



ACANTHOCEPHALAN
WORKSHOP 2022
DIJON - FRANCE

Book of abstracts



BIOGÉOSCIENCES
unité mixte de recherche CNRS / uB / EPHE 6282



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Foreword

Welcome to the 10th International Workshop on Acanthocephalans, holds on August 29-31 2022 in University of Bourgogne Franche-Comté, Dijon, France.

Acanthocephala is a small monophyletic group of heteroxeuous parasites consisting exclusively of endoparasites of Arthropods and Vertebrates. Some remarkable features of acanthocephalans have long fascinated parasitologists, ecologists and evolutionists. They still stimulate the work of an international community of researchers in fields as diverse as the ecology and physiology of host-parasite interactions, community ecology, evolution of parasitic cycles, integrative taxonomy, phylogeography, ecotoxicology, behavioral ecology, etc...The purpose of this workshop is to bring together researchers from around the world to evaluate the current state of research, exchange ideas, and envision new areas of research.

The Organizing Committee

History of the workshop

Workshop sequence	Continent	Country	Area	Year	Date
10	Europe	France	Dijon	2022	28 Aug. - 1 Sept.
9	Europe	Slovakia	Stara Lesna	2018	9-13 Sept.
8	Europe	Germany	Freudenstadt	2014	29 Sep - 1 Oct
7	North America	USA	Walpole, Maine	2009	14-17 Oct.
6	South America	Mexico	Jalisco	2005	7-12 Nov.
5	Europe	Finland	Yalaskaia	2001	23 – 27 May
4	Europe	Italy	Emilia-Romagna	1997	8-10 Sept.
3	Europe	UK	small meeting among few acantho folks		
2	Europe	UK	small meeting among few acantho folks		
1	North America	Texas	San Antonio	1983	December

Organizing committee

The coordinator of the organizing committee, Marie-Jeanne Perrot-Minnot, would like to thank all the staff members of the UMR Biogéosciences for their unvaluable involvement in organizing the 10th Acanthocephalan workshop.

Aude Balourdet
Alexandre Bauer
Edwijis Castro-Mercader
Camille-Sophie Cozzarolo (coordinator)
Sébastien Motreuil
Marie-Jeanne Perrot-Minnot (coordinator)
Thierry Rigaud (coordinator)
Jean-Emmanuel Rollin
Isabelle Santacroce
Maria Teixeira Brandao
Nicole Vitale
Rémi Wattier (coordinator)

Sunday August 28th

Registration (16.00-17.00 – Gabriel building)

Get together - Welcome drink (17.00-20.00 – Gabriel building)

Free evening (20.00- ...)

Dijon is a city of many gastronomies. You will find plenty of restaurants, brasseries and snacks downtown (circled area on the map below). Menus can range from both French cuisine (burgundy beef stew, snails, meurette eggs) to almost any type of worldwide cuisine. Some pizzerias even serve pizzas with snails!!!

Monday August 29th

Registration (08.30-09.00)

Opening session (09.00-09.30)

Organization of Wednesday's thematic round tables

Session 1a (09.30-10.30)

- 09.30** Ecotoxicological implications of cystacanths in their gammarid hosts (**Bernd Sures**, Louisa Rothe & Daniel Grabner)
- 10.10** Fish acanthocephalan (*Acanthocephalus lucii*) as a bioindicator of heavy metals in polluted aquatic environment (**D. Barčák** & T. Brázová)

Coffee break (10.30-11.00)

Session 1b (11.00-12.20)

- 11.00** Bioimaging of elements by NanoSIMS in different body parts of the acanthocephalan *Dentitruncus truttiae* (**Vlatka Filipović Marijić**, Dirk Schaumlöffel, Maria Angels Subirana, Josip Barišić, Etienne Gontier, Nesrete Krasnići, Tatjana Mijošek, Jesús S. Hernández-Orts, Tomáš Scholz & Marijana Erk)
- 11.30** Effects of cadmium exposure on biomarker responses and gene expression in acanthocephalans (**Sara Šariri**, Irena Vardić Smrzlić, Tatjana Mijošek, Zrinka Dragun, Ivana Karamatić, Damir Valić, Tomislav Kralj, Zuzana Redžović & Vlatka Filipović Marijić)

- 12.00** Karyotyping, chromosome mapping of 18S and H3 genes and occurrence of B chromosomes in *Acanthocephalus lucii* from PCB threatened water reservoir Zemplínska Šírava (**A. Marková**, M. Orosová & M. Zrzavá)

Lunch break (12.20-14.00)

Session 2 (14.00-14.20)

- 14.00** Approaching a better understanding of toxicant exposure and parasite infection complexity with mechanistic modelling (**T.T. Yen Le**, Daniel Grabner, Milen Nachev & Bernd Sures)

Plenary lecture 1 (14.40-15.20)

- 14.40** The rarity of Acanthocephalan reproduction: mysteries remain on copulatory cap-chastity belt and mode of insemination (**Bahram Sayyaf Dezfuli**, G. Servadei, F. Donzellini & G. Bosi)

Coffee break (15.20-16.00 – MSH)

Guided tour (16.30-19.00 – Beaux-Arts museum)

Free evening (19.00-...)

Tuesday August 30th

Session 3a (09.00-10.20)

- 09.00** A resilient acanthocephalan in the desert cichlid fish *Coptodon guineensis*: a successful transition to a simplified hypersaline environment (**Isaure de Buron**, Halima Louizi, Kristina Hill-Spanik, Abdeljebbar Qninba, Abdelaziz BenHoussa, Vincent A. Connors, Jean-François Agnese & Antoine Pariselle)
- 09.40** Contrasting the genetic structure of a generalist (*Southwellina hispida*) and specialist parasites (*Hexaglandula corynosoma*: Polymorphidae) distributed sympatrically along of the coasts from Gulf of Mexico and Pacific Ocean slopes from Mexico (**Martín García-Varela**, Alejandra López-Jiménez, Ana Lucia Sereno-Uribe, Marcelo Tonatiuh González-García & Leopoldo Andrade-Gómez)
- 10.00** Are DNA barcoding, iBLOs, BOLD-Systems and BINs opportunities or just fashionable gadgets for Acanthocephalans? (**Rémi Wattier**)

Coffee break (10.20-10.50)

Session 3b (10.50-12.10)

- 10.50** *Pomphorhynchus laevis* facing different environmental variations (temperature, resource quality) in their gammarid hosts (**Thierry Rigaud**, Sophie Labaude, Kévin Sanchez-Thirion, Jean-Nicolas Beisel, Frank Cézilly, Michael Danger & Vincent Felten)
- 11.30** A new methodological tool to address (ir)reversibility of behavioural manipulation by Acanthocephalan parasites: laser-based deparazitization of live hosts (Marie-Jeanne Perrot-Minnot, Aude Balourdet & Olivier Musset)

- 11.50** Don't worry be wormy: no anxiety-like behavior in amphipods infected by a manipulative parasite (**Camille-Sophie Cozzarolo** & Marie-Jeanne Perrot-Minnot)

Lunch break (12.10-13.40)

Session 4 (13.40-15.00)

- 13.40** The role of invasive gobies for transmission of acanthocephalans of the genus *Pomphorhynchus* (**Milen Nachev**, Michael Hohenadler, Nicklas Bröckers, Daniel Grabner & Bernd Sures)
- 14.00** Acanthocephalans of the genus *Neoechinorhynchus* of Tunisia, Southern Mediterranean Sea (**Halima Jmii Chine** & L. Gargouri)
- 14.20** Gammarids and acanthocephalans, a multi-(cryptic) hosts and multi-parasites system? (**Alexandre Bauer** & Thierry Rigaud)

Coffee break (15.00-15.30 – MSH)

Guided tour Clos Vougeot

15.30. A bus will bring you on a scenic route along famous vineyards to the Château du Clos de Vougeot, *headquarters of the climats de Bourgogne*.

16.00-18.45. Guided tour of the Château du Clos de Vougeot.



19.00. A bus will bring you to Glanon for the 'guinguette' conference diner at *Le Riva* restaurant.

'Guinguette' conference diner at Glanon beach (19.30-midnight)

23.30-midnight. Trip back by bus to Dijon.



La Guinguette, by Vincent van Gogh

Wednesday August 31th

Session 5 (09.00-10.40)

- 09.00** The mitochondrial genome in acanthocephalan phylogenetics (Liang Li)
- 09.40** Characterization of the complete mitochondrial genome of *Pseudoacanthocephalus bufonis* (Shiple, 1903) (Acanthocephala: Echinorhynchidae) and its phylogenetic implications (**Rui-Jia Yang**, Tian-You Zhao, Zhi Li, Liang Lü, S.-S. Ru, Hui-Xia Chen, Yuan-Hao Li & Liang Li)
- 10.00** Molecular phylogeny of the genera *Andracantha*, *Bolbosoma* and *Corynosoma* (Polymorphidae): challenging the current classification (**Jesús S. Hernández-Orts**, Martín García-Varela, Tomáš Scholz, Inga N. Martinek, Néstor A. García, Rocío Loizaga, Dennis N. Landete, Olga I. Lisitsyna, Tetiana A. Kuzmina & Francisco J. Aznar)
- 10.20** Phylogenetic analysis of North American species of *Neoechinorhynchus* Stiles & Hassall, 1905 (**Florian Reyda**, Margaret Doolin, Anindo Choudhury, Herman Wirshing & Anna Phillips)

Coffee break (10.40-11.10)

Plenary lecture 2 (11.10-12.00)

- 11.10** Variability of acanthocephalans described by Omar Amin (**Omar M. Amin**)

Lunch break & Poster session (12.00-14.00)

- P1** New records of acanthocephalans from fish-eating birds from the Western Mediterranean (**Jesús S. Hernández Orts**, Olga I. Lisitsyna & Ana Born-Torrijos)
- P2** Acanthocephalans from amphibians: novel molecular data for *Acanthocephalus ranae* (Schrank, 1788) (Echinorhynchida: Paracanthocephalidae) parasitising water frogs (*Pelophylax* spp.) in Slovakia (**Jesús S. Hernández-Orts**, Peter Mikulíček & Michal Benovics)
- P3** New data on the chromosomes of *Neoechinorhynchus rutili* and a brief overview of the cytogenetics of Acanthocephala (**Martina Orosová** & Anna Marková)
- P4** Matching host samples: inoculation of eggs of acanthocephalan parasites by faeces as a novel method for experimental infections in aquatic intermediate hosts (**Sara M. Rodríguez**, Katherine Burgos-Andrade & Nelson Valdivia)

Thematic round tables (14.00-15.30)

Coffee break (15.30-16.00)

Synthesis of thematic round tables (16.00-17.00)

Closing session of the 10th Acanthocephalan workshop (17.30)

Abstracts

Plenary Lectures

Variability of acanthocephalans described by Omar Amin

Omar M. Amin, Ph.D., D.N.M

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Unique and unusual features in the many species of acanthocephalans described and/or studied by Amin from fish, amphibians, reptiles, birds, and mammals, in various parts of the world including South America, Vietnam, Japan, the United States, the Middle East, and North and East Africa, are described. The presentation is in five parts. (1) An introductory section dealing with the classification, general morphology, ecology, and life cycles of the Acanthocephala. (2) Unusual anatomical features of taxonomic or of questionable taxonomic importance addressing variations in the proboscis, proboscis hooks, male and female reproductive organs, and lemnisci. Newly described structures including (a) Para-receptacle structure (PRS) and hoods in certain species as well as a new order of Acanthocephala from Vietnamese birds, are also featured. (3) Structural and functional relationships explaining the relationship between the metamorphosis of the giant nuclei in Eoacanthocephala and worm reproductive cycle. (4) Host-parasite relationships elucidating the relationships between worm anatomy and biology during worm growth. (5) Curiosities in reviews and revisions highlighting taxonomically based zoo-geographical patterns and trends in the genera *Neoechinorhynchus*, *Polymorphus*, and *Pallisentis*. A comprehensive treatment of the acanthocephalans of South America and those marine forms off the Eastern United States is also included here. A look at the September, 2013 classification scheme of the Acanthocephala is included covering 4 classes, 26 families, 157 genera, and 1298 species are included.

The rarity of Acanthocephalan reproduction: mysteries remain on copulatory cap-chastity belt and mode of insemination

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Reproduction of the Acanthocephala always received less attention. Many unanswered questions and mysteries on some processes in male and female reproductive apparatus and reproduction still waiting some responses. Among some taxa of invertebrates, such as the Insecta and Acanthocephala, several behavioral and physiological adaptations of males may help to avoid the second inseminations of the same female. One of these adaptations is a copulatory cap or chastity belt produced by the male accessory gland secretions as a plug within the female genital apparatus. All species of Acanthocephala are polygamous. The capping behaviour evolved in response to sexual selection and such a cap will delay or prevent the introduction of sperm by another male. The copulatory cap often noticed on the posterior end of female individuals and sometimes male worm having the genital region sealed off with cap and effectively removed from the reproductive population. The glands and their products have considerable importance in the reproductive process. Cement glands are one of the most conspicuous and distinctive elements of taxonomic interest in male Acanthocephala and glands vary in shape, number and arrangement in different classes of the taxon.

Very little work has been carried out on general structure and ultrastructure of the male reproductive apparatus. Here we present the aspect of cement glands of species belonging to 4 classes of this phylum. Indeed, insights on cement gland in male cystacanth of some species isolated from crustacean intermediate hosts and in immature male Pomphorhynchus laevis in its fish definitive host will be provided. Moreover, due to lack of information on relationship between different parts of male reproductive system herein ultrastructure of cement reservoir, seminal vesicle and Saefftigen's pouch in a palaeacanthocephalan will be detailed. In Acanthocephala, female reproductive system is simple and has less structures in

comparison to male counterpart. There is no agreement on mode of insemination in Acanthocephala, this study will furnish documentation on the presence of sperm flagellums, beneath the surface of the free ovarian balls in close proximity to unreleased mature oocyte as well as inside the copulatory cap over the female gonopore.

Abstracts

Oral Communications

In alphabetical order

Fish acanthocephalan (*Acanthocephalus lucii*) as a bioindicator of heavy metals in polluted aquatic environment

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Pollution of the aquatic environment is a global problem, and contamination with heavy metals is one of its most common type. For their detection, aquatic organisms, including fish, are known to be useful bioindicators. Recently, some fish acanthocephalans have also been included in ecotoxicological studies due to their exceptional bioaccumulation capacity. In this study, heavy metal pollution resulting from past mining activities was assessed in a water reservoir in Slovakia by examining the bioaccumulation capacity of *Acanthocephalus lucii*, which was compared with that of its fish host, the European perch (*Perca fluviatilis*), and the co-infecting tapeworm *Proteocephalus percae*. The concentrations of nine heavy metals were determined both in the parasites and in the host matrices (muscle, liver, kidney, brain, genital organs and adipose tissue) by ICP-MS. Considering statistically significant values of mean bioconcentration factors (BFs; $C_{[\text{parasite}]} / C_{[\text{fish tissue}]}$), *A. lucii* exhibited higher bioaccumulation capacity of As, Cd, Cr, Cu, Mn, Ni, Pb and Zn than its fish host. The highest BFs were calculated for Cd in fish muscle, hard roe and brain, Pb in hard roe and liver, and Cu in muscle. On the other hand, perch muscle showed the highest bioaccumulation capacity of Hg. Comparison of heavy metal concentrations in two intestinal parasites revealed that *A. lucii* is a more effective bioaccumulator for all elements analysed, except As and Mn, and finally *A. lucii* has the highest bioaccumulation capacity for Cd, Cr, Cu, Ni, and Pb, considering the host and both parasite species. Moreover, Ni concentration in *A. lucii* and intensity of infection of the acanthocephalan correlated negatively with Ni content in fish roe, liver and kidney, and concentration of Cr in hard roe and muscle, respectively. These data suggest that *A. lucii* could be used as a sensitive bioindicator of several heavy metals including toxic elements such as Cd and Pb, and intestinal helminths in polluted waters may have some benefit for their hosts.

This research was supported by the Slovak Research and Development Agency, project No APVV-18-0467 and Scientific Grant Agency of the Ministry of Education, Science, Research and Sport of the Slovak Republic and the Slovak Academy of Sciences (VEGA), project No 2/0126/20.

Gammarids and acanthocephalans, a multi- (cryptic) hosts and multi-parasites system?

Alexandre Bauer & Thierry Rigaud

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Multi-host parasites can exploit various host species that differ in abundance and susceptibility to infection, which will contribute unequally to their transmission and fitness. "Key hosts" are those contributing significantly more to the completion of the life cycle of such parasites. Several species of acanthocephalan manipulative parasites (among which *Pomphorhynchus laevis* and *P. tereticollis*) use amphipod crustaceans of the genus *Gammarus* as intermediate hosts. It has recently been shown that many *G. pulex* and *G. fossarum* cryptic lineages are living in sympatry in Eastern France rivers, bringing a new vision of the spectrum of intermediate hosts that acanthocephalans can use, and their relative contribution to the parasites life cycles. The aim of this work was to determine if this cryptic diversity may hide key intermediate hosts not previously detected. Using a combination of field samplings and experimental infections, we estimated the availability of the different *Gammarus* hosts over time, measured their relative susceptibility to infection, the virulence of parasites and manipulation intensity.

It was shown that the different host species living in sympatry in the studied rivers (the introduced *G. roeselii* and several lineages of the *G. pulex/fossarum* complex) are constantly available for acanthocephalans, but in relatively variable proportions. Different patterns of infections in acanthocephalans were highlighted during this temporal monitoring, suggesting variation in susceptibilities to infection by the different parasites between gammarid species, and therefore unequal contributions to the parasites life cycles. We showed that *G. roeselii* is less susceptible than *G. fossarum* morphospecies to infection by *P. laevis* in the laboratory. Differences were also revealed, in terms of both infectivity and virulence, between host cryptic lineages when submitted to infection by *P. laevis* and *P. tereticollis*.

This work shows that the system studied here is indeed a multi-host and multi-parasite system. Acanthocephalans, traditionally considered as generalists, actually show marked preferences, probably local, in the exploitation of certain intermediate hosts lineages which are not necessarily the most abundant ones.

Don't worry be wormy: no anxiety-like behavior in amphipods infected by a manipulative parasite

Camille-Sophie Cozzarolo & Marie-Jeanne Perrot-Minnot

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Parasites of diverse clades alter their host behaviour in ways that seemingly benefit their own life cycle. However, the cognitive processing and proximate mechanisms underlying parasitic manipulation are mostly unknown. One of the most striking alterations is the reversal of antipredator behaviour induced by some trophically-transmitted heteroxenous parasites in their intermediate host, thereby increasing their chances of reaching their final host. One hypothesis is the alteration of infected hosts cognitive ability in such way that fear- or more generally stress-related emotions are impaired. We tested this hypothesis in a key model system in the study of parasitic manipulation, the fish acanthocephalan parasite *Pomphorhynchus tereticollis* and its intermediate crustacean host *Gammarus fossarum*, using the threat of shock paradigm. We exposed uninfected and *P. tereticollis*-infected *G. fossarum* to chronic and/or acute stress and quantified their sheltering behaviour as a proxy for anxiety-like emotional state. The chronic treatment consisted of daily ten-minute sessions of electric shocks (9 or 15V) during the six days preceding a refuge use test and the acute treatment was a last session imposed ten minutes before the refuge use test. Uninfected gammarids that received the acute or both the acute and chronic treatments of both voltages, as well as those that received the chronic 15V treatment, hid more than their unshocked counterparts. On the other hand, treatments did not influence parasitized gammarids' refuge use. Our results support the hypothesis that acanthocephalan parasites hijack general anxiety-like circuitry of their intermediate host. Further studies are needed to investigate whether it involves inappropriate processing of information, impaired integration, or altered activation of downstream pathways initiating behavioural action.

A resilient acanthocephalan in the desert cichlid fish *Coptodon guineensis*: a successful transition to a simplified hypersaline environment

Isaure de Buron¹, Halima Louizi², Kristina Hill-Spanik¹, Abdeljebbar Qninba², Abdelaziz BenHoussa³, Vincent A. Connors⁴, Jean-François Agnese⁵ & Antoine Pariselle^{3,5}

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Sebkhât Imlili, is a salt flat in the Saharan desert of Southern Morocco with over 160 permanent pockets of hypersaline water generated in the Holocene and inhabited by a few organisms considered to be relics of the past (green Sahara period), including invertebrates and the cichlid fish, *Coptodon guineensis*. Fish were examined in December 2018 (winter), and April (spring), July (summer), and October (fall) of 2019. Over 60% of the fish were infected with acanthocephalans in their intestines. No seasonal variation in infection parameters was detected. Acanthocephalans were identified morphologically and molecularly (ITS rRNA gene) as *Acanthosentis* sp., which presumably uses the sole copepod encountered in the pockets of water as intermediate host - *Cletocamptus retrogressus*, a broadly distributed species highly tolerant of extreme environments and found in fresh as well as marine waters. Further study is required to identify the acanthocephalan as a taxonomic dilemma arose as to whether it is *A. papilio*, described from brackish mudskipper in the mangroves of Senegal, or *A. tilapiae*, possibly acquired from freshwater tilapia spp. (e.g., native *C. zillii* and *Oreochromis aureus*). In support for it being *A. papilio* is its common origin with *C. guineensis* along the coasts of West Africa and the occurrence of a marine copepod intermediate host of another *Acanthosentis* sp. However, neither molecular data or type specimens are available for *A. papilio*, whose description is poor and overlaps with *A. tilapiae*. Support for it being *A. tilapiae* is that the sebkha was once part of a riverine system and current co-infection of *C. guineensis* with heterophyid metacercariae *Pygidiopsis genata* reported from *C. zillii*. However, morphological discrepancies and limited molecular

data for *A. tilapiae* do not allow for confident identification of our specimens at this time. Significantly, whether it is one or the other species, this acanthocephalan illustrates the resilience of some parasites relative to environmental changes, likely tightly associated with their hosts' nature (here euryhaline), and which is crucial in view of climate change. Furthermore, the speed bump we encountered in this *C. guineensis* acanthocephalan exemplifies the need for complete descriptions, deposition of accessible vouchers, and molecular data.

Bioimaging of elements by NanoSIMS in different body parts of the acanthocephalan *Dentitruncus truttae*

Vlatka Filipović Marijić¹, Dirk Schaumlöffel², Maria Angels Subirana², Josip Barišić³, Etienne Gontier⁴, Nesrete Krasnići¹, Tatjana Mijošek¹, Jesús S. Hernández-Orts⁵, Tomáš Scholz⁵ & Marijana Erk¹

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³ Wellfish Diagnostics, Paisley, Scotland, UK

⁴ University of Bordeaux, Bordeaux Imaging Center, Bordeaux, France

⁵ Institute of Parasitology, Biology Centre, Czech Academy of Sciences, České Budějovice, Czech Republic

The nanoscale secondary ion mass spectrometry (NanoSIMS), as a type of imaging mass spectrometry, represents a technique for elemental imaging that has not been widely applied in parasitology. Acanthocephalans are intestinal parasites of Mandibulata and Gnathostomata, and are recognised as bioindicators of metal exposure due to their effective metal accumulation. It was reported that acanthocephalans accumulate metals, especially toxic ones, at much higher concentrations than other indicator organisms, like fish, bivalves or crustaceans. Till now, there are no data on metal localisation in different body parts of acanthocephalans, even though these data may help us in understanding their successful metal uptake and storage.

In the present study, the NanoSIMS was applied for localization of 11 elements, N, Fe, S, P, O, C, Na, Ca, Cu, Tl and Pb in three body parts of an acanthocephalan: trunk spines, inner part of the proboscis receptacle and the outer layer of the tegument. The chemical composition of the acanthocephalan body indicated complex structures that differed in the localization of the studied elements. In trunk spines, the highest element accumulation was found in the middle part, especially of S and Na, which were highly concentrated in the central ring, while Ca and C were mostly

found in the whole spine area. The lower content of S, Ca and P in the proximal spine region might provide spine flexibility. In the proboscis receptacle the most dominant element was Ca, mostly located in the cytoplasmic inner lining and proboscis retractor muscles. The same pattern was found for Na, Fe, Cu, S, C and P, but in much lower concentrations. Considering outer layers of the acanthocephalan body, S and Na were mostly localised in contractile filaments of the muscle and intracellular matrix, while Ca was mostly found in the central cytoplasmic core of longitudinal muscles and neck retractor. Toxic metals were distributed along investigated structures without clear patterns and in very small quantities. The highest density in all studied structures was confirmed for Na, Ca, S, C, N, O, as important and constitutive elements in living cells, and among metals Fe was predominant in all analysed structures.

Contrasting the genetic structure of a generalist
(*Southwellina hispida*) and specialist parasites
(*Hexaglandula corynosoma*: Polymorphidae)
distributed sympatrically along of the coasts from
Gulf of Mexico and Pacific Ocean slopes from
Mexico

Martín García-Varela, Alejandra López-Jiménez, Ana Lucia
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Polymorphidae is a monophyletic group of acanthocephalans distributed worldwide. Within family, *Southwellina hispida* is a generalist species that use a wide spectrum of fish-eating birds as herons, pelicans, cormorans, gulls, eagles, and hawks as definitive hosts. In contrast, *Hexaglandula corynosoma* is a specialist species that use only the Yellow-crowned night-heron as definitive host. Both species of acanthocephalans have a wide range of distribution, and nothing is known about their populations genetic structure. In the current research, sequences of the cytochrome *c* oxidase subunit 1 (*cox 1*) from mitochondrial DNA were generated of 76 specimens of *S. hispida* and 44 of *H. corynosoma* distributed sympatrically in the coasts from Gulf of Mexico and Pacific Ocean slopes from Mexico. The haplotype network reconstruction of *S. hispida* retrieved 43 haplotypes divided in two separates networks (star-shaped), with a few mutations steps and several common haplotypes surrounded both networks with high haplotype diversity and low nucleotide diversity. The haplotype network of *H. corynosoma* retrieved 40 haplotypes, no apparent strict phylogenetic structure with high haplotype diversity and relative high nucleotide diversity. Our analyses suggest that both populations of acanthocephalans are expansion and that the biology of the definitive hosts have played a key role in the population genetic structure and distribution of both species in the coasts of Mexico.

Molecular phylogeny of the genera *Andracantha*, *Bolbosoma* and *Corynosoma* (Polymorphidae): challenging the current classification

Jesús S. Hernández-Orts¹, Martín García-Varela², Tomáš Scholz¹, Inga N. Martinek¹, Néstor A. García³, Rocío Loizaga³, Dennis N. Landete⁴, Olga I. Lisitsyna^{5,6}, Tetiana A. Kuzmina⁵ & Francisco J. Aznar⁷

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Polymorphid acanthocephalans of the genera *Bolbosoma* and *Andracantha* reproduce exclusively in cetaceans and piscivorous birds, respectively, whereas species of *Corynosoma* typically use pinnipeds as definitive hosts, with 7 species having been described as specific to cetaceans, aquatic birds, the sea otter or the Australian water rat. Early multi-gene molecular phylogenetic analyses placed species from these three genera in a well-supported clade sister to other polymorphid genera. These analyses also supported the monophyly of *Andracantha*, but that of *Bolbosoma* and *Corynosoma* remained controversial. Here, we present the most comprehensive multi-gene phylogenetic analysis to date based on sequences of small and large nuclear ribosomal RNA subunits, and cytochrome *c* oxidase subunit I, from 15 of the 29 nominal species of *Corynosoma*, 5 of the 12 of *Bolbosoma* and 4 of the 9 of *Andracantha*. Maximum likelihood and Bayesian inference analyses failed to resolve the relationships between the three genera, thus more representatives are required to clarify this issue. Furthermore,

Andracantha emerged as a paraphyletic taxon, and *Corynosoma* as non-monophyletic taxon although with low support. Species of *Bolbosoma* formed a well-supported monophyletic group. However, the phylogenetic relationships of *Bolbosoma* with *Andracantha* and *Corynosoma* are not resolved. The results of our analyses challenge the current morphological classification of *Andracantha* thus a new diagnosis of this genus is warranted. *Andracantha sigma* from fish-eating birds of New Zealand should be transferred to *Corynosoma*, as this species appears in a strongly supported association with some Antarctic species of *Corynosoma* from penguins and seals. Finally, morphological and molecular data suggest the possible existence of a cryptic species complex within *Corynosoma* parasitising penguins.

This study was supported by the Institute of Parasitology, BC CAS (RVO: 60077344), the National Research Foundation of Ukraine (project 2020.02/0074), and the Joint Research Project between the National Academy of Science of Ukraine and the Czech Academy of Sciences 2020–2022 (NASU-20-05).

Acanthocephalans of the genus Neoechinorhynchus of Tunisia, Southern Mediterranean Sea

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Neoechinorhynchus Stiles & Hassal, 1905 is one of the largest acanthocephalan genera that show a worldwide distribution and can be found in several vertebrates mainly fish. Recently, in the Mediterranean area, the parasite was collected off the Tunisian coast at Bizerte Lagoon from *Chelon auratus* (Gargouri et al., 2015) and at Ichkeul Lagoon northern Tunisia from *Mugil cephalus* (Amin et al., 2020, Jmii Chine et al., 2020). During a research study that has been conducted since 2015, we examined specimens of this genus in various mugilid fish (*Mugil cephalus*, *Chelon ramada*, *C. auratus*, and *C. labrosus*) in Ichkeul Lagoon. Two species were recovered, *N. (N) agilis* (in *M. cephalus*, *C. auratus* and *C. ramada*) and *N. (H). personatus* (in *M. cephalus* and *C. labrosus*). In this presentation, we explore the evolution of *N. agilis* in its host *C. ramada* according to the size class distribution and to the seasons. Initial results showed that, on the one hand, the highest prevalence values were obtained in medium-sized fish (32-36 cm), however, abundance and mean intensity values generally increase with size classes. On the other hand, the study of the seasonal evolution of *N. (N) agilis* in *C. ramada* revealed that the acanthocephalan was present in the Ichkeul Lagoon throughout the year. A gradual increase in the prevalence value according to the seasons was observed; this parameter goes from 16% in winter to reach 32% in spring and 46% in summer then 47.27% in autumn. The values of the mean intensity showed fluctuation, which do not agree with those of the prevalence. The highest value (=12) of this parasitological parameter was retained during the winter season. All these changes in parasitological parameters could be most affected by the variation in the host diet and its physiological condition aside from several environmental factors such as the temperature and the salinity of the water. Our results can somewhat elucidate the ecological evolution of these parasites in brackish environments and gain a broad understanding of host-parasite interactions across spatial and temporal scales.

The mitochondrial genome in acanthocephalan phylogenetics

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Our present knowledge regarding the basic molecular phylogenetic framework of acanthocephalans remains far from complete. Mitochondrial genomes (mitogenomes) play important roles in phylogenetics, phylogeography, population genetics, and DNA identification of animals. However, to date, only 24 acanthocephalan species, representing 13 families belonging to 6 orders, with their mitochondrial genome data available (representing about 1.85 % acanthocephalan species, 60 % order-level taxa and 50 % family-level taxa of Acanthocephala). In the past 2 years, we sequenced and annotated the complete mitogenomes of 40 acanthocephalan species belonging to 10 families in 7 orders, using next-generation sequencing technology based on newly collected acanthocephalan specimens worldwide. The mitogenomes of these acanthocephalan species obtained herein are 13360 bp (*Acanthogyrus bilaspurensis*) to 17,272 bp (*Micracanthorhynchina* sp.) in size, and include 36 genes [12 protein-coding genes (PCGs), 22 tRNA genes and 2 rRNA genes (*rrnL* and *rrnS*)] and two non-coding regions (NCR1 and NCR2). The gene arrangement of PCGs and rRNAs is rather conserved in these acanthocephalans, but some tRNAs are more variability in translocation.

In order to construct the basic molecular phylogenetic framework of the phylum Acanthocephala, phylogenetic analyses were performed based on the amino acid sequences of 12 protein-coding genes using maximum likelihood (ML) and Bayesian inference (BI), respectively. Our phylogenetic results showed that the phylum Acanthocephala were divided into three monophyletic classes Archiacanthocephala, Eoacanthocephala and Palaeacanthocephala. The present analyses further confirmed the class Archiacanthocephala as the sister clade to the remaining Acanthocephala, and also challenged the validity of the class Polyacanthocephala. Our results indicated that the orders Gyraacanthocephala and Echinorhynchida are not monophyletic. The monophyly of the order Polymorphida was strongly supported, and the families Centrorhynchidae and Polymorphidae have closer relationships than the Plagiorhynchidae. However, the present

molecular phylogenetic framework of Acanthocephala based on the mitogenome data is still preliminary, because the representatives of some important orders or families have not been included. We hope to deepen collaborations with all researchers of Acanthocephala worldwide for increasing resources in mitogenome and genome science of Acanthocephala.

Keywords: Acanthocephala, mitochondrial genome, molecular phylogeny, systematics, evolutionary history

Karyotyping, chromosome mapping of 18S and H3 genes and occurrence of B chromosomes in *Acanthocephalus lucii* from PCB threatened water reservoir Zemplínska Šírava

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Acanthocephalus lucii is a common endoparasite of fish populations in eastern Slovakia. Our study focused on the classical and molecular cytogenetic analysis of *A. lucii* from the Zemplínska Šírava reservoir, one of the most polluted sites with PCBs (polychlorinated biphenyls) in Europe and worldwide.

The karyotype of *A. lucii* consists of six autosomal chromosomes (metacentric, submetacentric) and two (female) or one (male) sex chromosome X (acrocentric) on which a secondary constriction was found ($2n = 7/8$, $n = 2m + 1sm + 1a$ (X) + 1-3B). Cytogenetic examination revealed the presence of 1 to 3 supernumerary B chromosomes in most of the acanthocephalan individuals examined, what might be connected with polluted environment. Staining with chromomycin A3 showed CMA₃⁺ bands (GC-rich heterochromatin blocks) at the ends of all chromosomes, while DAPI⁺ bands (AT-rich heterochromatin blocks) were found in different regions of all chromosomes. Fluorescence in situ hybridization (FISH) allows identification of particular chromosomes and mapping of different cytogenetic markers. We have evaluated in our work applicability of two universal markers, namely 18S genes and histone H3 genes. FISH with an 18S rDNA probe revealed a single cluster of major rDNA (nuclear organizer region, NOR) at the site of the secondary constriction of sex chromosome X. FISH with the H3 probe showed a single hybridization signal in the pericentromeric region on the short arms of the largest chromosome pair. The process of spermatocyte meiosis and oogenesis was also scrutinized. The aim of our work was to expand the minimal knowledge of

chromosomes in *Acanthocephala* species, where only 14 species have been studied karyologically.

The novelty of our work is the new methodological approach to investigate the possible effects of a polluted environment on the distribution and quantity of repetitive DNA. In this pilot study, we found several clusters of repetitive DNA using the bioinformatics tool RepeatExplorer. In the future, a comparative analysis of populations from water bodies with and without pollution could reveal a difference in the load of DNA repeats as a possible response to pollution.

Acknowledgements: The study was supported by the Slovak Research and Development Agency (No. APVV 18-0467), by the Slovak Grant Agency VEGA (No. 2/0126/20) and Bilateral Mobility Plus Project (No. SAV-AV ČR-21-03).

The role of invasive gobies for transmission of acanthocephalans of the genus *Pomphorhynchus*

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Ponto-Caspian gobies became highly abundant in many regions outside their native distribution range such as the Rhine River system or the Elbe River. In the newly invaded habitats, the parasite communities of the invasive gobies are characterized by a lower species richness compared to their native range. However, some parasites such as preadult acanthocephalans of the genus *Pomphorhynchus* are highly abundant. Interestingly, it is still unknown which role the gobies play for the transmission of *Pomphorhynchus* species. Individuals of *Pomphorhynchus* sp. do not become mature in gobies and mostly remain encapsulated in the abdominal cavity as preadults. In view of these observations, gobiids could either represent a dead-end host leading to a decline of the *Pomphorhynchus* sp. population (dilution effect), or act as a paratenic host that could increase the infection pressure to the original host community (spill back). To be able to determine the importance of gobiids for one or the other process mentioned, we conducted two infection experiments using smaller and larger individuals of an appropriate definitive host (chub, *Squalius cephalus*), which were infected with preadults of *P. bosniacus* collected from the abdominal cavity of *Neogobius melanostomus*.

The results showed that preadults obtained from gobiids can develop and mature in the definitive host with mean recovery rates of 17.9 % (\pm 18.6) in smaller and 27.0 % (\pm 25.8) in larger chubs. Maximum recovery rates were 50.0 % and 70.0 % for smaller and larger chubs respectively, while no infection was observed in 38.0 % of smaller and 20.0 % of larger chub. Our study clearly demonstrates that gobies serve as a paratenic host for the acanthocephalan of the genus *Pomphorhynchus* and thus spill back the infection into the local fish community. However, comparisons with previous experimental studies conducted with cystacanths isolated from intermediate gammarid hosts showed that the preadults have significantly lower recovery rates than cystacanths.

A new methodological tool to address (ir)reversibility of behavioural manipulation by Acanthocephalan parasites: laser-based deparazitization of live hosts

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Several parasite species can modify their host's phenotype to their own advantage thereby increasing the probability of transmission from one host to another. Acanthocephalan parasites and their arthropod intermediate hosts are one of the few historical models in the study of Parasite-Induced-Phenotypic-Alteration (PIPA). Whether the alteration of intermediate hosts phenotype by acanthocephalan parasites is reversible or not is a core issue, to understand the mechanisms underlying PIPA, its multidimensional aspect as well as its fitness costs to the host. To address this issue, curing a "manipulated" infected host from its parasite is both an essential step and a methodological challenge. The technology of lasers could offer promising tool to clear endoparasites because it provides a contactless mean to deliver lethal high-energy beam, offers great versatility and could be highly specific to the target. We will present here some preliminary and original data on the efficiency and optimization of laser irradiation on the cystacanths' survival of *Pomphorhynchus tereticollis* and *Polymorphus minutus*, and on the consequence of deparazitization on the behaviour of *Gammarus fossarum*.

Aim

- Multidimensionality : although most studies have considered a single phenotypic alteration at a time, it is increasingly acknowledged that, most often, manipulative parasites affect more than one phenotypic dimension in their hosts

Phylogenetic analysis of North American species of *Neoechinorhynchus* Stiles & Hassall, 1905

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Neoechinorhynchus Stiles & Hassall, 1905 is one of the most diverse genera of acanthocephalans. With ~131 species worldwide, it represents ~10% of the diversity of Phylum Acanthocephala. In the USA and Canada, 8 species are reported from turtles and 27 species from fish. Towards a revision of the genus, our phylogenetic analyses presented here are based on nuclear DNA from the internal transcribed spacer (ITS) and large ribosomal subunit (LSU) regions of 20 species from fish from the USA and Canada and congeners from South America, Europe, and Asia after several years of field work. Multiple specimens of *Neoechinorhynchus* from the USA and Canada were included to address intra-specific and geographic variation. Specimens from 8 species in the analysis were collected from their respective type localities in the USA. Representatives of several other genera from Neoechinorhynchidae were also included to address paraphyly of *Neoechinorhynchus*. Our results recovered 3 novel lineages that correspond to putative new species of *Neoechinorhynchus*, requiring further morphological examination. Some of the clades recovered correspond to morphological characters that have been emphasized in species descriptions from the 20th Century, such as a markedly thicker dorsal body wall and markedly unequal lemnisci, while other characters, such as eggs with polar prolongations, appear not to. Specimens of European *Neoechinorhynchus rutili*, the type species of the genus, grouped with a species from western North America. The conspecificity of specimens from localities throughout eastern and central USA identified as *Neoechinorhynchus cylindratus*, a species reported from centrarchid fishes and multiple other fish families, was supported. However, some previously made records of this species are instead suspected to be *Neoechinorhynchus tenellus*, its closest relative. The four *Neoechinorhynchus* species from turtles from Oklahoma

formed a monophyletic group whose position within the tree suggests that turtle parasitism is derived from fish parasitism within Neoechinorhynchidae. *Octospinifer macilentus*, a neoechinorhynchid from catostomid fishes, nested in a clade of multiple *Neoechinorhynchus* species also from catostomid fishes, augmenting evidence from a previous study that *Neoechinorhynchus* is paraphyletic. Future studies will build on these results by including more neoechinorhynchid genera, more samples from other continents, and additional DNA loci.

Pomphorhynchus laevis facing different environmental variations (temperature, resource quality) in their gammarid hosts

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Variations in environmental conditions are known to affect host–parasite associations. In particular, understanding the precise impact of temperature has become a major challenge in a context of global warming. In addition, since numerous parasites, including acanthocephalans, are embedded in food webs, nutrient availability and the quality of resources available in ecosystems also modulates host-parasite relationships. Gammarids are ecologically important freshwater crustaceans and serve as intermediate hosts for several acanthocephalan species. These parasites induce multiple effects on gammarids, including alterations of their behavior, ultimately leading to modifications in their functional role. During these last years, experimental infections were used to assess the effect of temperature and food quality on several traits of the association between *Pomphorhynchus laevis* and *Gammarus pulex* (during the theses of Sophie Labaude in Dijon and Kevin Sanchez-Thirion in Metz). Elevated temperature affected hosts and parasites in multiple ways (decreased host survival, increased gammarids activity, faster parasites development and proboscis eversion). However, behavioral manipulation induced by these parasites was unaffected by temperature. The same types of results were found during two independent experiments where food quality was manipulated. High food quality increase several infection components (eg. Improve host survival, increase prevalence, increase multiple infections), but we found no impact on behavioral manipulation. These results suggest that predicted change in temperature and variations in resource availability may have little consequences on the trophic transmission of

parasites through changes in manipulation, although it may modify it through increased infection success and faster parasites development.

Effects of cadmium exposure on biomarker responses and gene expression in acanthocephalans

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Acanthocephalans are increasingly used as bioindicators of environmental quality due to effective metal accumulation, orders of magnitude higher than in other bioindicator organisms (such as fish, crustaceans, and mollusks). However, the mechanism of metal homeostasis and accumulation in acanthocephalans is still unexplained. Therefore we investigated the effects of in vitro Cd²⁺ exposure on metal accumulation, biomarkers of metal exposure (metallothioneins, MT) and general stress (total cytosolic proteins, TP) and gene expression in *Dentitruncus truttae* Sinzar, 1955 hosted in brown trout (*Salmo trutta* Linnaeus, 1758) from the Krka River. Parasites were exposed in culture to 2 and 10 mg Cd²⁺ l⁻¹ for 3 and 5 days and Cd²⁺ levels in the organisms were determined using HR ICP-MS. Measurement of TP was performed spectrophotometrically by the Lowry method and of MT by differential pulse voltammetry (DVP). Next-generation RNA-Seq technology was utilized to explore genome-wide differentially expressed gene profiles between control and Cd²⁺ treated acanthocephalan specimens.

Our results confirmed efficient Cd²⁺ accumulation in parasites, which levels in unexposed parasites and fish intestine were 391.90 µg kg⁻¹ and 196.80 µg kg⁻¹, respectively. When exposed to 10 mg Cd²⁺ l⁻¹, acanthocephalans accumulated 188.6 mg g⁻¹ after three days and 267 mg g⁻¹ after five days. Both biomarkers decreased with increasing Cd²⁺ concentration, probably due to the inhibitory effect of Cd²⁺ on protein synthesis. Concentrations of MT and TP represent one of the first data reported for acanthocephalans and their baseline levels (mg g⁻¹ wet tissue) were 0.80 and 56.3, respectively. High number of differentially expressed genes was identified for 2 mg l⁻¹ (4826 genes) and 10 mg l⁻¹ (5809 genes) dose of Cd²⁺. Gene ontology analysis showed that the top enriched biological processes included protein digestion and absorption, gap junction, focal adhesion, apoptosis and genes connected with infections and diseases.

Our results confirmed the ability of acanthocephalans to accumulate high concentrations of metals, but at the same time toxic effect on their metabolism was recorded. Exposure of acanthocephalans to high concentrations of Cd²⁺ altered the expression of thousands of genes and simultaneously several stress response systems.

Ecotoxicological implications of cystacanths in their gammarid hosts

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Amphipods are commonly used test organisms in ecotoxicological studies. Interestingly, their naturally occurring parasites have mostly been neglected in these investigations, even though several groups of parasites can have a multitude of effects, e.g. on host survival, physiology, or behavior. In the present talk, effects of acanthocephalan larvae, i.e. cystacanths on the outcome of ecotoxicological studies will be presented based on data taken from the literature as well as own experimental data. Cystacanths can have significant effects on toxicological endpoints (e.g. mortality, biochemical markers) that are unexpected in some cases (e.g. down-regulation of heat shock protein 70 response in infected individuals). Therefore, acanthocephalan larvae can bias the interpretation of results, for example if populations with different parasite profiles are compared, or if toxicological effects are masked by parasite effects.

As an example, data will be presented from a study which we performed at a wastewater treatment plant and where we were interested in biological effects of micropollutants such as pharmaceuticals or pesticides following exposure to conventionally-treated wastewater as opposed to river water as a reference. Accordingly, we exposed specimens of *Gammarus fossarum* infected and uninfected with cystacanths of *Polymorphus minutus* to different water qualities in a 22-day flow channel experiment. As biological responses we measured mortality rates, concentrations or activities of five biomarkers, and overall locomotor activity. No significant differences were found between mortality rates of different exposure conditions. Contrastingly, three biomarkers (phenoloxidase activity, glycogen, and lipid concentrations) showed a significant increase in infected gammarids, while the effect of the water type was not significant. Infected gammarids also showed a significantly higher locomotor activity in both water types. Our results suggest that the response of *G. fossarum* during the exposure experiments was mainly driven by parasite infection. This implies that parasites may act as additional biotic stressors in multiple stressor scenarios, and therefore, might play an important role when measuring the response of organisms to chemical stressors.

Are DNA barcoding, iBLOs, BOLD-Systems and BINs opportunities or just fashionable gadgets for Acanthocephalans?

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In 2003 DNA barcoding was introduced as a taxonomic tool. The principle was simple: 1) specimens of clearly established species would be identified by expert taxonomists, 2) a barcode region sequence produced (e.g. the Cytochrome Oxidase I), 3) such sequences being made publicly available to serve as reference libraries and 4) then, any research handling a specimen tricky to be determined as based on morphology could produce barcode sequence to be compared to the relevant reference library. Low molecular divergence would serve as a surrogate to ascribe/assign the specimen to one species.

Since 2003, DNA barcoding developed exponentially. The Barcode of Life initiatives (iBOLs) are involving millions of dollars and hundreds of countries. Barcode Index Numbers (BINs) were suggested to be unique for each species named by taxonomists based on morphology. The Barcode of life Data – Systems (BOLD-Systems) a cloud-based data storage and analysis platform (<https://v4.boldsystems.org/index.php>) is currently hosting 11,297k barcodes, clustered in 779k BINs for 242k animal + 71k plant + 24k fungi and others species.

This communication will be based on a literature review and data available in BOLD for acanthocephalans. It will challenge the fact that DNA barcoding, iBOL, BOLD-Systems and BINs could be either opportunities or simply useless gadgets for the community of scientists working on acanthocephalans.

Many issues are indeed to be discussed for acanthocephalans. Hereafter is a list which is far from being exhaustive: 1) is the congruence between morphology-based and phylogeny-based definition of a species fulfilled? 2) The BOLD-Systems is not curated, but is open to the idea. Who within the acanthocephalan community would be interested in managing such time-consuming task? 3) Only few institutions are uploading barcodes to BOLD, most data being directly “mined” by BOLD from GenBank. Again, some curation is needed! 4) What about pseudogenes

for COI? 5) Several BINs have been observed for the same nominal species, a case of cryptic diversity or an over-estimation of diversity by BINs?

Characterization of the complete mitochondrial genome of *Pseudoacanthocephalus bufonis* (Shiple, 1903) (Acanthocephala: Echinorhynchidae) and its phylogenetic implications

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The phylum Acanthocephala is an important group of obligate endoparasites, occurring in the alimentary canal of all major vertebrate groups. The current genetic data of Acanthocephala, especially the mitochondrial genomes, are still very limited. Moreover, our present knowledge regarding the basic molecular phylogenetic framework of acanthocephalans remains far from complete. In the present study, the complete mitochondrial genome of *Pseudoacanthocephalus bufonis* (Shiple, 1903), the first representative of the genus *Pseudoacanthocephalus* in the family Echinorhynchidae, was sequenced and annotated for the first time based on specimens collected from the spot-legged tree frog *Polypedates megacephalus* Hallowell (Amphibia: Rhacophoridae) in China. The mitogenome of *P. bufonis* is 14,056 bp in length, including 36 genes [12 protein-coding genes (PCGs) (lacking *atp8*), 22 tRNA genes and 2 rRNA genes (*rrnL* and *rrnS*)] and two non-coding regions (NCR1 and NCR2). Phylogenetic analyses based on the amino acid sequences of 12 protein-coding genes using maximum likelihood (ML) and Bayesian inference (BI) in different models revealed that the order Echinorhynchida and the family Echinorhynchidae are both paraphyletic. The current systematic status of the genus *Pseudoacanthocephalus* in the Echinorhynchidae was challenged, due to *P. bufonis* clustered together with *Cavisoma magnum* (Cavisomidae) rather than *Echinorhynchus truttae* (Echinorhynchidae). Our phylogenetic results also strongly supported the

monophyly of the order Polymorphida, and indicated that the Polymorphidae have closer relationship with the Centrorhynchidae, rather than the Plagiorhynchidae. The phylogenetic results provided a new insight into the evolutionary relationships of family-above taxa within Acanthocephala.

Keywords: parasite, Acanthocephala, mitochondrial genome, phylogeny, wildlife

Approaching a better understanding of toxicant exposure and parasite infection complexity with mechanistic modelling

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Enormous studies have indicated complexity in the interactions between environmental pollution and parasite infection. One factor contributes to such complexity is the specificity of pollutants. For instance, the partitioning between parasites and their hosts vary among metals. We have developed a physiologically-based pharmacokinetic (PBPK) model to delineate the factors that contribute to the variations. The model was characterised based on metal-specific parameters (i.e. the fraction in blood plasma and the tissue-blood partition coefficient) and physiological characteristics of fish (i.e. the blood flow and the tissue weight). The model was calibrated for the accumulation of various metals (silver, cobalt, copper, and zinc) in the system of the acanthocephalan *Pomphorhynchus tereticollis* and the chub *Squalius cephalus*. The results of model calibration show that metal availability to parasites, which is controlled by organotropism and fate, is critical to metal accumulation in acanthocephalans. Another factor that account for the complicated interactions between environmental pollution and parasite is the complex life cycle of parasites and diverse effects of pollutants on each stage. Metal exposure might interfere with parasite development and transmission by affecting the survival, activity, and infectivity of different states, on the one hand, and the sensitivity of the host, on the other hand. We developed a conceptual model for simulating the development and transmission of acanthocephalans during their life cycle. The number of parasites is determined by immigration (that is the infection in the host) and emigration (including predation, transmission, and mortality). For the intermediate host, its population is assumed to be composed of three groups: uninfected, non-infective (i.e. containing acanthocephalans at the non-effective stage), and infective (i.e. containing the effective stage, cystacanth). For the definite host, three groups are distinguished: uninfected, latent (containing adult acanthocephalans at the pre-patent stage), and shedding (containing adult acanthocephalans that are shedding eggs).

Abstracts

Posters

In alphabetical order

New records of acanthocephalans from fish-eating birds from the Western Mediterranean

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Acanthocephalans from fish-eating birds from the Iberian Peninsula have been generally neglected. Here, we explore the diversity of acanthocephalans infecting fish-eating birds in an area with high fishing and aquaculture activity in the western Mediterranean coast. More than 150 fish-eating birds belonging to 3 families and 8 species were examined for acanthocephalans. Birds were found dead in L'Albufera National Park and El Saler located near Valencia (Spain) between 2012 and 2016. Using an integrative approach, combining morphological (light and scanning electron microscopy) and molecular (partial *cox1* sequences) data, we identified three acanthocephalan species: *i*) Adults of *Southwellina hispida* (Van Cleave, 1925) (Polymorphidae) from the grey heron *Ardea cinerea* L. (2 out of 3 herons) and an immature female from the great cormorant *Phalacrocorax carbo* (L.) (1 out of 10); *ii*) Immature forms of *Plagiorhynchus* (*Prosthorhynchus*) *cylindraceus* (Goeze, 1782) (Plagiorhynchidae) from the Audouin's gull *Ichthyaeetus audouinii* Payraudeau (1 out of 13); and *iii*) cystacanths of *Centrorhynchus* sp. (Centrorhynchidae) from the yellow-legged gull *Larus michahellis* Naumann (3 out of 45 gulls). Novel sequences were generated for *S. hispida* (three adult isolates ex. *A. cinerea* and one immature isolate ex. *P. carbo*) and *P. (P.) cylindraceus* (on immature isolate ex. *I. audouinii*). These sequences represent the first molecular data for *S. hispida* in Europe and the first for *P. (P.) cylindraceus* in Spain. Our study suggests that seagulls may act as accidental hosts for polymorphid acanthocephalans with terrestrial life cycles (i.e., *Plagiorhynchus* spp. and *Centrorhynchus* spp.). The presence of terrestrial acanthocephalans in seagulls may be related to their habits of preying near waste disposal centers, where they could ingest the intermediate hosts, e.g., terrestrial isopods. This first exploration on the diversity of acanthocephalans from fish-eating birds from the Western

Mediterranean indicates much lower species richness compared with other regions of the world.

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Acanthocephalans from amphibians: novel
molecular data for *Acanthocephalus ranae*
(Schrank, 1788) (Echinorhynchida:
Paracanthocephalidae) parasitising water frogs
(*Pelophylax* spp.) in Slovakia

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Acanthocephalus ranae (Schrank, 1788) is widely distributed acanthocephalan of anurans in Europe and Asia. Adults of this species have also been reported in reptiles, birds and mammals from several European countries, but infections in these hosts may be a result of post-cyclic parasitism. Despite the broad spectrum of hosts and wide geographical distribution of *A. ranae*, only a partial sequence of the 18S rRNA gene from a cystacanth isolate of this species ex. *Asellus aquaticus* from France (GenBank LS991433) is publicly available. A large number of *A. ranae* adults were collected during the survey of parasitic helminths of two water frog species (*Pelophylax esculentus* and *P. ridibundus*) at five localities in Slovakia (Gbelce, Rusovce, Šaštín-Stráže, Šulianske jazero and Veľký Lél). Here, we provide the first molecular characterization (18S, 28S rRNA and *cox1* genes) associated with detailed morphological identification of adults of this typically amphibian acanthocephalan. In addition, *cox1* sequences were used to investigate the haplotype diversity of *A. ranae* at small geographical scale. The phylogenetic reconstructions for the concatenated three-gene dataset placed with strong support *A. ranae* as a sister lineage to *A. lucii*. Unexpectedly, our newly-generated *cox1* sequences formed a strongly-supported clade with an unpublished sequence from an isolate of an unidentified *Acanthocephalus* sp. ex. *Anguilla anguilla* from Austria (GenBank MT682935). Six haplotypes were identified in the 32 *cox1* sequences (five shared haplotypes and one singleton), and one haplotype was

shared by isolates from Slovakia and Austria. The intraspecific genetic divergence (uncorrected p -distance) observed within the *cox1* sequences ranged between 0.00 to 1.04% for *A. ranae*. Our novel molecular data open new opportunities for future ecological studies on the distribution, host ranges, and genetic structure of this acanthocephalan in Europe.

This study was supported by Institute of Parasitology, BC CAS (RVO: 60077344) and the Scientific Grant Agency of the Slovak Republic (VEGA 1/0583/22).

New data on the chromosomes of *Neoechinorhynchus rutili* and a brief overview of the cytogenetics of Acanthocephala

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Acanthocephalans are one of the least karyologically studied group, for which only partial and incomplete publications are available. Here we review the literature from 1891 to 2022 and the current state of knowledge of chromosomes within all orders of Acanthocephala. So far, only 13 thorny-headed worms have been studied (~1% of known species), whose chromosome number varies between 5 and 16, and whose sex-determining mechanisms are XX in females and XY or XO in males. Most studies focused on classical cytogenetics and examined only chromosome number and, in only 3 species, also morphology. The modern molecular approach has been neglected and so far only applied to *Pomphorhynchus* spp. where the species-specific location of the rDNA loci distinguishes the two sibling species *P. laevis* and *P. tereticollis*. So far, no chromosome end composition has been identified.

For the first time, a detailed karyotype analysis of *Neoechinorhynchus rutili* was performed using classical and molecular cytogenetic methods. The karyotype consists of six autosomal chromosomes (metacentric and acrocentric) and two (female) or one (male) sex chromosomes X (acrocentric), on which an apparent secondary constriction was found ($2n = 7/8$, $n = 2m + 1a + 1a(X)$). The AT-rich heterochromatin blocks were located in the centromeric regions of all chromosomes, but in addition were also found in the pericentromeric regions of the metacentric chromosome pair #2. The major rDNA sites detected by fluorescence in situ hybridization (FISH) with an 18S rDNA probe were situated in three loci and were found to be polymorphic in size. Clusters of ribosomal genes were located interstitially in pericentromeric regions of the long arms of metacentric chromosomes #1 and in pericentromeric regions of the short and long arms of metacentric chromosomes #2. Hybridization signals at the long chromosome arms were stronger in both chromosome pairs, indicating a greater

amount of rDNA at these sites compared to the NOR site at the short arms of chromosomes #2.

The karyological analysis of thorny-headed worms deserves more attention than it has received so far, as the characters obtained by cytogenetic techniques could be useful in solving taxonomic and evolutionary problems in Acanthocephala.

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Matching host samples: inoculation of eggs of acanthocephalan parasites by faeces as a novel method for experimental infections in aquatic intermediate hosts.

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Matching the number of samples regarding to infection probability or parasite burden is problematic, even more in intermediate hosts which are part of complex life cycles. These individuals accumulate parasites across its ontogeny and hence to obtain samples from nature of parasitized and no-parasitized individuals to different size becomes a challenge for ecological studies. Here, we report the eggs inoculation through infected feces as globally applicable methodology for to infect for first time to intermediate host and re-infect them. The experiment includes non-parasitized hosts of small size and maintained for 18 months in controlled laboratory conditions. We aimed to experimentally infect individuals and repeatability the infection over time and then to quantify the parasite occurrence, development larval time and parasite burden post infections. Using a series of experimental aquariums, we demonstrate the reliability of our experimental egg's infections. We apply this methodology in a complex parasite system that include the acanthocephalan parasite *Profilicollis altmani*, the mole crabs *Emerita analoga* and fresh feces of one of their definitive seagull hosts, the Grey gull *Leucophaeus modestus*. Our results showed that experimental infections were successful; 55% of inoculated hosts were parasitized with the first infection, and all of them were parasitized with the second infection. The small size crabs harbored less parasites than large crabs. The acanthellae larvae appear after seven days post-first inoculation and it was developed to cystacanth six days after the acanthellae. This study provides experimental evidence to open a way to balance the number of samples and repeat in other experiments that use parasites with complex life cycles.

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Mornings schedule

Sunday 28

Monday 29

Tuesday 30

Wednesday 31

	8h30 Registrations		
	9h Opening session Organisation of thematic round tables	9h Isaure de Buron	9h Liang Li
	9h30 Bernd Sures	9h40 Martín García Varela	9h40 Rui-Jia Yang
	10h10 Daniel Barčák	10h Rémi Wattier	10h10 Jesús S. Hernández-Orts
	10h30 Coffee break	10h20 Coffee break	10h20 Florian Reyda
	11h Vlatka Filipović Marijić	10h50 Thierry Rigaud	10h40 Coffee break
	11h30 Sara Šariri	11h30 Marie-Jeanne Perrot-Minnot	11h10 Omar M. Amin
	12h Anna Marková	11h50 Camille-Sophie Cozzarolo	12h Lunch break
	12h20 Lunch break	12h10 Lunch break	Poster session

Afternoons schedule

Sunday 28	Monday 29	Tuesday 30	Wednesday 31
		13h40 Milen Nachev	
	14h T.T. Yen Le	14h Halima Jmii Chine	14h Thematic round tables (parallel sessions)
		14h20 Alexandre Bauer	
	14h40 Bahram Sayyaf Dezfuli		
	15h20 Coffee break	15h Coffee break	
	16h30 Guided tour	15h30 Clos Vougeot guided tour	15h30 Coffee break
16h Registrations (Gabriel building)	"The Dukes of Burgundy" Beaux-Arts museum		
17h Welcome aperitif on campus (Gabriel building)			17h Closing session
	19h Free evening		17h30 Free evening
		19h30 Conference dinner, Guinguette at Glanon beach	