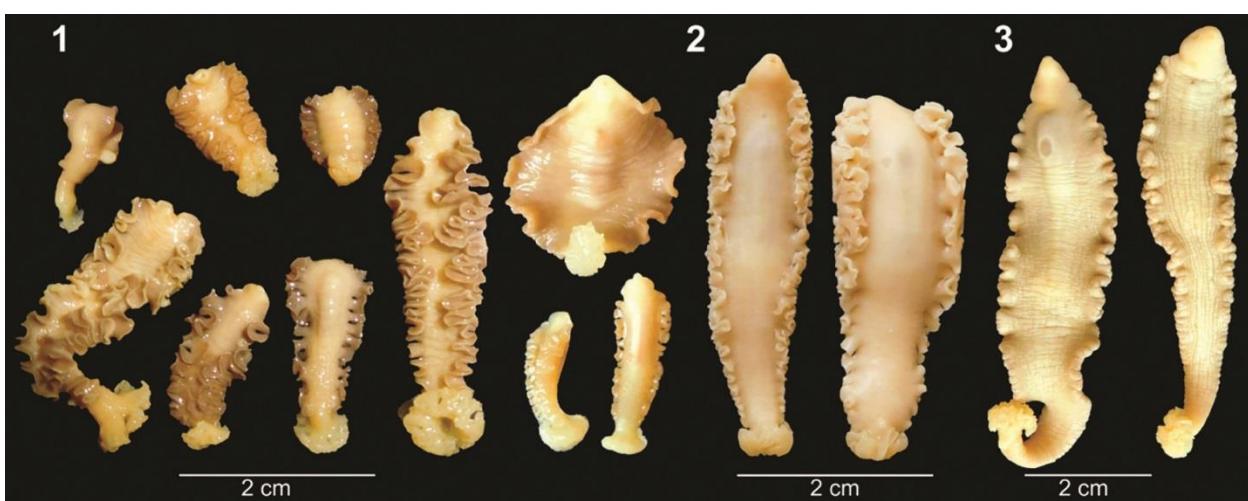
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**Figure 1.** General structure of *Gyrocotyle urna* eggs prepared for light microscopy (LM) and field emission scanning electron microscopy (FESEM). a Eggs under LM. E embryo, ES egg shell, Vi vitellocyte. Bar 20 lm. b Egg using FESEM. Bar 20  $\mu$ m.



**Figure 2.** Photomicrographs of gyrocotylideans obtained from North Atlantic. (1) *Gyrocotyle urna* from *Chimaera monstrosa*. (2) *Gyrocotyle* sp. from *Hydrolagus pallidus*. (3) *Gyrocotyle* sp. from *Harriotta raleighana*.

# First record of metacercariae of North American digenean, *Posthodiplostomum cf. minimum* (Strigeiformes: Diplostomidae) in Europe

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The American digenean *Posthodiplostomum cf. minimum* (MacCallum, 1921) (Strigeiformes: Diplostomidae) is a parasite of herons (Pelecaniformes, Ardeidae) in North America (Miller, 1954; Lumsden and Zischke, 1963). Its metacercariae cause "white grub disease" in fish, mainly centrarchids (Perciformes, Centrarchidae), such as sunfishes (*Lepomis* spp.) and crappies (*Pomoxis* spp.); Lewis and Nickum, 1964).

Here we report the first occurrence of the metacercariae of *P. minimum* in Europe. The cysts of the parasites were discovered in the mesentery and internal organs of two invasive Centrarchid fishes, *Lepomis gibbosus* and *Micropterus salmoides*, in Bulgaria, Czech Republic and Portugal. The analysis of ITS1-ITS2-28S sector of rDNA confirmed close relation between collected worms to American species of *Posthodiplostomum*, distancing them from common European species, *Posthodiplostomum cuticola*.

Out of the 11 localities investigated, the white grub disease was observed in seven of them: in Bulgaria (3 localities), Czech Republic (2 localities) and Portugal (2 localities). Metacercariae were completely absent in sites located in the Elbe River basin. The metacercariae of *Posthodiplostomum minimum* in white cysts were located mainly in liver, lesser in mesentery, kidneys, and rarely in spleen and brain of fish. The highest infestation of the fish with *P. minimum* was observed at two localities, Kula Reservoir (Danube River basin, Bulgaria) and Landeira Reservoir (Sado River basin, Portugal) and reached prevalence of 100% and 90% and maximum intensity of infection of 85 and 115, respectively. The lowest infestation was observed in the Struma River, Bulgaria, where only two specimens were found. Occurrence of *P. minimum* in the first intermediate host was not confirmed.

There are two possible ways of introduction of *P. minimum* to Europe: with the fish-host (Centrarchids) or with the first intermediate host (Physid snails). The American bladder snails, *Physella gyrina* (Say, 1821) and *Physella acuta* (Draparnaud, 1805), introduced to Europe, are confirmed as first intermediate host of *P. minimum* in America. The indigenous *Ph. fontinalis*, widely distributed in Europe, seems to be suitable intermediate host of *P. minimum* in Europe as well.

## Future plans

The non-indigenous digeneans will be cultivated to adults *in vitro* (on NCTC medium) and *in vivo* (in ducklings).

## 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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# Detecting myxozoan biodiversity using eDNA

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Myxozoa are cnidarian endoparasites that elude scientific study by having a two-host life cycle including an invertebrate and a vertebrate host with highly divergent morphologies. Furthermore, spores and developmental stages are microscopically small with sizes usually between 5µm to 100µm. To verify presence of infection in hosts trained experts with high quality microscopes are needed using histological and molecular methods for species identification. Species are known from all aquatic habitats all around the world but geographic sampling is naturally still patchy. Due to the paucity of our knowledge the overall biodiversity of this taxon is largely unknown and sounds estimates are not sound.

Myxozoan infection is transmitted between hosts through the water by spores. Even though spore densities can be very high in fishtanks and around aquaculture the amount of myxozoan DNA in natural environments is arguably much lower than that of the combined protist and eukaryote plankton.

The advent of high-throughput sequencing made the use of DNA extracted from environmental samples, such as water filtrates or sediment samples, possible. These techniques make it possible to sequence all DNA present in such a sample and can be tweaked to efficiently amplify taxonomically informative regions for certain lineages. For the latter approach primer design is a crucial, including all known myxozoan diversity to the exclusion of all non-myxozoan taxa.

We designed three primer pairs for all Myxozoa to the exclusion of all non-myxozoan taxa. These were compared to a universal eukaryote assay using Illumina MiSeq runs and three eDNA samples. Two of the samples were from the same site and date but had been spiked with i) low numbers of spores and ii) high number of spores before DNA extraction. Results showed that while the primers were able to pick up DNA even from spore counts less than five, they appeared restrictive with respect to the overall myxozoan diversity. Compared to the universal assay, the newly designed primers succeeded in reducing the amplification of non-myxozoan taxa. To overcome the taxonomic restrictiveness new primer pairs were designed for each of the four major lineages of Myxozoa independently, which are currently tested using Illumina MiSeq and IonProton.

With the increased use of high-throughput DNA sequencing of environmental DNA there are also more and more large datasets available online, e.g from studies designed to reveal and compare the taxonomic composition of higher taxa in different ecoregions and depths (e.g. Logares et al. 2014, deVargas et al. 2015). Using taxon specific queries and the stand-alone version of Blast we mine these for Myxozoa. The analyses of the results obtained by mining the 336 TARA datasets (de Vargas et al. 2105) are ongoing. Potential new lineages were found but amplification and verification of these sequences has not yet been successful. Since individual datasets can be very large suitable bioinformatic tools are necessary to make data mining and analyses of the results time-efficient. We are developing customised new scripts in order to mine further datasets as well as our own generated data. Mining 32 datasets published by Logares et al. (2014, Biomarks) has not resulted in any hits for Myxozoa.

## Future plans

Primer testing will continue. Suitable primer pairs will be used on environmental DNA from different localities around the world to facilitate a sound estimate of myxozoan biodiversity. Other published datasets will be mined for Myxozoan presence.

## 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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# New records and phylogenetic position of African freshwater camallanid nematodes

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We focused on nematodes mainly from the family Camallanidae from different host and localities in Africa (Lake Tanganyika and temporary seasonal water bodies of southern and central Mozambique) collected during different field trips. The obtained nematodes were measured and documented by light microscopy (drawings and micrographs) as well as scanning electron microscopy.

The first study concerns nematode larvae of four species of African annual killifish of the genus *Nothobranchius* (*Nothobranchius furzeri*, *N. kadleci*, *N. pienaari*, *N. orthonotus*) from Mozambique. Fish generations as well as their habitats are separated which limits parasite infection and transmission. Two species of camallanid nematodes and two morphotypes of gnathostomatid larvae were revealed in muscles and intestine of host fish. The morphological evaluation of gnathostomatid larvae *Spiroxys* sp. 2 and camallanid larvae *Camallanus* sp. was complemented with molecular analyses of nuclear ribosomal DNA sequence data (18S and ITS rDNA). The phylogenetic position of *Spiroxys* sp. 2 within the suborder Spirurina using 18S sequences was investigated for the first time. The results support the placement of *Spiroxys* spp. into the family Gnathostomatidae, monophyly of this genus and its close relationships with representatives of the family Anguillicolidae.

The second study concerns nematodes of the subgenus *Procamallanus* (*Spirocammallanus*) collected from six host cichlid species from Lake Tanganyika (lakeside of Burundi). Prevalence and intensity: fish infected/ fish examined, intensity of infection – *Cyprichromis microlepidotus*: 3/3, 1–5; *Boulengerochromis micolepis*: 2/4, 1–6; *Eretmodus marksmithi*: 2/36, 1; *Neolamprologus mondabu*: 5/17, 1; *Bathybates graueri*: 1/1, 1; *Trematocara unimaculatum*: 1/6, 2. Some of these nematodes are shown in Fig. 1. Currently, they are in the stage of identification. The specimens from the present material are biometrically more or less in agreement with the original description of marine or brackish-water species *P. (S.) olseni*. However they have ecological and morphological differences and does not fit exactly to the “Key to *Procamallanus* (*Spirocammallanus*) spp. parasitic in freshwater fishes of Africa” [1]. It will be probably new species from cichlids in Lake Tanganyika.

## Future plans

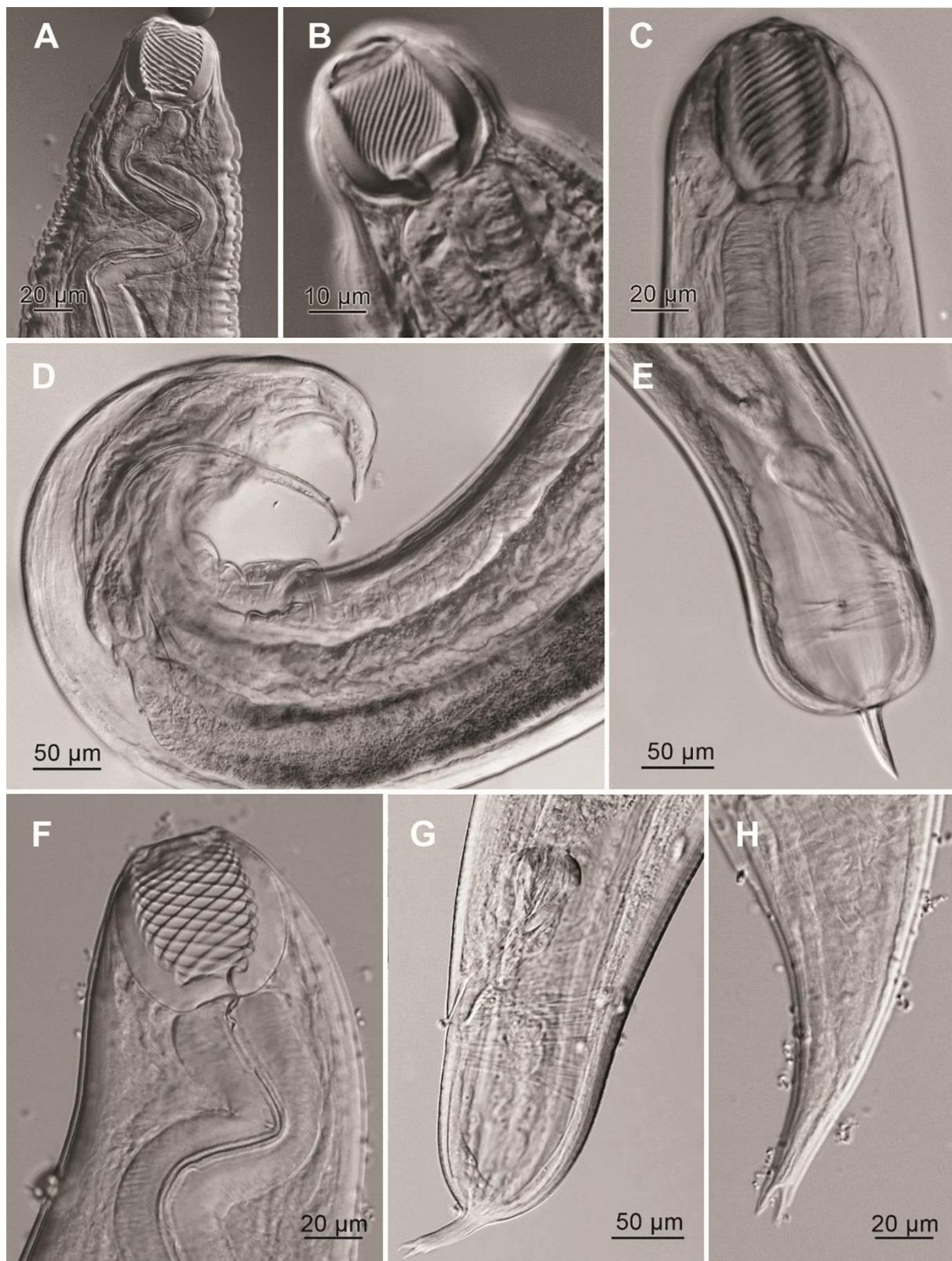
The results of the systematic evaluation of above mentioned nematodes will be presented. Detailed morphological and molecular description which can contribute to a better understanding of the comparative morphology and phylogenetic interactions of camallanid and gnathostomatid nematodes in Africa will be finished. Specimens of *Procamallanus* (*Spirocammallanus*) spp. will be part of a High School Scientific Acitivity (SOČ) defended in 2017.

## Acknowledgement

This study was supported by the Czech Science Foundation (project P505/12/G112).

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**Figure 1.** Overview of recorded *Procamallanus (Spirocammallanus)* spp. from different hosts: A, B – from *Trematocara unimaculatum*; C,D,E from *Cyprichromis microlepidotus*; F, G, H – from *Eretmodus marksmithi*. **A** – anterior part; **B**, **C**, **F** – buccal capsules with spirals; **D** – posterior part of male; **E**, **G**, **H** – posterior parts of females.

# New species of dactylogyrid monogeneans of the genera *Ameloblastella* and *Cosmetocleithrum* from Iquitos, Peru

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During a research on gill ectoparasites of siluriform fishes from the Peruvian Amazonia, the following monogeneans were found: *Ameloblastella edentensis* n. sp. from *Hypophthalmus edentatus* Spix & Agassiz; *Ameloblastella peruensis* n. sp. from *Hypophthalmus* sp.; *Ameloblastella formatrium* n. sp. from Pimelodidae gen. sp. (type-host) and *Duopalatinus* cf. *peruanus* Eigenmann & Allen; *Ameloblastella unapioides* n. sp. from *Sorubim lima* (Bloch & Schneider) (type-host) and *Pimelodus* sp.; *Cosmetocleithrum tortum* n. sp. from *Nemadoras hemipeltis* (Eigenmann); and *Cosmetocleithrum bifurcum* n. sp. from *Hassar orestis* (Steindachner) (both Doradidae) [1]. All new species described herein are mainly differentiated from their congeners based on the morphology of the copulatory complex. The pimelodids *H. edentatus* and *S. lima*, and the doradids *N. hemipeltis* and *H. orestis* represent new hosts species for species of *Ameloblastella* Kritsky, Mendoza-Franco & Scholz, 2000 and *Cosmetocleithrum* Kritsky, Thatcher & Boeger, 1986, respectively [2,3]. The morphological diagnoses of the present species of *Ameloblastella* and *Cosmetocleithrum* are also supported by a previous molecular analysis of these species [4].

## Acknowledgement

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# Alien gobiids in the diet of piscivorous fishes in a Central European lowland river

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Invasive fish, including gobies, have the potential to severely affect ecosystems by causing changes to local food webs. By altering energy flow through the aquatic food web, gobies could potentially affect growth rate and body condition of native predators, while native fish production may change through top-down effects as predators alter their feeding behaviour. High predation could even contribute to eventual control of the invasive species. Inclusion into the food web may also have important knock-on effects on native predators should gobies introduce novel parasites or act as novel hosts to existing parasites. Several studies from the Laurentian Great Lakes have shown that the invasive round goby *Neogobius melanostomus* has become a common prey of northern pike *Esox lucius* and burbot *Lota lota*. While gobiid consumption has been quite widely studied in North America, it remains relatively understudied in Europe. Hence, the aim of this study was a) to increase our knowledge of gobiid consumption by native European piscivores and b) to assess the ability of native piscivores to control non-native goby abundance/spread through predation.

Between 2012 and 2013, we monitored consumption of gobies by native predatory fish in the River Dyje, a mid-sized lowland river in the Czech Republic. In total, we analysed 267 digestive tracts from six native predatory fish of different ages: perch *Perca fluviatilis*, burbot, wels *Silurus glanis*, pike, Volga zander *Stizostedion volgense* and European zander *Stizostedion lucioperca*. Despite their relatively recent introduction (round goby first recorded four years previously), our results show that gobies have been fully incorporated into the Dyje food web. Gobies were commonly taken by burbot (68 % frequency occurrence) and 1+ perch (53 %) and slightly less often by wels (42 %) and pike (29 %). On the other hand, Volga zander took gobies rarely (4 %), showing a clear preference for native species (65 %). Goby remains were never observed in the digestive tracts of 0+ perch or zander. Perch >1+ and wels showed positive selection for tubenose goby *Proterorhinus semilunaris*, while burbot and pike showed a preference for round goby. A rough estimate of predation pressure suggests that predatory fish may be consuming around 16 % of all gobies on the river and are unlikely to be having any significant impact on numbers in the Dyje or preventing them spreading further. Consumption appears to be most related to encounter rate, i.e. predators feeding mainly along the rip-rap bank tend to consume them more often, and size. As such, adult burbot and perch are most likely to show most impact through changes in parasite loading.

## Future plans:

To further document those processes affecting predation of gobies by specific predators and to monitor the transfer of parasites to native predatory fish species in the River Dyje in relation to predation pressure.

## Acknowledgments:

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# **Descriptions of *Philometra aenei* n. sp. and *P. tunisiensis* n. sp. (Nematoda: Philometridae) from *Epinephelus* spp. (Osteichthyes: Serranidae) off Tunisia confirm a high degree of host specificity of gonad-infecting species of *Philometra* in groupers (Serranidae)**

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Based on light and electron microscopical studies of males and mature females, two new gonad-infecting species of *Philometra* Costa, 1845 (Nematoda: Philometridae) are described from the ovary of groupers, *Epinephelus* spp. (Serranidae, Perciformes), in the Mediterranean Sea off Tunisia (near Sfax): *Philometra aenei* n. sp. from the white grouper *E. aeneus* (Geoffroy Saint-Hilaire) and *P. tunisiensis* n. sp. from the goldblotch grouper *E. costae* (Steindachner). *Philometra aenei* is mainly characterised by the length of conspicuously distended spicules (108–123 µm), presence of a distinct dorsal barb at the middle region of the gubernaculum and a distinct protuberance consisting of two dorsolateral lamellar parts separated from each other by a smooth median field at its distal tip, a V-shaped mound on the male caudal extremity and by the body length of the males (2.34–3.05 mm). The male of this species was found to possess minute deirids in the cervical region, which is quite exceptional within the Philometridae. *Philometra tunisiensis* is distinguished from other gonad-infecting congeneric species parasitising serranids by the length of needle-like spicules and gubernaculum (201–219 µm and 78–87 µm), spicules representing 9–11% of the body length, the gubernaculum/spicules length ratio 1 : 2.52–2.77, the length of the male oesophagus comprising 15–16% of the body length, the absence of a dorsal protuberance on the distal lamellar part of the gubernaculum and a pair of large papillae posterior to the cloaca, a dorsally interrupted mound on the male caudal extremity and the body length of the male (2.01–2.42 mm). The presence of three morphologically very different species of *Philometra* in congeneric hosts in the Mediterranean Sea confirms a high degree of host specificity of these gonad-infecting nematodes parasitising groupers.

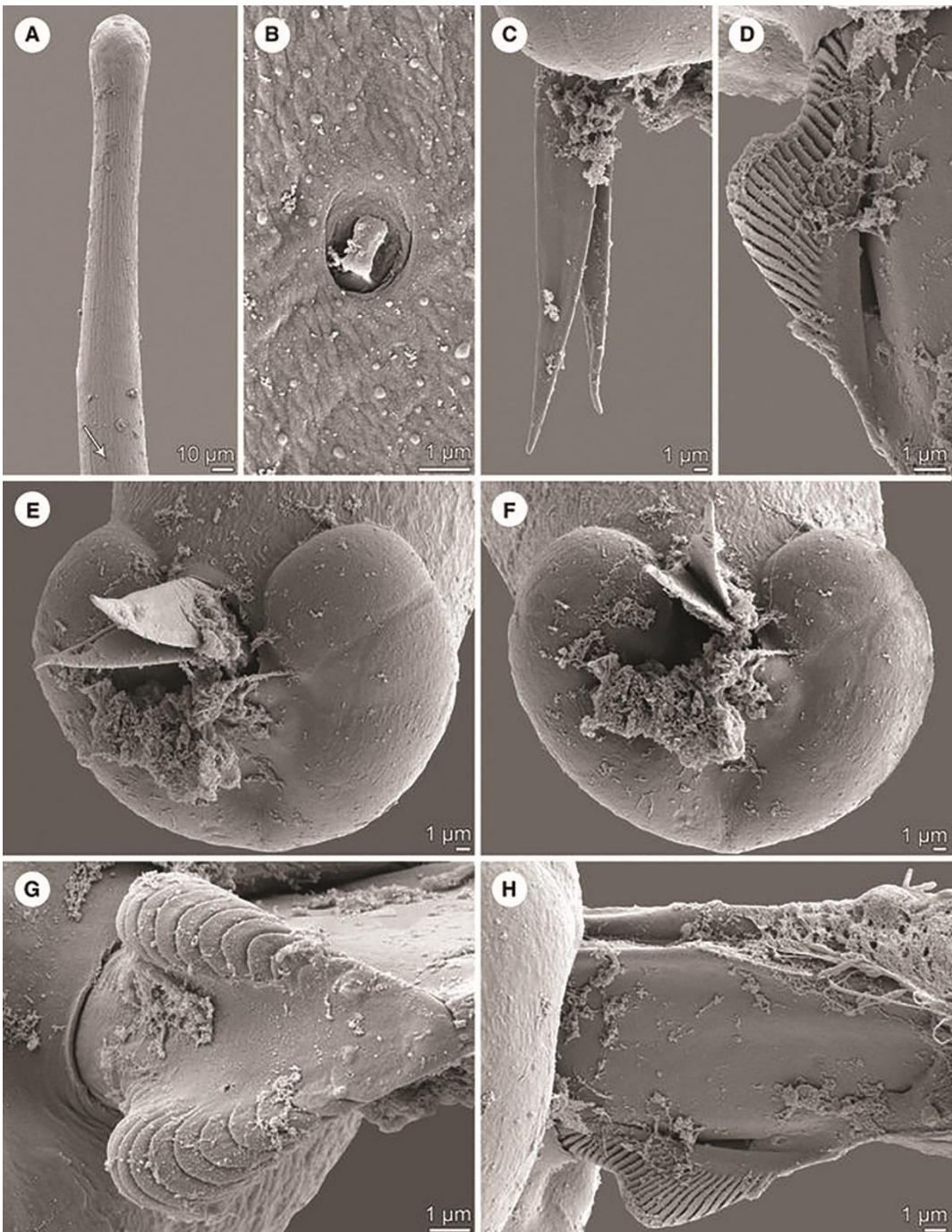
## **Acknowledgement**

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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**Figure 1.** *Philometra aenei* n. sp., scanning electron micrographs. A, Anterior end of body, lateral view (arrow indicates location of deirid); B, Deirid; C, Distal ends of spicules extruded from body, lateral view; D, Distal end of gubernaculum, lateral view; E, F, Caudal end, apical views (note the shape of extruded spicules in different positions); G, Distal end of gubernaculum, dorsal view; H, Extruded spicules and distal end of gubernaculum, lateral view.

# The effects of eye fluke *Diplostomum pseudospathaceum* on metabolism of European bitterling *Rhodeus amarus*

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Parasites are known to affect their hosts to increase the probability of successful transmission to subsequent hosts. Following our previous studies concerning effects of *Diplostomum* parasites on biology and behaviour of European bitterling, we now investigate the metabolic rate of *Diplostomum*-infected and control bitterling using intermittent flow respirometry in order to study the physiological consequences of parasitic infection.

European bitterling *Rhodeus amarus* L. is a small freshwater fish using living unionid mussels as a spawning substrate during reproduction. Males defend territories around the mussels and attract females to spawn into the mussels. Metacercariae of a trematode *Diplostomum pseudospathaceum* located in fish eye lenses may cause cataract formation and blindness and may also affect the physiology and behaviour of infected fish. To study the possible effect of *D. pseudospathaceum* on basal metabolism of European bitterling, we measured the metabolic rate of both males and females 1) infected several months ago (long-term infection), 2) infected during the first measurements in breeding season (short-term infection) and 3) uninfected control. Respiration experiments were carried out during the breeding season (May-June) and in autumn time (October-November). The fish were individually marked by visible implant elastomer tags in order to evaluate the possible temporal metabolic alteration and the relationship between the intensity of parasite infection and changes in host metabolic rate.

## Future plans

Completion of respiration experiments, the intensity of *Diplostomum* metacercariae infection in eye lenses of experimental fish assessment and results analysis.

## Acknowledgement

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

# Distribution of *Bucephalus polymorphus* in fish hosts after expansion of non-native gobies in the Morava River

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Introduced species may modify the local host-parasite dynamics by amplifying the infection which can 'spill-back' to native fauna, whether they are competent hosts for local parasites, or by acting as sinks with the 'dilution' of infection decreasing the parasite burdens of native hosts. Recently the infection of the trematode *Bucephalus polymorphus* has increased in several European rivers, being attributed to the introduction of intermediate host species from the Ponto-Caspian region.

*Bucephalus polymorphus* is a trematode parasite infecting the intestines of predatory fish (e.g. Percidae, Esocidae, etc.). Within its life cycle, unionid and dreissenid bivalves serve as the first intermediate host, a wide range of fish species serve as the second intermediate host, and piscivorous fish serve as a definitive host. Previous experimental studies showed that considerable 'spill-back' of *B. polymorphus* due to higher transmission rates after establishment of non-native gobies is partially buffered by decreased fitness of *B. polymorphus* that underwent development in gobies. As occurrence of this parasite among fish intermediate and definitive hosts was rather rare prior the introduction of gobiid fish in the Morava basin, we aimed to assess the host species spectrum and infection intensity after the goby expansion.

In the Morava River, we first tested the distribution of natural *B. polymorphus* metacercariae infection among the juvenile fish, the cohort highly susceptible to predation by predatory fish. Lower Morava represents a region inhabited by non-native gobies for almost ten years and shows significant fluctuation in juvenile fish community. We focused on the species dependent susceptibility to *B. polymorphus* infection, as well as ability to eliminate the parasite. Moreover, definitive hosts were examined for infection by adult trematodes to confirm successful completion of parasite life cycle in nature.

Out of the 10 and 6 juvenile fish species examined in 2015 and 2016, respectively, all except one (*Carassius gibelio*) was infected by *B. polymorphus* metacercariae. *Leuciscus idus*, *Abramis bjoerkna* and *Rhodeus amarus* were the most susceptible species, with *R. amarus* being the dominant species in juvenile fish community in both years. In contrary, the less susceptible species were *Rutilus rutilus*, *Alburnus alburnus*, *Barbus barbus*, *Neogobius melanostomus* and *Proterorhinus semilunaris*. Out of the species examined, *A. alburnus*, *A. bjoerkna*, *Squalius cephalus* and *P. semilunaris* exhibited high ability to eliminate the parasite and showed over 50% of dead parasites. *Sander lucioperca* examined for adult *B. polymorphus* showed high prevalence (67%) and abundance indicating the successful completion of parasite life cycle and increase in population density.

Our results showed similar trend in wide distribution of *B. polymorphus* among fish intermediate hosts as it was observed in 2011, shortly after the expansion of goby fishes in the lower Morava. However, the parasite abundance appeared to decrease in less abundant species, besides others reflecting the variation in composition of juvenile fish community.

## Acknowledgement:

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# Revision of the genus *Promonobothrium* (Cestoda: Caryophyllidea), tapeworm parasites of suckers (Catostomidae) in North America

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Species of *Promonobothrium* Mackiewicz, 1968 (Cestoda: Caryophyllidea), parasites of suckers (Cypriniformes: Catostomidae) in North America, are reviewed, with information on their taxonomic status, definitive hosts, life cycle and geographical distribution, together with new data on scolex morphology observed using scanning electron microscopy (SEM). Evaluation of type and voucher specimens from museum collections and newly collected material made it possible to recognize the following valid nominal species: *Promonobothrium minytremi* Mackiewicz, 1968 (type species); *P. ingens* (Hunter, 1927); *P. hunteri* (Mackiewicz, 1963); *P. ulmeri* (Calentine and Mackiewicz, 1966); *P. fossae* (Williams, 1974) and *P. mackiewiczi* (Williams, 1974). *Rogersus rogersi* Williams, 1980 is transferred to *Promonobothrium* based on morphological and molecular data. Moreover, two new species, *Promonobothrium currani* sp. n. and *P. papiliovarium* sp. n., are described from smallmouth buffalo *Ictiobus bubalus* (Rafinesque) and black buffalo *Ictiobus niger* (Rafinesque) in Mississippi, and from eastern creek chubsucker *Erimyzon oblongus* (Mitchill) in North Carolina, USA. The newly described species can be distinguished from other congeners by morphology of the scolex, position of the anteriormost vitelline follicles and testes, the presence of postovarian vitelline follicles, and morphology of the ovary. Molecular phylogenetic analyses of six species based on sequences of small and large subunits of nuclear ribosomal RNA genes (ssrDNA, lsrdNA) confirmed the monophyletic status of the genus and supported the validity of the species analysed.

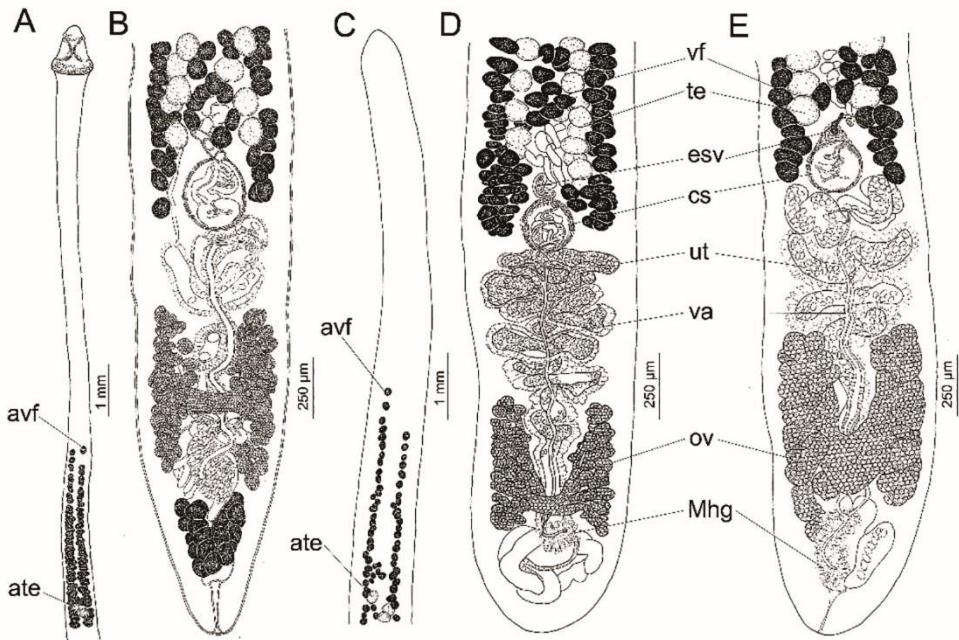
## Acknowledgement:

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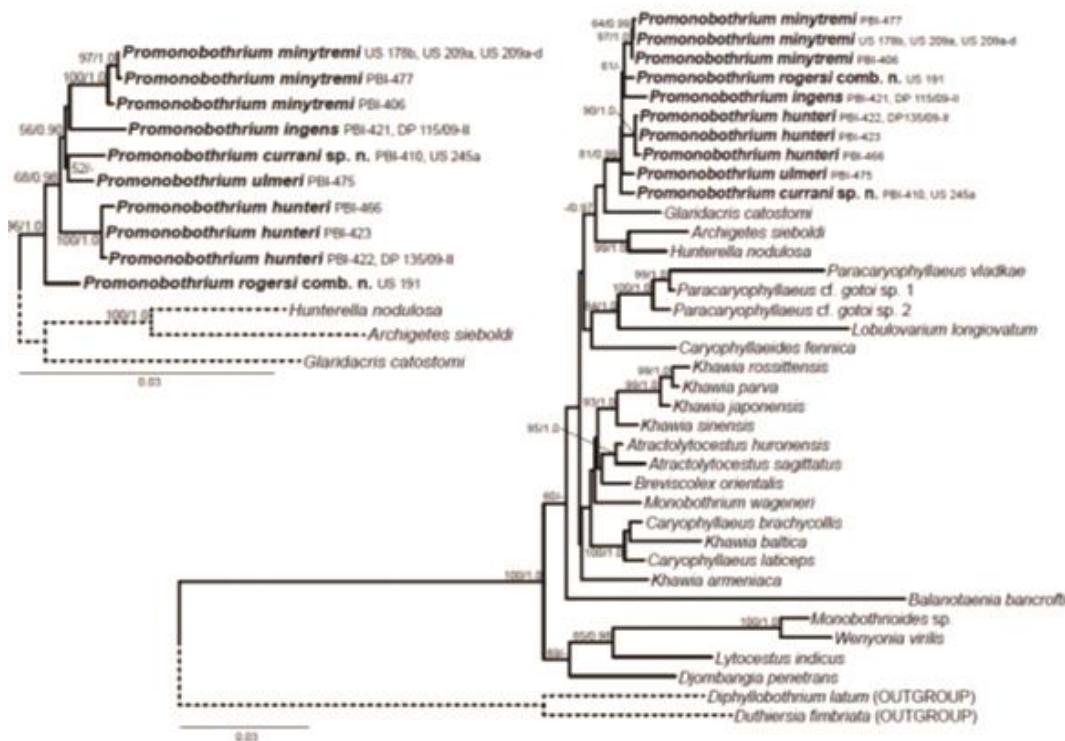
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**Figure 1.** Line drawings of new species of *Promonobothrium* Mackiewicz, 1968. A–B – *Promonobothrium currani* sp. n. from *Ictalurus bubalus*; C–D – *Promonobothrium papiliovarium* sp. n. from *Erimyzon oblongus*. E – *Promonobothrium rogersi* comb. n.. Abbreviations: ate – anteriormost testes, avf – anteriormost vitelline follicles, cs – cirrus-sac, esv – external seminal vesicle, Mhg – Mehlis glands, ov – ovary, te – testes, ut – uterus, va – vagina, vf – vitelline follicles.



**Figure 2.** Best Maximum likelihood estimates based on analyses of concatenated ssrDNA + lsrdNA genes analysed as separate partitions in GARLI. Phylogenetic position of *Promonobothrium* within the Caryophyllidea (right) and the interrelationships of *Promonobothrium* species (left).

# Evolution and genetic variability of the *Carassius auratus* complex

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The *Carassius auratus* complex is invasive species originating from East Asia. This species entered in the rivers of the Czech Republic in 1975. *C. auratus* is very unique cyprinid fish species with dual form of reproductive strategies i.e. sexual gonochoristic pathway and asexual pathway so-called gynogenesis. Gynogenesis is a special asexual reproduction strategy without the fusion of the egg and sperm cell to produce a mother clone with triple chromosomal set. In our study, we focused on the comparison of selected aspects of fish physiology, specific and nonspecific immunity (such as the activity of phagocytes, complement, lysozyme and IgM antibodies as part of specific immunity), as well as parasite load to explain the evolutionary strategies of this diploid-polyploid *Carassius auratus* complex. Finally, we focused on the genetic variability (haplotype variability) in diploid (sexual) population and polyploid (gynogenetic) females. Fish individuals were caught in the selected locality in the connecting channels Lednice-South Moravia situated on the Dyje River. Samples were collected in august in four consecutive years. Our samples of *Carassius auratus* complex were assigned to 16 mitochondrial haplotypes (selected control region mitochondrial DNA (D-loop) was used for this analysis). We reconstructed a haplotype tree where the specimens of *C. auratus* complex were designated to *Carassius auratus auratus*, *C. auratus gibelio*, *C. auratus M line* and *C. auratus langsdorfi*.

The study of genetic variability revealed that the majority of specimens (96%) belong to the *Carassius auratus gibelio* (represented by 13 different haplotypes with haplotype G02 being dominant). Three other forms of *C. auratus* complex were rare: *Carassius auratus auratus* (2%), *Carassius auratus M line* (1%) and *Carassius auratus langsdorfi* (0.5%). The species of 7 groups of metazoan parasites with the largest proportion of Monogenea were determined. Results of this study showed no significant difference in parasite abundance between diploid specimens and triploid females. Only Nematoda exhibited lower abundance in diploid males when compared to both females. Our analyses revealed significantly higher specific immunity analyzed by IgM for 3n females when compared to 2n males. Concerning the hematological parameters, only erythrocyte count was clearly affected by ploidy status and hematocrit was influenced by sex.

## Future plans:

To analyse MHC variability and the selection pressure acting on MHC genes in sexual diploids and gynogenetic triploids.

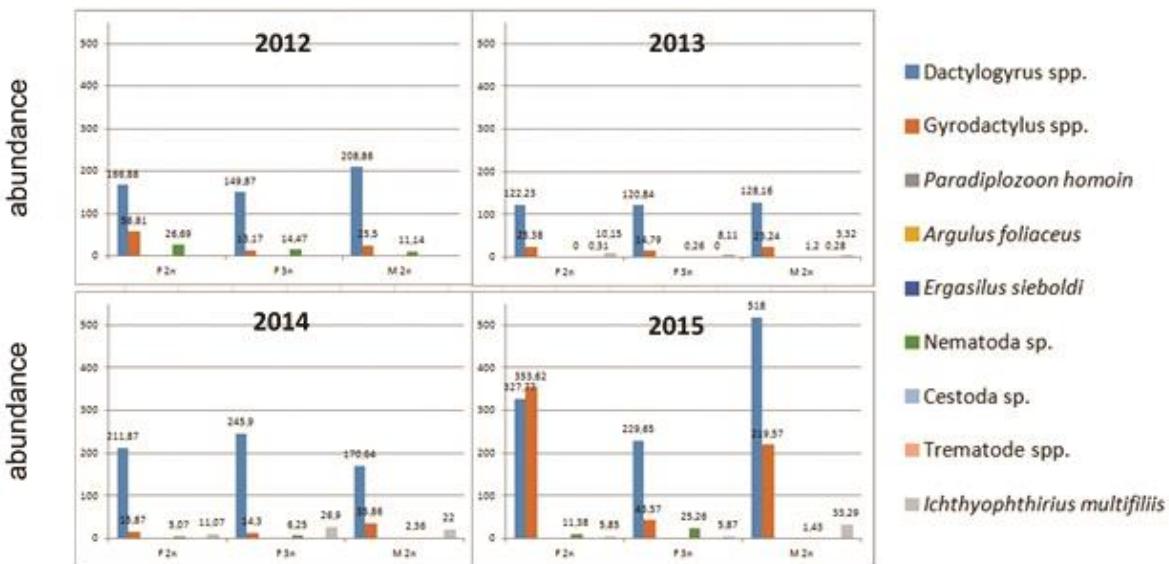
## Acknowledgement:

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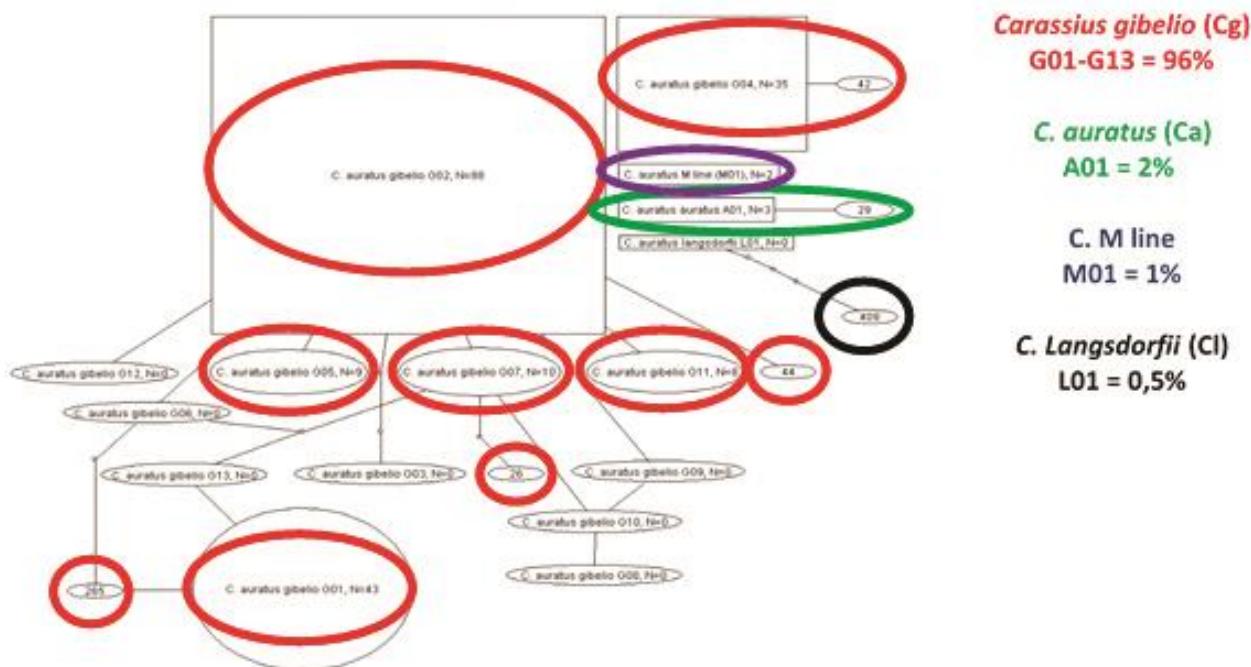
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**Figure 1.** Bar graph of parasites abundance in *Carassius auratus* complex (data collected in four consecutive years).



**Figure 2.** Haplotype scheme using D-loop sequences of *Carassius auratus* complex in this experiment (data collected in 2012 to 2015).

# Molecular diversity of new members of the *Sphaerospora sensu stricto* clade (Cnidaria: Myxozoa)

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Myxozoans are microscopic parasites belonging to the cnidarians. Phylogenetically, they cluster into four clades, the most basal Malacosporea, and three clades of Myxosporea, composed of the so-called ‘marine clade’, ‘freshwater clade’ and a clade composed of members of *Sphaerospora sensu stricto*. Around 80 *Sphaerospora* spp. have been described morphologically but 18S rDNA data is available on GenBank for only 17 species. The *Sphaerospora* s. s. clade is molecularly characterized by extensive insertions in the variable regions of 18S rDNA [1]. Presently, *Sphaerospora molnari* has the longest 18S rDNA sequence within in *Sphaerospora* s. s. clade which is composed of 3.7 kbp and is also one of the longest sequences amongst eukaryotes [2].

Over last four years, we have screened over 500 fish from total 11 fish families for *Sphaerospora* infection. We succeeded in various morphological descriptions and successfully amplified 11 complete and 4 partial new *Sphaerospora* s. s. 18S rDNA sequences, hence almost duplicating the presently available molecular information on members of *Sphaerospora* s. s. Amplification of sequences from members of this phylogenetic clade was notoriously difficult and various primer sets and combinations had to be designed and tested to obtain them as the long inserts in the variable regions of the 18S rDNA are unique for each species and the parasite’s mono- or disporous pseudoplasmodia, which are usually found in the renal tubules, cannot be physically isolated. Phylogenetic analyses using maximum likelihood and maximum parsimony methods produced an 18S rDNA based tree including all newly obtained sequences. We analysed the patterns of phylogenetic clustering within the *Sphaerospora* s. s. clade by annotating a number of biological characteristics such as aquatic environment, geography and fish host phylogeny.

We demonstrate that the only factor that is able to explain the clustering of the sphaerosporids is the phylogeny of the host and we propose a cophylogenetic and coevolutionary scenario, a hypothesis which is currently tested using relevant tree topology-based and distance based cophylogenetic methods. The detected patterns represent a new discovery as a previous dataset did not show the same cophylogenetic trend [3], likely due to the availability of limited data.

## Future plans

We would like to determine cophylogenetic links between the members of the *Sphaerospora* s. s. clade and their vertebrate host and estimate when host-switches have taken place. This would considerably aid our understanding of the evolution of the fast evolving *Sphaerospora* spp. Unfortunately, the unknown definitive (invertebrate) host for this clade of myxozoans leaves us unable to determine the relationship of sphaerosporids with their definitive hosts, a more distant future aim, once data become available.

## Acknowledgement

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

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# **Costly defence in a fluctuating environment – sensitivity of annual *Nothobranchius* fishes to predator kairomones**

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Anti-predator strategies increase the chances of survival of prey species but are subject to trade-offs and always come at a cost; one specific category being the “missed opportunity”. Some animals that can modulate the timing of life-cycle events, can also de-synchronise this timing with the occurrence of a predator. In an unpredictable environment, such a modification may result in a mismatch with prevailing conditions, consequently leading to reproductive failure. We examined the capability of multiple *Nothobranchius* populations and species to a) cease hatching when exposed to chemical cues from native predators and adult conspecifics and b) the ability of *N. furzeri* to modulate their growth rate in the presence of a gape-limited predator. As *N. furzeri* originate from a region with extreme environmental fluctuations where the cost of a missed opportunity can be serious, we predicted an inability to cease hatching but accelerated growth as both the predator’s gape limitation and the environment select for the same adaptation. Our results showed only one *N. furzeri* population ceased hatching (induced by adult conspecifics) and that killifish growth rate was not influenced by predator cues. This suggests that, in an unpredictable environment, the costs of a missed opportunity are substantial enough to prevent evolution of some anti-predator defence strategies.

## **Acknowledgement**

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# The relationship between fish host personality and susceptibility to parasite infection

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Consistent individual differences in behaviour, termed personality, are common in animal populations. Personalities are assumed to result from underlying tendencies that affect behaviour in different contexts, that vary across individuals and that are reasonably stable across time. Parasitism as an important factor in host behaviour may play a potentially important, but largely overlooked, role in the evolution of animal personalities. Parasite infection often leads to specific changes in axes of personality, having the potential to decouple behavioural syndromes. On the other hand, behavioural avoidance has often been observed as an efficient defensive response against parasites.

In our study, we focused on the impact of the host personality on susceptibility to parasitic infection in experimental conditions. We tested whether given host personality is associated with the susceptibility to parasitic infection and whether parasitic infection change the personality of particular hosts. The variability in the behaviour of the host may result in different opportunity for exposure to parasitism, wherein the susceptibility to parasites reflects primarily the immune response of the host.

As model organisms, we used artificially bred European bitterling (*Rhodeus amarus*, Cyprinidae) as a host and larval trematode *Diplostomum pseudospathaceum* as a parasite. One year old fish were tested for personality characteristics such as shyness-boldness continuum, exploration-avoidance continuum, general activity level and behavioural plasticity in three replications. After the first set of tests, half of the fish was selected based on the observation and experimentally infected by cercariae of *D. pseudospathaceum*. Subsequently fish were again tested during maturation of the parasite to detect potential change in their behaviour with respect to parasite infection. Observation of fish with mature parasites will be finished this autumn and consequently fish will be dissected for assessment of parasite infection level.

## Future plans

Tests in experimental conditions will be supplemented by tests in natural conditions, in order to effectively determine the relationship between the personality and parasitic infection.

## Acknowledgement

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# Monogenean parasites in South Africa: new challenges and many possibilities

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The largemouth bass *Micropterus salmoides* (Lacepède, 1802) is an alien invasive species in South Africa (SA) that was introduced into the country in 1928 from England. Following introduction it was stocked for angling throughout SA. To shed some light on the ecological status of parasite species present on alien invasive hosts, four largemouth bass, *Micropterus salmoides* (Lacepède, 1802), populations from four provinces (Eastern Cape, Western Cape, North West and KwaZulu-Natal) in South Africa were sampled to assess their parasite faunal composition. Morphological evaluation of the collected parasite specimens confirmed the presence of four monogeneans from the family Ancyrocephalidae: *Clavunculus bursatus* (Mueller, 1963), *Onchocleidus furcatus* (Mueller, 1937), *Onchocleidus principalis* (Mizelle, 1936) and *Syncleithrium fusiformis* (Mueller, 1934). Prevalence for these specimens were 100% at all three sites, but intensity of infection varied between the sites, with that of the North West and Western cape population being significantly lower ( $p = <0.0001$ ) than the other two sites. The presence of *O. furcatus* at all sampled sites indicates a strong parasite-host relationship and potential co-invader status. While the presence and/or absence of the other three specimens found suggest several introductions of largemouth bass stock of different origin [1].

Small size hosts are often neglected for the survey of monogenean parasites. The recent investigation at Ramsar wetland in South Africa during October 2014 and March 2015 showed that more attention should be given to these hosts. The cichlid *Pseudocrenilabrus philander* (Weber, 1897) was found to be parasitised by the monogenean *Gyrodactylus thlapi* Christison, Shinn and van As, 2005 and the cyprinid *Enteromius paludinosus* (syn. *Barbus paludinosus*) (Peters, 1852) was infected with the monogenean parasites *Dogielius intorquens* Crafford, Luus-Powell and Avenant-Oldewage, 2012, *Dactylogyrus teresae* Mashego, 1983, and three *Dactylogyrus* spp. [2]. An additional sampling, performed in October 2015, recorded the finding of *Gyrodactylus* sp. on *E. paludinosus* and this one is currently being described as a new species [3].

Species of the genus *Enteromius* Cope, 1867 were studied for their monogenean parasites at several spots along Limpopo and Olifants River systems, Limpopo Province, South Africa, from April 2015 till May 2016. Studied hosts, *Enteromius afrohamiltoni* (Crass, 1960), *Enteromius trimaculatus* (Peters, 1852) and *Enteromius unitaeniatus* (Günther, 1867) were infected by seven *Dactylogyrus* species, of which 4 are known to the science, *Dactylogyrus afrolongicornis afrolongicornis* Paperna, 1973; *Dactylogyrus afrolongicornis alberti* Paperna, 1973; *Dactylogyrus allalongionchus* Paperna, 1973 and *Dactylogyrus myersi* Price, McClellan, Druckenmiller & Jacobs 1969. Remaining three species are new for science and their description will be finished soon [4]. Morphometrical evaluation of the material from the study provided opportunity to study two subspecies *D. afrolongicornis afrolongicornis* and *D. afrolongicornis alberti*, and as a results of our observation we propose to synonymise them in a single species *D. afrolongicornis* only.

## Plans for next year:

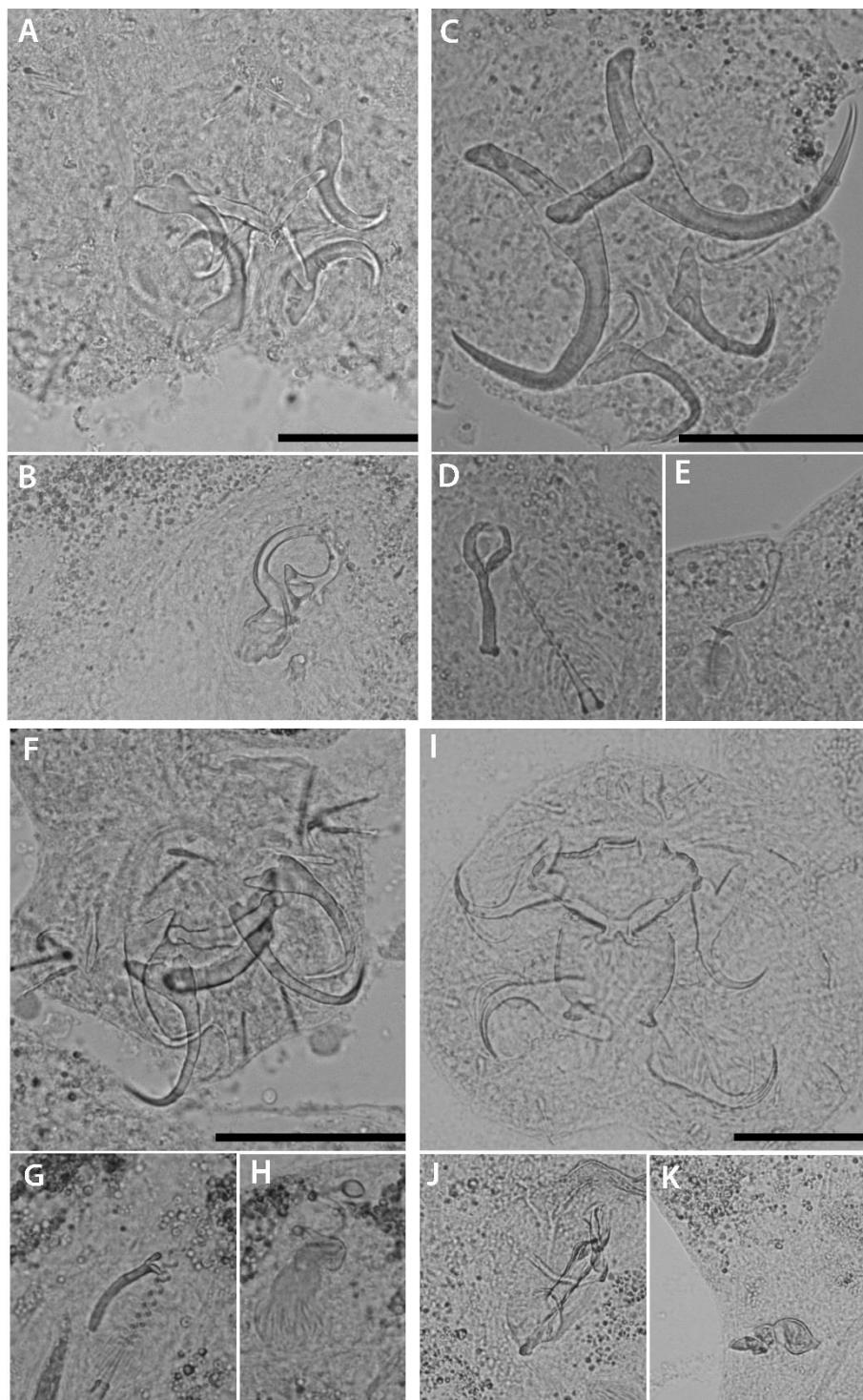
- Description of a new genus of Gyrodactylidae
- Finalise MSs on new Gyrodactylus species descriptions of various origin (South Africa, Antarctica, Adriatic Sea)
- Morphometrical evaluation of the marine monogenean collectd off shore South Africa

## Acknowledgement

The studies have been supported from the project no. GBP505/12/G112 from the Czech Sciences Fundation, and the bass sampling was financially supported from the DST/NRF Centre of Excellence for Invasion Biology.

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# Morphological diversity of *Cichlidogyrus* species (Dactylo-gyridae) in Tanganyikan and non-Tanganyikan cichlids

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Cichlidae is one of the most species-rich families of vertebrates with more than 3 000 species widely distributed in Africa and the Neotropics, and characterized by a high diversity in morphology, colors and behavior. Lake Tanganyika, the deepest and oldest lake in Africa, counts the genetically, morphologically and ecologically most diverse cichlid assemblages of the East African Great lakes and holds about 75 non-cichlid and 250 endemic cichlid species, the latter belonging to more than fifty genera and 14 to 16 tribes.

Currently the knowledge on parasite diversity in cichlid fish is very restricted. About 100 African and Levantine cichlid species from more than 1 500 cichlids have been investigated for the presence of monogenean parasites. These African cichlids are known to host five genera of dactylogyrideans, *Cichlidogyrus* being the most species-rich genus with more than 100 species described from more than 100 cichlid hosts. However, only 24 *Cichlidogyrus* species were described from Tanganyikan cichlids so far.

Haptoral structures (anchors, hooks and transversal bars) and male copulatory organ (MCO) structures in *Cichlidogyrus* species present a high morphological diversity in terms of shape and size. The haptor in *Cichlidogyrus* comprises of two pairs of anchors (one dorsal and one ventral), two transversal bars (the dorsal one with two auricles and the ventral one which is V-shaped) and seven pairs of hooks. Four main morphological groups of *Cichlidogyrus* are recognized based on the configuration of the hook pairs. The MCO consists of a copulatory tube with a more or less ovoid basal bulb in the proximal part prolonged into a tube variable in size and shape with a simple or garnished distal end, and an accessory piece extending from the basal bulb with a simple or complicated structure. The heel, the basal sclerotized part of the bulb, is one of the features of MCO which is important for *Cichlidogyrus* species identification. This structure was evidenced in the original descriptions of *Cichlidogyrus* species from various cichlid hosts. However, some species form an exception and lack a heel in their MCO. From Lake Tanganyika, only a single *Cichlidogyrus* species is known not to possess a heel within its MCO.

The main goal of this study is to evaluate the morphological diversity of the hook pairs configuration and the heel structure in *Cichlidogyrus* species from Tanganyikan and non-Tanganyikan cichlid hosts, and to highlight the importance of these sclerotized parts in the intraspecific variability within the hosts. For this purpose, we used original descriptions and drawings of 78 non Tanganyikan and 24 Tanganyikan *Cichlidogyrus* species in addition to unpublished data recently obtained on *Cichlidogyrus* from Burundese and Congolese cichlid hosts. Haptoral characteristics are usually used to differentiate between major lineages within *Cichlidogyrus* whereas the copulatory organs are more appropriate to distinguish between closely related species. *Cichlidogyrus* species isolated from Tanganyikan cichlid tribes exhibit similar morphotypes as previously reported for non-Tanganyikan species and present a high intraspecific variation in the morphology of the Haptoral and reproductive organ structures. Morphological characterization of the sclerotized parts in *Cichlidogyrus* represents a potential tool to investigate cichlid diversity. Their high morphological diversity suggests that species richness of cichlid monogeneans in Lake Tanganyika is higher than the diversity of originally described cichlid species in this lake.

## Future plans

- Investigation of the *Cichlidogyrus* species-rich diversity from cichlid communities from the Congolese shorelines of Lake Tanganyika;
- Investigation of the intraspecific variability and biogeographical diversity of *Cichlidogyrus* species from representative cichlid species belonging to Haplochromini and Tropheini tribes

## Acknowledgment:

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# Evolutionary ecology of annual fishes

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We studied several aspects of ecology and evolution of annual fishes in Africa and the Neotropics. In the first study [1], we link genetic and morphological data on a widely distributed species of African killifish, *Nothobranchius orthonotus*. The fragmented distribution and low dispersal ability of annual fish result in high genetic clustering of their populations. We tested whether genetic divergence of major mitochondrial lineages forming two candidate species is congruent with phenotypic diversification, using linear and geometric morphometry analyses of body shape in 23 wild populations. We also conducted a common garden experiment with two wild-derived populations to control for the effect of local environmental conditions on body shape. Our data were consistent with *N. orthonotus* being a single species with an extensive geographic range, strong population genetic structure and high morphological variability.

In a second study [2], we compared female fecundity traits in wild populations of two species of *Nothobranchius* that inhabit a region with gradient of aridity, with pools persisting for only 2 to 10 months. We demonstrated that local conditions were important determinants for *Nothobranchius* growth and fecundity that has overridden any major larger-scale patterns.

In the Neotropics, we studied seasonal dynamics in community structure, abundance, body size and sex ratio in two species of Neotropical annual fishes, each from a different phylogenetic clade [3]. Across seven ephemeral pools on the coastal plain of southern Brazil, we found three annual and 22 non-annual fish species. The annual species exhibited clear seasonal dynamics, with the appearance of young fish in the austral autumn (May, June) and a decline in abundance over the seasonal cycle; in contrast non-annual fishes used pools opportunistically. A single age cohort of annual fish female-biased sex ratios were recorded in most pools. Notably, annual fish disappeared before habitat desiccation (while non-annual fishes were present), indicating that their mortality is not driven by desiccation but by deterioration of the environment (high temperature).

Finally, we also associated male alternative mating behaviour, personality traits and morphological characters in Endler's guppy, *Poecilia wingei*. We found that alternative mating behaviour is more flexible than personality traits, and independent of them. Non-colour based morphological traits (gonopodium length, body length, caudal straps length, dorsal fin length) were not correlated with any particular mating behaviour.

## Future plans

In 2017, we plan to submit papers arising from experimental work in Brazil and long-term field studies in Mozambique.

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# Strigeid trematodes (Digenea) from Iceland – an integrative taxonomic approach

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The ecosystems at high latitudes, including the sub-Arctic Iceland, are considered as simple, low-diversity systems with short trophic linkages, few pathogens and limited resilience for adaptation to environmental change [1]. However, recent molecular studies have revealed considerable unrecognised species diversity within digenetic trematodes in northern Europe [2,3]. Trematodes (Digenea) are now known to play a fundamental role in functioning of ecosystems [4]. Therefore, host-parasite systems at high latitudes are a promising model for predicting the impact of accelerated environmental change. Our understanding of the diversity of northern parasites, host-parasite associations and the detection of disease emergence strongly depends on accurate parasite identification. Therefore, the application of comparative morphological and molecular approaches to species delimitation is of substantial importance.

We collected and examined for the presence of trematodes 11 bird, four fish and three snail species in Iceland. In the present study, 66 isolates of representatives of the family Strigeidae Railliet, 1919 were subjected to morphological and molecular assessment. Partial sequences of the mitochondrial cytochrome c oxidase subunit 1 (cox1) gene were used for molecular identification.

Integrated morphological and molecular analyses revealed the presence of eight species represented by larval or adult isolates, two of them using fishes as intermediate hosts during their life-cycle: *Apatemon gracilis* (Rudolphi, 1819) with a fully resolved life-cycle, using *Radix peregra* (Müller) as first intermediate host; *Salvelinus alpinus* (L.) and/or *Salmo trutta* L. as second intermediate hosts and *Somateria mollissima* (L.) as definitive host, and a second species of *Apatemon* Szidat, 1928 from *Gyraulus albus* (Müller) with a so far unknown life-cycle. The other related species, *Australapatemon Sudarikov*, 1959 (3 species) and *Cotylurus Szidat*, 1928 (3 species), use leeches or snails as intermediate hosts (see Fig. 1).

Therefore, we consider *Apatemon* Szidat, 1928, together with *Diplostomum* von Nordmann, 1832, of which we previously found an unexpected diversity (one described and five putative new species), the major metazoan parasites of freshwater fishes so far known from Iceland. Species of both genera may exert pathological impacts on fish wildlife populations in the northern ecosystems. Our finding of a second cryptic species of *Apatemon* indicates that species diversity of this genus is probably underestimated in the freshwater ecosystems at high latitudes. Further studies focused on metacercariae in fishes may shed light on the actual diversity of these fish pathogens in Iceland.

## Future plans

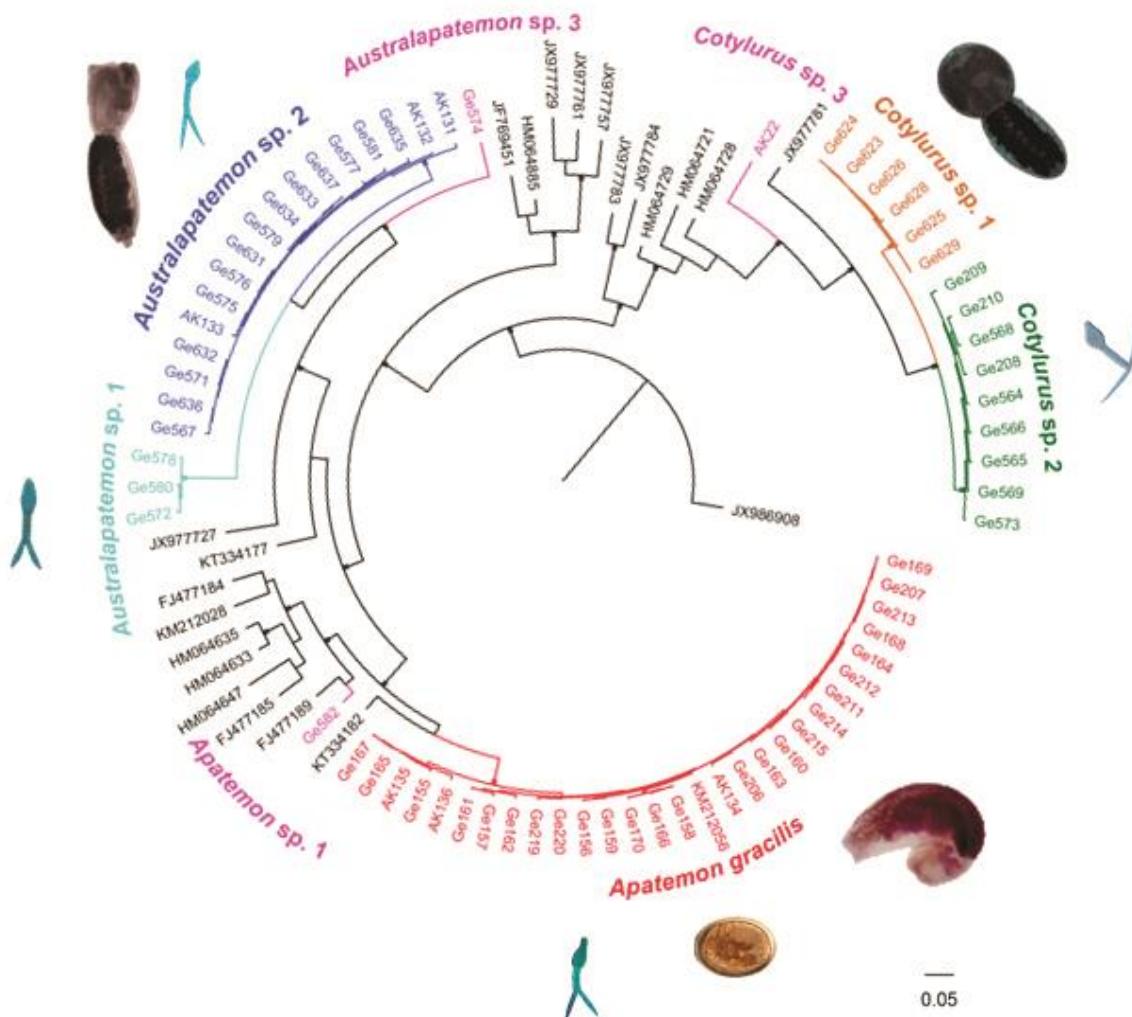
Morphological and molecular characterisation of newly obtained trematodes from fish-eating birds, fish and snail hosts from Iceland.

## 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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**Figure 1.** Bayesian inference (BI) phylogram for the Strigeidae inferred from partial *cox1* sequences constructed with MrBayes, v3.2.6 (Ronquist et al., 2012) under the GTR+I+Γ model. Outgroup: *Diplostomum pseudospathaceum*. BI analysis was run for 10,000,000 generations, with 25% of the sampled trees discarded as 'burn-in'. Black dots indicate nodes with posterior probability values > 0.95. The scale-bar indicates the expected number of substitutions per site.

# Developmental and ontogenetic effects of sexual parasitism, polyploidy and hybridisation

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In spite of its ubiquity, sexual reproduction has been disrupted many times during the evolution leading to formation of the so called asexual taxa. Ever since their discovery, unanswered questions remain whether and how these forms may establish, persist or why they do not replace sexual ones. While many studies attempted to answer these questions, the main problem is that asexual reproduction is often tightly associated with phenomena of hybridisation and polyploidy making it very difficult to disentangle the proximate effects of asexuality from the effects of its correlates (Choleva *et al.* 2012)

The main objective of this complex study is to test the extent to which the developmental and ontogenetic processes are affected by the switch to asexual reproduction, hybridisation and polyploidy. As a model species we use Cobitis loaches, where two sexual species (*C. elongatoides* and *C. taenia*) hybridize to produce clonally reproducing diploid (*elongatoides-taenia*) as well as triploid (2\**elongatoides*-1\**taenia*, 1\**elongatoides*-2\**taenia*) hybrid forms. Our hybrid model of gynogenetically reproducing fish might suit very well in order to resolve several complex questions concerning transcriptional regulation in hybrids, not only those reproducing clonally. Using RNAseq, we search for specific effects of asexuality and disentangle those from effects of hybridity and polyploidy. Specifically, we test whether hybrid expression profiles suggest transgressive, dominant or additive regulation of transcription and whether the changes are due to cis- or trans- regulation (Wittkopp *et al.* 2004, 2008; Zhang & Borevitz 2009). Results of gene-expression study have been compared to morphological and microhabitat data of hybrids and their parental species in order to reveal global pattern affecting hybrid phenotypes and behaviour.

We have isolated RNA from 48 samples (oocyte, liver) from *C. taenia*, *C. elongatoides*, *C. tanaitica*. Parental species were sampled in order to maximally cover existing genetic variability. Morphological dataset comprised several hundreds of samples of all biotypes. In addition, we analysed ecological preference of sexual and hybrid asexual forms using point sampling methodology in selected

mRNA was captured and transcribed based on polyA primers. Absolute quantification of RNA was performed by mapping libraries (Illumina highseq™, single-end 50 bp sequencing) by mosaik (Lee *et al.* 2014) and Bedtools 2.26 (Quinlan & Hall 2010) software with parameter of hash length 21 bp. Reads were further processed in order to deplete artificial PCR duplicates by Picard tool 1.9 (McKenna *et al.* 2010). Expression was processed by DESeq2 package (Love *et al.* 2014). We applied custom python scripts based on binomial test to reveal if allele expression is balanced. We applied dimension reduction methods (PCA, CA) and glm model fitting of factors related to gene dose of *C. taenia* (tt) and *C. elongatoides* (ee) genome ratio to test if gene expression profiles, geometrical morphometry and habitat characteristics conform to differences in reproductive mode, ploidy or hybrid nature of biotypes.

Results: We revealed that relative small amount of traits that were affected by explicit effects of switch to asexuality or ploidy. In general, hybrids tend to preserve parentally inherited regulation pathways suggesting that cis regulation of trait expression prevail. Changes in trans regulation do not generally cause allelic dominance which is rather surprising given the estimated age of the species split (~9 Mya). Global hybrid transcription is gene dose dependent, and hybrid expression profiles appear intermediate. Same pattern has also been confirmed in morphometry and microhabitat preferences where hybrids phenotypes and preferences significantly correlated with their gene copy number. However, we also observed an indication that *C. taenia* genome induces moderate imprinting of *C. elongatoides* genome because *C. taenia* alleles were generally more intensively transcribed than *C. elongatoides* ones. Such a slight dominance of *C. taenia* genome is apparent also in morphometric data.

### **The state of the art and Future plans**

Currently, one manuscript is at the preparation stage which will describe the basic RNAseq results and identify genes and pathways whose expression are affected by asexuality, polyploidy and hybridisation. The full amount of data directly testing the gene dose effect, and transcription regulation will follow. We still need to accomplish the final step, consisting of analysis of artificial F1 generation that will resolve proportion of cis/trans regulation. This is in the final stage of processing.

### **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.

# ***Eudiplozoon nipponicum* (Monogenea): analysis of secretome and characterization of selected proteins – serpins**

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Our experimental organisms *Eudiplozoon nipponicum* (family Diplozoidae, Polyopisthocotylea) is hematophagous ectoparasite from the gills of cyprinid fish (*Cyprinus carpio*).

The properties of proteins (e.g. function) of the members from the family Monogenea are among the less investigated in whole phylum Platyhelminthes.

During the previous experimental work, interesting results concerning the bioactive molecules of *E. nipponicum* (endopeptidases and their inhibitors) were recorded. Some of these molecules are probably presented in excretory-secretory products (secretome) of the parasite and thus potentially involved in parasite-host interactions. Therefore, we performed the complete mass spectrometry analysis of *E. nipponicum* secretome (LC MS/MS).

Some of the identified protein molecules, such as e.g. serpins, were selected and further characterized. In general, serpins play a role in many physiological processes of higher organisms, such as complement cascade activation, blood clotting, fibrinolysis, inflammation and programmed apoptosis.

The results of our experiments with serpin in recombinant form indicates that its function could be similar to other blood feeding worms. It was proved, that recombinant *E. nipponicum* serpin is e.g. able to inhibit the factor Xa which is the important member of blood coagulation cascade.

## **Future plans**

Finish the biochemical characterization of serpin and evaluation of proteomic (secretome) data of *E. nipponicum*.

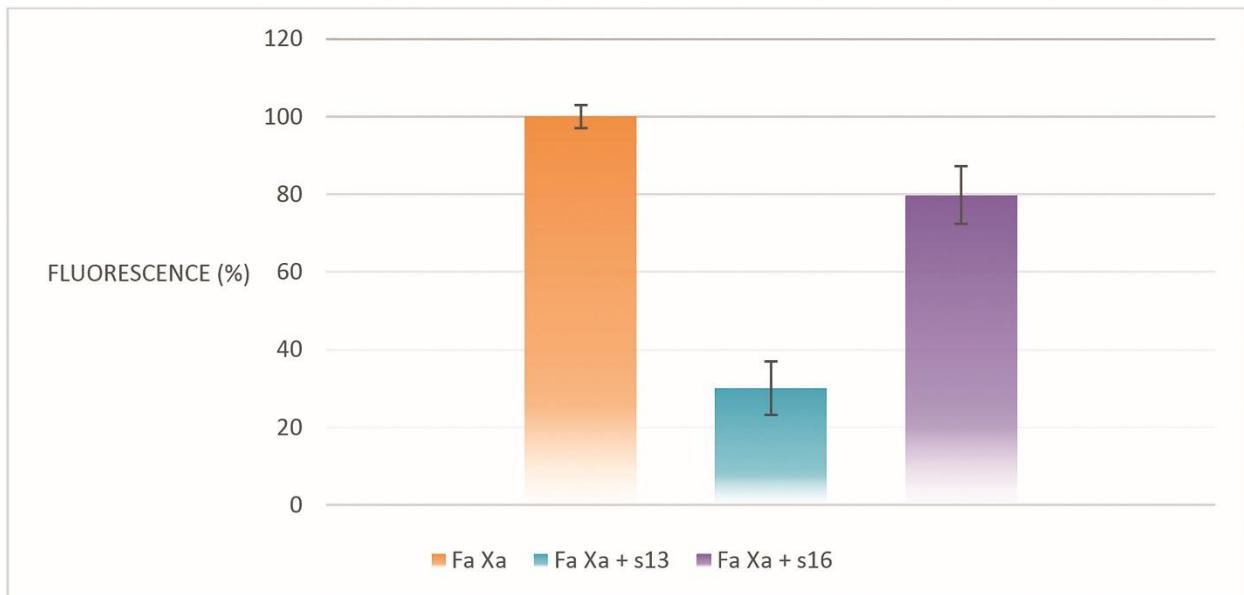
## **Acknowledgement**

This research was supported by Czech Science Foundation (GBP505/12/G112, P506/12/1258) and grants of the Masaryk University (MUNI/A/1325/2015).

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**Figure 1.** Inhibitory effect of *E. nipponicum* serpins (s13 and s16) on activated factor X (Xa).

# Diversity and interrelations of dactylogyrid monogeneans (Platyhelminthes) parasitizing African fishes

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In 2016, my research activities were a combination of continued and new projects examining the diversity of African monogeneans. These activities can be divided into the following parts:

**1.** *Quadriacanthus* species from catfishes. Seven species (four new) of *Quadriacanthus* were reported/described from the gills of three species of catfishes from Lake Turkana (Kenya) and the Nile River Basin (Sudan). Taxonomic evaluation of the monogeneans found was made on the basis of both morphometrical observation and molecular methods. The interspecific relationships among *Quadriacanthus* species parasitizing catfishes in the African region inferred from ribosomal DNA sequences were investigated for the first time. *Quadriacanthus clariadis* from a clariid fish is a sister species to *Q. bagrae* from a bagrid host, indicating a possible host-switching event in the evolutionary history of the genus [1].

**2.** *Cichlidogyrus* species from cichlids. Description of 8 new species of *Cichlidogyrus* collected from two species of the lamprologine cichlids, *Lamprologus callipterus* (Burundi) and *Neolamprologus fasciatus* (Zambia), and proposal of *Apocichlidogyrus* as a subgenus within *Cichlidogyrus* is being finalized. Examination of internal structures in these parasites revealed that errors in the original diagnosis of *Cichlidogyrus* had been made.

**3.** Fieldwork in Madagascar (7–27 April) in cooperation with Malagasy colleagues from the University of Antananarivo. The main goals was sampling parasite diversity across cichlid fishes of Madagascar, with special emphasis on monogeneans parasitizing endemic cichlids. A preliminary phylogenetic analysis using LSU rDNA showed that *Insulacleidus* (endemic monogenean genus to Madagascar) is not monophyletic, suggesting a need for further taxonomic revision. *Insulacleidus paretropli* collected from *Paretroplus polyactis* clusters with species of *Cichlidogyrus* and *Scutogyrus*, while the phylogenetic position of *I. ptychochromidis* parasitizing *Ptychochromis grandidieri* was not resolved. Also, the haptoral bars of *I. paretropli* are of a different morphotype than those of the latter parasite species. The basal position of *I. paretropli* within the clade formed by dactylogyrids parasitizing African cichlids may suggest that this species represents the ancestor from which members of *Cichlidogyrus* and *Scutogyrus* were derived. Indeed, unlike *I. ptychochromidis*, the haptoral bars of *I. paretropli* possess similar morphological features that are characteristic for members of *Cichlidogyrus*, i.e. V-shaped ventral bar with medial and distal portions reduced in diameter and saddle-shaped dorsal bar with two submedial projections. However, whereas in *I. paretropli* the projections are formed as bulges, in species of *Cichlidogyrus* are expanded into conspicuous auricle-like structures.

## Other activities

Teaching participation in the course “Summer School of fish Parasitology 2016: Parasites of Fish in Africa”, including authorship of the chapters “Ectoparasitic helminths (Monogenea)” and “Monogenean parasites in Africa” in the course handbook “Program and Manual of laboratory methods”.

## Plans for 2017

Publishing the above results and finalising/submitting manuscripts of a Ph.D. student, Maria Lujza Kičinjaová, dealing with morphological and molecular characterization of monogeneans parasitizing African tetras. A manuscript on phylogenetic relationships among dactylogyrids from African tetras based on nuclear gene sequences will be prepared.

## 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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# Study of the genetic relationships among parasite species of African freshwater fishes using nuclear ribosomal DNA

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The interspecific relationships among *Quadriacanthus* spp. parasitizing catfishes (Siluriformes) in the African region inferred from ribosomal DNA sequences were investigated for the first time [1]. Molecular analysis was conducted using two nuclear ribosomal DNA fragments; fragment spanning the partial 18S and complete internal transcribed spacer 1 (18S-ITS1), and fragment of partial 28S rDNA. For both 18S-ITS1 and 28S, *Q. clariadis* was found to be most closely related to *Q. bagrae*. *Quadriacanthus mandibulatus* was observed to be the most distant species from others. The separation of *Q. mandibulatus* n. sp. from the other species corresponds with the different morphology of its copulatory tube. *Quadriacanthus clariadis* from a clariid fish is a sister species to *Q. bagrae* from a bagrid host indicating a possible host-switching event in the evolutionary history of the genus.

The morphological evaluation of gnathostomatid larvae *Spiroxys* sp. 2 and camallanid larvae *Camallanus* sp. from the killifish of the genus *Nothobranchius* from Mozambique was complemented with molecular analyses of nuclear ribosomal DNA sequence data (18S and ITS-rDNA). The phylogenetic position of *Spiroxys* sp. 2 within suborder Spirurina nematodes using 18S sequences was also investigated for the first time [2]. The present results support the placement of *Spiroxys* spp. into family Gnathostomatidae, monophyly of this genus and its close relationships with representatives of family Anguillicolidae.

## Future plans

Main objectives for the next year are to perform the complete molecular characterization of African dactylogyridean species belonging to genera *Protoancylodiscoides*, *Schilbetrema*, *Schilbetrematoides* and *Synodontella* and to analyse the phylogenetic relationships among representatives of dactylogyrids parasitizing catfishes from Ethiopian, Oriental and Neotropical regions. The phylogenetic analyses of the *Afrocleidodiscus*, *Annulotrema* and *Characidotrema* species parasitizing African tetras (Characiformes: Alestidae) will be also completed.

## Acknowledgement

These studies are supported from the project no. GBP505/12/G112 from the Grant Agency of the Academy of Sciences of the Czech Republic.

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2. Nezhybová V., Seifertová M., Mašová Š. Morphological and molecular study on nematode larvae of *Nothobranchius* fishes (Cyprinodontiformes, Nothobranchiidae) from Mozambique (in prep.)

# Proteocephalidean tapeworms (Cestoda: Onchoproteocephalidea) of loaches (Cobitoidea): evidence for monophyly and high endemism of parasites in the Far East

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The parasite fauna of loaches (Cypriniformes: Cobitoidea), a group of small bottom-dwelling freshwater fishes with a mostly Eurasian distribution, remains a largely unknown quantity. Here we revise the taxonomy of tapeworms of the genus *Proteocephalus* Weinland, 1858 (Cestoda: Proteocephalidae) that had been found in loaches from the Palaearctic Region (Central Europe, Japan and Russia [Primorsky Region]). Molecular phylogenetic analysis based on two nuclear (*ssr*- and *lsrDNA*) and two mitochondrial genes (*cox1* and *rrnL*) revealed a monophyletic group consisting of four valid species nesting within the *Proteocephalus*-aggregate: (i) *Proteocephalus sagittus* (Grimm, 1872) from *Barbatula barbatula* (Europe, Russia and Tadzhikistan), (ii) *Proteocephalus* n. sp. 1 from *Barbatula toni* (Russian Far East – Primorsky Region), (iii) *Proteocephalus midoriensis* Shimazu, 1990 from *Lefua echigonia* (Japan) and *L. costata* (Russia) (new host and geographical record), and (iv) *Proteocephalus* n. sp. 2 from *Misgurnus anguillicaudatus* (Russia; Primorsky Region). *Proteocephalus sagittus* and *Proteocephalus* n. sp. 1, and *P. midoriensis* and *Proteocephalus* n. sp. 2 were recovered as sister taxa, respectively. *Proteocephalus sagittus* and *Proteocephalus* n. sp. 1 are characterized by having proglottids that are wider than long, an elongate to pyriform cirrus-sac and the vitelline follicles that form wide lateral bands. *Proteocephalus midoriensis* and *Proteocephalus* n. sp. 2 are characterized by having proglottids that are more elongate and an ovoid to almost spherical cirrus-sac and the vitelline follicles forming narrow lateral bands. *Proteocephalus* n. sp. 1 differs from *P. sagittus* in the posterolateral extent of the vitelline follicles, whereas *Proteocephalus* n. sp. 2 can be distinguished from *P. midoriensis* mainly by the relative size of the ovary, posterior extent of the vitelline follicles and width of the scolex. Unlike most species of the *Proteocephalus*-aggregate that possess an apical sucker, all species from loaches are devoid of any apical organ. The existence of two new species in loaches from the Primorsky Region of Russia indicates high endemism of fish parasites in this region. A key to the identification of recognised species from loaches is provided.

## Acknowledgement

This study was supported financially by the Institute of Parasitology (RVO: 60077344) and the Czech Science Foundation (project No. P505/12/G112). Collecting trip of A. C. and T. S. to Russia in June, 2011 and DNA sequencing were supported by the National Science Foundation, USA (PBI award Nos. 0818696 and 0818823) and the Institute of Biology and Soil Science, Russian Academy of Sciences, Vladivostok, Russia.

# Does presence of pharmaceuticals in effluent from sewage treatment plant influence the parasite infection in brown trout (*Salmo trutta m. fario*)?

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Presence of pharmaceuticals in effluent of sewage treatment plants (STPs) is a worldwide problem. These compounds are not effectively removed during the treatment processes and thus persist in aquatic environment. Recently, several studies described close relationship between parasitism in aquatic species and water/sediment contamination including a range of organic and anorganic pollutants. However, studies on the effects of drugs on parasites of aquatic species are still rare, focused mainly on the estrogenic compounds.

The aim of our study was to investigate effect of selected pharmaceuticals on parasitic abundance in brown trout (*Salmo trutta* var. *furio*). Fish and benthos were sampled at two localities of Živný brook (Blanice tributary, Elbe basin). At the same sites the chemical and toxicological analysis of water were made. At the control site, upstream the STP, the total concentration of pharmaceuticals was 790.1 ng/l. At the polluted site, downstream the STP, the total concentration of pharmaceuticals was 6995.3 ng/l. Fish length and condition parameters (CF, HSI, SSI, GSI) did not differ between the sites. Analysis of blood samples showed higher proportion of monocytes and myelocytes alongside with lower proportion of neutrophilic segments in leucogram at control site. Lysozyme concentration in mucus was higher at control site.

Parasitological investigation showed presence of two nematode species (*Salmonema ephemeridarum* and *Agamospirura* sp.), one digenetic (*Crepidostomum metoecus*) and monogeneans *Gyrodactylus* spp. Trout from control site were significantly more parasitized than that from polluted site. Whilst endoparasites were more abundant at control site, polluted site was characterised by higher abundance of ectoparasitic monogeneans. Higher endoparasite infection at control site probably reflected the higher density of endoparasites' intermediate hosts rather than presence of pharmaceutical residues.

A detailed analysis of pharmaceutical contents in fish tissues will help to shed light on the association between the differences in parasite infection.

## Future plans:

1. Analysis of bile and muscle samples with respect on pharmaceuticals content.
2. RNA analysis of liver samples.

## Acknowledgement:

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

# Patterns of cercarial emission of *Diplostomum* spp. in a subarctic lake ecosystem as adaptation for trematode transmission under subarctic climatic conditions

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Digenean trematodes are ubiquitous and abundant in aquatic ecosystems. Thousands of their free-swimming larvae, cercariae, are released from their molluscan first intermediate hosts into the environment every day and infect the next host in the life cycle. The cercarial emergence is regulated mainly by exogenous abiotic factors such as photo- and thermoperiod, and by biotic factors such as the activity and/or behaviour of the hosts. The natural effects of thermo- and photoperiod on cercarial emission have previously only been investigated in a single marine arctic system, whereas no similar study of trematode transmission patterns has been conducted in freshwater systems in these northern geographical areas.

This study aims to assess the extent of adaptability of cercarial emission to the unique climatic conditions of subarctic Europe by investigating the daily patterns of cercarial emergence of the fish pathogens *Diplostomum* spp. from the lymnaeid snails, *Radix balthica*, in a subarctic freshwater ecosystem. Sampling was conducted in August 2016 in Lake Takvatn, an oligotrophic dimictic lake in northern Norway (69°07'N, 19°05'E); with maximum epilimnetic and average air temperatures in summer of 14.0 °C and 13.2 °C, respectively. A total of 285 snails were collected along the littoral zones and examined for patent trematode infections. Altogether, trematodes of six different genera (*Apateomon*, *Diplostomum*, *Echinoparyphium*, *Plagiorchis*, *Trichobilharzia* and *Notocotylus*) were identified. Five species of *Diplostomum* infect *R. balthica* in Takvatn (Soldánová et al., under review) and at least two were present in the sampled snails, both of which were included in the experiments since cercarial emission strategies are characteristic at the genus level. Two types of experiments using 12 naturally infected snails were performed: (i) a field experiment following natural photo- (19 : 5 light : dark cycle) and thermoperiod (water temperature range 8–10°C) with cercarial counts carried out every 6 and 12 hours over two consecutive days; and (ii) a laboratory experiment simulating field light conditions with a temperature 5°C higher than measured in the lake. In the field, snails produced cercariae in beakers with a defined water volume, attached to specifically designed metal constructions, covered with a transparent lid and submerged in the lake. The mean daily emergence rate of *Diplostomum* spp. was 278 cercariae snail<sup>-1</sup>day<sup>-1</sup> in the field (minimum of 14; maximum of 1.707) and 3.593 cercariae snail<sup>-1</sup>day<sup>-1</sup> in the laboratory (minimum of 1.292; maximum of 7.312). Preliminary results suggest an adaptive response to generally lower temperature conditions in subarctic areas as well as a prolonged period of cercarial emergence throughout the polar day conditions as an adaptive compensation for a narrow temporal transmission window for parasites. An increased rate of cercarial emergence with increased water temperature in the laboratory experiment confirms the generally observed extreme sensitivity of trematodes to temperature changes.

## Acknowledgement

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# Parasite diversity of cichlid fish in Madagascar

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Madagascar is well known for the diversity of the highly endemic flora and fauna. It is estimated that 80 % to 90 % of its indigenous species are not found anywhere in the World (de Rham & Nourissat, 2004). The fishes living in Madagascar belong to the secondary freshwater families, mainly Cichlidae and Cyprinodontidae. The endemic cichlid species of Madagascar represent 5 following genera: *Paretroplus*, *Paratilapia*, *Ptychochromis*, *Ptychochromoides* and *Oxykolpia* with 20 valid species and other probably 10 undescribed species.

Recently we know practically nothing about original parasitofauna of Malagasy freshwater fish. Concerning parasites of endemic cichlid fish in Madagascar, Rakotofiringa and Euzet (1983) described the gill monogeneans of the genus *Insulacleidus* (Dactylogyridae, Monogenea) including three species: *Insulacleidus paratilapiaiae* from *Paratilapia polleni*, *I. paretropoli* from *Paretroplus polyacris* and *I. ptychochromidis* from *Ptychochromis oligacanthus*.

We performed three-week field study to investigate the metazoan parasites of native and endemic cichlid species living in North part of Madagascar. Four regions were visited and sampled for cichlid species as follows: National Park Ankarafantsika, Anjingo, Lakes Mont Passot (Nosy Be Island) and Andevoranto. A total of 89 specimens of cichlid fish and 9 specimens of 5 non-cichlid fish (*Heterotis niloticus*, *Liza macrolepis*, *Mugil robustus*, *Pachypanchax malonotus*, *Glossogobius giuri*) were examined for the presence of metazoan parasites. Concerning the cichlids introduced from Africa, three species were identified and investigated for parasites: *Oreochromis niloticus*, *O. mossambicus* a *Coptodon rendalli*. Other 7 cichlid species endemic to Madagascar were investigated for the presence of metazoan parasites: *Paratilapia* sp. 1, *Paratilapia* sp. 2, *Ptychochromis grandidieri*, *Ptychochromis* sp. 1, *Ptychochromis* sp. 2, *Paretroplus polyactis* and *Paretroplus lampanebe*.

The morphological and molecular identification of all metazoan parasites is currently in process. Monogenea was the parasite group with higher prevalence in cichlid species but generally with low or moderate intensity of infection. On the other hand, Cestoda reached generally moderate prevalence but in some cases very high intensity of infection in cichlids introduced from Africa when compared to endemic cichlids. Only endemic cichlids in last region were highly infected by copepods. Five *Cichlidogyrus* species (*C. halli*, *C. sclerosus*, *C. thurstonae*, *C. tiberianus* and *C. tilapiaiae*) and *Scutogyrus longicornis* were introduced to Madagascar with their African cichlid hosts. In general, our study indicates that the presence of endemic cichlids is weak and they are likely outcompeted by cichlid species introduced from Africa. Our study highlights the risk of cichlid introduction for native endemic cichlid fauna as we found that native endemic cichlids of *Paratilapia* and *Paretroplus polyactis* are highly susceptible to *Cichlidogyrus* infection. Three *Cichlidogyrus* species were identified on endemic cichlids with *C. tilapiaiae* even exceeding the prevalence and intensity of infection on cichlids introduced from Africa. Endemic monogeneans were present strictly on endemic cichlids only in one region investigated: *Insulacleidus paretropoli* (intensity of infection ranging from 1 to 5, prevalence 75%) on *Paretroplus polyactis* and *I. ptychochromidis* on *Ptychochromis grandidieri* (intensity of infection ranging from 1 to 24, prevalence 37%) were identified.

## Future plans

(1) Morphological identification of all metazoan parasites in collaboration with the Institute of Parasitology, Biological centre CAS in České Budějovice. (2) Molecular identification of parasite specimens, description/redescription of species (genera), (3) Phylogenetic analyses to investigate the origin of cichlid monogeneans and to test biogeographical hypotheses of historical dispersion of cichlids.

## Acknowledgement

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# Early life stages of exotic gobiids represent attractive new hosts for unionid glochidia

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Introduction of an exotic species has the potential to alter interactions between fish and bivalves; yet our knowledge in this field is limited, not least by lack of studies involving fish early life stages (ELS).

Here, for the first time, we examine glochidial infection of fish ELS by native and exotic bivalves in a system recently colonised by two exotic gobiid species (round goby *Neogobius melanostomus*, tubenose goby *Proterorhinus semilunaris*) and the exotic Chinese pond mussel *Anodonta woodiana*.

The ELS of native fish were only rarely infected by native glochidia. By contrast, exotic fish displayed significantly higher native glochidia prevalence and mean intensity of infection than native fish (17 versus 2% and 3.3 versus 1.4 respectively), inferring potential for a parasite spillback/dilution effect. Exotic fish also displayed a higher parasitic load for exotic glochidia, inferring potential for invasional meltdown. Compared to native fish, presence of gobiids increased the total number of glochidia transported downstream on drifting fish by approximately 900%.

We show that gobiid ELS are a novel, numerous and ‘attractive’ resource for unionid glochidia. As such, unionids could negatively affect gobiid recruitment through infection-related mortality of gobiid ELS and/or reinforce downstream unionid populations through transport on drifting gobiid ELS. These implications go beyond what is suggested in studies of older life stages, thereby stressing the importance of an holistic ontogenetic approach in ecological studies.

## Future plans

We plan to process data about host-parasite interaction on adult level of exotic gobiids and unionid mussels.

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# New data on vahlkampfiid amoebae (Heterolobosea Page and Blanton, 1985)

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Heterolobosea Page et Blanton, 1985, a major group of diverse eukaryotic microorganisms that belongs to the supergroup Excavata (Adl et al. 2005) includes, in addition to many others, amoebae that until recently were considered members of the family Vahlkampfiidae Jollos, 1917. Currently, the reconstructions of phylogenetic relationships within Heterolobosea place sequences of the former vahlkampfiid amoebae to three of seven highly diverse subclades of Tetramitia Cavalier-Smith, 1993 (Pánek and Cepicka 2012). Here we present our research output that contributes to the knowledge of Heterolobosea in general and vahlkampfiid amoebae in special.

Based on detailed light microscopical, ultrastructural and molecular analyses, an amoeba strain isolated from gill tissue of farmed rainbow trout sampled from a stock affected by nodular gill disease (NGD) represents the type strain of a newly described species, *Neovahlkampfia nana* (Tyml et al. 2016a in publications record). This species reinforces a well-supported but as for the number of sequences underrepresented subclade of Heterolobosea (Tetramitia I). *Neovahlkampfia nana*, the second nominal species of Tetramitia I, is reported more than 40 years after description of the only other species of this subclade, *Vahlkampfia damariscotae* Page, 1974, currently classified as *Neovahlkampfia damariscottae* (Brown et De Jonckheere, 1999). Details recognized in the ultrastructure of the type strain of *N. nana* deserve to be studied in other heterolobosean amoebae.

New data collected on 18 strain representatives of three genera of vahlkampfiid amoebae isolated from Arctic and Antarctic extremes contribute substantially to the knowledge of diversity of Heterolobosea in under-explored high latitudes (Tyml et al 2016b in publications record). Our phylogenetic analyses assigned one strain to *Allovahlkampfia* Walochnik et Mulec, 2009, two strains to *Vahlkampfia* Chatton et Lalung-Bonnaier, 1912 and 15 strains to *Naegleria* Alexeieff, 1912. Of these numerous *Naegleria* strains, 7 were of the Arctic and 7 of the Antarctic origin. Identity of sequences obtained from newly isolated strains of *N. polaris* and *N. neopolaris* with those previously described from opposite polar regions (De Jonckheere 2006) and the fact that identical species were not found in temperate zone supplements phylogeographically important although still rare data on bipolar distribution of amoebae (Tyml et al. 2015).

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# Diversity and invasion biology of cichlid parasites, with focus on a case study in Madagascar

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Thanks to their high species diversity and their diverse spectrum of speciation mechanisms, cichlid fishes (Teleostei, Cichlidae) represent a textbook model in evolutionary biology. Many cichlids are also of substantial economic value (fisheries, aquaculture, ornamental fish sector...). Despite this importance of the hosts, cichlid parasites remain understudied to date, even though some have been shown to be more species-rich than their host taxa. Furthermore they may offer important insights into the evolution of cichlids, and of host-parasite interactions in general. In collaboration with Belgian and French colleagues, we reviewed the state-of-the-art in cichlid parasitology. We will present some resulting trends, and some questions that came to mind. For several of these questions, our recent expedition to Madagascar, in collaboration with Malagasy colleagues, provides an excellent case study. We will discuss our results on the species diversity, evolutionary history and host-specificity of dactylogyridean monogenean gill parasites on endemic and introduced cichlid fishes of Madagascar. These will be compared with the findings from comparable work in the Congo Basin, carried out together with Belgian, French and Congolese colleagues.

## Future plans

Situating the parasites of Malagasy cichlids phylogenetically in comparison with other cichlid parasites. Publishing the results on parasite co-introduction and transmission in Madagascar. Finishing ongoing work on species descriptions/morphology of monogeneans from various parts of the Congo Basin.

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# ***Eudiplozoon nipponicum* (Monogenea): the molecules transcribed by hematophagous parasite of fish**

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Ectoparasitic flatworms from the family Diplozoidae (Platyhelminthes, Monogenea) represent a serious bloodfeeding fish pathogens. Until now, the running research is 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.

Therefore we started with a first complex transcriptomic followed by genomic analysis of monogenean representative - *Eudiplozoon nipponicum* Goto 1891 (Monogenea, Diplozoidae), which was performed by three sequencing strategies - 454/Roche, MiSeq Illumina and HiSeq Illumina.

The mRNA of *E. nipponicum* adults, in the form cDNA was sequenced (454/Roche, MiSeq Illumina) and raw reads were processed in order to get high quality transcriptomics sequences for further annotation. Raw reads were filtered and low quality bases were removed as well as contaminations – reads related to host organisms (common carp). Sequencing errors and mismatches were corrected and finally the processed reads were assembled into the form individual transcripts. 454/Roche and Illumina sequences were pooled and several further bioinformatics approaches (especially searching for the closest homologous in non-redundant databases and prediction of proteins key properties) were used. We identified number of molecules of our interest (e.g. those related to hematophagy – proteolytic enzymes and their inhibitors, anticoagulant agents and immunomodulators). Some of these molecules are subjects of our further research.

In order to reach the complete information of all *E. nipponicum* genes, the reads related to genomic DNA were generated by adoption of Illumina HiSeq platform. Quality of reads was checked, low quality reads were removed and total genome size and sequencing coverage were estimated. The estimation of genome size was performed also experimentally using DNA fluorescent double staining method, which represents simple and easy pointbased measurement of intensity of fluorescent signal given by amount of DNA. This method was originally designed for single-celled organisms and therefore we are optimizing the protocol for multicellular organism *E. nipponicum*.

All obtained sequences on *E. nipponicum* have been compared with sporadic nucleotide sequential datasets from other members of the class Monogenea.

## **Future plans**

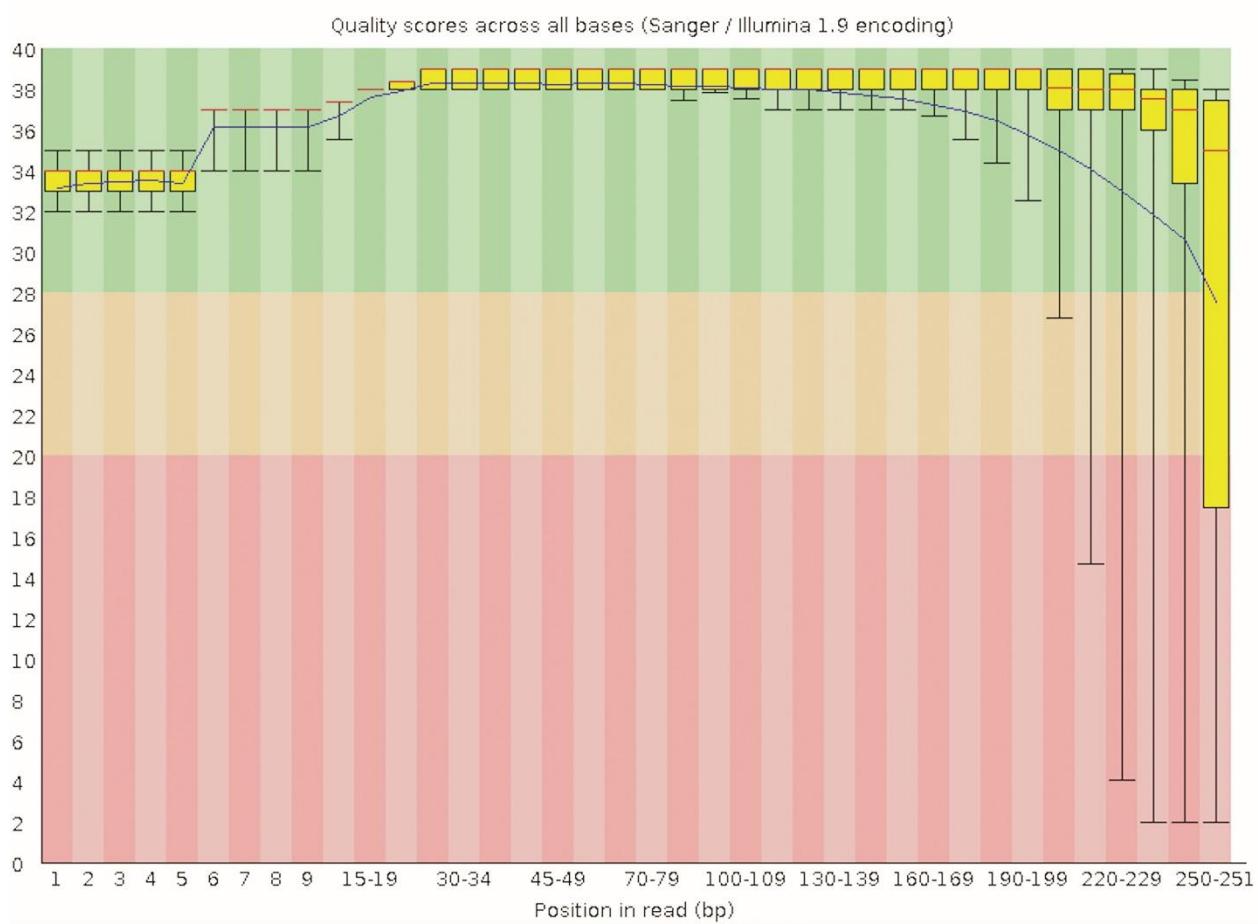
Finish the complete transcriptomic analysis of *E. nipponicum* and prepare a draft of its genome.

## **Acknowledgement**

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**Figure 1.** Quality analysis of genomic raw reads.

# Repatriation of an extinct fish host: An opportunity for myxozoan parasite diversity

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Wildlife repatriation represents an opportunity for parasites. Repatriated hosts are expected to accumulate generalist parasites via spillover from reservoir hosts, whereas colonization with specialist parasites is unlikely. We investigated the case of the anadromous allis shad, *Alosa alosa* (L.), which was reintroduced into the Rhine approximately 70 years after its extinction in this river system [1, 2]. In the course of the EU-LIFE project “The reintroduction of Allis shad (*Alosa alosa*) in the Rhine system” (2007–2010) and the follow-on EU-LIFE+ project “Conservation and Restoration of the Allis shad in the Gironde and Rhine watersheds” (2011–2015) allis shad broodstock from a natural population in France were spawned in captivity and 10.66 million larvae reared in aquaculture facilities were released between 2008 and 2014. Adult shad first returned to spawn in the Rhine six years after the release of young-of-the-year in these waters. We address the question of how myxozoan parasites, which are characterized by a complex life-cycle alternating between annelids and fish, can invade a reintroduced fish species and determine the impact of a *de novo* invasion on parasite diversity.

Using light microscopy, we screened 196 allis shad from (i) established populations in the French rivers Garonne and Dordogne and (ii) repatriated populations in the Rhine, represented by the first adults returning to spawn in 2014. Following microscopical detection of myxozoan infections general myxozoan primers were used for SSU rDNA amplification and sequencing. Phylogenetic analyses were performed and cloned sequences were analyzed from individuals of different water sources to better understand the diversity and population structure of myxozoan isolates in long-term coexisting vs recently established host-parasite systems.

We describe a new species, *Hoferellus alosae* n. sp. from the renal tubules of allis shad by use of morphological and molecular methods. A species-specific PCR assay was designed and determined that the prevalence of *H. alosae* n. sp. is 100 % in sexually mature fish in the Garonne/Dordogne river systems and 22 % in the first mature shad returning to spawn in the Rhine. The diversity of SSU rDNA clones of the parasite was up to four times higher in the Rhine and lacked a site-specific signature of SNPs such as in the French rivers. A second myxozoan, *Ortholinea* sp., was detected exclusively in allis shad from the Rhine.

Our data [3] demonstrate that the *de novo* establishment of myxozoan infections in rivers is slow but of great genetic diversity, which can only be explained by the introduction of spores from genetically diverse sources, predominantly via straying fish or by migratory piscivorous birds. Long-term studies will show if and how the high diversity of a *de novo* introduction of host-specific myxozoans succeeds into the establishment of a local successful strain in vertebrate and invertebrate hosts.

## Acknowledgement

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# **Species of *Gyrodactylus* von Nordmann, 1832 (Platyhelminthes: Monogenea) from cichlids from Zambezi and Limpopo river basins in Zimbabwe and South Africa: evidence for unexplored species richness**

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New findings on *Gyrodactylus* spp. parasitizing African cichlids in southern Africa are presented, comprising data from Zimbabwe and South Africa. Morphometry of opisthaptoral hard parts in combination with nuclear ribosomal DNA sequences confirmed the presence of six species of *Gyrodactylus* von Nordmann, 1832. Three new species are described from fishes in Zimbabwe: *Gyrodactylus chitandiri* n. sp. from the gill arches of *Coptodon rendalli* (Boulenger) and *Pseudocrenilabrus philander* (Weber); *Gyrodactylus occupatus* n. sp. from the fins of *Oreochromis niloticus* (L.), *Pharyngochromis acuticeps* (Steindachner) and *P. philander*; and *Gyrodactylus parisellei* n. sp. from the fins of *O. niloticus*, *P. philander* and *Tilapia* sp. *Gyrodactylus nyanzae* Paperna, 1973 was also identified from the gills of *O. niloticus* and *C. rendalli* collected from two localities in Zimbabwe; these findings represent new host and locality records for this parasite. *Gyrodactylus sturmbaueri* Vanhove, Snoeks, Volckaert & Huyse, 2011 was identified from *P. philander* collected in South Africa and Zimbabwe thereby providing new host and locality records for this parasite. Finally, *Gyrodactylus yacatli* Garcí'a-Vásquez, Hansen, Christison, Bron & Shinn, 2011 was collected from the fins of *O. niloticus* and *P. philander* studied in Zimbabwe; this represents the first record of this species from the continent of Africa. Notably, this study improves upon the knowledge of *Gyrodactylus* spp. parasitising cichlids from these southern African regions. All species studied were recorded from at least two different cichlid host species indicating trend for a wide range of *Gyrodactylus* hosts in Africa. Accordingly, this supports the idea of intensive this supports the idea of intensive host switching in the course of their evolution.

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# Preliminary list of publications dedicated to ECIP – 2016

Actual or past members of ECIP are presented in **bold**.

- Adámek Z., Mrkvová M., Zukal J., Roche K., Mikl L., Šlapanský L., Janáč M., Jurajda P.** (2016) Environmental quality and natural food performance at feeding sites in a carp (*Cyprinus carpio*) pond, *Aquaculture International* (in press). (IF<sub>2015</sub> = 0.960)
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- » Snižte své náklady na sekvenační běh – sdílejte jej
- » Volte pouze takovou kapacitu, jakou právě potřebujete

Jaké množství  
readů opravdu  
potřebuji?

35 %  
stále  
**VOLNÝCH**

17 %  
alokováno  
Jiřím O.

33 %  
alokováno  
Milošem Z.

15 %  
alokováno  
Janou N.

Vysokokapacitní sekvenační čip

» DNA fragmenty libovolného typu  
» Volitelná sekvenační kapacita  
» Široké možnosti multiplexingu  
» Objednávky i výsledky on-line

### SPECIFIKACE:

Vzorky:	PCR fragmenty, amplikonové směsi (AmpliSeq), fragmentovaná DNA
Délka čtení:	Až 200 bp, single-end
Platforma:	Ion Proton (Thermo Fisher Scientific)
Kapacita:	1 – 70 milionů readů
Alokace:	Volitelná – minimálně 1 milion readů
Přesnost čtení:	Typicky $\geq 99\%$
Analýza dat:	Basecalling, odstranění adapterů, kvalitativní filtrování
Volitelné služby:	Efektivní multiplexing, fragmentace a velikostní selekce, AmpliSeq knihovny, bioinformatické řešení na míru
Frekvence:	Analýza prováděna měsíčně

### Příklad nákladů projektu:

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1.000.000 readů

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